



# **TOXICOLOGICAL REVIEW**

**OF**

# **TRICHLOROETHYLENE**

## **CHAPTER 4**

(CAS No. 79-01-6)

**In Support of Summary Information on the  
Integrated Risk Information System (IRIS)**

*September 2011*

## **4. HAZARD CHARACTERIZATION**

This section presents the hazard characterization of TCE health effects. Because of the number of studies and their relevance to multiple endpoints, the evaluation of epidemiologic studies of cancer and TCE is summarized in Section 4.1 (endpoint-specific results are presented in subsequent sections). Genotoxicity data are discussed in Section 4.2. Due to the large number of endpoints and studies in the toxicity database, subsequent sections (see Sections 4.3–4.10) are organized by tissue/organ system. Each section is further organized by noncancer and cancer endpoints, discussing data from human epidemiologic and laboratory experimental studies. In cases where there is adequate information, the role of metabolism in toxicity, comparisons of toxicity between TCE and its metabolites, and carcinogenic mode of action are also discussed. Finally, Section 4.11 summarizes the overall hazard characterization and the weight of evidence for noncancer and carcinogenic effects.

### **4.1. EPIDEMIOLOGIC STUDIES ON CANCER AND TCE—METHODOLOGICAL OVERVIEW**

This brief overview of the epidemiologic studies on cancer and TCE below provides background to the discussion contained in Sections 4.4–4.10. Over 50 epidemiologic studies on cancer and TCE exposure (see Tables 4-1 through 4-3) were examined to assess their ability to inform weight-of-evidence evaluation (i.e., to inform the cancer hazard from TCE exposure) according to 15 standards of study design (see Table 4-4), conduct, and analysis. The analysis of epidemiologic studies on cancer and TCE serves to document essential design features, exposure assessment approaches, statistical analyses, and potential sources of confounding and bias. This analysis, furthermore, supports the discussion of site-specific cancer observations in Sections 4.4–4.9. In those sections, study findings are presented with an assessment and discussion of their observations according to a study's weight of evidence and the potential for alternative explanations, including bias and confounding. Tables containing observed findings for site-specific cancers are also found in Sections 4.4–4.9. Full details of the weight-of-evidence-review to identify a cancer hazard and study selections for meta-analysis may be found in Appendix B.

**Table 4-1. Description of epidemiologic cohort and proportionate mortality ratio (PMR) studies assessing cancer and TCE exposure**

Reference	Description	Study group (N) comparison group (N)	Exposure assessment and other information
<b>Aircraft and aerospace workers</b>			
Radican et al. (2008); Blair et al. (1998)	Civilian aircraft-maintenance workers with at least 1 yr in 1952–1956 at Hill Air Force Base, Utah. VS to 1990 (Blair et al., 1998) or 2000 (Radican et al., 2008); cancer incidence 1973–1990 (Blair et al., 1998)	14,457 (7,204 ever exposed to TCE). Incidence (Blair et al., 1998) and mortality rates (Radican et al., 2008; Blair et al., 1998) of nonchemical exposed subjects.	Most subjects (n = 10,718) with potential exposure to 1–25 solvents. Cumulative TCE assigned to individual subjects using JEM. Exposure-response patterns assessed using cumulative exposure, continuous or intermittent exposures, and peak exposure. TCE replaced in 1968 with 1,1,1-trichloroethane and was discontinued in 1978 in vapor degreasing activities. <b>Median TCE exposures were about 10 ppm for rag and bucket; 100–200 ppm for vapor degreasing.</b> Poisson regression analyses controlled for age, calendar time, sex (Blair et al., 1998), or Cox proportional hazard model for age and race.
Krishnadasan et al. (2007)	Nested case-control study within a cohort of 7,618 workers employed between 1950 and 1992, or who had started employment before 1980 at Boeing/Rockwell/Rocketdyne [SSFL, the UCLA cohort of Morgenstern (1997)]. Cancer incidence 1988–1999.	326 prostate cancer cases, 1,805 controls. Response rate: cases, 69%; controls, 60%.	JEM for TCE, hydrazine, PAHs, benzene, and mineral oil constructed from company records, walk-through, or interviews. Lifestyle factors obtained from living subjects through mail and telephone surveys. Conditional logistic regression controlled for cohort, age at diagnosis, physical activity, SES, and other occupational exposure (benzene, PAHs, mineral oil, hydrazine).

**Table 4-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)**

Reference	Description	Study group (N) comparison group (N)	Exposure assessment and other information
Ritz et al. (1999); Zhao et al. (2005)	Aerospace workers with $\geq 2$ yrs of employment at Rockwell/Rocketdyne (now Boeing) and who worked at SSFL, Ventura, California, from 1950 to 1993 [the UCLA cohort of (Morgenstern et al., 1997)]. Cancer mortality as of December 31, 2001. Cancer incidence 1988–2000 for subjects alive as of 1988.	6,044 (2,689 with high cumulative exposure to TCE). Mortality rates of subjects in lowest TCE exposure category. 5,049 (2,227 with high cumulative exposure to TCE). Incidence rates of subjects in lowest TCE exposure category.	JEM for TCE, hydrazine, PAHs, mineral oil, and benzene. IH ranked each job title ranked for presumptive TCE exposure as high (3), medium (2), low (1), or no (0) exposure for three time periods (1951–1969, 1970–1979, 1980–1989). Cumulative TCE score: low ( $<3$ ), medium ( $>3-12$ ), high ( $>12$ ) assigned to individual subjects using JEM. Cox proportional hazard, controlled for time, since 1 <sup>st</sup> employment, SES, age at diagnosis, and hydrazine exposure.
Boice et al. (2006b)	Aerospace workers with $\geq 6$ mo employment at Rockwell/Rocketdyne (SSFL and nearby facilities) from 1948 to 1999 [IEI cohort (IEI, 2005)]. VS to 1999.	41,351, 1,642 male hourly test stand mechanics (1,111 with potential TCE exposure). Mortality rates of U.S. population and California population. Internal referent groups including male hourly nonadministrative Rocketdyne workers; male hourly, nonadministrative SSFL workers; and test stand mechanics with no potential exposure to TCE.	Potential TCE exposure assigned to test stands workers only whose tasks included the cleaning or flushing of rocket engines (engine flush) (n = 639) or for general utility cleaning (n = 472); potential for exposure to large quantities of TCE was much greater during engine flush than when TCE used as a utility solvent. JEM for TCE and hydrazine without semiquantitative intensity estimates. Exposure to other solvents not evaluated due to low potential for confounding (few exposed, low exposure intensity, or not carcinogenic). Exposure metrics included employment duration, employment decade, year worked with potential TCE exposure, and year worked with potential TCE exposure via engine cleaning, weighted by number of tests. Lifetable (SMR); Cox proportional hazard controlling for birth year, hire year, and hydrazine exposure.
Boice et al. (1999)	Aircraft-manufacturing workers with at least 1 yr $\geq 1960$ at Lockheed Martin (Burbank, California). VS to 1996.	77,965 (2,267 with potential routine TCE exposures and 3,016 with routine or intermittent TCE exposure). Mortality rates of U.S. population (routine TCE exposed subjects) and nonexposed internal referents (routine and intermittent TCE exposed subjects).	12% with potential routine mixed solvent exposure and 30% with route or intermittent solvent exposure. JEM for potential TCE exposure on (1) routine basis or (2) intermittent or routine basis without semiquantitative intensity estimate. Exposure-response patterns assessed by any exposure or duration of exposure and internal control group. Vapor degreasing with TCE before 1966 and perchloroethylene, afterwards. Lifetable analyses; Poisson regression analysis adjusting for birth date, starting employment date, finishing employment date, sex, and race.

**Table 4-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)**

Reference	Description	Study group (N) comparison group (N)	Exposure assessment and other information
Morgan et al. (1998)	Aerospace workers with ≥6 mo 1950–1985 at Hughes (Tucson, Arizona). VS to 1993.	20,508 (4,733 with TCE exposures). Mortality rates of U.S. population for overall TCE exposure; mortality rates of all-other cohort subjects (internal referents).	TCE exposure intensity assigned using JEM. Exposure-response patterns assessed using cumulative exposure (low vs. high) and job with highest TCE exposure rating (peak, medium/high exposure vs. no/low exposure). <b>“High exposure” job classification defined as &gt;50 ppm.</b> Vapor degreasing with TCE 1952–1977, but limited IH data <1975. Limited IH data before 1975 and medium/low rankings likely misclassified given temporal changes in exposure intensity not fully considered (NRC, 2006).
Costa et al. (1989)	Aircraft manufacturing workers employed 1954–1981 at plant in Italy. VS to 1981.	8,626 subjects Mortality rates of the Italian population.	No exposure assessment to TCE and job titles grouped into one of four categories: blue- and white-collar workers, technical staff, and administrative clerks. Lifetable (SMR).
Garabrant et al. (1988)	Aircraft manufacturing workers ≥4 yrs employment and who had worked at least 1 d at San Diego, California, plant 1958–1982. VS to 1982.	14,067 Mortality rates of U.S. population.	TCE exposure assessment for 70 of 14,067 subjects; 14 cases of esophageal cancer and 56 matched controls. For these 70 subjects, company work records identified 37% with job title with potential TCE exposure without quantitative estimates. Lifetable (SMR).
<b>Cohorts identified from biological monitoring (U-TCA)</b>			
Hansen et al. (2001)	Workers biological monitored using U-TCA and air-TCE, 1947–1989. Cancer incidence from 1964 to 1996.	803 total. Cancer incidence rates of the Danish population.	712 with U-TCA, 89 with air-TCE measurement records, two with records of both types. U-TCA from 1947 to 1989; air TCE measurements from 1974. Historic median exposures estimated from the U-TCA concentrations were: 9 ppm for 1947–1964, 5 ppm for 1965–1973, 4 ppm for 1974–1979, and 0.7 ppm for 1980–1989. Air TCE measurements from 1974 onward were 19 ppm (mean) and 5 ppm (median). <b>Overall, median TCE exposure to cohort as extrapolated from air TCE and U-TCA measurements was 4 ppm (arithmetic mean, 12 ppm).</b> Exposure metrics: year 1 <sup>st</sup> employed, employment duration, mean exposure, cumulative exposure. Exposure metrics: employment duration, average TCE intensity, cumulative TCE, period 1 <sup>st</sup> employment. Lifetable analysis (SIR).
Anttila et al. (1995)	Workers biological monitored using U-TCA, 1965–1982. VS 1965–1991 and cancer incidence 1967–1992.	3,974 total (3,089 with U-TCA measurements). Mortality and cancer incidence rates of the Finnish population.	Median U-TCA, 63 µmol/L for females and 48 µmol/L for males; mean U-TCA was 100 µmol/L. Average 2.5 U-TCA measurements per individual. Using the Ikeda et al. (1972) relationship for TCE exposure to U-TCA, <b>TCE exposures were roughly 4 ppm (median) and 6 ppm (mean).</b> Exposure metrics: year since 1 <sup>st</sup> measurement. Lifetable analysis (SMR, SIR).

**Table 4-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)**

Reference	Description	Study group (N) comparison group (N)	Exposure assessment and other information
Axelsson et al. (1994)	Workers biological monitored using U-TCA, 1955–1975. VS to 1986 and cancer incidence 1958–1987.	1,421 males. Mortality and cancer incidence rates of Swedish male population.	Biological monitoring for U-TCA from 1955 and 1975. <b>Roughly ¾ of cohort had U-TCA concentrations equivalent to &lt;20 ppm TCE.</b> Exposure metrics: duration exposure, mean U-TCA. Lifetable analysis (SMR, SIR).
<b>Other cohorts</b>			
Clapp and Hoffman (2008)	Deaths between 1969 and 2001 among employees ≥5 yrs employment duration at an IBM facility (Endicott, New York).	360 deaths. Proportion of deaths among New York residents during 1979–1998.	No exposure assessment to TCE. PMR analysis.
Sung et al. (2008; 2007)	Female workers 1 <sup>st</sup> employed 1973–1997 at an electronics (RCA) manufacturing factory (Taoyuan, Taiwan). Cancer incidence 1979–2001 (Sung et al., 2007). Childhood leukemia 1979–2001 among first born of female subjects in Sung et al. (2008).	63,982 females and 40,647 females with 1 <sup>st</sup> live born offspring. Cancer incidence rates of Taiwan population (Sung et al., 2007). Childhood leukemia incidence rates of first born live births of Taiwan population (Sung et al., 2008).	No exposure assessment. Chlorinated solvents including TCE and perchloroethylene found in soil and groundwater at factory site. Company records indicated TCE not used 1975–1991 and perchloroethylene 1975–1991 and perchloroethylene after 1981. No information for other time periods. Exposure-response using employment duration. Lifetable analysis (SMR, SIR) (Sung et al., 2007; Chang et al., 2005; Chang et al., 2003) or Poisson regression adjusting for maternal age, education, sex, and birth year (Sung et al., 2008).
Chang et al. (2003; 2005)	Male and female workers employed 1978–1997 at electronics factory as studied by Sung et al. (2007). VS from 1985 to 1997 and cancer incidence 1979 to 1997.	86,868 total. Incidence (Chang et al., 2005) mortality (Chang et al., 2003) rates Taiwan population.	
ATSDR (2004a)	Workers 1952–1980 at the View-Master factory (Beaverton, Oregon).	616 deaths 1989–2001. Proportion of deaths between 1989 and 2001 in Oregon population.	No exposure information on individual subjects. TCE and other VOCs detected in well water at the time of the plant closure in 1998 were TCE, 1,220–1,670 µg/L; 1,1-DCE, up to 33 µg/L; and, perchloroethylene up to 56 µg/L. PMR analysis.
Raaschou-Nielsen et al. (2003)	Blue-collar workers employed >1968 at 347 Danish TCE-using companies. Cancer incidence through 1997.	40,049 total (14,360 with presumably higher level exposure to TCE). Cancer incidence rates of the Danish population.	Employers had documented TCE usage. Blue-collar vs. white-collar workers and companies with <200 workers were variables identified as increasing the likelihood for TCE exposure. Subjects from iron and metal, electronics, painting, printing, chemical, and dry cleaning industries. <b>Median exposures to TCE were 40–60 ppm for the year before 1970, 10–20 ppm for 1970–1979, and approximately 4 ppm for 1980–1989.</b> Exposure metrics: employment duration, year 1 <sup>st</sup> employed, and # employees in company. Lifetable (SIR).

**Table 4-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)**

<b>Reference</b>	<b>Description</b>	<b>Study group (N) comparison group (N)</b>	<b>Exposure assessment and other information</b>
Ritz ( <a href="#">1999a</a> )	Male uranium-processing plant workers $\geq 3$ mo employment 1951–1972 at DOE facility (Fernald, Ohio). VS 1951–1989, cancer.	3,814 white males monitored for radiation (2,971 with potential TCE exposure). Mortality rates of the U.S. population; non-TCE exposed internal controls for TCE exposure-response analyses.	JEM for TCE, cutting fluids, kerosene, and radiation generated by employees and industrial hygienists. Subjects assigned potential TCE according to intensity: light (2,792 subjects), moderate (179 subjects), heavy (no subjects). Lifetable (SMR) and conditional logistic regression adjusted for pay status, date first hire, radiation.
Henschler et al. ( <a href="#">1995</a> )	Male workers $\geq 1$ yr 1956–1975 at cardboard factory (Arnsberg region, Germany). VS to 1992.	169 exposed; 190 unexposed. Mortality rates from German Democratic Republic (broad categories) or RCC incidence rates from Danish population, German Democratic, or non-TCE exposed subjects.	Walk-through surveys and employee interviews used to identify work areas with TCE exposure. TCE exposure assigned to renal cancer cases using workman’s compensation files. Lifetable (SMR, SIR) or Mantel-Haenszel.
Greenland et al. ( <a href="#">1994</a> )	Cancer deaths, 1969–1984, among pensioned workers employed <1984 at GE transformer manufacturing plant (Pittsfield, Massachusetts), and who had job history record; controls were noncancer deaths among pensioned workers.	512 cases, 1,202 controls. Response rate: cases, 69%; controls, 60%.	IH assessment from interviews and position descriptions. TCE (no/any exposure) assigned to individual subjects using JEM. Logistic regression.
Sinks et al. ( <a href="#">1992</a> )	Workers employed 1957–1980 at a paperboard container manufacturing and printing plant (Newnan, Georgia). VS to 1988. Kidney and bladder cancer incidence through 1990.	2,050 total. Mortality rates of the U.S. population, bladder and kidney cancer incidence rates from the Atlanta-SEER registry for the years 1973–1977.	No exposure assessment to TCE; analyses of all plant employees including white- and blue-collar employees. Assignment of work department in case-control study based upon work history; Material Safety Data Sheets identified chemical usage by department. Lifetable (SMR, SIR) or conditional logistic regression adjusted for hire date and age at hire, and using 5- and 10-yr lagged employment duration.
Blair et al. ( <a href="#">1989</a> )	Workers employed 1942–1970 in U.S. Coast. VS to 1980.	3,781 males of whom 1,767 were marine inspectors (48%). Mortality rates of the U.S. population. Mortality rates of marine inspectors also compared to that of noninspectors.	No exposure assessment to TCE. Marine inspectors worked in confined spaces and had exposure potential to multiple chemicals. TCE was identified as one of 10 potential chemical exposures. Lifetable (SMR) and directly adjusted RRs.

**Table 4-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)**

<b>Reference</b>	<b>Description</b>	<b>Study group (N) comparison group (N)</b>	<b>Exposure assessment and other information</b>
Shannon et al. (1988)	Workers employed $\geq 6$ mo at GE lamp manufacturing plant, 1960–1975. Cancer incidence from 1964 to 1982.	1,870 males and females, 249 (13%) in coiling and wire-drawing area. Cancer incidence rates from Ontario Cancer Registry.	No exposure assessment to TCE. Workers in CWD had potential exposure to many chemicals including metals and solvents. A 1955-dated engineering instruction sheet identified TCE used as degreasing solvent in CWD. Lifetable (SMR).
Shindell and Ulrich (1985)	Workers employed $\geq 3$ mo at a TCE manufacturing plant 1957–1983. VS to 1983.	2,646 males and females. Mortality rates of the U.S. population.	No exposure assessment to TCE; job titles categorized as either white- or blue-collar. Lifetable analysis (SMR).
Wilcosky et al. (1984)	Respiratory, stomach, prostate, lymphosarcoma, and lymphatic leukemia cancer deaths 1964–1972 among 6,678 active and retired production workers at a rubber plant (Akron, Ohio); controls were a 20% age-stratified random sample of the cohort.	183 cases (101 respiratory, 33 prostate, 30 stomach, 9 lymphosarcoma, and 10 lymphatic leukemia cancer deaths).	JEM without quantitative intensity estimates for 20 exposures including TCE. Exposure metric: ever held job with potential TCE exposure.

CWD = coiling and wire drawing; DOE = U.S. Department of Energy; GE = General Electric; IBM = International Business Machines Corporation; IEI = International Epidemiology Institute; IH = industrial hygienist; JEM = job-exposure matrix; PAH = polycyclic aromatic hydrocarbon; RCC = renal cell carcinoma; RR = relative risk; SES = socioeconomic status; SIR = standardized incidence ratio; SMR = standardized mortality ratio; SSFL = Santa Susanna Field Laboratory; U-TCA = urinary TCA; UCLA = University of California, Los Angeles; VS = vital status

**Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure**

Reference	Population	Study group (N) comparison group (N) response rates	Exposure assessment and other information
<b>Bladder</b>			
Pesch et al. (2000a)	Histologically confirmed urothelial cancer (bladder, ureter, renal pelvis) cases from German hospitals (five regions) in 1991–1995; controls randomly selected from residency registries matched on region, sex, and age.	1,035 cases. 4,298 controls. Cases, 84%; controls, 71%.	Occupational history using job title or self-reported exposure. JEM and JTEM to assign exposure potential to metals and solvents (chlorinated solvents, TCE, perchloroethylene). Lifetime exposure to TCE exposure examined as 30 <sup>th</sup> , 60 <sup>th</sup> , and 90 <sup>th</sup> percentiles (medium, high, and substantial) of exposed control exposure index. Duration used to examine occupational title and job task duties and defined as 30 <sup>th</sup> , 60 <sup>th</sup> , and 90 <sup>th</sup> percentiles (medium, long, and very long) of exposed control durations. Logistic regression with covariates for age, study center, and smoking.
Siemiatycki et al. (1994); (1991)	Male bladder cancer cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	484 cases. 533 population controls; 740 other cancer controls. Cases, 78%; controls, 72%.	JEM to assign 294 exposures including TCE on semiquantitative scales categorized as any or substantial exposure. Other exposure metrics included exposure duration in occupation or job title. Logistic regression adjusted for age, ethnic origin, SES status, smoking, coffee consumption, and respondent status (occupation or job title) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, and respondent status (TCE).
<b>Brain</b>			
DeRoos et al. (2001); Olshan et al. (1999)	Neuroblastoma cases in children of <19 yrs selected from Children’s Cancer Group and Pediatric Oncology Group with diagnosis in 1992–1994; population controls (random digit dialing) matched to control on birth date.	504 cases. 504 controls. Cases, 73%; controls, 74%.	Telephone interview with parent using questionnaire to assess parental occupation and self-reported exposure history and judgment-based attribution of exposure to chemical classes (halogenated solvents) and specific solvents (TCE). Exposure metric was any potential exposure. Logistic regression with covariate for child’s age and material race, age, and education.

**Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)**

Reference	Population	Study group (N) comparison group (N) response rates	Exposure assessment and other information
Heineman et al. (1994)	White, male cases, age $\geq 30$ yrs, identified from death certificates in 1978–1981; controls identified from death certificates and matched for age, year of death and study area.	300 cases. 386 controls. Cases, 74%; controls, 63%.	In-person interview with next-of-kin; questionnaire assessing lifetime occupational history using job title and JEM of Gomez et al. (1994). Cumulative exposure metric (low, medium, or high) based on weighted probability and duration. Logistic regression with covariates for age and study area.
<b>Colon and rectum</b>			
Goldberg et al. (2001); Siemiatycki (1991)	Male colon cancer cases, 35–75 yrs, from 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	497 cases. 533 population controls and 740 cancer controls. Cases, 82%; controls, 72%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, ethnic origin, birthplace, education, income, parent's occupation, smoking, alcohol consumption, tea consumption, respondent status, heating source SES status, smoking, coffee consumption, and respondent status (occupation, some chemical agents) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, and respondent status (TCE).
Dumas et al. (2000); Simeiatycki (1991)	Male rectal cancer cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	292 cases. 533 population controls and 740 other cancer controls. Cases, 78%; controls, 72%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, education, respondent status, cigarette smoking, beer consumption and BMI (TCE) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, ethnic origin, and beer consumption (TCE).
Fredriksson et al. (1989)	Colon cancer cases aged 30–75 yrs identified through the Swedish Cancer Registry among patients diagnosed in 1980–1983; population-based controls were frequency-matched on age and sex and were randomly selected from a population register.	329 cases. 658 controls. Not available.	Mailed questionnaire assessing occupational history with telephone interview follow-up. Self-reported exposure to TCE defined as any exposure. Mantel-Haenszel stratified on age, sex, and physical activity.

**Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)**

Reference	Population	Study group (N) comparison group (N) response rates	Exposure assessment and other information
<b>Esophagus</b>			
Parent et al. (2000b); Siemiatycki (1991)	Male esophageal cancer cases, 35–75 yrs, diagnosed in 19 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	292 cases. 533 population controls; 740 subjects with other cancers. Cases, 78%; controls, 72%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, education, respondent status, cigarette smoking, beer consumption and BMI (solvents) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, ethnic origin, and beer consumption (TCE).
<b>Lymphoma</b>			
Purdue et al. (2011)	Cases aged 20–74 with histologically-confirmed NHL (B-cell diffuse and follicular, T-cell, lymphoreticular) without HIV in 1998–2000 and identified from four SEER areas (Los Angeles County and Detroit metropolitan area, random sample; Seattle_Puget Sound and Iowa, all consecutive cases); population controls aged 20–74 with no previous diagnosis of HIV infection or NHL, identified through (1) if >65 yrs of age, random digit dialing, or (2) if ≥65 yrs, identified from Medicare eligibility files and stratified on geographic area, age, and race.	1,321 cases. 1,057 controls. Cases, 76%; controls, 78%.	In-person interview using questionnaire or computer-assisted personal interview questionnaire specific for jobs held for >1 yr since the age of 16 yrs, hobbies, and medical and family history. For occupational history, 32 job- or industry-specific interview modules asked for detailed information on individual jobs and focused on solvents exposure, including TCE, assessment by expert industrial hygienist blinded to case and control status by levels of probability, frequency, and intensity. Exposure metric of overall exposure, average weekly exposure, year exposed, average exposure intensity, and cumulative exposure. Logistic regression adjusted for sex, age, race, education and SEER site.

**Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)**

Reference	Population	Study group (N) comparison group (N) response rates	Exposure assessment and other information
Gold et al. (2011)	Cases aged 35–74 with histologically-confirmed multiple myeloma in 2000–2002 and identified from Seer areas (Detroit, Seattle-Puget Sound); population controls.	181 cases. 481 controls. Cases, 71%; controls, 52%.	In-person interview using computer-assisted personal interview questionnaire for jobs held $\geq 1$ yr since 1941 (cases) or 1946 (controls) and since age 18 yrs. For occupational history, 20 occupations, job- or industry-specific interview modules asked for detailed information on individual jobs held at least 2 yrs and focused on solvents exposure, including TCE, assessment by expert industrial hygienist blinded to case and control status by levels of probability, duration, and cumulative exposure. Logistic regression adjusted for sex, age, race, education, and SEER site.
Cocco et al. (2010)	Histologically or cytologically confirmed cases aged $\geq 17$ yrs with lymphoma (B-cell, T-cell, CLL, multiple myeloma, Hodgkin) in 1998–2004 and residents of referral areas from seven European countries (Czech Republic, Finland, France, Germany, Ireland, Italy, and Spain); hospital (four participating countries) or population controls (all others); controls from: (1) Germany and Italy selected by random digit dialing from general population and matched (individually in German and group-based in Italy) to cases by sex, age, and residence area and (2) for all other countries, matched hospital controls with diagnoses other than cancer, infectious diseases, and immunodeficient diseases (individually in Czech Republic group-based in all other countries).	2,348 cases. 2,462 controls. Cases, 88%; controls, 81% hospital and 52% population.	In-person interviews using same structured questionnaire translated to the local language for information on sociodemographic factors, lifestyle, health history, and all full-time job held $\geq 1$ yr. Assessment by industrial hygienists in each participating center to 43 agents, including TCE, by confidence, exposure intensity, and exposure frequency. Exposure metric of overall TCE exposure and cumulative TCE exposure for subjects assessed with high degree of confidence. Logistic regression adjusted for age, gender, education, and study center.

**Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)**

Reference	Population	Study group (N) comparison group (N) response rates	Exposure assessment and other information
German centers: Seidler et al. (2007); Mester et al. (2006); Becker et al. (2004)	NHL and Hodgkin lymphoma cases aged 18–80 yrs identified through all hospitals and ambulatory physicians in six regions of Germany between 1998 and 2003; population controls were identified from population registers and matched on age, sex, and region.	710 cases. 710 controls. Cases, 87%; controls, 44%.	In-person interview using questionnaire assessing personal characteristics, lifestyle, medical history, UV light exposure, and occupational history of all jobs held for ≥1 yr. Exposure of a prior interest were assessed using job task-specific supplementary questionnaires. JEM used to assign cumulative quantitative TCE exposure metric, categorized according to the distribution among the control persons (50 <sup>th</sup> and 90 <sup>th</sup> percentile of the exposed controls). Conditional logistic regression adjusted for age, sex, region, smoking, and alcohol consumption.
Wang et al. (2009)	Cases among females aged 21 and 84 yrs with NHL in 1996–2000 and identified from Connecticut Cancer Registry; population-based female controls: (1) if <65 yrs of age, having Connecticut address stratified by 5-yr age groups identified from random digit dialing or (2) ≥65 yrs of age, by random selection from Centers for Medicare and Medicaid Service files.	601 cases. 717 controls. Cases, 72%; controls, 69% (<65 yrs), 47% (≥65 yrs)	In-person interview using questionnaire assessment specific for jobs held for >1 yr. Intensity and probability of exposure to broad category of organic solvents and to individual solvents, including TCE, estimated using JEM (Dosemeci et al., 1994; Gómez et al., 1994) and assigned blinded. Exposure metric of any exposure, exposure intensity (low, medium/high), and exposure probability (low, medium/high). Logistic regression adjusted for age, family history of hematopoietic cancer, alcohol consumption, and race.
Costantini et al. (2008); Miligi et al. (2006)	Cases aged 20–74 with NHL, including CLL, all forms of leukemia, or MM in 1991–1993 and identified through surveys of hospital and pathology departments in study areas and in specialized hematology centers in eight areas in Italy; population-based controls stratified by 5-yr age groups and by sex selected through random sampling of demographic or of National Health Service files.	1,428 NHL + CLL, 586 Leukemia, 263, MM. 1,278 controls (leukemia analysis). 1,100 controls (MM analysis). Cases, 83%; controls, 73%.	In-person interview primarily at interviewee’s home (not blinded) using questionnaire assessing specific jobs, extra occupational exposure to solvents and pesticides, residential history, and medical history. Occupational exposure assessed by job-specific or industry-specific questionnaires. JEM used to assign TCE exposure and assessed using intensity (two categories) and exposure duration (two categories). All NHL diagnoses and 20% sample of all cases confirmed by panel of three pathologists. Logistic regression with covariates for sex, age, region, and education. Logistic regression for specific NHL included an additional covariate for smoking.

**Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)**

Reference	Population	Study group (N) comparison group (N) response rates	Exposure assessment and other information
Persson and Fredriksson (1999); combined analysis of NHL cases in Persson et al. (1993; 1989)	Histologically confirmed cases of B-cell NHL, age 20–79 yrs, identified in two hospitals in Sweden: Oreboro in 1964–1986 (Persson et al., 1989) and in Linköping between 1975 and 1984 (Persson et al., 1993); controls were identified from previous studies and were randomly selected from population registers.	NHL cases, 199. 479 controls. Cases, 96% (Oreboro), 90% (Linköping); controls, not reported.	Mailed questionnaire to assess self reported occupational exposures to TCE and other solvents. Unadjusted Mantel-Haenszel $\chi^2$ .
Nordstrom et al. (1998)	Histologically-confirmed cases in males of hairy-cell leukemia reported to Swedish Cancer Registry in 1987–1992 (includes one case latter identified with an incorrect diagnosis date); population-based controls identified from the National Population Registry and matched (1:4 ratio) to cases for age and county.	111 cases. 400 controls. Cases, 91%; controls, 83%.	Mailed questionnaire to assess self reported working history, specific exposure, and leisure time activities. Univariate analysis for chemical-specific exposures (any TCE exposure).
Fritschi and Siemiatycki (1996b); Siemiatycki (1991)	Male NHL cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	215 cases. 533 population controls (Group 1) and 1,900 subjects with other cancers (Group 2). Cases, 83%; controls, 71%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales). Exposure metric defined as any or substantial exposure. Logistic regression adjusted for age, proxy status, income, and ethnicity (solvents) or Mantel-Haenszel stratified by age, BMI, and cigarette smoking (TCE).

**Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)**

Reference	Population	Study group (N) comparison group (N) response rates	Exposure assessment and other information
Hardell et al. (1994; 1981)	Histologically-confirmed cases of NHL in males, age 25–85 yrs, admitted to Swedish (Umea) hospital between 1974 and 1978; living controls (1:2 ratio) from the National Population Register, matched to living cases on sex, age, and place of residence; deceased controls from the National Registry for Causes of Death, matched (1:2 ratio) to dead cases on sex, age, place of residence, and year of death.	105 cases. 335 controls. Response rate not available.	Self-administered questionnaire assessing self-reported solvent exposure; phone follow-up with subject, if necessary. Unadjusted Mantel-Haenszel $\chi^2$ .
Persson et al. (1993; 1989)	Histologically confirmed cases of Hodgkin lymphoma, age 20–80 yrs, identified in two hospitals in Sweden: Oreboro in 1964–1986 (Persson et al., 1989) and in Linkoping between 1975 and 1984 (Persson et al., 1993); controls randomly selected from population registers.	54 cases (1989 study); 31 cases (1993 study). 275 controls (1989 study); 204 controls (1993 study). Response rate not available.	Mailed questionnaire to assess self reported occupational exposures to TCE and other solvents. Logistic regression with adjustment for age and other exposure; unadjusted Mantel-Haenszel $\chi^2$ .
<b>Childhood leukemia</b>			
Shu et al. (2004; 1999)	Childhood leukemia cases, <15 yrs, diagnosed between 1989 and 1993 by a Children’s Cancer Group member or affiliated institute; population controls (random digit dialing), matched for age, race, and telephone area code and exchange.	1,842 cases. 1,986 controls. Cases, 92%; controls, 77%.	Telephone interview with mothers, and whenever available, fathers, using questionnaire to assess occupation using job-industry title and self-reported exposure history. Questionnaire included questions specific for solvent, degreaser, or cleaning agent exposures. Logistic regression with adjustment for maternal or paternal education, race, and family income. Analyses of paternal exposure also included age and sex of the index child.

**Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)**

Reference	Population	Study group (N) comparison group (N) response rates	Exposure assessment and other information
Costas et al. (2002); MDPH (1997c, b)	Childhood leukemia (<19 yrs age) diagnosed in 1969–1989 and who were resident of Woburn, Massachusetts; controls randomly selected from Woburn public school records, matched for age.	19 cases. 37 controls. Cases, 91%; controls, not available.	Questionnaire administered to parents separately assessing demographic and lifestyle characteristics, medical history information, environmental and occupational exposure, and use of public drinking water in the home. Hydraulic mixing model used to infer delivery of TCE and other solvents water to residence. Logistic regression with composite covariate, a weighted variable of individual covariates.
McKinney et al. (1991)	Incident childhood leukemia and NHL cases, 1974–1988, ages not identified, from three geographical areas in England; controls randomly selected from children of residents in the three areas and matched for sex and birth health district.	109 cases. 206 controls. Cases, 72%; controls, 77%.	In-person interview with questionnaire with mother to assess maternal occupational exposure history, and with father and mother, as surrogate, to assess paternal occupational exposure history. No information provided in paper whether interviewer was blinded as to case and control status. Matched pair design using logistic regression for univariate and multivariate analysis.
Lowengart et al. (1987)	Childhood leukemia cases aged ≤10 yrs and identified from the Los Angeles (California) Cancer Surveillance Program in 1980–1984; controls selected from random digit dialing or from friends of cases and matched on age, sex, and race.	123 cases. 123 controls. Cases, 79%; controls, not available.	Telephone interview with questionnaire to assess parental occupational and self-reported exposure history. Matched (discordant) pair analysis.
<b>Melanoma</b>			
Fritschi and Siemiatycki (1996b); Siemiatycki (1991)	Male melanoma cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	103 cases. 533 population controls and 533 other cancer controls. Cases, 78%; controls, 72%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, education, and ethnic origin (TCE) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, and ethnic origin (TCE).

**Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)**

Reference	Population	Study group (N) comparison group (N) response rates	Exposure assessment and other information
<b>Pancreas</b>			
Kernan et al. (1999)	Pancreatic cancer deaths from 1984 to 1993 in 24 U.S. states; age-, sex-, race-, and state-matched noncancer deaths, excluding other pancreatic diseases and pancreatitis, controls.	63,097 cases. 252,386 population controls. Response rates not identified.	Exposure surrogate assigned for 111 chlorinated hydrocarbons, including TCE, and two broad chemical categories using usual occupation on death certificate and job-exposure-matrix of Gomez et al. (1994). Race and sex-specific mortality ORs from logistic regression analysis adjusted for age, marital status, metropolitan area, and residential status.
<b>Prostate</b>			
Aronson et al. (1996); Siemiatycki (1991)	Male prostate cancer cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	449 cases. 533 population controls (Group 1) and other cancer cases from same study (Group 2). Cases, 81%; controls, 72%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales). Logistic regression adjusted for age, ethnic origin, SES status, Quetlet, and respondent status (occupation) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, ethnic origin, and respondent status (TCE).
<b>Renal cell</b>			
Moore et al. (2010)	Cases aged 20–74 yrs from four European countries (Russia, Romania, Poland, Czech Republic) with histologically confirmed kidney cancer in 1999–2003; hospital controls with diagnoses unrelated to smoking or genitourinary disorders in 1998–2003 and frequency matched by sex, age, and study center.	1,097 cases (825 RCCs). 1,184 controls. Cases, 90–99%; controls, 90.3–96%.	In-person interview using questionnaire for information on lifestyle habits, smoking, anthropometric measures, personal and family medical history, and occupational history. Specialized job-specific questionnaire for specific jobs or industries of interest focused on solvents exposure, including TCE, with exposure assignment by expert blinded to case and control status by frequency, intensity, and confidence of TCE exposure. Exposure metric of overall exposure, duration (total hr, yr), and cumulative exposure. Logistic regression adjusted for sex, age, and study center. BMI, hypertension, smoking, residence location also included in initial models but did not alter ORs by >10%.

**Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)**

Reference	Population	Study group (N) comparison group (N) response rates	Exposure assessment and other information
Charbotel et al. (2009; 2006)	Cases from Arve Valley region in France identified from local urologists files and from area teaching hospitals; age- and sex-matched controls chosen from file of same urologist as who treated case or recruited among the patients of the case's general practitioner.	87 cases. 316 controls. Cases, 74%; controls, 78%.	Telephone interview with case or control, or, if deceased, with next-of-kin (22% cases, 2% controls). Questionnaire assessing occupational history, particularly, employment in the screw cutting jobs, and medical history. Semiquantitative TCE exposure assigned to subjects using a task/TCE-Exposure Matrix designed using information obtained from questionnaires and routine atmospheric monitoring of workshops or biological monitoring (U-TCA) of workers carried out since the 1960s. Cumulative exposure, cumulative exposure with peaks, and TWA. Conditional logistic regression with covariates for tobacco smoking and BMI.
Brüning et al. (2003)	Histologically-confirmed cases 1992–2000 from German hospitals (Arnsberg); hospital controls (urology department) serving area, and local geriatric department, for older controls, matched by sex and age.	134 cases. 401 controls. Cases, 83%; controls, not available.	In-person interviews with case or next-of-kin; questionnaire assessing occupational history using job title. Exposure metrics included longest job held, JEM of Pannett et al. (1985) to assign cumulative exposure to TCE and perchloroethylene, and exposure duration. Logistic regression with covariates for age, sex, and smoking.
Pesch et al. (2000b)	Histologically-confirmed cases from German hospitals (five regions) in 1991–1995; controls randomly selected from residency registries matched on region, sex, and age.	935 cases. 4,298 controls. Cases, 88%; controls, 71%.	In-person interview with case or next-of-kin; questionnaire assessing occupational history using job title (JEM approach), self-reported exposure, or job task (JTEM approach) to assign TCE and other exposures. Logistic regression with covariates for age, study center, and smoking.
Parent et al. (2000a); Siemiatycki (1991)	Male RCC cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	142 cases. 533 population controls (Group 1) and other cancer controls (excluding lung and bladder cancers) (Group 2). Cases, 82%; controls, 71%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (about 300 exposures on semiquantitative scales); TCE defined as any or substantial exposure. Mantel-Haenszel stratified by age, BMI, and cigarette smoking (TCE) or logistic regression adjusted for respondent status, age, smoking, and BMI (occupation, job title).

**Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)**

Reference	Population	Study group (N) comparison group (N) response rates	Exposure assessment and other information
Dosemeci et al. (1999)	Histologically-confirmed cases, 1988–1990, white males and females, 20–85 yrs, from Minnesota Cancer Registry; controls stratified for age and sex using random digit dialing, 21–64 yrs, or from HCFA records, 64–85 yrs.	438 cases. 687 controls. Cases, 87%; controls, 86%.	In-person interviews with case or next-of-kin; questionnaire assessing occupational history of TCE using job title and JEM of Gomez et al. (1994). Exposure metric was any TCE exposure. Logistic regression with covariates for age, smoking, hypertension, and BMI.
Vamvakas et al. (1998)	Cases who underwent nephrectomy in 1987–1992 in a hospital in Arnsberg region of Germany; controls selected accident wards from nearby hospital in 1992.	58 cases. 84 controls. Cases, 83%; controls, 75%.	In-person interview with case or next-of-kin; questionnaire assessing occupational history using job title or self-reported exposure to assign TCE and perchloroethylene exposure. Logistic regression with covariates for age, smoking, BMI, hypertension, and diuretic intake.
<b>Multiple or other sites</b>			
Lee et al. (2003)	Liver, lung, stomach, colorectal cancer deaths in males and females between 1966 and 1997 from two villages in Taiwan; controls were cardiovascular and cerebral-vascular disease deaths from same underlying area as cases.	53 liver, 39 stomach, 26 colorectal, 41 lung cancer cases. 286 controls. Response rate not reported.	Residence as recorded on death certificate. Mantel-Haenszel stratified by age, sex, and time period.
Siemietycki (1991)	Male cancer cases, 1979–1985, 35–75 yrs, diagnosed in 16 Montreal-area hospitals, histologically confirmed; cancer controls identified concurrently; age-matched, population-based controls identified from electoral lists and random digit dialing.	857 lung and 117 pancreatic cancer cases. 533 population controls (Group 1) and other cancer cases from same study (Group 2). Cases, 79% (lung), 71% (pancreas); controls, 72%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); TCE defined as any or substantial exposure. Mantel-Haenszel stratified on age, income, index for cigarette smoking, ethnic origin, and respondent status (lung cancer) and age, income, index for cigarette smoking, and respondent status (pancreatic cancer).

**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

BMI = body mass index; CLL = chronic lymphocytic leukemia; HCFA = Health Care Financing Administration; JTEM = job-task exposure matrix; MM = multiple myeloma; NCI = National Cancer Institute; NHL = non-Hodgkin lymphoma; OR = odds ratio; UV = ultra-violet

**Table 4-3. Geographic-based studies assessing cancer and TCE exposure**

Reference	Description	Analysis approach	Exposure assessment
<b>Broome County, New York studies</b>			
ATSDR (2006a), (2008b)	Total, 22 site-specific, and childhood cancer incidence from 1980 to 2001 among residents in two areas in Endicott, New York.	SIR among all subjects (ATSDR, 2006a) or among white subjects only (ATSDR, 2008b) with expected numbers of cancers derived using age-specific cancer incidence rates for New York State, excluding New York City. Limited assessment of smoking and occupation using medical and other records in lung and kidney cancer subjects (ATSDR, 2008b).	Two study areas, Eastern and Western study areas, identified based on potential for soil vapor intrusion exposures as defined by the extent of likely soil vapor contamination. Contour lines of modeled VOC soil vapor contamination levels based on exposure model using GIS mapping and soil vapor sampling results taken in 2003. The study areas were defined by 2000 Census block boundaries to conform to model predicted areas of soil vapor contamination. TCE was the most commonly found contaminant in indoor air in Eastern study area at levels ranging from 0.18 to 140 µg/m <sup>3</sup> , with tetrachloroethylene, cis-1,2-dichloroethene, 1,1,1-trichloroethane, 1,1-DCE, 1,1-dichloroethane, and Freon 113 detected at lower levels. Perchloroethylene was most common contaminant in indoor air in Western study area with other VOCs detected at lower levels.
<b>Maricopa County, Arizona studies</b>			
Aickin et al. (1992); Aickin (2004)	Cancer deaths, including leukemia, 1966–1986, and childhood (≤19 yrs old) leukemia incident cases (1965–1986), Maricopa County, Arizona.	Standardized mortality rate ratio from Poisson regression modeling. Childhood leukemia incidence data evaluated using Bayes methods and Poisson regression modeling.	Location of residency in Maricopa County, Arizona, at the time of death as surrogate for exposure. Some analyses examined residency in West Central Phoenix and cancer. Exposure information is limited to TCE concentration in two drinking water wells in 1982.
<b>Pima County, Arizona studies</b>			
ADHS (1995, 1990)	Cancer incidence in children (≤19 yrs old) and testicular cancer in 1970–1986 and 1987–1991, Pima County, Arizona.	Standardized incidence RR from Poisson regression modeling using method of Aickin et al. (Aickin et al., 1992). Analysis compares incidence in Tucson Airport Area to rate for rest of Pima County.	Location of residency in Pima, County, Arizona, at the time of diagnosis or death as surrogate for exposure. Exposure information is limited to monitoring since 1981 and include VOCs in soil gas samples (TCE, perchloroethylene, 1,1-DCE, 1,1,1-trichloroacetic acid); PCBs in soil samples, and TCE in municipal water supply wells.

**Table 4-3. Geographic-based studies assessing cancer and TCE exposure (continued)**

Reference	Description	Analysis approach	Exposure assessment
<b>Other</b>			
Coyle et al. (2005)	Incident breast cancer cases among men and women, 1995–2000, reported to Texas Cancer Registry.	Correlation study using rank order statistics of mean average annual breast cancer rate among women and men and atmospheric release of 12 hazardous air pollutants.	Reporting to EPA Toxic Release Inventory the number of pounds released for 12 hazardous air pollutants, (carbon tetrachloride, formaldehyde, methylene chloride, styrene, tetrachloroethylene, TCE, arsenic, cadmium, chromium, cobalt, copper, and nickel).
Morgan and Cassady (2002)	Incident cancer cases, 1988–1989, among residents of 13 census tracts in Redlands area, San Bernardino County, California.	SIR for all cancer sites and 16 site-specific cancers; expected numbers using incidence rates of site-specific cancer of a four-county region between 1988 and 1992.	TCE and perchlorate detected in some county wells; no information on location of wells to residents, distribution of contaminated water, or TCE exposure potential to individual residents in studied census tracts.
Vartiainen et al. (1993)	Total cancer and site-specific cancer cases (lymphoma sites and liver) from 1953 to 1991 in two Finnish municipalities.	SIR with expected number of cancers and site-specific cancers derived from incidence of the Finnish population.	Monitoring data from 1992 indicated presence of TCE, tetrachloroethylene and 1,1,1,-trichloroethane in drinking water supplies in largest towns in municipalities. Residence in town used to infer exposure to TCE.
Cohn et al. (1994b); Fagliano et al. (1990)	Incident leukemia and NHL cases, 1979–1987, from 75 municipalities and identified from the New Jersey State Cancer Registry. Histological type classified using WHO scheme and the classification of NIH Working Formulation Group for grading NHL.	Logistic regression modeling adjusted for age.	Monitoring data from 1984 to 1985 on TCE, trihalomethanes, and VOCs concentrations in public water supplies, and historical monitoring data conducted in 1978–1984.
Mallin (1990)	Incident bladder cancer cases and deaths, 1978–1985, among residents of nine northwestern Illinois counties.	SIR and SMR by county of residence and zip code; expected numbers of bladder cancers using age-race-sex specific incidence rates from SEER or bladder cancer mortality rates of the U.S. population from 1978 to 1985.	Exposure data are lacking for the study population with the exception of noting one of two zip code areas with observed elevated bladder cancer rates also had groundwater supplies contaminated with TCE, perchloroethylene, and other solvents.
Isacson et al. (1985)	Incident bladder, breast, prostate, colon, lung and rectal cancer cases reported to Iowa cancer registry between 1969 and 1981.	Age-adjusted site-specific cancer incidence in Iowa towns with populations of 1,000–10,000 and who were serviced by a public drinking water supply.	Monitoring data of drinking water at treatment plant in each Iowa municipality with populations of 1,000–10,000 used to infer TCE and other VOC concentrations in finished drinking water supplies.

GIS = geographic information system; NIH = National Institutes of Health; PCB = polychlorinated biphenyl; SEER = Surveillance, Epidemiology, and End Results; WHO = World Health Organization

**Table 4-4. Standards of epidemiologic study design and analysis use for identifying cancer hazard and TCE exposure**

<p><b>Category A: STUDY DESIGN</b></p> <p><b>Clear articulation of study objectives or hypothesis.</b> The ideal is a clearly stated hypothesis or study objectives and the study is designed to achieve the identified objectives.</p> <p><b>Selection and characterization in cohort studies of exposure and control groups and of cases and controls (case-control studies) is adequate.</b> The ideal is for selection of cohort and referents from the same underlying population and differences between these groups to be due to TCE exposure or level of TCE exposure and not to physiological, health status, or lifestyle factors. Controls or referents are assumed to lack or to have background exposure to TCE. These factors may lead to a downward bias including one of which is known as “healthy worker bias,” often introduced in analyses when mortality or incidence rates from a large population such as the U.S. population are used to derive expected numbers of events. The ideal in case-control studies is cases and controls are derived from the same population and are representative of all cases and controls in that population. Any differences between controls and cases are due to exposure to TCE itself and not to confounding factors related to both TCE exposure and disease. Additionally, the ideal is for controls to be free of any disease related to TCE exposure. In this latter case, potential bias is toward the null hypothesis.</p>
<p><b>Category B: <u>ENDPOINT MEASURED</u></b></p> <p><b>Levels of health outcome assessed.</b> Three levels of health outcomes are considered in assessing the human health risks associated with exposure to TCE: biomarkers of effects and susceptibility, morbidity, and mortality. Both morbidity, as enumerated by incidence, and mortality, as identified from death certificates, are useful indicators in risk assessment for hazard identification. The ideal is for accurate and predictive indicator of disease. Incidence rates are generally considered to provide an accurate indication of disease in a population and cancer incidence is generally enumerated with a high degree of accuracy in cancer registries. Death certifications are readily available and have complete national coverage but diagnostic accuracy is reduced and can vary by specific diagnosis. Furthermore, diagnostic inaccuracies can contribute to death certificates as a poor surrogate for disease incidence. Incidence, when obtained from population-based cancer registries, is preferred for identifying cancer hazards.</p> <p><b>Changes in diagnostic coding systems for lymphoma, particularly NHL.</b> Classification of lymphomas today is based on morphologic, immunophenotypic, genotypic, and clinical features using the WHO classification, introduced in 2001, and incorporation of WHO terminology into International Classification of Disease (ICD)-0-3. ICD Versions 7 and earlier had rubrics for general types of lymphatic and hematopoietic cancer, but no categories for distinguishing specific types of cancers, such as acute leukemia. Epidemiologic studies based on causes of deaths as coded using these older ICD classifications typically grouped together lymphatic neoplasms instead of examining individual types of cancer or specific cell types. Before the use of immunophenotyping, these grouping of ambiguous diseases such as NHL and Hodgkin lymphoma may be have misclassified. With the introduction of ICD-10 in 1990, lymphatic tumors coding, starting in 1994 with the introduction of the Revised European-American Lymphoma classification, the basis of the current WHO classification, was more similar to that presently used. Misclassification of specific types of cancer, if unrelated to exposure, would have attenuated estimate of RR and reduced statistical power to detect associations. When the outcome was mortality, rather than incidence, misclassification would be greater because of the errors in the coding of underlying causes of death on death certificates (<a href="#">IOM, 2003</a>). Older studies that combined all lymphatic and hematopoietic neoplasms must be interpreted with care.</p>

**Table 4-4. Standards of epidemiologic study design and analysis use for identifying cancer hazard and TCE exposure (continued)**

<p><b>Category C: TCE-EXPOSURE CRITERIA</b></p> <p><b>Adequate characterization of exposure.</b> The ideal is for TCE exposure potential known for each subject and quantitative assessment (job-exposure-matrix approach) of TCE exposure assessment for each subject as a function of job title, year exposed, duration, and intensity. The assessment approach is accurate for assigning TCE intensity (TCE concentration or a TWA) to individual study subjects and estimates of TCE intensity are validated using monitoring data from the time period. For the purpose of this report, the objective for cohort and case-controls studies is to differentiate TCE-exposed subjects from subjects with little or no TCE exposure. A variety of dose-metrics may be used to quantify or classify exposures for an epidemiologic study. They include precise summaries of quantitative exposure, concentrations of biomarkers, cumulative exposure, and simple qualitative assessments of whether exposure occurred (yes or no). Each method has implicit assumptions and potential problems that may lead to misclassification. Studies in which it was unclear that the study population was actually exposed to TCE are excluded from analysis.</p>
<p><b>Category D: FOLLOW-UP (COHORT)</b></p> <p><b>Loss to follow-up.</b> The ideal is complete follow-up of all subjects; however, this is not achievable in practice, but it seems reasonable to expect loss to follow-up not to exceed 10%. The bias from loss to follow-up is indeterminate. Random loss may have less effect than if subjects who are not followed have some significant characteristics in common.</p> <p><b>Follow-up period allows full latency period for over 50% of the cohort.</b> The ideal to follow all study subjects until death. Short of the ideal, a sufficient follow-up period to allow for cancer induction period or latency over 15 or 20 yrs is desired for a large percentage of cohort subjects.</p>
<p><b>Category E: INTERVIEW TYPE (CASE-CONTROL)</b></p> <p><b>Interview approach.</b> The ideal interviewing technique is face-to-face by trained interviewers with &gt;90% of interviews with cases and control subjects conducted face-to-face. The effect on the quality of information from other types of data collection is unclear, but telephone interviews and mail-in questionnaires probably increase the rate of misclassification of subject information. The bias is toward the null hypothesis if the proportion of interview by type is the same for case and control, and of indeterminate direction otherwise.</p> <p><b>Blinded interviewer.</b> The ideal is for the interviewer to be unaware whether the subject is among the cases or controls and the subject to be unaware of the purpose and intended use of the information collected. Although desirable for case-control studies, blinding is usually not possible to fully accomplish because subject responses during the interview provide clues as to subject status. The potential for bias from face-to-face interviews is probably less than with mail-in interviews. Some studies have assigned exposure status in a blinded manner using a JEM and information collected in the unblinded interview. The potential for bias in this situation is probably less with this approach than for nonblinded assignment of exposure status.</p>

**Table 4-4. Standards of epidemiologic study design and analysis use for identifying cancer hazard and TCE exposure (continued)**

<p><b>Category F: PROXY RESPONDENTS</b></p> <p><b>Proxy respondents.</b> The ideal is for data to be supplied by the subject because the subject generally would be expected to be the most reliable source; &lt;10% of either total cases or total controls for case-control studies. A subject may be either deceased or too ill to participate, however, making the use of proxy responses unavoidable if those subjects are to be included in the study. The direction and magnitude of bias from use of proxies is unclear, and may be inconsistent across studies.</p>
<p><b>Category G: SAMPLE SIZE</b></p> <p>The ideal is for the sample size is large enough to provide sufficient statistical power to ensure that any elevation of effect in the exposure group, if present, would be found, and to ensure that the confidence bounds placed on RR estimates can be well characterized.</p>
<p><b>Category H: ANALYSIS ISSUES</b></p> <p><b>Control for potentially confounding factors of importance in analysis.</b> The ideal in cohort studies is to derive expected numbers of cases based on age-sex- and time-specific cancer rates in the referent population and in case-control studies by matching on age and sex in the design and then adjusting for age in the analysis of data. Age and sex are likely correlated with exposure and are also risk factors for cancer development. Similarly, other factors such as cigarette smoking and alcohol consumption are risk factors for several site-specific cancers reported as associative with TCE exposure. To be a confounder of TCE, exposure to the other factor must be correlated, and the association of the factor with the site-specific cancer must be causal. The expected effect from controlling for confounders is to move the estimated RR estimate closer to the true value.</p> <p><b>Statistical methods are appropriate.</b> The ideal is that conclusions are drawn from the application of statistical methods that are appropriate to the problem and accurately interpreted.</p> <p><b>Evaluation of exposure-response.</b> The ideal is an examination of a linear exposure-response as assessed with a quantitative exposure metric such as cumulative exposure. Some studies, absent quantitative exposure metrics, examine exposure response relationships using a semiquantitative exposure metric or by duration of exposure. A positive dose-response relationship is usually more convincing of an association as causal than a simple excess of disease using TCE dose-metric. However, a number of reasons have been identified for a lack of linear exposure-response finding and the failure to find such a relationship mean little from an etiological viewpoint.</p> <p><b>Documentation of results.</b> The ideal is for analysis observations to be completely and clearly documented and discussed in the published paper, or provided in supplementary materials accompanying publication.</p>

Twenty-four of the studies identified in a systematic review were selected for inclusion in the meta-analysis through use of the following meta-analysis inclusion criteria: (1) cohort or case-control designs; (2) evaluation of incidence or mortality; (3) adequate selection in cohort studies of exposure and control groups and of cases and controls in case-control studies; (4) TCE exposure potential inferred to each subject and quantitative assessment of TCE exposure assessment for each subject by reference to industrial hygiene records indicating a high probability of TCE use, individual biomarkers, job-exposure matrices (JEMs), water distribution models, or obtained from subjects using questionnaire (case-control studies); and (5) relative risk (RR) estimates for kidney cancer, liver cancer, or non-Hodgkin lymphoma (NHL) adjusted, at minimum, for possible confounding of age, sex, and race (see Table 4-5). This evaluation is summarized below, separately for cohort and case-control studies. Appendix C contains a full discussion of the meta-analysis, its analytical methodology, including sensitivity analyses, and findings. The meta-analysis focuses on kidney cancer, liver cancer, and NHL, as most studies reported RRs for these sites. Fewer numbers of studies reported RRs for other site-specific cancers and TCE exposure and examination of these site-specific cancers and TCE exposure using meta-analysis was not attempted.

**Table 4-5. Summary of criteria for meta-analysis study selection**

Decision outcome	Studies	Primary reason(s)
<b>Studies recommended for meta-analysis:</b>		
	Axelson et al. (1994); Greenland et al. (1994); Hardell et al. (1994); Siemiatycki (1991); Anttila et al. (1995); Morgan et al. (1998); Nordstrom et al. (1998); Boice et al. (1999); Boice et al. (2006b); Dosemeci et al. (1999); Persson and Fredriksson (1999); Pesch et al. (2000b); Hansen et al. (2001); Brüning et al. (2003); Raaschou-Nielsen et al. (2003); Zhao et al. (2005); Miligi et al. (2006); Charbotel et al. (2006); Blair et al. (1998); its follow-up Radican et al. (2008); Wang et al. (2009); Cocco et al. (2010); Moore et al. (2010); Purdue et al. (2011)	Analytical study designs of cohort or case-control; evaluation of incidence or mortality; adequate selection in cohort studies of exposure and control groups and of cases and controls in case-control studies; TCE exposure potential inferred to each subject and quantitative assessment of TCE exposure assessment for each subject by reference to industrial hygiene records indicating a high probability of TCE use, individual biomarkers, JEMs, water distribution models, or obtained from subjects using questionnaire (case-control studies); RR estimates for kidney cancer, liver cancer, or NHL adjusted, at minimum, for possible confounding of relevant risk factors (e.g., age, sex, and race).

**Table 4-5. Summary of criteria for meta-analysis study selection (continued)**

Decision outcome	Studies	Primary reason(s)
<b>Studies not recommended for meta-analysis:</b>		
	Clapp and Hoffman ( <a href="#">2008</a> ); ATSDR ( <a href="#">2004a</a> ; <a href="#">Cohn et al., 1994b</a> )	Weakness with respect to analytical study design (i.e., geographic-based, ecological or PMR design).
	Garabrant et al. ( <a href="#">1988</a> ); Isacson et al. ( <a href="#">1985</a> ); Shindell and Ulrich ( <a href="#">1985</a> ); Wilcosky et al. ( <a href="#">1984</a> ); Shannon et al. ( <a href="#">1988</a> ); Blair et al. ( <a href="#">1989</a> ); Costa et al. ( <a href="#">1989</a> ); ( <a href="#">ADHS, 1995, 1990</a> ); Mallin ( <a href="#">1990</a> ); Aickin et al. ( <a href="#">1992</a> ); Sinks et al. ( <a href="#">1992</a> ); Vartiainen et al. ( <a href="#">1993</a> ); Morgan and Cassady ( <a href="#">2002</a> ); Lee et al. ( <a href="#">2003</a> ); Aickin ( <a href="#">2004</a> ); Chang et al. ( <a href="#">2005</a> ; <a href="#">Chang et al., 2003</a> ); Coyle et al. ( <a href="#">2005</a> ); ATSDR ( <a href="#">2006a</a> ); ATSDR ( <a href="#">2008b</a> ); Sung et al. ( <a href="#">2008</a> ; <a href="#">2007</a> )	TCE exposure potential not assigned to individual subjects using JEM, individual biomarkers, water distribution models, or industrial hygiene data from other process indicating a high probability of TCE use (cohort studies).
	Lowengart et al. ( <a href="#">1987</a> ); Fredriksson et al. ( <a href="#">1989</a> ); McKinney et al. ( <a href="#">1991</a> ); Heineman et al. ( <a href="#">1994</a> ); Siemiatycki et al. ( <a href="#">1994</a> ); Aronson et al. ( <a href="#">1996</a> ); Fritschi and Siemiatycki ( <a href="#">1996b</a> ); Dumas et al. ( <a href="#">2000</a> ); Kernan et al. ( <a href="#">1999</a> ); Shu et al. ( <a href="#">2004</a> ; <a href="#">1999</a> ); Parent et al. ( <a href="#">2000b</a> ); Pesch et al. ( <a href="#">2000a</a> ); DeRoos et al. ( <a href="#">2001</a> ); Goldberg et al. ( <a href="#">2001</a> ); Costas et al. ( <a href="#">2002</a> ); Krishnadasan et al. ( <a href="#">2007</a> ) Costantini et al. ( <a href="#">2008</a> ); Gold et al. ( <a href="#">2011</a> )	Cancer incidence or mortality reported for cancers other than kidney, liver, or NHL.
	Ritz ( <a href="#">1999a</a> )	Subjects monitored for radiation exposure with likelihood for potential confounding; cancer mortality and TCE exposure not reported for kidney cancer and all hemato- and lymphopoietic cancer reported as broad category.
	Henschler et al. ( <a href="#">1995</a> )	Incomplete identification of cohort and index kidney cancer cases included in case series.

The cohort studies ([Clapp and Hoffman, 2008](#); [Radican et al., 2008](#); [Sung et al., 2008](#); [Krishnadasan et al., 2007](#); [Sung et al., 2007](#); [Boice et al., 2006b](#); [Chang et al., 2005](#); [Zhao et al., 2005](#); [ATSDR, 2004a](#); [Chang et al., 2003](#); [Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#); [Boice et al., 1999](#); [Ritz, 1999a](#); [Blair et al., 1998](#); [Morgan et al., 1998](#); [Anttila et al., 1995](#); [Henschler et al., 1995](#); [Axelson et al., 1994](#); [Greenland et al., 1994](#); [Sinks et al., 1992](#); [Blair et al., 1989](#); [Costa et al., 1989](#); [Garabrant et al., 1988](#); [Shannon et al., 1988](#); [Shindell and Ulrich, 1985](#); [Wilcosky et al., 1984](#)) (see Table 4-1), with data on the incidence or morality of site-specific cancer in relation to TCE exposure, range in size 803 ([Hansen et al., 2001](#)) to 86,868 ([Chang et al., 2005](#); [Chang et al., 2003](#)), and were conducted in Denmark, Sweden, Finland, Germany, Taiwan, and the United States (see Table 4-1). Three case-control studies nested within cohorts ([Krishnadasan et al., 2007](#); [Greenland et al., 1994](#); [Wilcosky et al., 1984](#)) are considered as cohort studies because the summary risk estimate from a nested case-control study, the odds ratio (OR), was estimated from incidence density sampling. This is considered an unbiased estimate of the hazard ratio, similar to a RR estimate from a cohort study, if, as is the

case for these studies, controls are selected from the same source population as the cases, the sampling rate is independent of exposure status, and the selection probability is proportional to time-at-risk ([IOM, 2003](#)). Cohort and nested case-control study designs are analytical epidemiologic studies and are generally relied on for identifying a causal association between human exposure and adverse health effects ([U.S. EPA, 2005b](#)).

While all of these cohort studies are considered in the overall weight of evidence, 11 of them met all meta-analysis inclusion criteria: the cohorts of Blair et al. ([1998](#)) and its follow-up by Radican et al. ([2008](#)); Morgan et al. ([1998](#)), Boice et al. ([Boice et al., 2006b; 1999](#)), and Zhao et al. ([2005](#)), of aerospace workers or aircraft mechanics; and Axelson et al. ([1994](#)), Anttila et al. ([1995](#)), Hansen et al. ([2001](#)), and Raaschou-Nielsen et al. ([2003](#)) of Nordic workers in multiple industries with TCE exposure; and Greenland et al. ([1994](#)) of electrical manufacturing workers. Subjects or cases and controls in these studies are considered to sufficiently represent the underlying population, and the bias associated with selection of referent populations is considered minimal. The exposure-assessment approaches included detailed JEM, biomonitoring data, or use of industrial hygiene data on TCE exposure patterns and factors that affect such exposure, with high probability of TCE exposure potential to individual subjects. The statistical analyses methods were appropriate and well documented, the measured endpoint was an accurate indicator of disease, and the follow-up was sufficient for cancer latency. These studies are also considered as strong studies for identifying kidney, liver, and NHL cancer hazard. The remaining cohort studies less satisfactorily meet identified criteria or standards of epidemiologic design and analysis, having deficiencies in multiple criteria ([Clapp and Hoffman, 2008](#); [Sung et al., 2008](#); [Sung et al., 2007](#); [Chang et al., 2005](#); [ATSDR, 2004a](#); [Chang et al., 2003](#); [Ritz, 1999a](#); [Henschler et al., 1995](#); [Sinks et al., 1992](#); [Costa et al., 1989](#); [Garabrant et al., 1988](#); [Shindell and Ulrich, 1985](#); [Wilcosky et al., 1984](#)). Krishnandansen et al. ([2007](#)), who reported on prostate cancer, met four of the five meta-analysis inclusion criteria except that for reporting an RR estimate cancer of the kidney, liver, or NHL, the site-specific cancers examined using meta-analysis.

The case-control studies on TCE exposure are of several site-specific cancers, including bladder (Pesch et al., [2000a](#); [Siemiatycki et al., 1994](#); [Siemiatycki, 1991](#)); brain ([De Roos et al., 2001](#); [Heineman et al., 1994](#)); childhood lymphoma or leukemia ([Shu et al., 2004](#); [Costas et al., 2002](#); [Shu et al., 1999](#); [McKinney et al., 1991](#); [Lowengart et al., 1987](#)); colon cancer ([Goldberg et al., 2001](#); [Siemiatycki, 1991](#)); esophageal cancer ([Parent et al., 2000b](#); [Siemiatycki, 1991](#)); liver cancer ([Lee et al., 2003](#)); lung cancer ([Siemiatycki, 1991](#)); adult lymphoma or leukemia ([Hardell et al., 1994](#)) [NHL, Hodgkin lymphoma]; ([Fritschi and Siemiatycki, 1996a](#); [Siemiatycki, 1991](#)) [NHL]; ([Nordström et al., 1998](#)) [hairy cell leukemia]; ([Persson and Fredrikson, 1999](#)) [NHL]; ([Miligi et al., 2006](#)) [NHL and chronic lymphocytic leukemia (CLL)]; ([Seidler et al., 2007](#)) [NHL, Hodgkin lymphoma and subjects included in ([Cocco et al., 2010](#); [Costantini et al., 2008](#)) [leukemia types, CLL included with NHL] ([Wang et al., 2009](#); [Miligi et al., 2006](#)) [NHL];

([Cocco et al., 2010](#)) [B-cell including CLL and multiple myeloma, T-cell, and Hodgkin lymphomas]; ([Purdue et al., 2011](#)) [NHL]; Gold et al. ([2011](#)) [multiple myeloma]; melanoma ([Fritschi and Siemiatycki, 1996b](#); [Siemiatycki, 1991](#)); rectal cancer ([Dumas et al., 2000](#); [Siemiatycki, 1991](#)); renal cell carcinoma (RCC), a form of kidney cancer ([Moore et al., 2010](#); [Charbotel et al., 2006](#); [Brüning et al., 2003](#); [Parent et al., 2000a](#); [Pesch et al., 2000b](#); [Dosemeci et al., 1999](#); [Vamvakas et al., 1998](#); [Siemiatycki, 1991](#)); pancreatic cancer ([Siemiatycki, 1991](#)); and prostate cancer ([Aronson et al., 1996](#); [Siemiatycki, 1991](#)) (see Table 4-2). No case-control studies of reproductive cancers (breast or cervix) and TCE exposure were found in the peer-reviewed literature.

While all of these case-control studies are considered in the overall weight of evidence, 13 of them met the meta-analysis inclusion criteria identified in Section B.2.9 ([Purdue et al., 2011](#); [Cocco et al., 2010](#); [Moore et al., 2010](#); [Wang et al., 2009](#); [Charbotel et al., 2006](#); [Miligi et al., 2006](#); [Brüning et al., 2003](#); [Pesch et al., 2000b](#); [Dosemeci et al., 1999](#); [Persson and Fredrikson, 1999](#); [Nordström et al., 1998](#); [Hardell et al., 1994](#); [Siemiatycki, 1991](#)). They were of analytical study design, cases and controls were considered to represent underlying populations and selected with minimal potential for bias; exposure assessment approaches included assignment of TCE exposure potential to individual subjects using information obtained from face-to-face, mailed, or telephone interviews; analyses methods were appropriate, well-documented, included adjustment for potential confounding exposures, with RR estimates and associated CIs reported for kidney cancer, liver cancer or NHL.

These studies were also considered, to varying degrees, as strong studies for weight-of-evidence characterization of hazard. Both Brüning et al. ([2003](#)) and Charbotel et al. ([2006](#)) had a priori hypotheses for examining RCC and TCE exposure. Strengths of both studies are in their examination of populations with potential for high exposure intensity and in areas with high frequency of TCE usage and their assessment of TCE potential. An important feature of the exposure assessment approach of Charbotel et al. ([2006](#)) is their use of a large number of studies on biological monitoring of workers in the screw-cutting industry, a predominant industry with documented TCE exposures, as support. Charbotel et al. ([2006](#)) is preferred to Charbotel et al. ([2009](#)), who examined kidney cancer risk and TCE exposure at the existing French occupational exposure limit for cases and controls from their earlier publication (Charbotel et al., [2009](#)); the earlier publication contained more extensive analyses and included exposure-response analyses using several exposure metrics and multiple exposure categories. Other studies were either large multiple-center studies ([Purdue et al., 2011](#); [Cocco et al., 2010](#); [Moore et al., 2010](#); [Wang et al., 2009](#); [Miligi et al., 2006](#); [Pesch et al., 2000b](#)) or reporting from one location of a larger international study ([Seidler et al., 2007](#); [Dosemeci et al., 1999](#)). Cocco et al. ([2010](#)) includes subjects in Seidler et al. ([2007](#)) and is preferred because of the larger number of subjects from four other European countries. In contrast to Brüning et al. ([2003](#)) and Charbotel et al. ([2006](#)), two studies conducted in geographical areas with widespread TCE usage and potential for

exposure to higher intensity; in these other studies, a lower exposure prevalence to TCE is found [any TCE exposure: 15% of cases ([Dosemeci et al., 1999](#)); 6% of cases ([Miligi et al., 2006](#)); 13% of cases ([Wang et al., 2009](#)); 4% of cases ([Cocco et al., 2010](#))], and most subjects were identified as exposed to TCE probably had minimal contact (3% of cases with moderate/high TCE exposure ([Miligi et al., 2006](#)); 2% of cases with high intensity, but of low probability of TCE exposure ([Wang et al., 2009](#)). This pattern of lower exposure prevalence and intensity is common to community-based, population case-control studies ([Teschke et al., 2002](#)).

Fourteen case-control studies did not meet specific meta-analysis inclusion criterion ([Gold et al., 2011](#); [Shu et al., 2004](#); [Lee et al., 2003](#); [Costas et al., 2002](#); [Goldberg et al., 2001](#); [Dumas et al., 2000](#); [Parent et al., 2000b](#); [Pesch et al., 2000a](#); [Kernan et al., 1999](#); [Shu et al., 1999](#); [Vamvakas et al., 1998](#); [Aronson et al., 1996](#); [Fritschi and Siemiatycki, 1996b](#); [Siemiatycki et al., 1994](#)). Twelve studies reported RR estimates for site-specific cancers other than kidney, liver, and NHL ([Gold et al., 2011](#); [Shu et al., 2004](#); [Costas et al., 2002](#); [Goldberg et al., 2001](#); [Dumas et al., 2000](#); [Parent et al., 2000b](#); [Pesch et al., 2000a](#); [Kernan et al., 1999](#); [Shu et al., 1999](#); [Aronson et al., 1996](#); [Fritschi and Siemiatycki, 1996b](#); [Siemiatycki et al., 1994](#)). [Vamvakas et al. \(1998\)](#) has been the subject of considerable controversy ([Cherrie et al., 2001](#); [Mandel, 2001](#); [Green and Lash, 1999](#); [McLaughlin and Blot, 1997](#); [Bloemen and Tomenson, 1995](#); [Swan, 1995](#)) with questions raised on the potential for selection bias related to the study's controls. This study was deficient in the criterion for adequacy of case and control selection. [Brüning et al. \(2003\)](#), a study from the same region as [Vamvakas et al. \(1998\)](#), is considered a stronger study for identifying cancer hazard since it addresses many of the deficiencies of [Vamvakas et al. \(1998\)](#). [Lee et al. \(2003\)](#), in their study of hepatocellular cancer, assigns one level of exposure to all subjects in a geographic area, an inherent measurement error and misclassification bias because not all subjects are exposed uniformly. Additionally, statistical analyses in this study did not control for hepatitis viral infection, a known risk factor for hepatocellular cancer and of high prevalence in the study area.

The geographic-based studies ([ATSDR, 2008b](#), 2006a; [Aickin, 2004](#); [Morgan and Cassady, 2002](#); [ADHS, 1995](#); [Cohn et al., 1994b](#); [Vartiainen et al., 1993](#); [Aickin et al., 1992](#); [ADHS, 1990](#); [Mallin, 1990](#); [Isacson et al., 1985](#)) with data on cancer incidence are correlation studies to examine cancer outcomes of residents in communities with TCE and other chemicals detected in groundwater wells or in municipal drinking water supplies (see Table 4-3). These studies did not meet all five meta-analysis inclusion criteria. The geographic-base studies are not of analytical designs such as cohort and case-control designs. Another deficiency in all studies is their low level of detail to individual subjects for TCE. One level of exposure to all subjects in a geographic area is assigned without consideration of water distribution networks, which may influence TCE concentrations delivered to a home, or a subject's ingestion rate to estimate TCE exposure to individual study subjects. Some inherent measurement error and misclassification bias is likely in these studies because not all subjects are exposed uniformly. Additionally, in

contrast to case-control studies, the geographic-based studies, including the Agency for Toxic Substances and Disease Registry ([ATSDR, 2008b](#)), had limited accounting for other potential risk factors. These studies are of low sensitivity for weight-of-evidence characterization of hazard compared to other cohort and case-control studies.

## 4.2. GENETIC TOXICITY

This section discusses the genotoxic potential of TCE and its metabolites. A summary is provided at the end of each section for TCE or its metabolite for their mutagenic potential in addition to an overall synthesis summary at the end of the genotoxicity section. The liver and kidney are subjects of study for the genotoxic potential of TCE and its metabolites, and are discussed more in-depth in Sections 4.4.3, 4.4.7, 4.5.6.2.7, 4.5.7, E.2.3, and E.2.4.

The application of genotoxicity data to predict potential carcinogenicity is based on the principle that genetic alterations are found in all cancers. Genotoxicity is the ability of chemicals to alter the genetic material in a manner that permits changes to be transmitted during cell division. Although most tests for mutagenicity detect changes in DNA or chromosomes, some specific modifications of the epigenome including proteins associated with DNA or RNA, can also cause transmissible changes. Changes that occur due to the modifications in the epigenome are discussed in endpoint-specific Sections 4.3–4.9 as well as Sections E.3.1–E.3.4.

Genetic alterations can occur through a variety of mechanisms including gene mutations, insertions, deletions, translocations, or amplification; evidence of mutagenesis provides mechanistic support for the inference of potential for carcinogenicity in humans.

Evaluation of genotoxicity data entails a weight-of-evidence approach that includes consideration of the various types of genetic damage that can occur. In acknowledging that genotoxicity tests are by design complementary evaluations of different mechanisms of genotoxicity, a recent International Programme on Chemical Safety (IPCS) publication ([Eastmond et al., 2009](#)) notes that “multiple negative results may not be sufficient to remove concern for mutagenicity raised by a clear positive result in a single mutagenicity assay.” These considerations inform the present approach. In addition, consistent with U.S. EPA’s *Guidelines on Carcinogenic Risk Assessment and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* ([U.S. EPA, 2005c, b](#)), the approach does not address relative potency (e.g., among TCE metabolites, or of such metabolites with other known genotoxic carcinogens) per se, nor does it consider quantitative issues related to the probable production of these metabolites in vivo. Instead, the analysis of genetic toxicity data presented here focuses on the identification of a genotoxic hazard of these metabolites; a quantitative analysis of TCE metabolism to reactive intermediates, via PBPK modeling, is presented in Section 3.5.

TCE and its known metabolites, TCA, DCA, CH, TCOH, DCVC, and DCVG, have been studied to varying degrees for their genotoxic potential. The following section summarizes

available data on genotoxicity for both TCE and its metabolites for each potential genotoxic endpoints, when available, in different organisms.

#### 4.2.1. TCE

##### 4.2.1.1. DNA Binding Studies

Covalent binding of TCE to DNA and protein in cell-free systems has been studied by several investigators. Incubation of [ $^{14}\text{C}$ ]-TCE with salmon sperm DNA in the presence of microsomal preparations from B6C3F<sub>1</sub> mice resulted in dose-related covalent binding of TCE to DNA. The binding was enhanced when the microsomes were taken from mice pretreated with phenobarbital, which induces CYP enzymes, suggesting that the binding may be related to an oxidative metabolite, or when 1,2-epoxy-3,3,3-trichloropropane, an inhibitor of epoxide hydrolase, was added to the incubations ([Banerjee and Van Duuren, 1978](#)). In addition, covalent binding of [ $^{14}\text{C}$ ]-TCE with microsomal proteins was detected after incubation with microsomal preparations from mouse lung, liver, stomach, and kidney, and rat liver ([Banerjee and Van Duuren, 1978](#)). Furthermore, incubation of [ $^{14}\text{C}$ ]-TCE with calf thymus DNA in the presence of hepatic microsomes from phenobarbital-pretreated rats yielded significant covalent binding ([DiRenzo et al., 1982](#)).

A number of studies have also examined the role of TCE metabolism in covalent binding to DNA and proteins. Miller and Guengerich ([1983](#)) used liver microsomes from control, b-naphthoflavone- and phenobarbital-induced B6C3F<sub>1</sub> mice, Osborne-Mendel rats, and human liver microsomes. Significant covalent binding of TCE metabolites to calf thymus DNA and proteins was observed in all experiments. Phenobarbital treatment increased the formation of chloral and TCE oxide formation, DNA, and protein adducts. In contrast, b-naphthoflavone treatment did not induce the formation of any microsomal metabolite, suggesting that the forms of CYP induced by phenobarbital are primarily involved in TCE metabolism while the b-naphthoflavone-inducible forms of CYP have only a minor role in TCE metabolism. TCE metabolism (based on TCE-epoxide and DNA-adduct formation) was 2.5–3-fold higher in mouse than in rat microsomes due to differences in rates and clearance of metabolism (discussed in Section 3.3.3.1). The levels of DNA and protein adducts formed in human liver microsomal system approximated those observed in liver microsomes prepared from untreated rats. It was also shown that whole hepatocytes of both untreated mice and phenobarbital-induced rats and mice could activate TCE into metabolites able to covalently bind extracellular DNA. A study by Cai and Guengerich ([2001a](#)) postulates that TCE oxide (an intermediate in the oxidative metabolism of TCE in rat and mouse liver microsomes) is responsible for the covalent binding of TCE with protein, and to a lesser extent, DNA. Mass spectrometry was used to analyze the reaction of TCE oxide (synthesized by m-chloroperbenzoic acid treatment of TCE) with nucleosides, oligonucleotides, and protein to understand the transient nature of the inhibition of enzymes in the context of adduct formation. Protein amino acid adducts were observed during

the reaction of TCE oxide with the model peptides. The majority of these adducts were unstable under physiological conditions. Results using other peptides also indicate that adducts formed from the reaction of TCE oxide with macromolecules and their biological effects are likely to be relatively short-lived.

Studies have been conducted using *in vitro* and *in vivo* systems to understand the DNA and protein binding capacity of TCE. In a study in male mice, after repeated *i.p.* injections of [<sup>14</sup>C]-TCE, radioactivity was detected in the DNA and RNA of all organs studied (kidney, liver, lung, spleen, pancreas, brain, and testis) ([Bergman, 1983](#)). However, *in vivo* labeling was shown to be due to metabolic incorporation of C1 fragments, particularly in guanine and adenine, rather than to DNA-adduct formation. In another study ([Stott et al., 1982](#)), following *i.p.* injection of [<sup>14</sup>C]-TCE in male Sprague-Dawley rats (10–100 mg/kg) and B6C3F<sub>1</sub> mice (10–250 mg/kg), high liver protein labeling was observed while very low DNA labeling was detected. Stott et al. ([1982](#)) also observed very low levels of DNA binding ( $0.62 \pm 0.43$  alkylation/ $10^6$  nucleotides) in mice administered 1,200 mg/kg of TCE. In addition, a dose-dependent binding of TCE to hepatic DNA and protein at low doses in mice was demonstrated by Kautiainen et al. ([1997](#)). In their dose-response study (doses between 2 µg/kg and 200 mg/kg body weight), the highest level of protein binding (2.4 ng/g protein) was observed 1 hour after the treatment followed by a rapid decline, indicating pronounced instability of the adducts and/or rapid turnover of liver proteins. Highest binding of DNA (120 pg/g DNA) was found between 24 and 72 hours following treatment. Dose-response curves were linear for both protein and DNA binding. In this study, the data suggest that TCE does bind to DNA and proteins in a dose-dependent fashion; however, the type and structure of adducts were not determined.

Mazzullo et al. ([1992](#)) reported that TCE was covalently bound *in vivo* to DNA, RNA, and proteins of rat and mouse organs 22 hours after *i.p.* injection. Labeling of proteins from various organs of both species was higher than that of DNA. Bioactivation of TCE to its intermediates using various microsomal fractions was dependent on CYP enzyme induction and the capacity of these intermediates to bind to DNA. It appeared that mouse lung microsomes were more efficient in forming the intermediates than rat lung microsomes, although no other species specific differences were found ([Mazzullo et al., 1992](#)). This also supports the results described by Miller and Guengerich ([1983](#)). The authors suggest some binding ability of TCE to interact covalently with DNA ([Mazzullo et al., 1992](#)).

In summary, studies report that TCE exposure *in vivo* can lead to binding to nucleic acids and proteins, and some authors have suggested that such binding is likely due to conversion to one or more reactive metabolites.

#### **4.2.1.2. Bacterial Systems—Gene Mutations**

Gene mutation studies (Ames assay) in various *Salmonella typhimurium* strains of bacteria exposed to TCE both in the presence and absence of stabilizing agent have been

conducted by different laboratories ([McGregor et al., 1989](#); [Mortelmans et al., 1986](#); [Shimada et al., 1985](#); [Crebelli et al., 1982](#); [Baden et al., 1979](#); [Waskell, 1978](#); [Henschler et al., 1977](#); [Simmon et al., 1977](#)) (see Table 4-6). It should be noted that these studies have tested TCE samples of different purities using various experimental protocols. In all in vitro assays, volatilization is a concern when TCE is directly administered.

Waskell ([1978](#)) studied the mutagenicity of several anesthetics and their metabolites. Included in their study was TCE (and its metabolites) using the Ames assay. The study was conducted both in the presence and absence of a metabolic activation system, S9, and caution was exercised to perform the experiment under proper conditions (incubation of reaction mixture in sealed dessicator vials). This study was performed in both TA98 and TA100 *S. typhimurium* strains at a dose range of 0.5–10% between 4 and 48 hours. No change in revertant colonies was observed in any of the doses or time courses tested. No information either on the presence or absence of stabilizers in TCE obtained commercially nor its effect on cytotoxicity was provided in the study.

In other studies, highly purified, epoxide-free TCE samples were not mutagenic in experiments with and without exogenous metabolic activation by S9 in *S. typhimurium* strain TA100 using the plate incorporation assay ([Henschler et al., 1977](#)). Furthermore, no mutagenic activity was found in several other strains including TA1535, TA1537, TA97, TA98, and TA100 using the preincubation protocol ([Mortelmans et al., 1986](#)). Simmon et al. ([1977](#)) observed a less than twofold but reproducible and dose-related increase in *his* + revertants in plates inoculated with *S. typhimurium* TA100 and exposed to a purified, epoxide-free TCE sample. The authors observed no mutagenic response in strain TA1535 with S9 mix and in either TA1535 or TA100 without rat or mouse liver S9. Similar results were obtained by Baden et al. ([1979](#)), Bartsch et al. ([1979](#)), and Crebelli et al. ([1982](#)). In all of these studies, purified, epoxide-free TCE samples induced slight but reproducible and dose-related increases in *his* + revertants in *S. typhimurium* TA100 only in the presence of S9. No mutagenic activity was detected without exogenous metabolic activation or when liver S9 from naïve rats, mice, and hamsters ([Crebelli et al., 1982](#)) was used for activation. Therefore, a number of these studies showed positive results in TA100 with metabolic activation, but not in other strains or without metabolic activation.

**Table 4-6. TCE genotoxicity: bacterial assays**

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
<i>S. typhimurium</i> (TA100)	0.1–10 µL (epoxide-free)	–	–	Plate incorporation assay	Henschler et al. (1977)
<i>S. typhimurium</i> (TA1535, TA100)	1–2.5% (epoxide-free)	+ (TA100) – (TA1535)			Simmon et al. (1977)
<i>S. typhimurium</i> (TA98, TA100)	0.5–10%	–	–	The study was conducted in sealed dessicator vials	Waskell (1978)
<i>S. typhimurium</i> (TA100, TA1535)	1–3% (epoxide-free)	+ (TA100) ± (TA1535)	–		Baden et al. (1979)
<i>S. typhimurium</i> (TA100)	5–20% (v/v)	–	–	Negative under normal conditions, but twofold increase in mutations in a preincubation assay	Bartsch et al. (1979)
	0.33–1.33% (epoxide-free)	+	–		Crebelli et al. (1982)
<i>S. typhimurium</i> (TA1535, TA100)	1–5% (higher and lower purity)	– (higher purity) + (lower purity)	–	Extensive cytotoxicity	Shimada et al. (1985)
<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA97)	10–1,000 µL/plate	–	–	Preincubation protocol	Mortelmans et al. (1986)
<i>S. typhimurium</i> (TA98, TA100, TA1535)	≤10,000 µg/plate (unstabilized)	–	Not determined	Vapor assay	McGregor et al. (1989)
	≤10,000 µg/plate (oxirane-stabilized)	+	+	Vapor assay	McGregor et al. (1989)
<i>S. typhimurium</i>	≤10,000 µg/plate (epoxybutane stabilized)	Not determined	+	Preincubation assay	McGregor et al. (1989)
	≤10,000 µg/plate (epichlorohydrin stabilized)	Not determined	+	Vapor assay	McGregor et al. (1989)
<i>S. typhimurium</i> (YG7108)	1.000–3.000 µg/plate	Not determined	+	Microcolony assay/revertants	Emmert et al. (2006)
<i>Escherichia coli</i> (K12)	0.9 mM (analytical grade)	+	–	Revertants at arg56 but not nad113 or other loci	Greim et al. (1975)

Shimada et al. ([1985](#)) tested a low-stabilized, highly purified TCE sample in an Ames reversion test, modified to use vapor exposure, in *S. typhimurium* TA1535 and TA100. No mutagenic activity was observed—either in the presence or absence of S9 mix. However, at the same concentrations (1, 2.5, and 5%), a sample of lower purity, containing undefined stabilizers, was directly mutagenic in TA100 (>5-fold) and TA1535 (>38-fold) at 5% concentration regardless of the presence of S9. It should be noted that the doses used in this study resulted in extensive killing of bacterial population, particularly at 5% concentration; >95% toxicity was observed.

A series of studies evaluating TCE (with and without stabilizers) were conducted by McGregor et al. ([1989](#)). The authors tested high-purity and oxirane-stabilized TCE samples for their mutagenic potential in *S. typhimurium* strains TA1535, TA98, and TA100. Preincubation protocol was used to test stabilized TCE (up to 10,000 µg/plate). Mutagenic response was not observed either in the presence or absence of metabolic activation. When TCE was tested in a vapor delivery system without the oxirane stabilizers, the authors did not observe any mutagenic activity. However, TA1535 and TA100 produced a mutagenic response both in the presence and absence of S9 when exposed to TCE containing 0.5–0.6% 1,2-epoxybutane. Furthermore, exposure to epichlorohydrin also increased the frequency of mutants.

Emmert et al. ([2006](#)) used a CYP2E1-competent bacterial strain (*S. typhimurium* containing YG7108pin3ERb<sub>5</sub> plasmid) in their experiments. TCE was among several other compounds investigated and was tested at concentrations of 1,000–3,000 µg/plate. TCE induced toxicity and microcolonies  $\geq 1,000$  µg per plate. A study on *Escherichia coli* K12 strain was conducted by Greim et al. ([1975](#)) using analytical-grade TCE samples. Revertants were scored at two loci: *arg*<sub>56</sub>, sensitive to base-pair substitution and *nad*<sub>113</sub>, reverted by frameshift mutagens. In addition, forward mutations to 5-methyltryptophan resistance and galactose fermentation were selected. Approximately twofold increase in *arg* + colonies was observed. No change in other sites was observed. No definitive conclusion can be drawn from this study due to lack of information on reproducibility and dose-response.

In addition to the above studies, the ability of TCE to induce gene mutations in bacterial strains has been reviewed and summarized by several authors ([Clewell and Andersen, 2004](#); [Moore and Harrington-Brock, 2000](#); [Douglas et al., 1999](#); [Fahrig et al., 1995](#); [Crebelli and Carere, 1989](#)). In summary, TCE, in its pure form as a parent compound, is unlikely to induce point mutations in most bacterial strains. It is possible that some mutations observed in response to exposure to technical-grade TCE may be contributed by the contaminants/impurities such as 1,2-epoxybutane and epichlorohydrin, which are known bacterial mutagens. However, several studies of TCE reported low, but positive responses in the TA100 strain in the presence of S9 metabolic activation, even when genotoxic stabilizers were not present.

#### 4.2.1.3. Fungal and Yeast Systems—Gene Mutations, Conversions, and Recombination

Gene mutations, conversions, and recombinations have been studied to identify the effect of TCE in fungi and yeast systems (see Table 4-7).

Crebelli et al. (1985) studied the mutagenicity of TCE in *Aspergillus nidulans* both for gene mutations and mitotic segregation. No increase in mutation frequency was observed when *A. nidulans* was plated on selective medium and then exposed to TCE vapors. A small but statistically significant increase in mutations was observed when conidia of cultures were grown in the presence of TCE vapors and then plated on selective media. Since TCE required actively growing cells to exert its genotoxic activity and previous studies (Bignami et al., 1980) have shown activity in the induction of *methG1* suppressors by TCOH and CH, it is possible that endogenous metabolic conversion of TCE into TCOH or CH may have been responsible for the positive response.

To understand the CYP mediated genotoxic activity of TCE, Callen et al. (1980) conducted a study in two yeast strains (D7 and D4) CYP. The D7 strain in its log-phase had a CYP concentration up to 5 times higher than a similar cell suspension of D4 strain. Two different concentrations (15 and 22 mM) at two different time points (1 and 4 hours) were studied. A significant increase in frequencies of mitotic gene conversion and recombination was observed at 15 mM concentrations at 1-hour exposure period in the D7 strain; however, the 22 mM concentration was highly cytotoxic (only 0.3% of the total number of colonies survived). No changes were seen in D4 strain, suggesting that metabolic activation via CYP played an important role in both genotoxicity and cytotoxicity. However, marginal or no genotoxic activity was observed when incubation of cells and test compounds were continued for 4 hours in either strain, possibly because of increased cytotoxicity, or a destruction of the metabolic system.

Koch et al. (1988) studied the genotoxic effects of chlorinated ethylenes including TCE in various yeast *Saccharomyces cerevisiae* strains. Strain D7 was tested (11.1, 16.6, and 22.2 mM TCE) in stationary-phase cells without S9, stationary-phase cells with S9, and logarithmic-phase cells using different concentrations. No significant change in mitotic gene conversion or reverse mutation was observed in either the absence or presence of S9. In addition, there was a considerable increase in the induction of mitotic aneuploidy in strain D61.M, though no statistical analysis was performed.

**Table 4-7. TCE genotoxicity: fungal and yeast systems**

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
<b>Gene conversions</b>					
<i>Saccharomyces cerevisiae</i> D7 and D4	15 and 22 mM; 1 and 4 hrs	Not determined	+ at 1 hr, D7 strain; – at 4 hrs, both D7 and D4	Gene conversion; CYP content fivefold greater in D7 strain; high cytotoxicity at 22 mM	Callen et al. ( <a href="#">1980</a> )
<i>S. cerevisiae</i> D7	11.1, 16.6, and 22.2 mM	–	–	Both stationary and log phase/production of phototropic colonies	Koch et al. ( <a href="#">1988</a> )
<i>Schizosaccharomyces pombe</i>	0.2–200 mM (“pure” and technical-grade)	–	–	Forward mutation, different experiments with different doses and time	Rossi et al. ( <a href="#">1983</a> )
<i>S. cerevisiae</i> D7		+	–		Bronzetti et al. ( <a href="#">1980</a> )
<i>A. nidulans</i>		No data	+	Forward mutation	Crebelli et al. ( <a href="#">1985</a> )
<b>Recombination</b>					
<i>S. cerevisiae</i>		+	–	Gene conversion	Bronzetti et al. ( <a href="#">1980</a> )
<i>S. cerevisiae</i> D7 and D4	15 and 22 mM; 1 and 4 hrs	Not determined	+		Callen et al. ( <a href="#">1980</a> )
<i>A. nidulans</i>		Not determined	+	Gene cross over	Crebelli et al. ( <a href="#">1985</a> )
<b>Mitotic aneuploidy</b>					
<i>S. cerevisiae</i> D61.M	5.5, 11.1, and 16.6 mM	+	+	Loss of dominant color homolog	Koch et al. ( <a href="#">1988</a> )

Rossi et al. (1983) studied the effect of TCE on yeast species *Schizosaccharomyces pombe* both using in vitro and host-mediated mutagenicity studies and the effect of two stabilizers, epichlorohydrin and 1,2-epoxybutane, that are contained in technical-grade TCE. The main goal of this study was to evaluate the genotoxic activity of TCE samples of different purity and determine whether the effect was due to the additives present in the TCE or TCE itself. Forward mutations at five loci (*ade 1, 3, 4, 5, 9*) of the adenine pathway in the yeast, strain P1 was evaluated. The stationary-phase cells were exposed to 25 mM concentration of TCE for 2, 4, and 8 hours in the presence and absence of S9. No change in mutation frequency was observed either in pure- or technical-grade samples either in the presence or absence of S9 at any of the time-points tested. Interestingly, this suggests that the stabilizers used in technical-grade TCE are not genotoxic in yeast. In a follow-up experiment, the same authors studied the effect of different concentrations (0.22, 2.2, and 22.0 mM) in a host-mediated assay using liver microsome preparations obtained from untreated mice, from phenobarbital- and naphthoflavone-pretreated mice and rats, which also suggested that stabilizers were not genotoxic in yeast. This experiment is described in more detail in Section 4.2.1.4.1.

Furthermore, TCE was tested for its ability to induce both point mutation and mitotic gene conversion in diploid strain of yeast *S. cerevisiae* (strain D7) both with and without a mammalian microsomal activation system. In a suspension test with D7, TCE was active only with microsomal activation (Bronzetti et al., 1980).

These studies are consistent with those of bacterial systems in indicating that pure TCE as a parent compound is not likely to cause mutations, gene conversions, or recombinations in fungal or yeast systems. In addition, the data suggest that contaminants used as stabilizers in technical-grade TCE are not genotoxic in these systems, and that the observed genotoxic activity in these systems is predominantly mediated by TCE metabolites.

#### **4.2.1.4. Mammalian Systems Including Human Studies**

##### **4.2.1.4.1. Gene mutations (bacterial, fungal, or yeast with a mammalian host)**

Very few studies have been conducted to identify the effect of TCE, particularly on gene (point) mutations using mammalian systems (see Table 4-8). An overall summary of different endpoints using mammalian systems will be provided at the end of this section. In order to assess the potential mutagenicity of TCE and its possible contaminants, Rossi et al. (1983) performed genotoxicity tests using two different host-mediated assays with pure- and technical-grade TCE. Male mice were administered with one dose of 2 g/kg of pure or technical-grade TCE by gavage. Following the dosing, for the i.p. host-mediated assay, yeast cell suspensions ( $2 \times 10^9$  cells/mL) were inoculated into the peritoneal cavity of the animals. Following 16 hours, animals were sacrificed and yeast cells were recovered to detect the induction of forward mutations at five loci (*ade 1, 2, 4, 5, 9*) of the adenine pathway. A second host-mediated assay was performed by exposing the animals to 2 g/kg of pure or technical-grade TCE and

inoculating the cells into the blood system. Yeast cells were recovered from livers following 4 hours of exposure. Forward mutations in the five loci (*ade 1,2,4,5,9*) were not observed in host-mediated assay either with pure or technical-grade TCE. Genotoxic activity was not detected when the mutagenic epoxide stabilizers were tested for mutagenicity independently or in combination. To confirm the sensitivity of the assay, the authors tested a positive control, *N*-nitroso-dimethyl-nitrosamine (1 mg/kg), and found a mutation frequency of >20 times the spontaneous level. The authors suggested that the negative result could have been due to an inadequate incubation time of the sample with the yeast cells.

Male and female transgenic *lac Z* mice were exposed by inhalation to an actual concentrations of 0, 203, 1,153, and 3,141 ppm TCE, 6 hours/day for 12 days (Douglas et al., 1999). Following 14 and 60 days of last exposure, animals were sacrificed and the mutation frequencies were determined in various organs such as bone marrow, kidney, spleen, liver, lung, and testicular germ cells. No statistically significant increases in base-changes or small-deletions were observed at any of the doses tested in male or female lung, liver, bone marrow, spleen, and kidney, or in male testicular germ cells when the animals were sampled 60 days after exposure. In addition, statistically significantly increased gene mutations were not observed in the lungs at 14 days after the end of exposure (Douglas et al., 1999). The authors acknowledged that *lacZ* bacteriophage transgenic assay does not detect large deletions. The authors also acknowledged that their hypothesis does not readily explain the increases in small deletions and base-change mutations found in the von Hippel-Lindau (*VHL*) tumor suppressor gene in RCCs of the TCE-exposed population. DCA, a TCE metabolite has been shown to increase *lacI* mutations in transgenic mouse liver, however, only after 60-weeks-of-exposure to high concentration (>1,000 ppm) in drinking water (Leavitt et al., 1997). DCA induced relatively small increase in *lac I* mutations when the animals were exposed for 60 weeks, a significantly longer duration than the TCE exposure in the Douglas et al. (1999) study (<2 weeks). Because a relatively small fraction of TCE is metabolized to DCA (see Section 3.3), the mutagenic effect of DCA is unlikely to have been detected in the experiments in Douglas et al. (1999). GSH conjugation, which leads to the production of genotoxic metabolites (see Section 4.2.5), constitutes a relatively small (and relatively uncertain) portion of TCE metabolism in mice, with little data on the extent of renal DCVC bioactivation vs. detoxification in mice (see Sections 3.3 and 3.5). In addition, statistically significantly increased kidney tumors have not been reported in mice with TCE treatment, and the increased incidence of kidney tumors in rats, while considered biologically significant, are quite low and not always statistically significant (see Section 4.4). Therefore, although Douglas et al. (1999) did not detect increased mutations in the kidney, these results are not highly informative as to the role of mutagenicity in TCE-induced kidney tumors, given the uncertainties in the production in genotoxic GSH conjugation metabolites in mice and the low carcinogenic potency of TCE for kidney tumors in rodents relative to what is detectable in experimental bioassays.

**Table 4-8. TCE genotoxicity: mammalian systems—gene mutations and chromosome aberrations**

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
<b>Gene mutations (forward mutations)</b>					
<i>Schizosaccharomyces pombe</i>	2 g/kg, 4 and 16 hrs	Not determined	–	Host-mediated: i.v. and i.p. injections of yeast cells	Rossi et al. ( <a href="#">1983</a> )
<b>Gene mutations (mutations frequency)</b>					
<i>lac Z</i> transgenic mice	0, 203, 1,153, or 3,141 ppm	No base changes or small deletions	No base changes or small deletions	Lung, liver, bone marrow, spleen, kidney, testicular germ cells used	Douglas et al. ( <a href="#">1999</a> )
<b>Chromosomal aberrations<sup>a</sup></b>					
Chinese hamster ovary	745–14,900 µg/mL	Not determined	–	8–14 hrs	Galloway et al. ( <a href="#">1987</a> )
	499–14,900 µg/mL	–	Not determined	2 hrs exposure	Galloway et al. ( <a href="#">1987</a> )
C57BL/6J mice	5, 50, 500, or 5,000 ppm (6 hrs)	–	Not applicable	Splenocytes	Kligerman et al. ( <a href="#">1994</a> )
Sprague-Dawley rats	5, 50, 500, or 5,000 ppm (6 hrs, single and 4-d exposure)	–	Not applicable	Peripheral blood lymphocytes	Kligerman et al. ( <a href="#">1994</a> )

<sup>a</sup>It should be noted that results of most chromosomal aberration assays report the combined incidence of multiple effects, including chromatid breaks, isochromatid or chromosome breaks, chromatid exchanges, dicentric chromosomes, ring chromosomes, and other aberrations.

#### 4.2.1.4.2. *VHL* gene mutations

Studies have been conducted to determine the role of *VHL* gene mutations in RCC, with and without TCE exposure, and are summarized here. Most of these studies are epidemiologic, comparing *VHL* mutation frequencies of TCE-exposed to nonexposed cases from RCC case-control studies, or to background mutation rates among other RCC case series (described in Section 4.4.3). Inactivation of the *VHL* gene through mutations, loss of heterozygosity, and imprinting has been observed in about 70% of renal clear cell carcinomas ([Alimov et al., 2000](#); [Kenck et al., 1996](#)). Recent studies have also examined the role of other genes or pathways in RCC subtypes, including c-Myc activation and vascular endothelial growth factor (VEGF) ([Toma et al., 2008](#); [Furge et al., 2007](#)).

Several studies have examined the role of *VHL* gene inactivation in RCC, including a recent study that measured not only mutations but also promoter hypermethylation ([Nickerson et al., 2008](#)). This study focused on kidney cancer regardless of cause, and found that 91% of cc-RCC exhibited alterations of the *VHL* gene, suggesting a role for *VHL* mutations as an early event in clear cell-RCC. A recent analysis of current epidemiological studies of renal cell cancer suggests *VHL* gene alterations as a marker of clear cell-RCC, but that limitations of previous studies may make the results difficult to interpret ([Chow and Devesa, 2008](#)). Conflicting results have been reported in epidemiological studies of *VHL* mutations in TCE-exposed cases and are described in detail in Section 4.2.7. Both Brüning et al. ([1997b](#)) and Brauch et al. ([2004](#); [1999](#)) associated increased *VHL* mutation frequency in TCE-exposed RCC cases. The two other available studies of Schraml et al. ([1999](#)) and Charbotel et al. ([2007](#)), because of their limitations and lower mutation detection rate in the case of Charbotel et al. ([2007](#)) neither add nor detract to the conclusions from the earlier studies. Additional discussion of these data is provided in Section 4.4.3.

Limited animal studies have examined the role of TCE and *VHL* mutations, although Mally et al. ([2006](#)) have recently conducted both in vitro and in vivo studies using the Eker rat model (see Section 4.4.6.1.1). The Eker rat model (*Tsc-2*<sup>±</sup>) is at increased risk for the development of spontaneous RCC and as such, has been used to understand the mechanisms of renal carcinogenesis ([Stemmer et al., 2007](#); [Wolf et al., 2000](#)). One study has demonstrated similar pathway activation in Eker rats as that seen in humans with *VHL* mutations leading to RCC, suggesting that *Tsc-2* inactivation is analogous to inactivation of *VHL* in human RCC ([Liu et al., 2003](#)). In Mally et al. ([2006](#)), male rats carrying the Eker mutation were exposed to TCE (0, 100, 250, 500, or 1,000 mg/kg body weight by gavage, 5 days/week) for 13 weeks to determine the renal effects (additional data from this study on in vitro DCVC exposure are discussed below, Section 4.2.5). A significant increase in labeling index in kidney tubule cells was observed; however, no enhancement of preneoplastic lesions or tumor incidence was found in Eker rat kidneys compared to controls. In addition, no *VHL* gene mutations in exons 1–3 were

detected in tumors obtained from either control or TCE-exposed Eker rats. Although no other published studies have directly examined *VHL* mutations following exposure to TCE, two studies performed mutational analysis of archived formalin-fixed paraffin embedded tissues from renal carcinomas from previous rat studies. These carcinomas were induced by the genotoxic carcinogens potassium bromate ([Shiao et al., 2002](#)) or *N*-nitrosodimethylamine ([Shiao et al., 1998](#)). Limited mutations in the *VHL* gene were observed in all samples, but, in both studies, these were found only in the clear cell renal carcinomas. Limitations of these two studies include the small number of total samples analyzed, as well as potential technical issues with DNA extraction from archival samples (see Section 4.4.6.6.1). However, analyses of *VHL* mutations in rats may not be informative as to the potential genotoxicity of TCE in humans because the *VHL* gene may not be the target for nephrocarcinogenesis in rats to the extent that it appears to be in humans.

#### **4.2.1.4.3. Chromosomal aberrations**

A few studies were conducted to investigate the ability of TCE to induce chromosomal aberrations in mammalian systems (see Table 4-8). Galloway et al. ([1987](#)) studied the effect of TCE on chromosome aberrations in Chinese hamster ovary cells. When the cells were exposed to TCE (499–14,900 µg/mL) for 2 hours with metabolic activation, S9, no chromosomal aberrations were observed. Furthermore, without metabolic activation, no changes in chromosomal aberrations were found when the cells were exposed to TCE concentrations of 745–14,900 µg/mL for 8–14 hours. It should be noted that in this study, liquid incubation method was used and the experiment was part of a larger study to understand the genotoxic potential of 108 chemicals.

Three inhalation studies in mice and rats examined if TCE could induce cytogenetic damage ([Kligerman et al., 1994](#)). In the first two studies, CD rats or C57Bl/6 mice, were exposed to 0-, 5-, 500-, or 5,000-ppm TCE for 6 hours. Peripheral blood lymphocytes in rats and splenocytes in mice were analyzed for induction of chromosomal aberrations, sister chromatid exchanges (SCEs), and micronucleus formation. The results of micronucleus and SCEs will be discussed in the next sections (see Sections 4.2.1.4.4 and 4.2.1.4.5). No significant increase in chromosomal aberrations was observed in binucleated peripheral blood lymphocytes. In the third study, the authors exposed the same strain of rats for 6 hours/day over 4 consecutive days. No statistically significant concentration-related increases in chromosomal aberrations were observed. The limited results of the above studies have not reported TCE to cause chromosomal aberrations either in in vitro or in vivo mammalian systems.

#### 4.2.1.4.4. Micronucleus induction

The appearance of micronuclei is another endpoint that can demonstrate the genotoxic effect of a chemical. Several studies have been conducted to identify if TCE can cause micronucleus formation (see Table 4-9).

Wang et al. (2001) investigated micronucleus formation by TCE administered as a vapor in Chinese hamster ovary-K1 cells in vitro. Cells were grown in culture media with an inner Petri dish containing TCE that would evaporate into the media containing cells. The concentration of TCE in cultured medium was determined by gas chromatography. The actual concentration of TCE ranged from 0.8 and 1.4 ppm after a 24-hour treatment. A significant dose-dependent increase in micronuclei formation was observed. A dose-dependent decrease in cell growth and cell number was also observed. The authors did not test if the micronuclei formed were due to direct damage to the DNA or spindle formation.

Robbiano et al. (2004) conducted an in vitro study on DNA damage and micronuclei formation in rat and human kidney cells exposed to six carcinogenic chemicals including TCE. The authors examined for the ability of TCE to induce DNA fragmentation and formation of micronuclei in primary cultures of rat and human kidney cells derived from kidney cancer patients with 1–4 mM TCE concentrations. A significant dose-dependent increase in the frequency of micronuclei was obtained in primary kidney cells from both male rats and human of both genders. The authors acknowledged that the significance of the results should be considered in light of the limitations, including: (1) examination of TCE on cells from only three rats; (2) considerable variation in the frequency of DNA lesions induced in the cells; and (3) the possibility that kidney cells derived from kidney cancer patients may be more sensitive to DNA-damaging activity due to a more marked expression of enzymes involved in the metabolic activation of kidney procarcinogens and suppression of DNA repair processes. Nevertheless, this study is important and provides information of the possible genotoxic effects of TCE.

In the same study, Robbiano et al. (2004) administered rats a single oral dose of TCE (3,591 mg/kg) corresponding to  $\frac{1}{2}$  LD<sub>50</sub>, which had been pre-exposed to folic acid for 48 hours and the rats were euthanized 48 hours later following exposure to TCE. The frequency of binucleated cells was taken as an index of kidney cell proliferation. A statistically significant increase in the average frequency of micronucleus was observed.

Hu et al. (2008) studied the effect of TCE on micronuclei frequencies using human hepatoma HepG2 cells. The cells were exposed to 0.5, 1, 2, and 4 mM TCE for 24 hours. TCE caused a significant increase in micronuclei frequencies at all concentrations tested. It is important to note that similar concentrations were used in Robbiano et al. (2004).

**Table 4-9. TCE genotoxicity: mammalian systems—micronucleus, sister chromatic exchanges**

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
<b>Micronucleus</b>					
Human hepatoma HepG2 cells	0.5–4 mM, 24 hrs	Not applicable	+		Hu et al. (2008)
Primary cultures of human and rat kidney cells	1.0, 2.0, or 4.0 mM	Not applicable	+	Dose-dependent significant increase	Robbiano et al. (2004)
Sprague-Dawley rats	3,591 mg/kg	+	–		Robbiano et al. (2004)
Chinese hamster ovary-K1 cells	0.8–1.4 ppm		+	Dose-dependent significant increase	Wang et al. (2001)
Male CD-1 mice	457 mg/kg	+	Not applicable	Bone marrow, correlated with TCOH in urine	Hrelia et al. (1994)
C56BL/6J mice	5, 50, 500, or 5,000 ppm	–	Not applicable	Splenocytes	Kligerman et al. (1994)
Sprague-Dawley rats	5, 50, 500, or 5,000 ppm	+	Not applicable	Dose dependent; peripheral blood lymphocytes	Kligerman et al. (1994)
<b>SCEs</b>					
Chinese hamster ovary	0.17%	–	Not determined	1 hr (vapor)	White et al. (1979)
	17.9–700 µg/mL	Not determined	+	25 hrs (liquid)	Galloway et al. (1987)
	49.7–14,900 µg/mL	+	Not determined	2 hrs	Galloway et al. (1987)
Human lymphocytes	178 µg/mL	Not determined	+		Gu et al. (1981a; 1981b)
Sprague-Dawley rats	5, 50, 500, or 5,000 ppm	–	Not applicable	Peripheral blood lymphocytes	Kligerman et al. (1994)
Peripheral blood lymphocytes from humans occupationally exposed	Occupational exposure	–	Not applicable		Nagaya et al. (1989a)
C57BL/6J mice	5, 50, 500, or 5,000 ppm	–	Not applicable	Splenocytes	Kligerman et al. (1994)

As described in the chromosomal aberration section (see Section 4.2.1.4.3), inhalation studies were performed using male C57BL/6 mice and CD rats ([Kligerman et al., 1994](#)) to determine if TCE could induce micronuclei. In the first and second study, rats or mice respectively, were exposed to 0-, 5-, 500-, or 5,000 ppm TCE for 6 hours. Peripheral blood lymphocytes in rats and splenocytes in mice were cultured and analyzed for induction of micronuclei formation. Bone marrow polychromatic erythrocytes (PCEs) were also analyzed for micronuclei. TCE caused a statistically significant increase in micronuclei formation at all concentrations in rat bone marrow PCEs but not in mice. The authors note that TCE was significantly cytotoxic at the highest concentration tested as determined by significant concentration-related decrease in the ratio of PCEs/normochromatic erythrocytes. In the third study, to confirm the results of the first study, the authors exposed rats to one dose of 5,000 ppm for 6 hours. A statistical increase in bone marrow micronuclei-PCEs was observed confirming the results of the first study.

Hrelia et al. ([1994](#)) treated male CD-1 mice with TCE (457 mg/kg body weight; i.p.) for 30 hours. Bone marrow cells were harvested for determination of micronuclei frequencies in PCEs. An increase in micronuclei frequency at 30 hours after treatment was observed. Linear regression analysis showed that micronuclei frequency induced by TCE correlated with TCOH concentrations in urine, a marker of TCE oxidative metabolism ([Hrelia et al., 1994](#)).

In summary, based on the results of the above studies, TCE is capable of inducing micronuclei in different in vitro and in vivo systems tested. Specific methods were not used that could definitively identify the mechanism of micronuclei formation. These are important findings that indicate TCE has genotoxic potential as measured by the micronucleus formation.

#### **4.2.1.4.5. SCEs**

Studies have been conducted to understand the ability of TCE to induce SCEs both in vitro and in vivo systems (see Table 4-9). White et al. ([1979](#)) evaluated the possible induction of SCE in Chinese hamster ovary cells using a vapor exposure procedure by exposing the cells to TCE (0.17%) for 1 hour in the presence of S9 metabolic activation. No change in SCE frequencies were observed between the control and the treatment group. However, in another study by Galloway et al. ([1987](#)) a dose-related increase in SCE frequency in repeated experiments both with and without metabolic activation was observed. It should be noted that in this study, liquid incubation was used, and the exposure times were 25 hours without metabolic activation at a concentration between 17.9 and 700 µg/mL and 2 hours in the presence of S9 at a concentration of 49.7–14,900 µg/mL. Due to the difference in the dose, length of exposure, and treatment protocol (vapor exposure vs. liquid incubation), no direct comparison can be made. It should also be noted that inadequacy of dose selection and the absence of positive control in the White et al. ([1979](#)) makes it difficult to interpret the study. In another study ([Gu et al., 1981a](#)), a small but positive response was observed in assays with peripheral lymphocytes.

No statistically significant increase in SCEs was found when male C57Bl/6 mice or CD rats were exposed to TCE at concentrations of 5, 500, or 5,000 ppm for 6 hours ([Kligerman et al., 1994](#)). Furthermore, in another study by Nagaya et al. ([1989b](#)), lymphocytes of TCE-exposed workers (n = 22) and matched controls (n = 22) were analyzed for SCEs. The workers had constantly used TCE in their jobs, although the exact exposure was not provided. The duration of their employment ranged from 0.7 to 34 years, averaging about 10 years. It should be noted that there were both smokers and nonsmokers among the exposed population. If a subject had not smoked for at least 2 years before the samples were taken, then they were considered as nonsmokers. There were eight nonsmokers in the group. If they were classified as smokers, then they smoked between 10 and 50 cigarettes per day. No significant increase in mean SCE frequencies were found in exposed population compared to controls, though the study is relatively small.

In summary, induction of SCEs has been reported in several, though not all, paradigms of TCE exposure, consistent with the structural damage to DNA/chromosomes indicated by excess micronuclei formation.

#### **4.2.1.4.6.     Unscheduled DNA synthesis (UDS)**

In vitro studies are briefly described here, with additional discussion of effects related to TCE-induced UDS in the context of the liver in Section E.2.4.1. Perocco and Prodi ([1981](#)) studied UDS in human lymphocytes cultured in vitro (see Table 4-10). Three doses of TCE (2.5, 5.0, and 10  $\mu\text{L}/\text{mL}$ ) were used as final concentrations with and without S9. The results indicate that there was an increase in UDS only in the presence of S9, and in addition, the increase was maximal at the TCE concentration of 5  $\mu\text{L}/\text{mL}$ . Three chlorinated ethane and ethylene solvent products were examined for their genotoxicity in hepatocyte primary culture DNA repair assays using vapor-phase exposures. Rat hepatocytes primary cultures were initiated and exposed to low-stabilized or standard stabilized TCE (0.1–2.5%) for 3 or 18 hours. UDS or DNA repair was not observed using either low or standard stabilized TCE, even at vapor phase doses up to those that produced extensive cell killing after 3 or 18 hour exposure ([Shimada et al., 1985](#)). Costa and Ivanetich ([1984](#)) examined the ability of TCE to induce UDS hepatocytes isolated from phenobarbital treated rats. The UDS was assessed only at the highest concentration that is tolerated by the hepatocytes (2.8 mM TCE).

These results indicate that TCE stimulated UDS in isolated rodent hepatocytes, and, importantly, in human lymphocytes in vitro.

**Table 4-10. TCE genotoxicity: mammalian systems—UDS, DNA strand breaks/protein crosslinks, and cell transformation**

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
<b>UDS</b>					
Rat primary hepatocytes		Not determined	–		Shimada et al. (1985)
Human lymphocytes	2.5, 5, or 10 µL/mL	±	–	Increase was only in certain doses and maximum at 5 µL/mL concentration	Perocco and Prodi (1981)
Phenobarbital-induced rat hepatocytes	2.8 mM	Not determined	+		Costa and Ivanetich (1984)
<b>DNA strand breaks/protein crosslinks</b>					
Primary rat kidney cells	0.5, 1.0, 2.0, or 4.0 mM	Not applicable	+	Dose-dependent significant increase	Robbiano et al. (2004)
Primary cultures of human kidney cells	1.0, 2.0, or 4.0 mM	Not determined	+	Dose-dependent significant increase	Robbiano et al. (2004)
Sprague-Dawley rats	3,591 mg/kg	+	Not applicable	Single oral administration	Robbiano et al. (2004)
Sprague-Dawley rats	500, 1,000, and 2,000 ppm	–	Not applicable	Comet assay	Clay (2008)
<b>Cell transformation</b>					
BALB/c 3T3 mouse cells	4, 20, 100, or 250 µg/mL	Not applicable	+	Weakly positive compared to other halogenated compounds tested in the same experiment	Tu et al. (1985)
Rat embryo cells		Not applicable	+		Price et al. (1978)
Syrian hamster embryo cells	5, 10, or 25 µg/mL	Not applicable	–		Amacher and Zelljadt (1983)

#### 4.2.1.4.7. DNA strand breaks

DNA damage in response to TCE exposure was studied using comet assay in human hepatoma HepG2 cells ([Hu et al., 2008](#); see Table 4-10). The cells were exposed to 0.5, 1, 2, and 4 mM for 24 hours. TCE increased the DNA migration in a significant dose-dependent manner at all tested concentrations suggesting TCE caused DNA strand breaks and chromosome damage.

TCE (4–10 mmol/kg body weight) were given to male mice by i.p. injection. The induction of single-strand breaks (SSBs) in DNA of liver, kidney, and lung was studied by the DNA unwinding technique. There was a linear increase in the level of SSBs in kidney and liver DNA but not in lung DNA 1 hour after administration ([Walles, 1986](#)).

Robbiano et al. ([2004](#)) conducted an in vitro study on DNA damage in rat and human kidney cells exposed to six carcinogenic chemicals, including TCE, in the comet assay. The authors examined the ability of TCE to induce DNA fragmentation in primary cultures of rat and human kidney cells with 1–4 mM TCE concentrations. TCE was dissolved in ethanol with a maximum concentration of 0.3% and the rat cultures were exposed to 20 hours. Primary human kidney cells were isolated from fragments of kidney discarded during the course of surgery for carcinoma of both male and female donors with an average age of 64.2 years and were also exposed to 20 hours. Significant dose-dependent increases in the ratio of treated/control tail length (average 4–7  $\mu$ M compared to control) was observed as measured by comet assay in primary kidney cells from both male rats and human of both genders.

Clay et al. ([2008](#)) studied the DNA damage inducing capacity of TCE using the comet assay in rat kidney proximal tubules. Rats were exposed by inhalation to a range of TCE concentrations (500, 1,000, or 2,000 ppm) for 6 hours/day for 5 days. TCE did not induce DNA damage (as measured by tail length and percentage tail DNA and tail movement) in rat kidney proximal tubules in any of the doses tested possibly due to study limitations (small number of animals tested [ $n = 5$ ] and limited exposure time [6 hours/day for only 5 days]). These results are in contrast to the findings of Robbiano et al. ([2004](#)), which showed DNA damage and increased micronuclei in the rat kidney 20 hours following a single dose (3,591 mg/kg body weight) of TCE. The DNA damage reported by comet assay is consistent with results for other markers of chromosomal damage or DNA structural damage such as excess micronuclei formation and SCE induced by TCE exposure.

#### 4.2.1.4.8. DNA damage related to oxidative stress, polymorphisms

A detailed description of studies related to lipid peroxidation of TCE is presented in conjunction with discussion of liver toxicity (see Section 4.5, E.2.4.3, and E.3). A recent study reported on genetic polymorphism in solvent exposed population ([Kumar et al., 2009](#)). Normal ( $n = 220$ ) and solvent-exposed ( $n = 97$ ) populations were genotyped for CYP1A1, GSTM1, GSTT1 and GSTP1 polymorphisms. No exposure related differences were observed. In addition, the authors also examined TCE-exposed lymphocytes for the presence of chromosomal

aberrations and micronucleus at concentration of 2, 4 or 6 mM TCE. No significant changes in any of the parameters were observed.

#### 4.2.1.4.9. Cell transformation

In vitro cell transformation using BALB/c-3T3 cells was conducted using TCE with concentrations varying from 0 to 250 µg/mL in liquid phase exposed for 72 hours (see Table 4-10). The cytotoxicity of TCE at the concentration tested in the transformation assay was determined by counting cells from duplicate plates of each test conditions at the end of the treatment period. A dose-dependent increase in Type III foci was observed, although no statistical analysis was conducted ([Tu et al., 1985](#)). In another study by Amacher and Zelljadt ([1983](#)), Syrian hamster embryo cells were exposed to 5, 10, or 25 µg/mL of TCE. In this experiment, two different serums (horse serum and fetal bovine serum) were also tested to understand the importance of serum quality in the transformation assay. A preliminary toxicity assay was performed to select dose levels that had 50–90% cell survival. One week after dosing, the cell colonies were fixed and counted for variability determination and examination of individual colonies for the evidence of morphological transformation. No significant change in morphological cell transformation was obtained. Furthermore, no significant changes were seen in transformed colonies when tested in different serum. However, these studies are of limited use for determining the genotoxic potential of TCE because they did not examine the foci for mutations, for instance in oncogenes or tumor suppressor genes.

#### 4.2.1.5. Summary

Evidence from a number of different analyses and a number of different laboratories using a fairly complete array of endpoints suggests that TCE, following metabolism, has the potential to be genotoxic. A series of carefully controlled studies evaluating TCE itself (without mutagenic stabilizers and without metabolic activation) found it to be incapable of inducing gene mutations in most standard mutation bacterial assays ([Mortelmans et al., 1986](#); [Shimada et al., 1985](#); [Crebelli et al., 1982](#); [Baden et al., 1979](#); [Bartsch et al., 1979](#); [Waskell, 1978](#); [Henschler et al., 1977](#); [Simmon et al., 1977](#)). Therefore, it appears that it is unlikely that TCE is a direct-acting mutagen, though TCE has shown potential to affect DNA and chromosomal structure. Low, but positive, responses were observed in the TA100 strain in the presence of S9 metabolic activation, even when genotoxic stabilizers were not present, suggesting that metabolites of TCE are genotoxic. TCE is also positive in some but not all fungal and yeast systems ([Koch et al., 1988](#); [Crebelli et al., 1985](#); [Rossi et al., 1983](#); [Callen et al., 1980](#)). Data from human epidemiological studies support the possible mutagenic effect of TCE leading to *VHL* gene damage and subsequent occurrence of RCC. Association of increased *VHL* mutation frequency in TCE-exposed RCC cases has been observed ([Brauch et al., 2004](#); [Brauch et al., 1999](#); [Brüning et al., 1997b](#)).

TCE can lead to binding to nucleic acids and proteins ([Kautiainen et al., 1997](#); [Mazzullo et al., 1992](#); [Bergman, 1983](#); [Miller and Guengerich, 1983](#); [DiRenzo et al., 1982](#)), and such binding appears to be due to conversion to one or more reactive metabolites. For instance, increased binding was observed in samples bioactivated with mouse and rat microsomal fractions ([Mazzullo et al., 1992](#); [Miller and Guengerich, 1983](#); [DiRenzo et al., 1982](#); [Banerjee and Van Duuren, 1978](#)). DNA binding is consistent with the ability to induce DNA and chromosomal perturbations. Several studies report the induction of micronuclei in vitro and in vivo from TCE exposure ([Hu et al., 2008](#); [Robbiano et al., 2004](#); [Wang et al., 2001](#); [Hrelia et al., 1994](#); [Kligerman et al., 1994](#)). Reports of SCE induction in some studies are consistent with DNA effects, but require further study ([Kligerman et al., 1994](#); [Nagaya et al., 1989b](#); [Gu et al., 1981a](#); [Gu et al., 1981b](#); [White et al., 1979](#)).

Overall, evidence from a number of different analyses and a number of different laboratories using various genetic endpoints indicates that TCE has a potential to induce damage to the structure of the chromosome in a number of targets, but has a more limited ability to induce mutation in bacterial systems.

Below, the genotoxicity data for TCE metabolites, TCA, DCA, TCOH, CH, DCVC, and DCVG, are briefly reviewed. The contributions of these data are twofold. First, to the extent that these metabolites may be formed in the in vitro and in vivo test systems for TCE, they provide insight into what agent or agents may contribute to the limited activity observed with TCE in these genotoxicity assays. Second, because the in vitro systems do not necessarily fully recapitulate in vivo metabolism, the genotoxicity of the known in vivo metabolites themselves provide data as to whether one may expect genotoxicity to contribute to the toxicity of TCE following in vivo exposure.

#### **4.2.2. TCA**

The TCE metabolite, TCA, has been studied using a variety of genotoxicity assay for its genotoxic potential (see International Agency for Research on Cancer [IARC, 2004] for additional information). Evaluation of in vitro studies of TCA must consider toxicity and acidification of medium resulting in precipitation of proteins, as TCA is commonly used as a reagent to precipitate proteins.

##### **4.2.2.1. Bacterial Systems—Gene Mutations**

TCA has been evaluated in a number of in vitro test systems including the bacterial assays (Ames) using different *S. typhimurium* strains such as TA98, TA100, TA104, TA1535, and RSJ100 (see Table 4-11). The majority of these studies did not report positive findings for genotoxicity (([1976](#)) ([1980](#)) ([1983](#)) ([Kargalioglu et al., 2002](#); [Nelson et al., 2001](#); [DeMarini et al., 1994](#); [Rapson et al., 1980](#); [Waskell, 1978](#)). Waskell ([1978](#)) studied the effect of TCA (0.45 mg/plate) on bacterial strains TA98 and TA100 both in the presence and absence of S9.

The author did not find any revertants at the maximum nontoxic dose tested. Following exposure to TCA, Rapson et al. (1980) reported no change in mutagenic activity in strain TA100 in the absence of S9. DeMarini et al. (1994) performed different studies to evaluate the genotoxicity of TCA, including the Microscreen prophage-induction assay (TCA concentrations 0–10 mg/mL) and use of the *S. typhimurium* TA100 strain using bag vaporization technique (TCA concentrations 0–100 ppm), neither of which yielded positive results. Nelson et al. (2001) reported no positive findings with TCA using a *S. typhimurium* microsuspension bioassay (*S. typhimurium* strain TA104) following incubation of TCA for various lengths of time, with or without rat cecal microbiota. Similarly, no activity was observed in a study conducted by Kargalioglu et al. (2002) where *S. typhimurium* strains TA98, TA100, and RSJ100 were exposed to TCA (0.1–100 mM) either in the presence or absence of S9 (Kargalioglu et al., 2002).

**Table 4-11. Genotoxicity of TCA—bacterial systems**

Test system/endpoint	Doses (LED or HID) <sup>a</sup>	Results		Reference
		With activation	Without activation	
λ Prophage induction, <i>E. coli</i> WP2s	10,000	–	–	DeMarini et al. (1994)
SOS chromotest, <i>E. coli</i> PQ37	10,000	–	–	Giller et al. (1997)
<i>S. typhimurium</i> TA1535, 1536, 1537, 1538, reverse mutation	20 µg/plate	NT	–	Shirasu et al. (1976)
<i>S. typhimurium</i> TA100, 98, reverse mutation	450 µg/plate	–	–	Waskell (1978)
<i>S. typhimurium</i> TA100, 1535, reverse mutation	4,000 µg/plate	–	–	Nestmann et al. (1980)
<i>S. typhimurium</i> TA1537, 1538, 98, reverse mutation	2,000 µg/plate	–	–	Nestmann et al. (1980)
<i>S. typhimurium</i> TA100, reverse mutation	520 µg/plate	NT	–	Rapson et al. (1980)
<i>S. typhimurium</i> TA100, 98, reverse mutation	5,000 µg/plate	–	–	Moriya et al. (1983)
<i>S. typhimurium</i> TA100, reverse mutation	600 ppm	–	–	DeMarini et al. (1994)
<i>S. typhimurium</i> TA100, reverse mutation, liquid medium	1,750	+	+	Giller et al. (1997)
<i>S. typhimurium</i> TA104, reverse mutation, microsuspension	250 µg/plate	–	–	Nelson et al. (2001)
<i>S. typhimurium</i> TA100, RSJ100, reverse mutation	16,300	–	–	Kargalioglu et al. (2002)
<i>S. typhimurium</i> TA98, reverse mutation	13,100	–	–	Kargalioglu et al. (2002)
<i>S. typhimurium</i> TA1535, SOS DNA repair		+	–	Ono et al. (1991)

<sup>a</sup>LED = lowest effective dose; HID = highest ineffective dose; doses are in µg/mL for in vitro tests unless specified.

+ = positive; – = negative; NT = not tested

Source: Table adapted from IARC monograph (2004b) and modified/updated for newer references.

TCA was also negative in other bacterial systems. The SOS chromotest (which measures DNA damage and induction of the SOS repair system) in *E. coli* PQ37, ± S9 ([Giller et al., 1997](#)) evaluated the genotoxic activity of TCA ranging from 10 to 10,000 µg/mL and did not find any response. Similarly, TCA was not genotoxic in the Microscreen prophage-induction assay in *E. coli* with TCA concentrations ranging from 0 to 10,000 µg/mL, with and without S9 activation ([DeMarini et al., 1994](#)).

However, TCA induced a small increase in SOS DNA repair (an inducible error-prone repair system) in *S. typhimurium* strain TA1535 in the presence of S9 ([Ono et al., 1991](#)). Furthermore, Giller et al. ([1997](#)) reported that TCA demonstrated genotoxic activity in an Ames fluctuation test in *S. typhimurium* TA100 in the absence of S9 at noncytotoxic concentrations ranging from 1,750 to 2,250 µg/mL. The addition of S9 decreased the genotoxic response, with effects observed at 3,000–7,500 µg/mL. Cytotoxic concentrations in the Ames fluctuation assay were 2,500 and 10,000 µg/mL without and with microsomal activation, respectively.

#### **4.2.2.2. Mammalian Systems**

##### **4.2.2.2.1. Gene mutations**

The mutagenicity of TCA has also been tested in cultured mammalian cells (see Table 4-12). Harrington-Brock et al. ([1998](#)) examined the potential of TCA to induce mutations in L5178Y/TK<sup>±</sup>-3.7.2C mouse lymphoma cells. In this study, mouse lymphoma cells were incubated in culture medium treated with TCA concentrations up to 2,150 µg/mL in the presence of S9 metabolic activation and up to 3,400 µg/mL in the absence of S9 mixture. In the presence of S9, a doubling of mutant frequency was seen at concentrations of ≥2,250 µg/mL, including several concentrations with survival >10%. In the absence of S9, TCA increased the mutant frequency by twofold or greater only at concentrations of ≥2,000 µg/mL. These results were obtained at ≤11% survival rates. The authors noted that the mutants included both large- and small-colony mutants. The small-colony mutants are indicative of chromosomal damage. It should be noted that no rigorous statistical evaluation was conducted on these data. Cytotoxic and genotoxic effects of TCA were tested in a microplate-based cytotoxicity test and a HGPRT gene mutation assay using Chinese hamster ovary K1 cells, respectively ([Zhang et al., 2010](#)). TCA was the least cytotoxic when compared to six other haloacetic acids. TCA, at concentrations of 0, 200, 1,000, 5,000 and 10,000 µM, induced a visible increase in mutant frequency but did not show any statistically significant increase at any of the doses tested.

**Table 4-12. TCA Genotoxicity—mammalian systems (both in vitro and in vivo)**

Test system/endpoint	Doses (LED or HID) <sup>a</sup>	Results		Reference
		With activation	Without activation	
Gene mutation, mouse lymphoma L5178Y/TK ± cells, in vitro	3,000	(+)	?	Harrington-Brock et al. (1998)
Gene mutation, Chinese hamster ovary cells in vitro, HGPRT gene mutation assay	10,000 µM	NT	–	Zhang et al. (2010)
DNA strand breaks, B6C3F <sub>1</sub> mouse and F344 rat hepatocytes, in vitro	1,630	NT	–	Chang et al. (1992)
DNA strand breaks, human CCRF-CEM lymphoblastic cells, in vitro	1,630	NT	–	Chang et al. (1992)
DNA damage, Chinese hamster ovary cells, in vitro, comet assay	3 mM	NT	–	Plewa et al. (2002)
DNA strand breaks, B6C3F <sub>1</sub> mouse liver, in vivo	1.0, oral, × 1	+		Nelson and Bull (1988)
DNA strand breaks, B6C3F <sub>1</sub> mouse liver, in vivo	500, oral, × 1	+		Nelson et al. (1989)
DNA strand breaks, B6C3F <sub>1</sub> mouse liver, in vivo	500, oral, 10 repeats	–		Nelson et al. (1989)
DNA strand breaks, B6C3F <sub>1</sub> mouse liver and epithelial cells from stomach and duodenum, in vivo	1,630, oral, × 1	–		Chang et al. (1992)
DNA strand breaks, male B6C3F <sub>1</sub> mice, in vivo	500 (neutralized)	–		Styles et al. (1991)
Micronucleus formation, Swiss mice, in vivo	125, i.p., × 2	+		Bhunya and Behera (1987)
Micronucleus formation, female C57BL/6JfBL10/Alpk mouse bone-marrow erythrocytes, in vivo	1,300, i.p., × 2	–		Mackay et al. (1995)
Micronucleus formation, male C57BL/6JfBL10/Alpk mouse bone-marrow erythrocytes, in vivo	1,080, i.p., × 2	–		Mackay et al. (1995)
Micronucleus formation, <i>Pleurodeles waltl</i> newt larvae peripheral erythrocytes, in vivo	80	+		Giller et al. (1997)
Chromosomal aberrations, Swiss mouse bone-marrow cells in vivo	125, i.p., × 1	+		Bhunya and Behera (1987)
	100, i.p., × 5	+		Bhunya and Behera (1987)
	500, oral, × 1	+		Bhunya and Behera (1987)
Chromosomal aberrations, chicken <i>Gallus domesticus</i> bone marrow, in vivo	200, i.p., × 1	+		Bhunya and Jena (1996)

**Table 4-12. TCA Genotoxicity—mammalian systems (both in vitro and in vivo) (continued)**

Test system/endpoint	Doses (LED or HID) <sup>a</sup>	Results <sup>b</sup>		Reference
		With activation	Without activation	
Chromosomal aberrations, human lymphocytes, in vitro	5,000, (neutralized)	–		Mackay et al. ( <a href="#">1995</a> )
Sperm morphology, Swiss mice, in vivo	125, i.p., × 5	+		Bhunya and Behera ( <a href="#">1987</a> )

<sup>a</sup>LED = lowest effective dose; HID = highest ineffective dose; doses are in µg/mL for in vitro tests; mg/kg for in vivo tests unless specified.

+ = positive; (+) = weakly positive; – = negative; NT = not tested; ? = inconclusive

Source: Table adapted from IARC monograph ([2004b](#)) and modified/updated for newer references.

#### 4.2.2.2.2. Chromosomal aberrations

Mackay et al. (1995) investigated the ability of TCA to induce chromosomal damage in an in vitro chromosomal aberration assay using cultured human cells. The authors treated the cells with TCA as free acid, both in the presence and absence of metabolic activation. TCA induced chromosomal damage in cultured human peripheral lymphocytes at concentrations (2,000 and 3,500 µg/mL) that significantly reduced the pH of the medium. However, exposure of cells to neutralized TCA did not have any effect even at a cytotoxic concentration of 5,000 µg/mL. It is possible that the reduced pH was responsible for the TCA-induced clastogenicity in this study. To further evaluate the role of pH changes in the induction of chromosome damage, the authors isolated liver-cell nuclei from B6C3F<sub>1</sub> mice and suspended in a buffer at various pH levels. The cells were stained with chromatin-reactive (fluorescein isothiocyanate) and DNA-reactive (propidium iodide) fluorescent dyes. A decrease in chromatin staining intensity was observed with the decrease in pH, suggesting that pH changes, independent of TCA exposure, can alter chromatin conformation. It was concluded by the authors that TCA-induced pH changes are likely to be responsible for the chromosomal damage induced by un-neutralized TCA. In another in vitro study, Plewa et al. (2002) evaluated the induction of DNA strand breaks induced by TCA (1–25 mM) in Chinese hamster ovary cells and did not observe any genotoxicity.

#### 4.2.2.2.3. Micronucleus

Relative genotoxicity of TCA was tested in a mouse in vivo system (see Table 4-12) using three different cytogenetic assay (bone marrow chromosomal aberrations, micronucleus, and sperm-head abnormalities) (Bhunya and Behera, 1987) and for chromosomal aberrations in chicken (Bhunya and Jena, 1996). TCA induced a variety of anomalies including micronucleus in the bone marrow of mice and chicken. A small increase in the frequency of micronucleated erythrocytes at 80 µg/mL in a newt (*Pleurodeles waltl* larvae) micronucleus test was observed in response to TCA exposure (Giller et al., 1997). Mackay et al. (1995) investigated the ability of TCA to induce chromosomal DNA damage in the in vivo bone-marrow micronucleus assay in mice. C57BL mice were given TCA intraperitoneally at doses of 0, 337, 675, or 1,080 mg/kg-day for males and 0, 405, 810, or 1,300 mg/kg-day for females for two consecutive days, and bone-marrow samples were collected 6 and 24 hours after the last dose. The administered doses represented 25, 50, and 80% of the median lethal dose, respectively. No treatment-related increase in micronucleated PCEs was observed.

#### 4.2.2.2.4. Other DNA damage studies

DNA unwinding assays have been used as indicators of SSBs and are discussed in detail in Section E.2.3. Studies were conducted on the ability of TCA to induce SSBs (see Table 4-12)

([Chang et al., 1992](#); [Styles et al., 1991](#); [Nelson et al., 1989](#); [Nelson and Bull, 1988](#)). Nelson and Bull ([1988](#)) evaluated the ability of TCA and other compounds to induce single-strand DNA breaks in vivo in Sprague-Dawley rats and B6C3F<sub>1</sub> mice. Single oral doses were administered to three groups of three animals, with an additional group as a vehicle control. Animals were sacrificed after 4 hours, and 10% liver suspensions were analyzed for single-strand DNA breaks by the alkaline unwinding assay. Dose-dependent increases in single-strand DNA breaks were induced in both rats and mice, with mice being more susceptible than rats. The lowest dose of TCA that produced significant SSBs was 0.6 mmol/kg (98 mg/kg) in rats but 0.006 mmol/kg (0.98 mg/kg) in mice.

However, in a follow-up study, Nelson et al. ([1989](#)) male B6C3F<sub>1</sub> mice were treated with 500 mg/kg TCA, and SSBs in whole liver homogenate were examined, and no significant differences from controls were reported. Moreover, in the experiments in the same study with DCA, increased SSBs were reported, but with no dose-response between 10 and 500 mg/kg, raising concerns about the reliability of the DNA unwinding assay used in these studies. For further details, see Section E.2.3. In an additional follow-up experiment with a similar experimental paradigm, Styles et al. ([1991](#)) tested TCA for its ability to induce strand breaks in male B6C3F<sub>1</sub> mice in the presence and absence of liver growth induction. The test animals were given one, two, or three daily doses of neutralized TCA (500 mg/kg) by gavage and killed 1 hour after the final dose. Additional mice were given a single 500-mg/kg gavage dose and sacrificed 24 hours after treatment. Liver nuclei DNA were isolated, and the induction of SSBs was evaluated using the alkaline unwinding assay. Exposure to TCA did not induce strand breaks under the conditions tested in this assay. In a study by Chang et al. ([1992](#)), administration of single oral doses of TCA (1–10 mmol/kg) to B6C3F<sub>1</sub> mice did not induce DNA strand breaks in a dose-related manner as determined by the alkaline unwinding assay. No genotoxic activity (evidence for strand breakage) was detected in F344 rats administered by gavage up to 5 mmol/kg (817 mg/kg).

In summary, although Nelson and Bull ([1988](#)) report effects on DNA unwinding for TCE and its metabolites with DCA having the highest activity and TCA the lowest, Nelson et al. ([1989](#)), using the same assay, reported no effect for TCA and the same effect at 10 and 500 mg/kg for DCA in mice. Moreover, Styles et al. ([1991](#)) did not find a positive result for TCA using the same paradigm as Nelson and Bull ([1988](#)) and Nelson et al. ([1989](#)). Furthermore, Chang et al. ([1992](#)) also did not find increased SSBs for TCA exposure in rats. (see Section E.2.4.3).

#### **4.2.2.3. Summary**

In summary, TCA has been studied using a variety of genotoxicity assays, including the recommended battery. No mutagenicity was reported in *S. typhimurium* strains in the presence or absence of metabolic activation or in an alternative protocol using a closed system, except in

one study on strain TA100 using a modified protocol in liquid medium. This is largely consistent with the results from TCE, which was negative in most bacterial systems except some studies with the TA100 strain. Mutagenicity in mouse lymphoma cells was only induced at cytotoxic concentrations. Measures of DNA-repair responses in bacterial systems have been inconclusive, with induction of DNA repair reported in *S. typhimurium* but not in *E. coli*. TCA-induced clastogenicity may be secondary to pH changes and not a direct effect of TCA.

#### 4.2.3. DCA

DCA is another metabolite of TCE that has been studied using a variety of genotoxicity assay for its genotoxic potential (see Tables 4-13 and 4-14; see IARC (2004b) for additional information).

##### 4.2.3.1. Bacterial and Fungal Systems—Gene Mutations

Studies were conducted to evaluate mutagenicity of DCA in different *S. typhimurium* and *E. coli* strains ([Kargalioglu et al., 2002](#); [Nelson et al., 2001](#); [Giller et al., 1997](#); [Fox et al., 1996a](#); [Fox et al., 1996b](#); [DeMarini et al., 1994](#); [Herbert et al., 1980](#); [Waskell, 1978](#)). DCA was mutagenic in three strains of *S. typhimurium*: strain TA100 in three of five studies, strain RSJ100 in a single study, and strain TA98 in two of three studies. DCA failed to induce point mutations in other strains of *S. typhimurium* (TA104, TA1535, TA1537, and TA1538) or in *E. coli* strain WP2uvrA. In one study, DCA caused a weak induction of SOS repair in *E. coli* strain PQ37 ([Giller et al., 1997](#)).

DeMarini et al. (1994), in the same study as described in the TCA section of this section, also studied DCA as one of their compounds for analysis. In the prophage-induction assay using *E. coli*, DCA, in the presence of S9, was genotoxic producing 6.6–7.2 plaque-forming units (PFU)/mM and slightly less than threefold increase in PFU/plate in the absence of S9. In the second set of studies, which involved the evaluation of DCA at concentrations of 0–600 ppm for mutagenicity in *S. typhimurium* TA100 strain, DCA was mutagenic both in the presence and absence of S9, producing 3–5 times increases in the revertants/plate compared to the background. The lowest effective concentrations for DCA without S9 were 100 and 50 ppm in the presence of S9. In the third and most important study, mutation spectra of DCA were determined at the base-substitution allele *hisG46* of *S. typhimurium* TA100. DCA-induced revertants were chosen for further molecular analysis at concentrations that produced mutant yields that were two- to fivefold greater than the background. The mutation spectra of DCA were significantly different from the background mutation spectrum. Thus, despite the modest increase in the mutant yields (3–5 times) produced by DCA, the mutation spectra confirm that DCA is mutagenic. DCA primarily induced GC-AT transitions.

**Table 4-13. Genotoxicity of DCA (bacterial systems)**

Test system/endpoint	Doses (LED or HID) <sup>a</sup>	Results <sup>b</sup>		Reference
		With activation	Without activation	
$\lambda$ Prophage induction, <i>E. coli</i> WP2s	2,500	+	–	DeMarini et al. (1994)
SOS chromotest, <i>E. coli</i> PQ37	500	–	(+)	Giller et al. (1997)
<i>S. typhimurium</i> , DNA repair-deficient strains TS24, TA2322, TA1950	31,000	–	–	Waskell (1978)
<i>S. typhimurium</i> TA100, TA1535, TA1537, TA1538, reverse mutation		–	–	Herbert et al. (1980)
<i>S. typhimurium</i> TA100, reverse mutation	50	+	+	DeMarini et al. (1994)
<i>S. typhimurium</i> TA100,TA1535, TA1537, TA98, reverse mutation	5,000	–	–	Fox et al., (1996b)
<i>S. typhimurium</i> TA100, reverse mutation, liquid medium	100	+	+	Giller et al. (1997)
<i>S. typhimurium</i> RSJ100, reverse mutation	1,935	–	+	Kargalioglu et al. (2002)
<i>S. typhimurium</i> TA104, reverse mutation, microsuspension	150 µg/plate	–	–	Nelson et al. (2001)
	10 µg/plate	(+)	–	Herbert et al. (1980)
<i>S. typhimurium</i> TA98, reverse mutation	5,160	–	+	Kargalioglu et al. (2002)
	1,935	+	+	Kargalioglu et al. (2002)
<i>S. typhimurium</i> TA100, reverse mutation	1,935	+	+	Kargalioglu et al. (2002)
<i>E. coli</i> WP2uvrA, reverse mutation	5,000	–	–	Fox et al., (1996b)

<sup>a</sup>LED = lowest effective dose; HID = highest ineffective dose; doses are in µg/mL for in vitro tests unless specified.

+ = positive; (+) = weakly positive; – = negative

Source: Table adapted from IARC monograph (2004b) and modified/updated for newer references.

**Table 4-14. Genotoxicity of DCA—mammalian systems**

Test system/endpoint	Doses (LED or HID) <sup>a</sup>	Results		Reference
		With activation	Without activation	
Gene mutation, mouse lymphoma cell line L5178Y/TK ± in vitro	5,000	–	–	Fox et al., (1996b)
Gene mutation, mouse lymphoma cell line L5178Y/TK ± –3.7.2C in vitro	400	NT	+	Harrington-Brock et al. (1998)
Gene mutation, Chinese hamster ovary cells in vitro, HGPRT gene mutation assay	1,000 µM	NT	+	Zhang et al. (2010)
DNA strand breaks and alkali-labile damage, Chinese hamster ovary cells in vitro (single-cell gel electrophoresis assay)	3,225 µg/mL	NT	–	Plewa et al. (2002)
DNA strand breaks, B6C3F <sub>1</sub> mouse hepatocytes in vitro	2,580	NT	–	Chang et al. (1992)
DNA strand breaks, F344 rat hepatocytes in vitro	1,290	NT	–	Chang et al. (1992)
Micronucleus formation, mouse lymphoma L5178Y/TK ± –3.7.2C cell line in vitro	800	NT	–	Harrington-Brock et al. (1998)
Chromosomal aberrations, Chinese hamster ovary in vitro	5,000	–	–	Fox et al., (1996b)
Chromosomal aberrations, mouse lymphoma L5178Y/Tk ± –3.7.2C cell line in vitro	600	NT	+	Harrington-Brock et al. (1998)
Aneuploidy, mouse lymphoma L5178Y/Tk ± –3.7.2C cell line in vitro	800	NT	–	Harrington-Brock et al. (1998)
DNA strand breaks, human CCRF-CEM lymphoblastoid cells in vitro	1,290	NT	–	Chang et al. (1992)
DNA strand breaks, male B6C3F <sub>1</sub> mouse liver in vivo	13, oral, × 1	+		Nelson and Bull (1988)
DNA strand breaks, male B6C3F <sub>1</sub> mouse liver in vivo	10, oral, × 1	+		Nelson et al. (1989)
DNA strand breaks, male B6C3F <sub>1</sub> mouse liver in vivo	1,290, oral, × 1	–		Chang et al. (1992)
DNA strand breaks, male B6C3F <sub>1</sub> mouse splenocytes in vivo	1,290, oral, × 1	–		Chang et al. (1992)
DNA strand breaks, male B6C3F <sub>1</sub> mouse epithelial cells from stomach and duodenum in vivo	1,290, oral, × 1	–		Chang et al. (1992)
DNA strand breaks, male B6C3F <sub>1</sub> mouse liver in vivo	5,000, dw, × 7–14 d	–		Chang et al. (1992)
DNA strand breaks, alkali-labile sites, cross linking, male B6C3F <sub>1</sub> mouse blood leukocytes in vivo (single-cell gel electrophoresis assay)	3,500, dw, × 28 d	+		Fuscoe et al. (1996)

**Table 4-14. Genotoxicity of DCA—mammalian systems (continued)**

Test system/endpoint	Doses (LED or HID) <sup>a</sup>	Results		Reference
		With activation	Without activation	
DNA strand breaks, male Sprague-Dawley rat liver in vivo	30, oral, × 1		+	Nelson and Bull ( <a href="#">1988</a> )
DNA strand breaks, male F344 rat liver in vivo	645, oral, × 1		–	Chang et al. ( <a href="#">1992</a> )
DNA strand breaks, male F344 rat liver in vivo	2,000, dw, × 30 wks		–	Chang et al. ( <a href="#">1992</a> )
Gene mutation, lacI transgenic male B6C3F <sub>1</sub> mouse liver assay in vivo	1,000, dw, × 60 wks		+	Leavitt et al. ( <a href="#">1997</a> )
Micronucleus formation, male B6C3F <sub>1</sub> mouse peripheral erythrocytes in vivo	3,500, dw, × 9 d		+	Fuscoe et al. ( <a href="#">1996</a> )
Micronucleus formation, male B6C3F <sub>1</sub> mouse peripheral erythrocytes in vivo	3,500, dw, × 28 d		–	Fuscoe et al. ( <a href="#">1996</a> )
Micronucleus formation, male B6C3F <sub>1</sub> mouse peripheral erythrocytes in vivo	3,500, dw, × 10 wks		+	Fuscoe et al. ( <a href="#">1996</a> )
Micronucleus formation, male and female CrI:CD (Sprague-Dawley) BR rat bone-marrow erythrocytes in vivo	1,100, i.v., × 3		–	Fox et al., ( <a href="#">1996b</a> )
Micronucleus formation, Pleurodeles waltl newt larvae peripheral erythrocytes in vivo	80 d		–	Giller et al. ( <a href="#">1997</a> )

<sup>a</sup>LED = lowest effective dose; HID = highest ineffective dose; doses are in µg/mL for in vitro tests; mg/kg for in vivo tests unless specified; dw = drinking-water (in mg/L).

+ = positive; – = negative; NT = not tested

Source: Table adapted from IARC monograph ([2004b](#)) and modified/updated for newer references.

Kargalioglu et al. (2002) analyzed the cytotoxicity and mutagenicity of the drinking water disinfection byproducts including DCA in *S. typhimurium* strains TA98, TA100, and RSJ100 ± S9. DCA was mutagenic in this test although the response was low when compared to other disinfection byproducts tested in strain TA100. This study was also summarized in a review by Plewa et al. (2002). Nelson et al. (2001) investigated the mutagenicity of DCA using a *S. typhimurium* microsuspension bioassay following incubation of DCA for various lengths of time, with or without rat cecal microbiota. No mutagenic activity was detected for DCA with *S. typhimurium* strain TA104.

Although limited data, it appears that DCA has mutagenic activity in the *S. typhimurium* strains, particularly TA100.

#### **4.2.3.2. Mammalian Systems**

##### **4.2.3.2.1. Gene mutations**

The mutagenicity of DCA has been tested in mammalian systems, particularly, mouse lymphoma cell lines in vitro (Harrington-Brock et al., 1998; Fox et al., 1996b); and *lacI* transgenic mice in vivo (Leavitt et al., 1997). Harrington-Brock et al. (1998) evaluated DCA for its mutagenic activity in L5178Y/TK ± (-) 3.7.2C mouse lymphoma cells. A dose-related increase in mutation (and cytotoxic) frequency was observed at concentrations between 100 and 800 µg/mL. Most mutagenic activity of DCA at the *Tk* locus was due to the production of small-colony *Tk* mutants (indicating chromosomal mutations). Different pH levels were tested in induction of mutant frequencies and it was determined that the mutagenic effect observed was due to the chemical and not pH effects.

Mutation frequencies were studied in male transgenic B6C3F<sub>1</sub> mice harboring the bacterial *lacI* gene administered DCA at either 1.0 or 3.5 g/L in drinking water (Leavitt et al., 1997). No significant difference in mutant frequency was observed after 4 or 10 weeks of treatment in both of the doses tested as compared to control. However, at 60 weeks, mice treated with 1.0 g/L DCA showed a slight increase (1.3-fold) in the mutant frequency over the control, but mice treated with 3.5 g/L DCA had a 2.3-fold increase in the mutant frequency. Mutational spectra analysis revealed that ~33% had G:C-A:T transitions and 21% had G:C-T:A transversions and this mutation spectra was different than that was seen in the untreated animals, indicating that the mutations were likely induced by the DCA treatment. The authors conclude that these results are consistent with the previous observation that the proportion of mutations at T:A sites in codon 61 of the *H-ras* gene was increased in DCA-induced liver tumors in B6C3F<sub>1</sub> mice (Leavitt et al., 1997).

Zhang et al. (2010) tested the cytotoxic and genotoxic effects of DCA in a microplate-based cytotoxicity test and HGPRT gene mutation assay using Chinese hamster ovary K1 cells, respectively. The concentrations at which these tests were conducted were 0, 200, 1,000, 5,000 and 10,000 µM. Two parameters were used to indicate chronic cytotoxicity: the lowest

cytotoxic concentration and the percent C1/2 value. The lowest cytotoxic concentration for DCA was  $2.87 \times 10^{-3}$  M. Statistically significant increase in HGPRT mutant frequency was observed at concentrations  $\geq 1,000$   $\mu$ M.

#### **4.2.3.2.2. Chromosomal aberrations and micronucleus**

Harrington-Brock et al. (1998) evaluated DCA for its potential to induce chromosomal aberrations in DCA-treated (0, 600, and 800  $\mu$ g/mL) mouse lymphoma cells. A clearly positive induction of aberrations was observed at both concentrations tested. No significant increase in micronucleus was observed in DCA-treated (0, 600, and 800  $\mu$ g/mL) mouse lymphoma cells (Harrington-Brock et al., 1998). However, no chromosomal aberrations were found in Chinese hamster ovary cells exposed to DCA (Fox et al., 1996b)

Fusco et al. (1996) investigated in vivo genotoxic potential of DCA in bone marrow and blood leukocytes using the peripheral-blood-erythrocyte micronucleus assay (to detect chromosome breakage and/or malsegregation) and the alkaline single cell gel electrophoresis (comet) assay, respectively. Mice were exposed to DCA in drinking water, available ad libitum, for up to 31 weeks. A statistically significant dose-related increase in the frequency of micronucleated PCEs was observed following subchronic exposure to DCA for 9 days. Similarly, a significant increased was also observed when exposed for  $\geq 10$  weeks particularly at the highest dose of DCA tested (3.5 g/L). DNA cross-linking was observed in blood leukocytes in mice exposed to 3.5 g/L DCA for 28 days. These data provide evidence that DCA may have some potential to induce chromosome damage when animals were exposed to concentrations similar to those used in the rodent bioassay.

#### **4.2.3.2.3. Other DNA damage studies**

Nelson and Bull (1988) and Nelson et al. (1989) have been described in Sections 4.2.2.2.4 and E.2.3, with positive results for DNA unwinding for DCA, though Nelson et al. (1989) reported the same response at 10 and 500 mg/kg in mice, raising concerns about the reliability of the assay in these studies. Chang et al. (1992) conducted both in vitro and in vivo studies to determine the ability of DCA to cause DNA damage. Primary rat (F344) hepatocytes and primary mouse hepatocytes treated with DCA for 4 hours did not induce DNA SSBs as detected by alkaline DNA unwinding assay. No DNA strand breaks were observed in human CCRF-CEM lymphoblastoid cells in vitro exposed to DCA. Similarly, analysis of the DNA SSBs in mice killed 1 hour after a single dose of 1, 5, or 10 mM/kg DCA did not cause DNA damage. None of the F344 rats killed 4 hours after a single gavage treatment (1–10 mM/kg) produced any detectable DNA damage.

#### 4.2.3.3. Summary

In summary, DCA has been studied using a variety but limited number of genotoxicity assays. Within the available data, DCA has been demonstrated to be mutagenic in the *S. typhimurium* assay, particularly in strain TA100, the in vitro mouse lymphoma assay and in vivo cytogenetic and gene mutation assays. DCA can cause DNA strand breaks in mouse and rat liver cells following in vivo administration by gavage.

#### 4.2.4. CH

CH has been evaluated for its genotoxic potential using a variety of genotoxicity assays (see Tables 4-15, 4-16, and 4-17). These data are particularly important because it is known that a large flux of TCE metabolism leads to CH as an intermediate, so a comparison of their genotoxicity profiles is likely to be highly informative.

##### 4.2.4.1. DNA Binding Studies

Limited analysis has been performed examining DNA binding potential of CH ([Von Tungeln et al., 2002](#); [Ni et al., 1995](#); [Keller and Hd'A, 1988](#)). Keller and Heck ([1988](#)) conducted both in vitro and in vivo experiments using B6C3F<sub>1</sub> mouse strain. The mice were pretreated with 1,500 mg/kg TCE for 10 days and then given 800 mg/kg [<sup>14</sup>C] chloral. No detectable covalent binding of [<sup>14</sup>C] to DNA in the liver was observed. Another study with in vivo exposures to nonradioactive CH at a concentration of 1,000 and 2,000 nmol in mice B6C3F<sub>1</sub> demonstrated an increase in malondialdehyde-derived and 8-oxo-2'-deoxyguanosine adducts in liver DNA ([Von Tungeln et al., 2002](#)). Ni et al. ([1995](#)) observed malondialdehyde adducts in calf thymus DNA when exposed to CH and microsomes from male B6C3F<sub>1</sub> mouse liver.

Keller and Heck ([1988](#)) investigated the potential of chloral to form DNA-protein cross-links in rat liver nuclei using concentrations 25, 100, or 250 mM. No statistically significant increase in DNA-protein cross-links was observed. DNA and RNA isolated from the [<sup>14</sup>C] chloral-treated nuclei did not have any detectable [<sup>14</sup>C] bound. However, the proteins from chloral-treated nuclei did have a concentration-related binding of [<sup>14</sup>C].

**Table 4-15. CH genotoxicity: bacterial, yeast, and fungal systems**

Test system/endpoint	Doses (LED or HID) <sup>a</sup>	Results		Reference
		With activation	Without activation	
SOS chromotest, <i>Escherichia coli</i> PQ37	10,000	–	–	Giller et al. (1995)
<i>S. typhimurium</i> TA100, TA1535, TA98, reverse mutation	10,000	–	–	Waskell (1978)
<i>S. typhimurium</i> TA100, TA1537, TA1538, TA98, reverse mutation	1,000	+	+	Haworth et al. (1983)
<i>S. typhimurium</i> TA100, reverse mutation	5,000 µg/plate	–	–	Leuschner and Leuschner (1991)
<i>S. typhimurium</i> TA100, reverse mutation	2,000 µg/plate	+	+	Ni et al. (1994)
<i>S. typhimurium</i> TA100, reverse mutation, liquid medium	300	+	–	Giller et al. (1995)
<i>S. typhimurium</i> TA100, TA104, reverse mutation	1,000 µg/plate	+	+	Beland (1999)
<i>S. typhimurium</i> TA104, reverse mutation	1,000 µg/plate	+	+	Ni et al. (1994)
<i>S. typhimurium</i> TA1535, reverse mutation	1,850	–	–	Leuschner and Leuschner (1991)
<i>S. typhimurium</i> TA1535, TA1537 reverse mutation	6,667	–	–	Haworth et al. (1983)
<i>S. typhimurium</i> TA1535, reverse mutation	10,000	–	–	Beland (1999)
<i>S. typhimurium</i> TA98, reverse mutation	7,500	–	–	Haworth et al. (1983)
<i>S. typhimurium</i> TA98, reverse mutation	10,000 µg/plate	–	+	Beland (1999)
<i>A. nidulans</i> , diploid strain 35X17, mitotic cross-overs	1,650	Not tested	–	Crebelli et al. (1985)
<i>A. nidulans</i> , diploid strain 30, mitotic cross-overs	6,600	Not tested	–	Kafer (1986)
<i>A. nidulans</i> , diploid strain NH, mitotic cross-overs	1,000	Not tested	–	Kappas (1989)
<i>A. nidulans</i> , diploid strain P1, mitotic cross-overs	990	Not tested	–	Crebelli et al. (1991)
<i>A. nidulans</i> , diploid strain 35X17, nondisjunctions	825	Not tested	+	Crebelli et al. (1985)
<i>A. nidulans</i> , diploid strain 30, aneuploidy	825	Not tested	+	Kafer (1986)
<i>A. nidulans</i> , haploid conidia, aneuploidy, polyploidy	1,650	Not tested	+	Kafer (1986)
<i>A. nidulans</i> , diploid strain NH, nondisjunctions	450	Not tested	+	Kappas (1989)

**Table 4-15. CH genotoxicity: bacterial, yeast, and fungal systems (continued)**

Test system/endpoint	Doses (LED or HID) <sup>a</sup>	Results <sup>b</sup>		Reference
		With activation	Without activation	
<i>A. nidulans</i> , diploid strain P1, nondisjunctions	660	Not tested	+	Crebelli et al. (1991)
<i>A. nidulans</i> , haploid strain 35, hyperploidy	2,640	Not tested	+	Crebelli et al. (1991)
<i>S. cerevisiae</i> , meiotic recombination	3,300	Not tested	Inconclusive	Sora and Agostini Carbone (1987)
<i>S. cerevisiae</i> , disomy in meiosis	2,500	Not tested	+	Sora and Agostini Carbone (1987)
<i>S. cerevisiae</i> , disomy in meiosis	3,300	Not tested	+	Sora and Agostini Carbone (1987)
<i>S. cerevisiae</i> , D61.M, mitotic chr. malsegregation	1,000	Not tested	+	Albertini, (1990)
<i>Drosophila melanogaster</i> , somatic mutation wing spot test	825		+	Zordan et al. (1994)
<i>Drosophila melanogaster</i> , induction of sex-linked lethal mutation	37.2 feed		Inconclusive	Beland (1999)
<i>Drosophila melanogaster</i> , induction of sex-linked lethal mutation	67.5 inj		-	Beland (1999)

<sup>a</sup>LED = lowest effective dose; HID = highest ineffective dose; doses are in µg/mL for in vitro tests; inj = injection.

+ = positive; - = negative

Source: Table adapted from IARC monograph (2004b) and modified/updated for newer references.

**Table 4-16. CH genotoxicity: mammalian systems—all genetic endpoints, in vitro**

Test system/endpoint	Doses (LED or HID) <sup>a</sup>	Results		Reference
		With activation	Without activation	
DNA-protein cross-links, rat nuclei in vitro	41,250	NT	–	Keller and Heck (1988)
DNA SSBs, rat primary hepatocytes in vitro	1,650	NT	–	Chang et al. (1992)
Gene mutation, mouse lymphoma L5178Y/TK <sup>±</sup> , in vitro	1,000		(+)	Harrington-Brock et al. (1998)
SCEs, Chinese hamster ovary cells, in vitro	100	+	+	Beland (1999)
Micronucleus formation (kinetochore-positive), Chinese hamster C1 cells, in vitro	165	NT	+	Degrassi and Tanzarella (1988)
Micronucleus formation (kinetochore-negative), Chinese hamster C1 cells, in vitro	250	NT	–	Degrassi and Tanzarella (1988)
Micronucleus formation (kinetochore-positive), Chinese hamster LUC2 cells, in vitro	400	NT	+	Parry et al., (1990)
Micronucleus formation (kinetochore-positive), Chinese hamster LUC2 cells, in vitro	400	NT	+	Lynch and Parry (1993)
Micronucleus formation, Chinese hamster V79 cells, in vitro	316	NT	+	Seelbach et al. (1993)
Micronucleus formation, mouse lymphoma L5178Y/TK <sup>±</sup> , in vitro	1,300	NT	–	Harrington-Brock et al. (1998)
Micronucleus formation, mouse lymphoma L5178Y/TK <sup>±</sup> , in vitro	500	NT	+	Nesslany and Marzin (1999)
Chromosomal aberrations, Chinese Hamster CHED cells, in vitro	20	NT	+	Furnus et al. (1990)
Chromosomal aberrations, Chinese Hamster ovary cells, in vitro	1,000	+	+	Beland (1999)
Chromosomal aberrations, mouse lymphoma L5178Y/TK <sup>±</sup> cells line, in vitro	1,250	NT	(+)	Harrington-Brock et al. (1998)
Aneuploidy, Chinese hamster CHED cells, in vitro	10	NT	+	Furnus et al. (1990)
Aneuploidy, primary Chinese hamster embryonic cells, in vitro	250	NT	+	Natarajan et al. (1993)
Aneuploidy, Chinese hamster LUC2p4 cells, in vitro	250	NT	+	Warr et al. (1993)
Aneuploidy, mouse lymphoma L5178Y/TK <sup>±</sup> , in vitro	1,300	NT	–	Harrington-Brock et al. (1998)
Tetraploidy and endoreduplication, Chinese hamster LUC2p4cells, in vitro	500	NT	+	Warr et al. (1993)
Cell transformation, Syrian hamster embryo cells (24-hr treatment)	350	NT	+	Gibson et al. (1995)
Cell transformation, Syrian hamster dermal cell line (24-hr treatment)	50	NT	+	Parry et al. (1996)
DNA SSBs, human lymphoblastoid cells, in vitro	1,650	NT	–	Chang et al. (1992)

**Table 4-16. CH genotoxicity: mammalian systems—all genetic endpoints, in vitro (continued)**

Test system/endpoint	Doses (LED or HID) <sup>a</sup>	Results		Reference
		With activation	Without activation	
Gene mutation, <i>tk</i> and <i>hprt</i> locus, human lymphoblastoid	1,000	NT	+	Beland ( <a href="#">1999</a> )
SCEs, human lymphocytes, in vitro	54	NT	(+)	Gu et al. ( <a href="#">1981a</a> )
Micronucleus formation, human lymphocytes, in vitro	100	–	+	Van Hummelen and Kirsch-Volders ( <a href="#">1992</a> )
Micronucleus formation, human lymphoblastoid AHH-1 cell line, in vitro	100	NT	+	Parry et al. ( <a href="#">1996</a> )
Micronucleus formation, human lymphoblastoid maximum contaminant level-5 cell line, in vitro	500	NT	–	Parry et al. ( <a href="#">1996</a> )
Micronucleus formation (kinetochore-positive), human diploid LEO fibroblasts, in vitro	120	NT	+	Bonatti et al. ( <a href="#">1992</a> )
Aneuploidy (double Y induction), human lymphocytes, in vitro	250	NT	+	Vagnarelli et al. ( <a href="#">1990</a> )
Aneuploidy (hyperdiploidy and hypodiploidy), human lymphocytes in vitro	50	NT	+	Sbrana et al. ( <a href="#">1993</a> )
Polyploidy, human lymphocytes, in vitro	137	NT	+	Sbrana et al. ( <a href="#">1993</a> )
C-Mitosis, human lymphocytes, in vitro	75	NT	+	Sbrana et al. ( <a href="#">1993</a> )

<sup>a</sup>LED = lowest effective dose; HID = highest ineffective dose; doses are in µg/mL for in vitro tests.

+ = positive; (+) = weakly positive in an inadequate study; – = negative; NT = not tested

Source: Table adapted from IARC monograph ([2004b](#)) and modified/updated for newer references.

**Table 4-17. CH genotoxicity: mammalian systems—all genetic damage, in vivo**

Test system/endpoint	Doses (LED or HID) <sup>a</sup>	Results	Reference
DNA SSBs, male Sprague-Dawley rat liver	300, oral	+	Nelson and Bull (1988)
DNA SSBs, male F344 rat liver	1,650, oral	–	Chang et al. (1992)
DNA SSBs, male B6C3F <sub>1</sub> mouse liver	100, oral	+	Nelson and Bull (1988)
DNA SSBs, male B6C3F <sub>1</sub> mouse liver	825, oral	–	Chang et al. (1992)
Micronucleus formation, male and female NMRI mice, bone-marrow erythrocytes	500, i.p.	–	Leuschner and Leuschner (1991)
Micronucleus formation, BALB/c mouse spermatids	83, i.p.	–	Russo and Levis (1992b)
Micronucleus formation, male BALB/c mouse bone-marrow erythrocytes and early spermatids	83, i.p.	+	Russo and Levis (1992a)
Micronucleus formation, male BALB/c mouse bone-marrow erythrocytes	200, i.p.	+	Russo et al. (1992)
Micronucleus formation, male F1 mouse bone-marrow erythrocytes	400, i.p.	–	Leopardi et al. (1993)
Micronucleus formation, C57B1 mouse spermatids	41, i.p.	+	Allen et al., 1994
Micronucleus formation, male Swiss CD-1 mouse bone-marrow erythrocytes	200, i.p.	+	Marrazzini et al., (1994)
Micronucleus formation, B6C3F <sub>1</sub> mouse spermatids after spermatogonial stem-cell treatment	165, i.p.	+	Nutley et al. (1996)
Micronucleus formation, B6C3F <sub>1</sub> mouse spermatids after meiotic cell treatment	413, i.p.	–	Nutley et al. (1996)
Micronucleus formation, male F1, BALB/c mouse peripheral-blood erythrocytes	200, i.p.	–	Grawe et al. (1997)
Micronucleus formation, male B6C3F <sub>1</sub> mouse bone-marrow erythrocytes	500, i.p., × 3	+	Beland (1999)
Micronucleus formation, infants, peripheral lymphocytes	50, oral	+	Ikbal et al. (2004)
Chromosomal aberrations, male and female F1 mouse bone marrow cells	600, i.p.	–	Xu and Alder (1990)
Chromosomal aberrations, male and female Sprague-Dawley rat bone-marrow cells	1,000, oral	–	Leuschner and Leuschner (1991)
Chromosomal aberrations, BALB/c mouse spermatogonia treated	83, i.p.	–	Russo and Levis, (1992a)
Chromosomal aberrations, F1 mouse secondary spermatocytes	82.7, i.p.	+	Russo et al. (1984)
Chromosomal aberrations, male Swiss CD-1 mouse bone-marrow erythrocytes	400, i.p.	–	Marrazzini et al. (1994)
Chromosomal aberrations, ICR mouse oocytes	600, i.p.	–	Mailhes et al. (1993)

**Table 4-17. CH genotoxicity: mammalian systems—all genetic damage, in vivo (continued)**

Test system/endpoint	Doses (LED or HID) <sup>a</sup>	Results	Reference
Micronucleus formation, infants, peripheral lymphocytes	50, oral	+	Ikbal et al. ( <a href="#">2004</a> )
Polyploidy, male and female F1, mouse bone-marrow cells	600, i.p.	–	Xu and Alder ( <a href="#">1990</a> )
Aneuploidy F1 mouse secondary spermatocytes	200, i.p.	+	Miller and Adler ( <a href="#">1992</a> )
Aneuploidy, male F1 mouse secondary spermatocytes	400, i.p.	–	Leopardi et al. ( <a href="#">1993</a> )
Hyperploidy, male Swiss CD-1 mouse bone-marrow erythrocytes	200, i.p.	+	Marrazzini et al. ( <a href="#">1994</a> )

<sup>a</sup>LED = lowest effective dose; HID = highest ineffective dose; doses are in mg/kg body weight for in vivo tests.

+ = positive; – = negative

Source: Table adapted from IARC monograph ([2004b](#)) and modified/updated for newer references.

#### 4.2.4.2. Bacterial and Fungal Systems—Gene Mutations

CH induced gene mutations in *S. typhimurium* TA100 and TA104 strains, but not in most other strains assayed. Four of six studies of CH exposure in *S. typhimurium* TA100 and two of two studies in *S. typhimurium* TA104 were positive for revertants ([Beland, 1999](#); [Giller et al., 1995](#); [Ni et al., 1994](#); [Haworth et al., 1983](#)). Waskell ([1978](#)) studied the effect of CH along with TCE and its other metabolites. CH was tested at different doses (1.0–13 mg/plate) in different *S. typhimurium* strains (TA98, TA100, TA1535) for gene mutations using Ames assay. No revertant colonies were observed in strains TA98 or TA1535 both in the presence and absence of S9 mix. Similar results were obtained by Leuschner and Leuschner ([1991](#)). However, in TA100, a dose-dependent statistically significant increase in revertant colonies was obtained both in the presence and absence of S9. It should be noted that CH that was purchased from Sigma was recrystallized 1–6 times from chloroform and the authors describe this as crude CH. However, this positive result is consistent with other studies in this strain as noted above. Furthermore, Giller et al. ([1995](#)) studied CH genotoxicity in three short-term tests. Chloral-induced mutations in strain TA100 of *S. typhimurium* (fluctuation test). Similar results were obtained by Haworth et al. ([1983](#)). These are consistent with several studies of TCE, in which low, but positive responses were observed in the TA100 strain in the presence of S9 metabolic activation, even when genotoxic stabilizers were not present.

A significant increase in mitotic segregation was observed in *A. nidulans* when exposed to 5 and 10 mM CH ([Crebelli et al., 1985](#)). Studies of mitotic crossing-over in *A. nidulans* have been negative, while these same studies were positive for aneuploidy ([Crebelli et al., 1991](#); [Kappas, 1989](#); [Käfer, 1986](#); [Crebelli et al., 1985](#)).

Two studies were conducted in *S. cerevisiae* to understand the chromosomal malsegregation as a result of exposure to CH ([Albertini, 1990](#); [Sora and Agostini Carbone, 1987](#)). CH (1–25 mM) was dissolved in sporulation medium and the frequencies of various meiotic events such as recombination and disomy were analyzed. CH inhibited sporulation as a function of dose and increased diploid and disomic clones. CH was also tested for mitotic chromosome malsegregation using *S. cerevisiae* D61.M ([Albertini, 1990](#)). The tester strain was exposed to a dose range of 1–8 mg/mL. An increase in the frequency of chromosomal malsegregation was observed as a result of exposure to CH.

Limited analysis of CH mutagenicity has been performed in *Drosophila* ([Beland, 1999](#); [Zordan et al., 1994](#)). Of these two studies, CH was positive in the somatic mutation wing spot test ([Zordan et al., 1994](#)) and equivocal in the induction of sex-linked lethal mutation when in feed but negative when exposed via injection ([Beland, 1999](#)).

#### 4.2.4.3. Mammalian Systems

##### 4.2.4.3.1. Gene mutations

Harrington-Brock et al. (1998) noted that CH-induced concentration related cytotoxicity in TK± mouse lymphoma cell lines without S9 activation. A nonstatistical increase in mutant frequency was observed in cells treated with CH. The mutants were primarily small colony TK mutants, indicating that most CH-induced mutants resulted from chromosomal mutations rather than point mutations. It should be noted that in most concentrations tested (350–1,600 µg/mL), cytotoxicity was observed. Percentage cell survival ranged from 96 to 4%.

##### 4.2.4.3.2. Micronucleus

Micronuclei induction following exposure to CH is positive in most test systems in both in vitro and in vivo assays, although some negative tests also exist (Harrington-Brock et al., 1998; Allen et al., 1994; Marrazzini et al., 1994) (Iqbal et al., 2004; Beland, 1999; Nesslany and Marzin, 1999; Grawé et al., 1997; Nutley et al., 1996; Parry et al., 1996; Giller et al., 1995; Leopardi et al., 1993; Lynch and Parry, 1993; Seelbach et al., 1993; Bonatti et al., 1992; Russo and Levis, 1992b, a; Russo et al., 1992; Van Hummelen and Kirsch-Volders, 1992; Leuschner and Leuschner, 1991; Degrassi and Tanzarella, 1988). Some studies have attempted to make inferences regarding aneuploidy induction or clastogenicity as an effect of CH. Aneuploidy results from defects in chromosome segregation during mitosis and is a common cytogenetic feature of cancer cells (see Section E.3.1.5).

Giller et al. (1995) studied CH genotoxicity in three short-term tests. CH caused a significant increase in the frequency of micronucleated erythrocytes following in vivo exposure of the amphibian, *Pleurodeles waltl*, newt larvae.

CH induced aneuploidy in vitro in multiple Chinese hamster cell lines (Natarajan et al., 1993; Warr et al., 1993; Furnus et al., 1990) and human lymphocytes (Sbrana et al., 1993; Vagnarelli et al., 1990) but not in mouse lymphoma cells (Harrington-Brock et al., (1998)). In vivo studies performed in various mouse strains led to increased aneuploidy in spermatocytes (Miller and Adler, 1992; Liang and Pacchierotti, 1988; Russo et al., 1984), but not oocytes (Mailhes et al., (1993)) or bone marrow cells (Leopardi et al., 1993; Xu and Adler, 1990).

The potential of CH to induce aneuploidy in mammalian germ cells has been of particular interest since Russo et al. (1984) first demonstrated that CH treatment of male mice results in significant increase in frequencies of hyperploidy in metaphase II cells. This hyperploidy was thought to have arisen from chromosomal nondisjunction in premeiotic/meiotic cell division and may be a consequence of CH interfering with spindle formation (reviewed by Russo et al. (1984)] and Liang and Brinkley (1985])). CH also causes meiotic delay, which may be associated with aneuploidy (Miller and Adler, 1992). CH has been shown to induce micronuclei but not structural chromosomal aberrations in mouse bone-marrow cells. Micronuclei induced by nonclastogenic agents are generally believed to represent intact chromosomes that failed to

segregate into either daughter-cell nucleus at cell division ([Russo et al., 1992](#); [Xu and Adler, 1990](#)). Furthermore, CH-induced micronuclei in mouse bone-marrow cells ([Russo et al., 1992](#)) and in cultured mammalian cells ([Bonatti et al., 1992](#); [Degrassi and Tanzarella, 1988](#)) have shown to be predominantly kinetochore-positive in composition upon analysis with immunofluorescent methods. The presence of a kinetochore in a micronucleus is considered evidence that the micronucleus contains a whole chromosome lost at cell division ([Eastmond and Tucker, 1989](#); [Degrassi and Tanzarella, 1988](#); [Hennig et al., 1988](#)). Therefore, both TCE and CH appear to increase the frequency of micronuclei.

Allen et al. ([1994](#)) treated male C57B1/6J mice were given a single i.p. injection of 0, 41, 83, or 165 mg/kg CH. Spermatids were harvested at 22 hours, and 11, 13.5, and 49 days following exposure ([Allen et al., 1994](#)). Harvested spermatids were processed to identify both kinetochore-positive micronucleus (aneugen) and kinetochore-negative micronucleus (clastogen). All CH doses administered 49 days prior to cell harvest were associated with significantly increased frequencies of kinetochore-negative micronuclei in spermatids, however; dose dependence was not observed. This study is in contrast with other studies ([Bonatti et al., 1992](#); [Degrassi and Tanzarella, 1988](#)), which demonstrated predominantly kinetochore-positive micronucleus.

The ability of CH to induce aneuploidy and polyploidy was tested in human lymphocyte cultures established from blood samples obtained from two healthy nonsmoking donors ([Sbrana et al., 1993](#)). Cells were exposed for 72 and 96 hours at doses between 50 and 250 µg/mL. No increase in percentage hyperdiploid, tetraploid, or endoreduplicated cells were observed when cells were exposed to 72 hours at any doses tested. However, at 96 hours of exposure, significant increase in hyperdiploid was observed at one dose (150 µg/mL) and was not dose dependent. Significant increase in tetraploid was observed at a dose of 137 mg/mL, but again, no dose dependence was observed.

Ikbāl et al. ([2004](#)) assessed the genotoxic effects in cultured peripheral blood lymphocytes of 18 infants (age range of 31–55 days) before and after administration of a single dose of CH (50 mg/kg of body weight) for sedation before a hearing test for micronucleus frequency. A significant increase in micronuclei frequency was observed after administration of CH.

#### **4.2.4.3.3. Chromosomal aberrations**

Several studies have included chromosomal aberration analysis in both in vitro and in vivo systems exposed to CH and have had positive results in vitro—although not all studies had statistically significant increases (Harrington-Brock et al., ([1998](#)); ([Beland, 1999](#); [Furnus et al., 1990](#)).

Analysis of CH treated mouse lymphoma cell lines for chromosomal aberrations resulted in a nonsignificant increase in chromosomal aberrations (Harrington-Brock et al., ([1998](#)).

However, it should be noted that the concentrations tested (1,250 and 1,300 µg/mL) were cytotoxic (with a cell survival of 11 and 7%, respectively). Chinese hamster embryo cells were also exposed to 0.001, 0.002, and 0.003% CH for 1.5 hours ([Furnus et al., 1990](#)). A nonstatistically significant increase in frequency of chromosomal aberrations was observed only 0.002 and 0.003% concentrations, with the increase not dose-dependent. In this study, it should be noted that the cells were only exposed for 1.5 hours to CH and cells were allowed to grow for 48 hours (two cell cycles) to obtain similar mitotic index before analyzing for chromosomal aberrations. No information on cytotoxicity was provided except that higher doses decreased the frequency of mitotic cells at the time of fixation.

In vivo chromosome aberration studies have mostly reported negative or null results ([Mailhes et al., 1993](#); [Russo and Levis, 1992b, a](#); [Leuschner and Leuschner, 1991](#); [Xu and Adler, 1990](#); [Liang and Pacchierotti, 1988](#)) with the exception of one study ([Russo et al., 1984](#)) in an F1 cross of mouse strain between C57B1/Cne × C3H/Cne.

#### **4.2.4.3.4. SCEs**

SCEs were assessed by Ikbal et al. ([2004](#)) in cultured peripheral blood lymphocytes of 18 infants (age range of 31–55 days) before and after administration of a single dose of CH (50 mg/kg of body weight) for sedation before a hearing test. The authors report a significant increase in the mean number of SCEs, from before administration ( $7.03 \pm 0.18$  SCEs/cell) and after administration ( $7.90 \pm 0.19$  SCEs/cell), with each of the 18 individuals showing an increase with treatment. Micronuclei were also significantly increased. SCEs were also assessed by Gu et al. ([1981a](#)) in human lymphocytes exposed in vitro with inconclusive results, although positive results were observed by Beland ([1999](#)) in Chinese hamster ovary cells exposed in vitro with and without an exogenous metabolic system.

#### **4.2.4.3.5. Cell transformation**

CH was positive in the two studies designed to measure cellular transformation ([Parry et al., 1996](#); [Gibson et al., 1995](#)). Both studies exposed Syrian hamster cells (embryo and dermal) to CH and induced cellular transformation.

#### **4.2.4.4. Summary**

CH has been reported to induce micronuclei formation, aneuploidy, and mutations in multiple in vitro systems and in vivo. In vivo studies have limited results to an increased micronuclei formation mainly in mouse spermatocytes. CH was positive in some in vitro genotoxicity assays that detected point mutations, micronuclei induction, chromosomal aberrations, and/or aneuploidy. The in vivo data exhibited mixed results ([Allen et al., 1994](#)) ([Leuschner and Beuscher, 1998](#); [Nutley et al., 1996](#); [Adler, 1993](#); [Mailhes et al., 1993](#); [Russo et al., 1992](#); [Xu and Adler, 1990](#)). Most of the positive studies showed that CH induces

aneuploidy. Based on the existing array of data, CH has the potential to be genotoxic, particularly when aneuploidy is considered in the weight of evidence for genotoxic potential. Some have suggested that CH may act through a mechanism of spindle poisoning and resulting in numerical changes in the chromosomes, but some data also suggest induction of chromosomal aberrations. These results are consistent with TCE, albeit there are more limited data on TCE for these genotoxic endpoints.

#### 4.2.5. DCVC and DCVG

DCVC and DCVG have been studied for their genotoxic potential; however, since there is a limited number of studies to evaluate them based on each endpoint, particularly in mammalian systems, the following section has been combined to include all of the available studies for different endpoints of genotoxicity. Study details can be found in Table 4-18.

DCVC and DCVG, cysteine intermediates of TCE formed by the GST pathway, are capable of inducing point mutations as evidenced by the fact that they are positive in the Ames assay. Dekant et al. (1986c) demonstrated mutagenicity of DCVC in *S. typhimurium* strains (TA100, TA2638, and TA98) using the Ames assay in the absence of S9. The effects were decreased with the addition of a beta-lyase inhibitor aminooxyacetic acid, suggesting that bioactivation by this enzyme plays a role in genotoxicity. Vamvakas et al. (1987) tested NAcDCVC for mutagenicity following addition of rat kidney cytosol and found genotoxic activity. Furthermore, Vamvakas (1988b), in another experiment, investigated the mutagenicity of DCVG and DCVC in *S. typhimurium* strain TA2638, using kidney subcellular fractions for metabolic activation and AOAA (a beta-lyase inhibitor) to inhibit genotoxicity. DCVG and DCVC both exhibited direct-acting mutagenicity, with kidney mitochondria, cytosol, or microsomes enhancing the effects for both compounds and AOAA diminishing, but not abolishing the effects. Importantly, addition of liver subcellular fractions did not enhance the mutagenicity of DCVG, consistent with in situ metabolism playing a significant role in the genotoxicity of these compounds in the kidney.

**Table 4-18. TCE GSH conjugation metabolites genotoxicity**

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
<b>Gene mutations (Ames test)</b>					
<i>S. typhimurium</i> , TA100, 2638, 98	0.1–0.5 nmol	ND	+	DCVC was mutagenic in all three strains of <i>S. typhimurium</i> without the addition of mammalian subcellular fractions.	Dekant et al., (1986c)
<i>S. typhimurium</i> , TA2638	50–300 nmol	+	+	Increase in number of revertants in DCVC alone at low doses; further increase in revertants was observed in the presence of microsomal fractions. Toxicity as indicated by decreased revertants per plate were seen at higher doses.	Vamvakas et al. (1988b)
<b>Mutation analysis</b>					
In vitro—rat kidney epithelial cells, LOH in <i>Tsc</i> gene	10 µM	NA	–	Only 1/9 transformed cells showed LOH.	Mally et al. (2006)
In vitro—rat kidney epithelial cells, <i>VHL</i> gene (exons 1–3)	10 µM	NA	–	No mutations in <i>VHL</i> gene. <u>Note:</u> <i>VHL</i> is not a target gene in rodent models of chemical-induced or spontaneous renal carcinogenesis.	Mally et al. (2006)
<b>UDS</b>					
Porcine kidney tubular epithelial cell line (LLC-PK1)	2.5 µM–5, 10, 15, 24 hrs; 2.5–100 µM	NA	+	Dose-dependent in UDS up to 24 hrs tested at 2.5 µM. Also, there was a dose-dependent increase at lower concentrations. Higher concentrations were cytotoxic as determined by LDH release from the cells.	Vamvakas et al. (1989)
Syrian hamster embryo fibroblasts		NA	+	Increase in UDS in treatment groups.	Vamvakas et al. (1988a)

**Table 4-18. TCE GSH conjugation metabolites genotoxicity (continued)**

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
<b>DNA strand breaks</b>					
Male rabbit renal tissue (perfused kidneys and proximal tubules)	0–100 mg/kg or 10 µM to 10 mM	ND	+	Dose-dependent increase in strand breaks in both i.v. and i.p. injections (i.v. injections were done only for 10 and 20 mg/kg) were observed. Perfusion of rabbit kidney (45-min exposure) and proximal tubules (30-min exposure) experiment resulted in a dose-dependent difference in the amount of SSBs.	Jaffe et.al. (1985)
Primary kidney cells from both male rats and human	1–4 mM; 20 hrs exposure	NA	+	Statistically significant increase in all doses (1, 2, or 4 mM) both in rats and human cells.	Robbiano et al. (2004)
In vivo—male Sprague-Dawley rats exposed to TCE or DCVC—comet assay	TCE: 500–2,000 ppm, inhalation, 6 hrs/d, 5 d DCVC: 1 or 10 mg/kg, single oral dose for 16 hrs	+ (DCVC) – (TCE)	NA	No significant increase in tail length in any of the TCE exposed groups. In 1, 2 hrs exposure—1 or 10 mg to DCVC—resulted in significant increase with no dose-response, but not at 16 hrs. In 2, ND for 1 mg, significant increase at 10 mg.	Clay (2008)
<b>Micronucleus</b>					
Syrian hamster embryo fibroblasts		NA	–	No micronucleus formation.	Vamvakas et al. (1988a)
Primary kidney cells from both male rats and human	1–4 mM; 20 hrs exposure	NA	+	Statistically significant increase in all doses (1, 2, and 4 mM) both in rat and human cells.	Robbiano et al. (2004)
Male Sprague-Dawley rats; proximal tubule cells (in vivo)	4 mM/kg TCE exposure, single dose	NA	+	Statistically significant increase in the average frequency of micronucleated kidney cells was observed.	Robbiano et al. (1998)
<b>Cell transformation</b>					
Kidney tubular epithelial cell line (LLC-PK1)	1 or 5 µM; 7 wks	NA	+	Induced morphological cell transformation at both concentrations tested. Furthermore, cells maintained both biochemical and morphological alterations remained stable for 30 passages.	Vamvakas et al. (1996)
Rat kidney epithelial cells (in vitro)	10 µM; 24 hrs exposure, 7 wks post incubation	NA	+	Cell transformation was higher than control; however, cell survival percentage ranged from 39 to 64%, indicating cytotoxicity.	Mally et al. (2006)

**Table 4-18. TCE GSH conjugation metabolites genotoxicity (continued)**

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
<b>Gene expression</b>					
Kidney tubular epithelial cell line (LLC-PK1)	1 or 5 µM clones, 30, 60, or 90 min	NA	+	Increased c-Fos expression in 1 and 5 µM exposed clones at three different times tested.	Vamvakas et al. (1996)
Kidney tubular epithelial cell line (LLC-PK1)		NA	+	Expression of c-Fos and c-Myc increased in a time-dependent manner.	Vamvakas et al. (1993)

LDH = lactate dehydrogenase; ND = not determined; NA = not applicable

While additional data are not available on DCVC or NAcDCVC, the genotoxicity of DCVC is further supported by the predominantly positive results in other available in vitro and in vivo assays. Jaffe et al. (1985) reported DNA strand breaks due to DCVC administered in vivo, in isolated perfused kidneys, and in isolated proximal tubules of albino male rabbits. Vamvakas et al. (1989) reported dose-dependent increases in UDS in LLC-PK1 cell clones at concentrations without evidence of cytotoxicity. In addition, Vamvakas et al. (1996) reported that 7-week DCVC exposure to LLC-PK1 cell clones at noncytotoxic concentrations induces morphological and biochemical de-differentiation that persists for at least 30 passages after removal of the compound. This study also reported increased expression of the proto-oncogene c-Fos in the cells in this system. In a Syrian hamster embryo fibroblast system, DCVC did not induce micronuclei, but demonstrated a UDS response (Vamvakas et al., 1988a).

Two more recent studies are discussed in more detail. Mally et al. (2006) isolated primary rat kidney epithelial cells from *Tsc-2<sup>Ek/+</sup>* (Eker) rats, and reported increased transformation when exposed to 10  $\mu$ M DCVC, similar to that of the genotoxic renal carcinogens *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (Horesovsky et al., 1994). The frequency was variable but consistently higher than background. No loss-of-heterozygosity (LOH) of the *Tsc-2* gene was reported either in these DCVC transformants or in renal tumors (which were not increased in incidence) from TCE-treated Eker rats, which Mally et al. (2006) suggested support a nongenotoxic mechanism because a substantial fraction of spontaneous renal tumors in Eker rats showed LOH at this locus (Yeung et al., 1995; Kubo et al., 1994) and because LOH was exhibited both in vitro and in vivo with 2,3,4-tris(glutathion-S-yl)-hydroquinone treatment in Eker rats (Yoon et al., 2001). However, 2,3,4-tris(glutathion-S-yl)-hydroquinone is not genotoxic in standard mutagenicity assays (Yoon et al., 2001), and Kubo et al. (1994) also reported that none of renal tumors induced by the genotoxic carcinogen, *N*-ethyl-*N*-nitrosourea, showed LOH. Therefore, the lack of LOH at the *Tsc-2* locus induced by DCVC in vitro, or TCE in vivo, reported by Mally et al. (2006) is actually more similar to the response from the genotoxic carcinogen *N*-ethyl-*N*-nitrosourea than the nongenotoxic carcinogen 2,3,4-tris-(glutathion-S-yl)-hydroquinone. Therefore, these data do not substantially contradict the body of evidence on DCVC genotoxicity.

Finally, Clay (2008) evaluated the genotoxicity of DCVC in vivo using the comet assay to assess DNA breakage in the proximal tubules of rat kidneys. Rats were exposed orally to a single dose of DCVC (1 or 10 mg/kg). The animals were sacrificed either 2 or 16 hours after dosing and samples were prepared for detecting the DNA damage. DCVC (1 and 10 mg/kg) induced no significant DNA damage in rat kidney proximal tubules at the 16-hour sampling time or after 1 mg/kg DCVC at the 2-hour sampling time. While Clay et al. (2008) concluded that these data were insufficient to indicate a positive response in this assay, the study did report a statistically significant increase in percentage tail DNA 2 hours after treatment with 10 mg/kg

DCVC, despite the small number of animals at each dose (n = 5) and sampling time. Therefore, these data do not substantially contradict the body of evidence on DCVC genotoxicity.

Overall, DCVC, and to a lesser degree DCVG and NAcDCVC, have demonstrated genotoxicity based on consistent results in a number of available studies. While some recent studies ([Clay, 2008](#); [Mally et al., 2006](#)) have reported a lack of positive responses in some in vivo measures of genotoxicity with DCVC treatment, due to a number of limitations discussed above, these studies do not substantially contradict the body of evidence on DCVC genotoxicity. It is known that these metabolites are formed in vivo following TCE exposure, specifically in the kidney, so they have the potential to contribute to the genotoxicity of TCE, especially in that tissue. Moreover, DCVC and DCVG genotoxic responses were enhanced when metabolic activation using *kidney* subcellular fractions was used ([Vamvakas et al., 1988b](#)). Finally, the lack of similar responses in in vitro genotoxicity assays with TCE, even with metabolic activation, is likely the result of the small yield (if any) of DCVC under in vitro conditions, since in vivo, DCVC is likely formed predominantly in situ in the kidney while S9 fractions are typically derived from the liver. This hypothesis could be tested in experiments in which TCE is incubated with subcellular fractions from the kidney, or from both the kidney and the liver (for enhanced GSH conjugation).

#### 4.2.6. TCOH

Limited studies are available on the effect of TCOH on genotoxicity (see Table 4-19). TCOH is negative in the *S. typhimurium* assay using the TA100 strain ([DeMarini et al., 1994](#); [Bignami et al., 1980](#); [Waskell, 1978](#)). A study by Beland ([1999](#)) using *S. typhimurium* strain TA104 did not induce reverse mutations without exogenous metabolic activation; however, it did increase mutant frequency in the presence of exogenous metabolic activation at a dose >2,500 µg/plate. TCOH has not been evaluated in the other recommended screening assays. Therefore, the database is limited for the determination of TCOH genotoxicity.

**Table 4-19. Genotoxicity of TCOH**

Test system/endpoint	Doses (LED or HID) <sup>a</sup>	Results		Reference
		With activation	Without activation	
<i>S. typhimurium</i> TA100, 98, reverse mutation	7,500 µg/plate	–	–	Waskell (1978)
<i>S. typhimurium</i> TA100, reverse mutation	0.5 µg/cm <sup>3</sup> vapor	–	–	DeMarini et al. (1994)
<i>S. typhimurium</i> TA104, reverse mutation	2,500 µg/plate	+	–	Beland (1999)
<i>S. typhimurium</i> TA100, 1535 reverse mutation	NA	–	–	Bignami et al. (1980)
SCEs	NA	NA	+	Gu et al. (1981b)

<sup>a</sup>LED = lowest effective dose; HID = highest ineffective dose.

+ = positive; – = negative; NA = doses not available, results based on the abstract

#### 4.2.7. Synthesis and Overall Summary

TCE and its metabolites (TCA, DCA, CH, DCVC, DCVG, and TCOH) have been evaluated to varying degrees for their genotoxic activity in several in vitro systems such as bacteria, yeast, and mammalian cells, as well as in in vivo systems.

There are several challenges in interpreting the genotoxicity results obtained from TCE exposure. For example, some studies in bacteria should be interpreted with caution if conducted using technical-grade TCE since it may contain known bacterial mutagens in trace amounts as stabilizers (e.g., 1,2-epoxybutane and epichlorohydrin). Because of the volatile nature of TCE, there could be false negative results if proper precautions are not taken to limit evaporation, such as the use of a closed, sealed system. The adequacy of the enzyme-mediated activation of TCE in vitro tests is another consideration. For example, it is not clear if standard S9 fractions can adequately recapitulate the complex in vivo metabolism of TCE to reactive intermediates, which in some cases entails multiple sequential steps involving multiple enzyme systems (e.g., CYP, GST, etc.) and interorgan processing (as is described in more detail in Section 3.3). In addition, the relative potency of the metabolites in vitro may not necessarily inform their relative contribution to the overall mechanistic effects of the parent compound, TCE. Furthermore, although different assays provided data relevant to different types of genotoxic endpoints, not all effects that are relevant for carcinogenesis are encompassed. The standard battery of prokaryotic as well as mammalian genotoxicity test protocols typically specify the inclusion of significantly cytotoxic concentrations of the test compound.

With respect to potency, several TCE studies have been conducted along with numerous other chlorinated compounds and the results interpreted as a comparison of the group of compounds tested (relative potency). However, for the purposes of hazard characterization, such comparisons are not informative—particularly if they are not necessarily correlated with in vivo

carcinogenic potency. Also, differentiating the effects of TCE with respect to its potency can be influenced by many factors such as the type of cells, their differing metabolic capacities, sensitivity of the assay, need for greater concentration to show any effect, interpretation of data when the effects are marginal, and gradation of severity of effects.

Also, type of samples used, methodology used for the isolation of genetic material, and duration of exposure can particularly influence the results of several studies. This is particularly true for human epidemiological studies. For example, while some studies use tissues obtained directly from the patients, others use formalin fixed tissues sections to isolate DNA for mutation detection. Type of fixing solution, fixation time, and period of storage of the tissue blocks often affect the quality of DNA. Formic acid contained in the formalin solution or picric acid contained in Bouin's solution is known to degrade nucleic acids resulting in either low yield or poor quality of DNA. In addition, during collection of tumor tissues, contamination of neighboring normal tissue can easily occur if proper care is not exercised. This could lead to the 'dilution effect' of the results (i.e., because of the presence of some normal tissue) frequency of mutations detected in the tumor tissue can be lower than expected. Due to some of these technical difficulties in obtaining proper material (DNA) for the detection of mutation, the results of these studies should be interpreted cautiously.

The following synthesis, summary, and conclusions focus on the available studies that may provide some insight into the potential genotoxicity of TCE considering the above challenges when interpreting the mutagenicity data for TCE.

Overall, evidence from a number of different analyses and a number of different laboratories using a fairly complete array of endpoints suggests that TCE, following metabolism, has the potential to be genotoxic. TCE has a limited ability to induce mutation in bacterial systems, but greater evidence of potential to bind or to induce damage in the structure of DNA or the chromosome in a number of targets. A series of carefully controlled studies evaluating TCE itself (without mutagenic stabilizers and without metabolic activation) found it to be incapable of inducing gene mutations in most standard mutation bacterial assays ([Mortelmans et al., 1986](#); [Shimada et al., 1985](#); [Crebelli et al., 1982](#); [Baden et al., 1979](#); [Bartsch et al., 1979](#); [Waskell, 1978](#); [Henschler et al., 1977](#); [Simmon et al., 1977](#)). Therefore, it appears that it is unlikely that TCE is a direct-acting mutagen, though TCE has shown potential to affect DNA and chromosomal structure. TCE is also positive in some, but not all, fungal and yeast systems ([Koch et al., 1988](#); [Crebelli et al., 1985](#); [Rossi et al., 1983](#); [Callen et al., 1980](#)). Data from human epidemiological studies support the possible mutagenic effect of TCE leading to *VHL* gene damage and subsequent occurrence of RCC. Association of increased *VHL* mutation frequency in TCE-exposed RCC cases has been observed ([Brauch et al., 2004](#); [Brauch et al., 1999](#); [Brüning et al., 1997b](#)).

TCE can lead to binding to nucleic acids and proteins ([Kautiainen et al., 1997](#); [Mazzullo et al., 1992](#); [Bergman, 1983](#); [Miller and Guengerich, 1983](#); [DiRenzo et al., 1982](#)), and such

binding appears to be due to conversion to one or more reactive metabolites. For instance, increased binding was observed in samples bioactivated with mouse and rat microsomal fractions ([Mazzullo et al., 1992](#); [Miller and Guengerich, 1983](#); [DiRenzo et al., 1982](#); [Banerjee and Van Duuren, 1978](#)). DNA binding is consistent with the ability to induce DNA and chromosomal perturbations. Several studies report the induction of micronuclei in vitro and in vivo from TCE exposure ([Hu et al., 2008](#); [Robbiano et al., 2004](#); [Wang et al., 2001](#); [Hrelia et al., 1994](#); [Kligerman et al., 1994](#)). Reports of SCE induction in some studies are consistent with DNA effects, but require further study ([Kligerman et al., 1994](#); [Nagaya et al., 1989b](#); [Gu et al., 1981a](#); [Gu et al., 1981b](#); [White et al., 1979](#)).

TCA, an oxidative metabolite of TCE, exhibits little, if any genotoxic activity in vitro. TCA did not induce mutations in *S. typhimurium* strains in the absence of metabolic activation or in an alternative protocol using a closed system ([Kargalioglu et al., 2002](#); [Nelson et al., 2001](#); [Giller et al., 1997](#); [DeMarini et al., 1994](#); [Rapson et al., 1980](#); [Waskell, 1978](#)), but a mutagenic response was induced in TA100 in the Ames fluctuation test ([Giller et al., 1997](#)). However, in vitro experiments with TCA should be interpreted with caution if steps have not been taken to neutralize pH changes caused by the compound ([Mackay et al., 1995](#)). Measures of DNA-repair responses in bacterial systems have shown induction of DNA repair reported in *S. typhimurium* but not in *E. coli*. Mutagenicity in mouse lymphoma cells was only induced at cytotoxic concentrations ([Harrington-Brock et al., 1998](#)). TCA was positive in some genotoxicity studies in vivo mouse, newt, and chick test systems ([Giller et al., 1997](#); [Bhunya and Jena, 1996](#); [Birner et al., 1994](#); [Bhunya and Behera, 1987](#)). DNA unwinding assays have either shown TCA to be much less potent than DCA ([Nelson and Bull, 1988](#)) or negative ([Styles et al., 1991](#); [Nelson et al., 1989](#)). Due to limitations in the genotoxicity database, the possible contribution of TCA to TCE genotoxicity is unclear.

DCA, a chloroacid metabolite of TCE, has also been studied using different types of genotoxicity assays. Although limited studies are conducted for different genetic endpoints, DCA has been demonstrated to be mutagenic in the *S. typhimurium* assays, in vitro ([Kargalioglu et al., 2002](#); [Plewa et al., 2002](#); [DeMarini et al., 1994](#)) in some strains, mouse lymphoma assay, ([Harrington-Brock et al., 1998](#)) in vivo cytogenetic tests ([Leavitt et al., 1997](#); [Fusco et al., 1996](#)), the micronucleus induction test, the Big Blue mouse system, and other tests ([Harrington-Brock et al., 1998](#); [Leavitt et al., 1997](#); [Fusco et al., 1996](#); [DeMarini et al., 1994](#); [Chang et al., 1992](#); [Nelson et al., 1989](#); [Nelson and Bull, 1988](#); [Bignami et al., 1980](#)). DCA can cause DNA strand breaks in mouse and rat liver cells following in vivo exposure in mice and rats ([Fusco et al., 1996](#)). Because of uncertainties as to the extent of DCA formed from TCE exposure, inferences as to the possible contribution from DCA genotoxicity to TCE toxicity are difficult to make.

CH is mutagenic in the standard battery of screening assays. Effects include positive results in bacterial mutation tests for point mutations and in the mouse lymphoma assay for

mutagenicity at the Tk locus ([Haworth et al., 1983](#)). In vitro tests showed that CH also induced micronuclei and aneuploidy in human peripheral blood lymphocytes and Chinese hamster pulmonary cell lines. Micronuclei were also induced in Chinese hamster embryonic fibroblasts. Several studies demonstrate that CH induces aneuploidy (loss or gain of whole chromosomes) in both mitotic and meiotic cells, including yeast ([Gualandi, 1987](#); [Sora and Agostini Carbone, 1987](#); [Käfer, 1986](#); [Singh and Sinha, 1979, 1976](#)), cultured mammalian somatic cells ([Degrassi and Tanzarella, 1988](#)), and spermatocytes of mice ([Liang and Pacchierotti, 1988](#); [Russo et al., 1984](#)). CH was negative for sex-linked recessive lethal mutations in *Drosophila* ([Yoon et al., 1985](#)). It induces SSBs in hepatic DNA of mice and rats ([Nelson and Bull, 1988](#)) and mitotic gene conversion in yeast ([Bronzetti et al., 1984](#)). Schatten and Chakrabarti ([1998](#)) showed that CH affects centrosome structure, which results in the inability to reform normal microtubule formations and causes abnormal fertilization and mitosis of sea urchin embryos. Based on the existing array of data, CH has the potential to be genotoxic, particularly when aneuploidy is considered in the weight of evidence for genotoxic potential. CH appears to act through a mechanism of spindle poisoning, resulting in numerical changes in the chromosomes. These results are consistent with TCE, albeit there are limited data on TCE for these genotoxic endpoints.

DCVC, and to a lesser degree DCVG, has demonstrated bacterial mutagenicity based on consistent results in a number of available studies ([Vamvakas et al., 1988b](#); [Vamvakas et al., 1987](#); [Dekant et al., 1986c](#)). DCVC has demonstrated a strong, direct-acting mutagenicity both with and without the presence of mammalian activation enzymes. It is known that these metabolites are formed in vivo following TCE exposure, so they have the potential to contribute to the genotoxicity of TCE. The lack of similar response in bacterial assays with TCE is likely the result of the small yield (if any) of DCVC under in vitro conditions, since in vivo, DCVC is likely formed predominantly in situ in the kidney (S9 fractions are typically derived from the liver). DCVC and DCVG have not been evaluated extensively in other genotoxicity assays, but the available in vitro and in vivo data are predominantly positive. For instance, several studies have reported that DCVC can induce primary DNA damage in mammalian cells in vitro and in vivo ([Clay, 2008](#); [Vamvakas et al., 1989](#); [Jaffe et al., 1985](#)). Long-term exposure to DCVC-induced de-differentiation of cells ([Vamvakas et al., 1996](#)). It has been shown to induce expression of the protooncogene c-Fos ([Vamvakas et al., 1996](#)) and cause cell transformation in rat kidney cells ([Mally et al., 2006](#)). In LLC-PK1 cell clones, DCVC was reported to induce UDS, but not micronuclei ([Vamvakas et al., 1988a](#)). Finally, DCVC-induced transformation in kidney epithelial cells isolated from Eker rats carrying the heterozygous *Tsc-2* mutations ([Mally et al., 2006](#)). Moreover, the lack of LOH at the *Tsc-2* locus observed in exposed cells does not constitute negative evidence of DCVC genotoxicity, as none of renal tumors induced in Eker rats by the genotoxic carcinogen *N*-ethyl-*N*-nitrosourea showed LOH ([Kubo et al., 1994](#)).

In support of the importance of metabolism, there is some concordance between effects observed from TCE and those from several metabolites. For instance, both TCE and CH have been shown to induce micronuclei in mammalian systems, but chromosomal aberrations have been more consistently observed with CH than with TCE. The role of TCA in TCE genotoxicity is less clear, as there is less concordance between the results from these two compounds. Finally, several other TCE metabolites show at least some genotoxic activity, with the strongest data from DCA, DCVG, and DCVC. While quantitatively smaller in terms of flux as compared to TCA and TCOH (for which there is almost no genotoxicity data), these metabolites may still be toxicologically important.

Thus, uncertainties with regard to the characterization of TCE genotoxicity remain, particularly because not all TCE metabolites have been sufficiently tested in the standard genotoxicity screening battery to derive a comprehensive conclusion. However, the metabolites that have been tested, particularly DCVC, have predominantly resulted in positive data, although to a lesser extent in DCVG and NAcDCVC. This supports the conclusion that these compounds are genotoxic, particularly in the kidney, where in situ metabolism produces and/or bioactivates these TCE metabolites.

#### **4.3. CENTRAL NERVOUS SYSTEM (CNS) TOXICITY**

TCE exposure results in CNS effects in both humans and animals that can result from acute, subchronic, or chronic exposure. There are studies indicating that TCE exposure results in CNS tumors and this discussion can be found in Section 4.9. The studies discussed in this section focus on the most critical neurological effects that were extracted from the neurotoxicological literature. Although there are several studies and reports that have evaluated TCE as an anesthetic, those studies were not included in this section because of the high exposure levels in comparison to the selected critical neurological effects described below. The critical neurological effects are nerve conduction changes, sensory effects, cognitive deficits, changes in psychomotor function, and changes in mood and sleep behaviors. The selection criteria that were used to determine study importance included study design and validity, pervasiveness of neurological effect, and for animal studies, the relevance of these reported outcomes in humans. More detailed information on human and animal neurological studies with TCE can be found in Appendix D.

##### **4.3.1. Alterations in Nerve Conduction**

###### **4.3.1.1. Trigeminal Nerve Function: Human Studies**

A number of human studies have been conducted that examined the effects of occupational or drinking water exposures to TCE on trigeminal nerve function (see Table 4-20). Many studies reported that humans exposed to TCE present trigeminal nerve function abnormalities as measured by blink reflex and masseter reflex test measurements ([Kilburn,](#)

2002a, b; Kilburn and Warshaw, 1993a; Feldman et al., 1992; Feldman et al., 1988). The blink and masseter reflexes are mediated primarily by the trigeminal nerve and changes in measurement suggest impairment in nerve conduction. Other studies measured the trigeminal somatosensory evoked potential (TSEP) following stimulation of the trigeminal nerve and reported statistically significantly delayed response on evoked potentials among exposed subjects compared to nonexposed individuals (Mhiri et al., 2004; Barret et al., 1987; Barret et al., 1984; Barret et al., 1982). Two studies that also measured trigeminal nerve function did not find any effect (Rasmussen et al., 1993a; El Ghawabi et al., 1973), but the methods were not provided in either study (Rasmussen et al., 1993a; El Ghawabi et al., 1973) or an appropriate control group was not included (Rasmussen et al., 1993a). These studies and results are described below and summarized in detail in Table 4-20.

**Table 4-20. Summary of human trigeminal nerve and nerve conduction velocity studies**

Reference <sup>a</sup>	Subjects	Exposure	Effect
Barret et al. (1982)	11 workers with chronic TCE exposure. Controls: 20 unexposed subjects.	Presence of TCE and TCA found through urinalysis. Atmospheric TCE concentrations and duration of exposure not reported in paper.	Following stimulation of the trigeminal nerve, significantly higher voltage stimuli was required to obtain a normal response and there was a significant increase in latency for response and decreased response amplitude.
Barret et al. (1984)	188 factory workers. No unexposed controls; lowest exposure group used as comparison.	>150 ppm; n = 54 < 150 ppm; n = 134. 7 hrs/d for 7 yrs.	Trigeminal nerve and optic nerve impairment, asthenia and dizziness were significantly increased with exposure.
Barret et al. (1987)	104 degreaser machine operators. Controls: 52 unexposed subjects Mean age 41.6 yrs.	Mean duration, 8.2 yrs, average daily exposure 7 hrs/d. Average TCOH range = 162–245 mg/g creatinine. Average TCA range = 93–131 mg/g creatinine.	Evoked trigeminal responses were measured following stimulation of the nerve and revealed increased latency to respond, amplitude or both and correlated with length of exposure ( $p < 0.01$ ) and with age ( $p < 0.05$ ), but not concentration.
El-Ghawabi et al. (1973)	30 money printing shop workers. Controls: 20 nonexposed males. 10 workers exposed to inks not containing TCE.	Mean TCE air concentrations ranged from 41 to 163 ppm. Exposure durations: <1 yr: n = 3 1 yr: n = 1 2 yrs: n = 2 3 yrs: n = 11 4 yrs: n = 4 ≥5 yrs: n = 9	No effect on trigeminal nerve function was noted.
Feldman et al. (1988)	21 Woburn, Massachusetts residents. 27 controls.	TCE maximum reported concentration in well water was 267 ppb; other solvents also present. Exposure duration 1–12 yrs.	Measurement of the blink reflex as mediated by the trigeminal nerve resulted in significant increases in the latency of reflex components ( $p < 0.001$ ).

**Table 4-20. Summary of human trigeminal nerve and nerve conduction velocity studies (continued)**

Reference <sup>a</sup>	Subjects	Exposure	Effect
Feldman et al. (1992)	18 workers. 30 controls.	TCE exposure categories of “extensive,” “occasional,” and “chemical other than TCE.” “Extensive” = chronically exposed ( $\geq 1$ yr) to TCE for 5 d/wk and >50% workday. “Occupational” = chronically exposed to TCE for 1–3 d/wk and >50% workday.	The blink reflex as mediated by the trigeminal was measured. The “extensive” group revealed latencies >3 SDs above the nonexposed group mean on blink reflex components.
Kilburn and Warshaw (1993a)	160 residents living in Southwest Tucson with TCE, other solvents, and chromium in groundwater. Control: 113 histology technicians from a previous study (Kilburn and Warshaw, 1992b; Kilburn et al., 1987).	>500 ppb of TCE in well water before 1981 and 25–100 ppb afterwards. Duration ranged from 1 to 25 yrs.	Significant impairments in sway speed with eyes open and closed and blink reflex latency (R-1), which suggests trigeminal nerve impairment.
Kilburn (2002b, 2002a)	236 residents near a microchip plant in Phoenix, Arizona. Controls: 161 regional referents from Wickenburg, Arizona and 67 referents in northeastern Phoenix.	<0.2–10,000 ppb of TCE, <0.2–260,000 ppb TCA, <0.2–6,900 ppb 1,1-DCE, <0.2–1,600 ppb 1,2-DCE, <0.2–23,000 ppb perchloroethylene, <0.02–330 ppb vinyl chloride in well water. Exposure duration 2–37 yrs.	Trigeminal nerve impairment as measured by the blink reflex test; both right and left blink reflex latencies (R-1) were prolonged. Exposed group mean 14.2 + 2.1 ms (right) or 13.9 + 2.1 ms (left) vs. referent group mean of 13.4 + 2.1 ms (right) or 13.5 + 2.1 ms (left), $p = 0.0001$ (right) and 0.008 (left).
Mhiri et al. (2004)	<b>23 phosphate industry workers.</b> <b>Controls: 23 unexposed workers.</b>	Exposure ranged from 50 to 150 ppm, for 6 hrs/d for at least 2 yrs. Mean urinary TCOH and TCA levels were $79.3 \pm 42$ and $32.6 \pm 22$ mg/g creatinine.	<b>TSEPs were recorded. Increase in the TSEP latency was observed in 15/23 (65%) workers.</b>
Rasmussen et al. (1993a)	96 Danish metal degreasers. Age range: 19–68. No unexposed controls; low exposure group used as comparison.	Average exposure duration: 7.1 yrs); range of full-time degreasing: 1 mo to 36 yrs. Exposure to TCE or to CFC113: (1) Low exposure: $n = 19$ , average full-time exposure 0.5 yr. (2) Medium exposure: $n = 36$ , average full-time exposure 2.1 yrs. (3) High exposure: $n = 41$ , average full-time exposure 11 yrs. TCA in high exposure group = 7.7 mg/L (maximum = 26.1 mg/L).	No statistically significant trend on trigeminal nerve function, although some individuals had abnormal function.

**Table 4-20. Summary of human trigeminal nerve and nerve conduction velocity studies (continued)**

Reference <sup>a</sup>	Subjects	Exposure	Effect
<b>Ruijten et al. (1991)</b>	<b>31 male printing workers. Mean age 44 yrs; mean duration 16 yrs. Controls: 28 unexposed; mean age 45 yrs.</b>	Mean cumulative exposure = 704 ppm × yr (SD 583, range: 160–2,150 ppm × yr. Mean, 17 ppm at time of study; historic TCE levels from 1976 to 1981, mean of 35 ppm. Mean duration of 16 yrs.	Measurement of trigeminal nerve function by using the blink reflex resulted in no abnormal findings. Increased latency in the masseter reflex is indicative of trigeminal nerve impairment.
Triebig et al. (1982)	24 workers (20 males, 4 females) occupationally exposed—ages 17–56. Controls: 144 individuals to establish normal nerve conduction parameters. Matched group: 24 unexposed workers (20 males, 4 females).	Exposure duration of 1–258 mo (mean 83 mo). Air exposures were between 5 and 70 ppm.	No statistically significant difference in nerve conduction velocities between the exposed and unexposed groups.
Triebig et al. (1983)	66 workers occupationally exposed. Control: 66 workers not exposed to solvents.	Subjects were exposed to a mixture of solvents, including TCE.	Exposure-response relationship observed between length of solvent exposure and statistically significant reduction in mean sensory ulnar nerve conduction velocities.

<sup>a</sup>**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

Integrity of the trigeminal nerve is commonly measured using blink and masseter reflexes. Five studies ([Kilburn, 2002b, a](#); [Kilburn and Warshaw, 1993a](#); [Feldman et al., 1992](#); [Feldman et al., 1988](#); [Barret et al., 1984](#)) reported a significant increase in the latency to respond to the stimuli generating the reflex. The latency increases in the blink reflex ranged from 0.4 ms ([Kilburn, 2002b, a](#)) to up to 3.44 ms ([Feldman et al., 1988](#)). The population groups in these studies were exposed by inhalation occupationally ([Barret et al., 1984](#)) and through drinking water environmentally ([Kilburn, 2002b, a](#); [Kilburn and Warshaw, 1993a](#); [Feldman et al., 1988](#)). Feldman et al. ([1992](#)) demonstrated persistence in the increased latency of the blink reflex response. In one subject, exposure to TCE (levels not reported by authors) occurred through a degreasing accident (high and acute exposure), and increased latency response times persisted 20 years after the accident. Another two subjects, evaluated at 9 months and 1 month following a high occupational exposure (exposure not reported by authors), also had higher blink reflex latencies with an average increase of 2.8 ms over the average response time in the control group used in the study. Although one study ([Ruijten et al., 1991](#)) did not find these increases in male printing workers exposed to TCE, this study did find a statistically significant average increase of 0.32 ms ( $p < 0.05$ ) in the latency response time in TCE-exposed workers on the masseter reflex test, another test commonly used to measure the integrity of the trigeminal nerve.

Three studies ([Mhiri et al., 2004](#); [Barret et al., 1987](#); [Barret et al., 1982](#)), adopting TSEPs to measure trigeminal nerve function, found significant abnormalities in these evoked potentials. These studies were conducted on volunteers who were occupationally exposed to TCE through metal degreasing operations ([Barret et al., 1987](#); [Barret et al., 1982](#)) or through cleaning tanks in the phosphate industry ([Mhiri et al., 2004](#)). Barret et al. ([1982](#)) reported that in 8/11 workers, an increased voltage ranging from a 25 to a 45 volt increase was needed to generate a normal TSEP and two of workers had an increased TSEP latency. Three out of 11 workers had increases in TSEP amplitudes. In a later study, Barret et al. ([1987](#)) also reported abnormal TSEPs (increased latency and/or increased amplitude) in 38% of the degreasers who were evaluated. The individuals with abnormal TSEPs were significantly older (45 vs. 40.1 years;  $p < 0.05$ ) and were exposed to TCE longer (9.9 vs. 5.6 years;  $p < 0.01$ ). Mhiri et al. ([2004](#)) was the only study to evaluate individual components of the TSEP and noted significant increases in latencies for all TSEP potentials (N1, P1, N2, P2, N3;  $p < 0.01$ ) and significant decreases in TSEP amplitude (P1,  $p < 0.02$ ; N2,  $p < 0.05$ ). A significant positive correlation was demonstrated between exposure duration and increased TSEP latency ( $p < 0.02$ ).

Two studies reported no statistically significant effect of TCE exposure on trigeminal nerve function ([Rasmussen et al., 1993a](#); [El Ghawabi et al., 1973](#)). El-Ghawabi et al. ([1973](#)) conducted a study on 30 money printing shop workers occupationally exposed to TCE. Trigeminal nerve involvement was not detected, but the authors did not include the experimental methods that were used to measure trigeminal nerve involvement and did not provide any data as to how this assessment was made. Rasmussen et al. ([1993a](#)) conducted a historical cohort study on 99 metal degreasers, 70 exposed to TCE and 29 to the fluorocarbon, CFC113. It was reported that 1/21 people (5%) in the low exposure group, 2/37 (5%) in the medium exposure group, and 4/41 (10%) in the high-exposure group experienced abnormalities in trigeminal nerve sensory function, with a linear trend test  $p$ -value of 0.42. The mean urinary TCA concentration was reported for the high-exposure group only and was 7.7 mg/L (maximum concentration, 26.1 mg/L). The trigeminal nerve function findings of high-exposure group subjects were compared to that of the low-exposure group since this study did not include an unexposed or non-TCE exposed group, and decreased the sensitivity of the study.

#### **4.3.1.2. Nerve Conduction Velocity—Human Studies**

Two occupational studies assessed ulnar and median nerve function using tests of conduction latencies ([Triebig et al., 1983](#); [Triebig et al., 1982](#)) (see Table 4-20). The ulnar nerve and median nerves are major nerves located in the arm and forearm. Triebig ([1982](#)) studied 24 healthy workers (20 males, 4 females) exposed to TCE occupationally (5–70 ppm) at three different plants and did not find statistically significant differences in ulnar or median nerve conduction velocities between exposed and unexposed subjects. This study measured exposure data, but exposures/responses were not reported by dose levels. The Triebig ([1983](#)) study is

similar in design to the previous study ([Triebig et al., 1982](#)), but with a larger number of subjects. In this study, a dose-response relationship was observed between lengths of exposure to mixed solvents that included TCE (at unknown concentration). A statistically significant reduction in nerve conduction velocities was observed for the medium- and long-term exposure groups for the sensory ulnar nerve as was a statistically significant reduction in mean nerve conduction velocity observed between exposed and control subjects.

#### 4.3.1.3. Trigeminal Nerve Function: Laboratory Animal Studies

There is little evidence that TCE disrupts trigeminal nerve function in animal studies. Two studies demonstrated that TCE produces morphological changes in the trigeminal nerve at a dose of 2,500 mg/kg-day for 10 weeks ([Barret et al., 1992](#); [Barret et al., 1991](#)). However, dichloroacetylene, a degradation product formed during the volatilization of TCE, was found to produce more severe morphological changes in the trigeminal nerve and at a lower dose of 17 mg/kg-day ([Barret et al., 1992](#); [Barret et al., 1991](#)). Only one study ([Albee et al., 2006](#)) evaluated the effects of TCE on trigeminal nerve function; a subchronic inhalation exposure did not result in any significant functional changes. A summary of these studies is provided in Table 4-21.

**Table 4-21. Summary of animal trigeminal nerve studies**

Reference <sup>a</sup>	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL <sup>b</sup>	Effects
<a href="#">Barret et al. (1991)</a>	Direct gastric administration	Rat, Sprague-Dawley, female, 7/group	0, 2.5 g/kg, acute administration. 17 mg/kg dichloroacetylene.	LOAEL: 2.5 g/kg	Morphometric analysis was used for analyzing the trigeminal nerve. Increases in external and internal fiber diameter as well as myelin thickness were observed in the trigeminal nerve after TCE treatment.
<a href="#">Barret et al. (1992)</a>	Direct gastric administration	Rat, Sprague-Dawley, female, 7/group	<b>0, 2.5 g/kg; one dose/d, 5 d/wk, 10 wks. 17 mg/kg dichloroacetylene</b>	LOAEL: 2.5 g/kg	<b>Trigeminal nerve analyzed using morphometric analysis. Increased internode length and fiber diameter in class A fibers of the trigeminal nerve observed with TCE treatment. Changes in fatty acid composition also noted.</b>
<a href="#">Albee et al. (1997)</a>	Inhalation	Rat, F344, male, 6	0 or 300 ppm dichloroacetylene, 2.25 hrs	LOAEL: 300 ppm dichloroacetylene	Dichloroacetylene (TCE byproduct) exposure impaired the TSEP up to 4 d postexposure.
<a href="#">Albee et al. (2006)</a>	Inhalation	Rat, F344, male and female, 10/sex/group	0, 250, 800, or 2,500 ppm	NOAEL: 2,500 ppm	No effect on TSEPs was noted at any exposure level.

<sup>a</sup>**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

<sup>b</sup>NOAEL = no-observed-adverse-effect level, LOAEL = lowest-observed-adverse-effect-level.

Barret et al. ([1992](#); [1991](#)) conducted two studies evaluating the effects of both TCE and dichloroacetylene on trigeminal nerve fiber diameter and internodal length as well as several markers for fiber myelination. Female Sprague-Dawley rats (n = 7/group) were dosed with 2,500 mg/kg TCE or 17 mg/kg-day dichloroacetylene by gavage for 5 days/week for 10 weeks. TCE-dosed animals only exhibited changes in the smaller Class A fibers where internode length increased marginally (<2%) and fiber diameter increased by 6%. Conversely, dichloroacetylene-treated rats exhibited significant and more robust decreases in internode length and fiber diameter in both fiber classes A (decreased 8%) and B (decreased 4%).

Albee et al. ([2006](#)) evaluated the effects of a subchronic inhalation TCE exposure in F344 rats (10/sex/group). Rats were exposed to 0, 250, 800, and 2,500 ppm TCE for 6 hours/day, 5 days/week for 13 weeks. TCE exposures were adequate to produce permanent auditory impairment even though TSEPs were unaffected. While TCE appears to be negative in disrupting the trigeminal nerve, the TCE breakdown product, dichloroacetylene, does impair trigeminal nerve function. Albee et al. ([1997](#)) showed that a single inhalation exposure of rats to 300-ppm dichloroacetylene, for 2.25 hours, disrupted trigeminal nerve evoked potentials for at least 4 days post exposure.

#### **4.3.1.4. Discussion and Conclusions: TCE-Induced Trigeminal Nerve Impairment**

Epidemiologic studies of exposure to TCE found impairment of trigeminal nerve function, assessed by the blink reflex test or the TSEP, in humans exposed occupationally by inhalation or environmentally by ingestion (see Table 4-20). Mean inhalational exposures inferred from biological monitoring or from a range of atmospheric monitoring in occupational studies was approximately 50–<150 ppm TCE exposure. Residence location is the exposure surrogate in geographical-based studies of contaminated water supplies with several solvents. Well water contaminant concentrations of TCE ranged from <0.2 to 10,000 ppb and do not provide an estimate of TCE concentrations in drinking water to studied individuals. Two occupational studies, each including >100 subjects, reported statistically significant dose-response trends based on ambient TCE concentrations, duration of exposure, and/or urinary concentrations of the TCE metabolite TCA ([Barret et al., 1987](#); [Barret et al., 1984](#)). Three geographical-based studies of environmental exposures to TCE via contaminated drinking water are further suggestive of trigeminal nerve function decrements; however, these studies are more limited than occupational studies due to questions of subject selection. Both exposed subjects, who were litigants, and control subjects may not be representative of exposed ([Kilburn, 2002b, a](#); [Kilburn and Warshaw, 1993a](#)); referents in Kilburn and Warshaw ([1993a](#)), were histology technicians and subjects in a previous study of formaldehyde and other solvent exposures and neurobehavioral effects ([Kilburn and Warshaw, 1992b](#); [Kilburn et al., 1987](#)). Results were mixed in a number of smaller studies. Two of these studies reported changes in

trigeminal nerve response ([Mhiri et al., 2004](#); [Barret et al., 1982](#)), including evidence of a correlation with duration of exposure and increased latency in one study ([Mhiri et al., 2004](#)). Ruijten et al. ([1991](#)) reported no significant change in the blink reflex, but did report an increase in the latency of the masseter reflex, which also may reflect effects on the trigeminal nerve. Two other studies reported no observed effect on trigeminal nerve impairment, but the authors failed to provide assessment of trigeminal nerve function ([Rasmussen et al., 1993a](#); [El Ghawabi et al., 1973](#)) or there was not a control (nonexposed) group included in the study ([Rasmussen et al., 1993a](#)). Therefore, because of limitations in statistical power, the possibility of exposure misclassification, and possible differences in measurement methods, these studies are not judged to provide substantial evidence against a causal relationship between TCE exposure and trigeminal nerve impairment. Overall, the weight of evidence supports a relationship between TCE exposure and trigeminal nerve dysfunction in humans.

Impairment of trigeminal nerve function is observed in studies of laboratory animal studies. Although one subchronic animal study demonstrated no significant impairment of trigeminal nerve function following TCE exposure up to 2,500 ppm (no observed-adverse-effect level [NOAEL]) ([Albee et al., 2006](#)), morphological analysis of the nerve revealed changes in its structure ([Barret et al., 1992](#); [Barret et al., 1991](#)). However, the dose at which an effect was observed by Barret et al. ([1992](#); [1991](#)) was high (2,500 mg/kg-day—lowest-observed-adverse-effect level [LOAEL]) compared to any reasonable occupational or environmental setting, although no lower doses were used. The acute or subchronic duration of these studies, as compared to the much longer exposure duration in many of the human studies, may also contribute to the apparent disparity between the epidemiologic and (limited) laboratory animal data.

The subchronic study of Barret et al. ([1992](#)) and the acute exposure study of Albee et al. ([Albee et al., 1997](#)) also demonstrated that dichloroacetylene, a (ex vivo) TCE degradation product, also induces trigeminal nerve impairment, at much lower doses than TCE. It is possible that under some conditions, co-exposure to dichloroacetylene from TCE degradation may contribute to the changes observed to be associated with TCE exposure in human studies, and this issue is discussed further below in Section 4.3.10.

Overall evidence from numerous epidemiologic studies supports a conclusion that TCE exposure induces trigeminal nerve impairment in humans. Laboratory animal studies provide limited additional support, and do not provide strong contradictory evidence. Persistence of these effects after cessation of exposure cannot be determined since exposure was ongoing in the available human and laboratory animal studies.

## **4.3.2. Auditory Effects**

### **4.3.2.1. Auditory Function: Human Studies**

The TCE Subregistry from the National Exposure Registry developed by the ATSDR was the subject of three studies ([ATSDR, 2002](#); [Burg and Gist, 1999](#); [Burg et al., 1995](#)). A fourth study ([Rasmussen et al., 1993a](#)) of degreasing workers exposed to either TCE or CFC113 also indirectly evaluated auditory function. These studies are discussed below and presented in detail in Table 4-22.

**Table 4-22. Summary of human auditory function studies**

Reference	Subjects	Exposure	Effect
ATSDR (2002)	116 children, under 10 yrs of age, residing near six Superfund sites. Further study of children in Burg et al. (1999; 1995). Control: 182 children.	TCE and other solvents in groundwater supplies. Exposures were modeled using tap water TCE concentrations and GIS for spatial interpolation, and LaGrange for temporal interpolation to estimate exposures from gestation to 1990 across the area of subject residences. Control = 0 ppb; low exposure group = 0–<23 ppb-yr; and high exposure group = >23 ppb-yr.	Auditory screening revealed increased incidence of abnormal middle ear function in exposed groups as indicated from acoustic reflex test. Adjusted ORs for right ear ipsilateral acoustic reflects control, OR: 1.0, low exposure group, OR: 5.1, $p < 0.05$ ; high exposure group, OR: 7.2, $p < 0.05$ . ORs adjusted for age, sex, medical history, and other chemical contaminants. No significant decrements reported in the pure tone and tympanometry screening.
Burg et al. (1995)	From an NHIS TCE subregistry of 4,281 (4,041 living and 240 deceased) residents.	Environmentally exposed to TCE and other solvents via well water in Indiana, Illinois, and Michigan.	Increase in self-reported hearing impairments for children $\leq 9$ yrs.
Burg et al. (1999)	3,915 white registrants. Mean age 34 yrs (SD = 19.9 yrs).	Cumulative TCE exposure subgroups: <50 ppb, n = 2,867; 50–500 ppb, n = 870; 500–5,000 ppb, n = 190; >5,000 ppb, n = 35. Exposure duration subgroups: <2, 2–5, 5–10, and >10 yrs.	A statistically significant association (adjusted for age and sex) between duration of exposure and self-reported hearing impairment was found.
Rasmussen et al. (1993c)	96 Danish metal degreasers. Age range: 19–68 yrs. No unexposed controls; low exposed group is referent.	Average exposure duration: 7.1 yrs range of full-time degreasing: 1 mo to 36 yrs. Exposure to TCE or and CFC113. (1) Low exposure: n = 19, average full-time exposure 0.5 yr. (2) Medium exposure: n = 36, average full-time exposure 2.1 yrs. (3) High exposure: n = 41, average full-time exposure 11 yrs. Mean U-TCA in high exposure group = 7.7 mg/L (maximum = 26.1 mg/L).	Auditory impairments noted through several neurological tests. Significant relationship of exposure was found with acoustic-motor function ( $p < 0.001$ ), Paced Auditory Serial Addition Test ( $p < 0.001$ ), and Rey Auditory Verbal Learning Test ( $p < 0.001$ ).

NHIS = National Health Interview Survey

Burg et al. ([1999](#); [1995](#)) reviewed the effects of TCE on 4,281 individuals (TCE Subregistry) residentially exposed to this solvent for more than 30 consecutive days. Face-to-face interviews were conducted with the TCE subregistry population and self-reported hearing loss was evaluated based on personal assessment through the interview (no clinical evaluation was conducted). TCE registrants who were  $\leq 9$  years old had a statistically significant increase in hearing impairment as reported by the subjects. The RR in this age group for hearing impairments was 2.13 (95% CI: 1.12–4.06), which decreased to 1.12 (95% CI: 0.52–2.24) for the 10–17-year-old age group and 0.32 (95% CI: 0.10–1.02) for all older age groups. A statistically significant association (when adjusted for age and sex) was found between duration of exposure (in these studies, this was length of residency) and reported hearing impairment. The ORs were 2.32 (95% CI: 1.18–4.56) for subjects exposed to TCE  $>2$ – $\leq 5$  years, 1.17 (95% CI: 0.55–2.49) for exposure  $>5$ – $\leq 10$  years, and 2.46 (95% CI: 1.30–5.02) for exposure durations  $>10$  years.

ATSDR ([2002](#)) conducted a follow-up study to the TCE subregistry findings ([Burg and Gist, 1999](#); [Burg et al., 1995](#)) and focused on the subregistry children located in Elkhart, Indiana, Rockford, Illinois, and Battle Creek, Michigan using clinical tests for oral motor, speech, and hearing function. Exposures were modeled using tap water TCE concentrations and geographic information system (GIS) for spatial interpolation, and LaGrange for temporal interpolation to estimate exposures from gestation to 1990 across the area of subject residences. Modeled data were used to estimate lifetime exposures (ppb-years) to TCE in residential wells. The median TCE exposure for the children was estimated from drinking water as 23 ppb/year of exposure (ranging from 0 to 702 ppb/year). Approximately 20% (17–21%, depending on ipsilateral or contralateral test reflex) of the children in the TCE subregistry and 5–7% in the control group exhibited an abnormal acoustic reflex (involuntary muscle contraction that measures movement of the stapedius muscle in the middle ear following a noise stimulus), which was statistically significant ( $p = 0.003$ ). Abnormalities in this reflex could be an early indicator of more serious hearing impairments. No significant decrements were reported in the pure tone and tympanometry screening.

Rasmussen et al. ([1993c](#)) used a psychometric test to measure potential auditory effects of TCE exposure in an occupational study. Results from 96 workers exposed to TCE and other solvents were presented in this study. Details of the exposure groups and exposure levels are provided in Table 4-22. The acoustic motor function test was used for evaluation of auditory function. Significant decrements ( $p < 0.05$ ) in acoustic motor function performance scores (average decrement of 2.5 points on a 10-point scale) were reported for TCE exposure.

#### **4.3.2.2. Auditory Function: Laboratory Animal Studies**

The ability of TCE to permanently disrupt auditory function and produce abnormalities in inner ear histopathology has been demonstrated in several studies using a variety of test methods.

Two different laboratories have identified NOAELs following inhalation exposure for auditory function of 1,600 ppm for 12 hours/day for 13 weeks in Long-Evans rats (n = 6–10) ([Rebert et al., 1991](#)) and 1,500 ppm for 18 hours/day, 5 days/week for 3 weeks in Wistar-derived rats (n = 12) ([Jaspers et al., 1993](#)). The LOAELs identified in these and similar studies are 2,500–4,000 ppm TCE for periods of exposure ranging from 4 hours/day for 5 days to 12 hours/day for 13 weeks (e.g., [Albee et al., 2006](#); [Boyes et al., 2000](#); [Muijser et al., 2000](#); [Fechter et al., 1998](#); [Crofton and Zhao, 1997](#); [Rebert et al., 1995](#); [Crofton et al., 1994](#); [Rebert et al., 1993](#)). Rebert et al. ([1993](#)) estimated acute blood TCE levels associated with permanent hearing impairment at 125 µg/mL by methods that probably underestimated blood TCE values (rats were anaesthetized using 60% carbon dioxide [CO<sub>2</sub>]). A summary of these studies is presented in Table 4-23.

**Table 4-23. Summary of animal auditory function studies**

Reference <sup>a</sup>	Exposure route	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL <sup>a</sup>	Effects
<a href="#">Rebert et al. (1991)</a>	Inhalation	Rat, Long-Evans, male, 10/group	Long-Evans: 0, 1,600, and 3,200 ppm; 12 hrs/d, 12 wks	Long-Evans: NOAEL: 1,600 ppm; LOAEL: 3,200 ppm	BAERs were measured. Significant decreases in BAER amplitude and an increase in latency of appearance of the initial peak (P1).
		Rat, F344, male, 4–5/group	F344: 0, 2,000, and 3,200 ppm; 12 hrs/d, 3 wks	F344: LOAEL: 2,000 ppm	
<a href="#">Rebert et al. (1993)</a>	Inhalation	Rat, Long-Evans, male, 9/group	0, 2,500, 3,000, and 3,500 ppm; 8 hrs/d, 5 d	NOAEL: 2,500 ppm; LOAEL: 3,000 ppm.	BAERs were measured 1–2 wks postexposure to assess auditory function. Significant decreases in BAERs were noted with TCE exposure.
<a href="#">Rebert et al. (1995)</a>	Inhalation	Rat, Long-Evans, male, 9/group	0 and 2,800 ppm; 8 hrs/d, 5 d	LOAEL: 2,800 ppm	BAER measured 2–14 ds postexposure at a 16 kHz tone. Hearing loss ranged from 55 to 85 dB.
<a href="#">Crofton et al. (1994)</a>	Inhalation	Rat, Long-Evans, male, 7–8/group	0 and 3,500 ppm TCE; 8 hrs/d, 5 d	LOAEL: 3,500 ppm	BAER measured and auditory thresholds determined 5–8 wks postexposure. Selective impairment of auditory function for mid-frequency tones (8 and 16 kHz).

**Table 4-23. Summary of animal auditory function studies (continued)**

Reference <sup>a</sup>	Exposure route	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL	Effects
Crofton and Zhou (1997); Boyes et al. (2000)	Inhalation	Rat, Long-Evans, male, 8–10/group	0, 800, 1,600, 2,400, and 3,200 ppm; 6 hrs/d, 5 d/wk, 13 wks	NOAEL: 1,600 ppm; LOAEL: 2,400 ppm	Auditory thresholds as measured by BAERs for the 16 kHz tone increased with TCE exposure. Measured 3–5 wks post exposure.
		Rat, Long-Evans, male, 8–10/group	0, 1,600, 2,400, and 3,200 ppm; 6 hrs/d, 5 d	NOAEL: 2,400 ppm; LOAEL: 3,200 ppm	
		Rat, Long-Evans, male, 8–10/group	0, 800, 1,600, 2,400, and 3,200 ppm; 6 hrs/d, 5 d/wk, 4 wks	NOAEL: 2,400 ppm; LOAEL: 3,200 ppm	
		Rat, Long-Evans, male, 9–12/group	0, 4,000, 6,000, and 8,000 ppm; 6 hrs	NOAEL: 6,000 ppm; LOAEL: 8,000 ppm	
Fechter et al. (1998)	Inhalation	Rat, Long-Evans, male, 12/group	0 and 4,000 ppm; 6 hrs/d, 5 d	LOAEL: 4,000 ppm	Cochlear function measured 5–7 wks after exposure. Loss of spiral ganglion cells noted. Auditory function was significantly decreased 3 wks postexposure, as measured by compound action potentials and reflex modification.
Jaspers et al. (1993)	Inhalation	Rat, Wistar derived WAG-Rii/MBL, male, 12/group	0, 1,500, and 3,000 ppm; 18 hrs/d, 5 d/wk, 3 wks	NOAEL: 1,500 ppm	Auditory function assessed repeatedly 1–5 wks postexposure for 5, 20, and 35 kHz tones; no effect at 5 or 35 kHz; decreased auditory sensitivity at 20 kHz, 3,000 ppm.
Muijser et al. (2000)	Inhalation	Rat, Wistar derived WAG-Rii/MBL, male, 8	0 and 3,000 ppm; 18 hrs/d, 5 d/wk, 3 wks	LOAEL: 3,000 ppm	Auditory sensitivity decreased with TCE exposure at 4, 8, 16, and 20 kHz tones. White noise potentiated the decrease in auditory sensitivity.
Albee et al. (2006)	Inhalation	Rat, F344, male and female, 10/sex/group	0, 250, 800, and 2,500 ppm; 6 hrs/d, 5 d/wk, 13 wks	NOAEL: 800 ppm; LOAEL: 2,500 ppm	Mild frequency specific hearing deficits; focal loss of cochlear hair cells.

**Table 4-23. Summary of animal auditory function studies (continued)**

Reference <sup>a</sup>	Exposure route	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL	Effects
Yamamura et al. (1983)	Inhalation	Guinea Pig, albino Hartley, male, 7–10/group	0, 6,000, 12,000, and 17,000 ppm; 4 hrs/d, 5 d	NOAEL: 17,000 ppm	No change in auditory sensitivity at any exposure level as measured by cochlear action potentials and microphonics. Study was conducted in guinea pig and species is less sensitive to auditory toxicity than rats. Studies were also not conducted in a sound-isolation chamber and effects may be impacted by background noise.

**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

BAER = brainstem auditory-evoked potential

Reflex modification was used in several studies to evaluate the auditory function in TCE-exposed animals (Boyes et al., 2000; Muijser et al., 2000; Fechter et al., 1998; Crofton and Zhao, 1997; Crofton et al., 1994; Crofton and Zhao, 1993; Jaspers et al., 1993; Yamamura et al., 1983). These studies collectively demonstrate significant decreases in auditory function at midfrequency tones (8–20 kHz tones) for TCE exposures >1,500 ppm after acute, short-term, and chronic durations. Only one study (Yamamura et al., 1983) did not demonstrate impairment in auditory function from TCE exposures as high as 17,000 ppm for 4 hours/day over 5 days. This was the only study to evaluate auditory function in guinea pigs, whereas the other studies used various strains of rats. Despite the negative finding in Yamamura et al. (1983), auditory testing was not performed in an audiometric sound attenuating chamber and extraneous noise could have influenced the outcome. It is also important to note that the guinea pig has been reported to be far less sensitive than the rat to the effects of ototoxic aromatic hydrocarbons such as toluene.

Crofton and Zhao (1997) also presented a benchmark dose (BMD) for which the calculated dose of TCE would yield a 15 dB loss in auditory threshold. This benchmark response (BMR) was selected because a 15 dB threshold shift represents a significant loss in threshold sensitivity for humans. The benchmark concentrations for a 15 dB threshold shift are 5,223 ppm for 1 day, 2,108 ppm for 5 days, 1,418 ppm for 20 days and 1,707 ppm for 65 days of exposure. While more sensitive test methods might be used and other definitions of a benchmark effect chosen with a strong rationale, these data provide useful guidance for exposure concentrations that yield hearing loss in rats.

Brainstem auditory-evoked responses (BAERs) were also measured in several studies (Albee et al., 2006; Rebert et al., 1995; Rebert et al., 1993; Rebert et al., 1991) following at exposures of 3–13 weeks. Rebert et al. (1991) measured BAERs in male Long-Evans rats

(n = 10) and F344 rats (n = 4–5) following stimulation with 4, 8, and 16 kHz sounds. The Long-Evans rats were exposed to 0, 1,600, or 3,200 ppm TCE, 12 hours/day for 12 weeks and the F344 rats were exposed to 0, 2,000, or 3,200 ppm TCE, 12 hours/day for 3 weeks. BAER amplitudes were significantly decreased at all frequencies for F344 rats exposed to 2,000 and 3,000 ppm TCE and for Long-Evans rats exposed to 3,200 ppm TCE. These data identify a LOAEL at 2,000 ppm for the F344 rats and a NOAEL at 1,600 ppm for the Long-Evans rats. In subsequent studies, Rebert et al. ([1995](#); [1993](#)) again demonstrated that TCE significantly decreases BAER amplitudes and also significantly increases the latency of appearance. Similar results were obtained by Albee et al. ([2006](#)) for male and female F344 rats exposed to TCE for 13 weeks. The NOAEL for this study was 800 ppm based on ototoxicity at 2,500 ppm.

Notable physiological changes were also reported in a few auditory studies. Histological data from cochleas in Long-Evans rats exposed to 4,000 ppm TCE indicated that there was a loss in spiral ganglion cells ([Fechter et al., 1998](#)). Similarly, there was an observed loss in hair cells in the upper basal turn of the cochlea in F344 rats exposed to 2,500 ppm TCE ([Albee et al., 2006](#)).

#### **4.3.2.3. Summary and Conclusion of Auditory Effects**

Human and animal studies indicated that TCE produces decrements in auditory function. In the human epidemiological studies ([ATSDR, 2002](#); [Burg and Gist, 1999](#); [Burg et al., 1995](#); [Rasmussen et al., 1993d](#)), it is suggested that auditory impairments result from both an inhalation and oral TCE exposure. A LOAEL of approximately 23 ppb-years TCE (extrapolated from  $\leq 23$  ppb-years group in ATSDR ([2002](#)) from oral intake is noted for auditory effects in children. The only occupational study where auditory effects were seen reported mean urinary trichloroacetic acid (U-TCA) concentration, a nonspecific metabolite of TCE, of 7.7 mg/L for the high cumulative exposure group only ([Rasmussen et al., 1993d](#)). A NOAEL or a LOAEL for auditory changes resulting from inhalational exposure to TCE cannot be interpolated from average U-TCA concentration of subjects in the high-exposure group because of a lack of detailed information on long-term exposure levels and duration ([Rasmussen et al., 1993d](#)). Two studies ([Burg and Gist, 1999](#); [Burg et al., 1995](#)) evaluated self-reported hearing effects in people included in the TCE subregistry comprised of people residing near Superfund sites in Indiana, Illinois, and Michigan. In Burg et al. ([1995](#)), interviews were conducted with the TCE-exposed population and it was found that children aged  $\leq 9$  years old had statistically significant hearing impairments in comparison to nonexposed children. This significant increase in hearing impairment was not observed in any other age group that was included in this epidemiological analysis. This lack of effect in other age groups may suggest association with another exposure other than drinking water; however, it may also suggest that children may be more susceptible than adults. In a follow-up analysis, Burg et al. ([1999](#)) adjusted the statistical analysis of the original data ([Burg et al., 1995](#)) for age and sex. When these adjustments were made, a

statistically significant association was reported self-reported for auditory impairment and duration of residence. These epidemiological studies provided only limited information given their use of an indirect exposure metric of residence location, no auditory testing of this studied population, and self-reporting of effects. ATSDR (2002) further tested the findings in the Burg studies (Burg and Gist, 1999; Burg et al., 1995) by contacting the children who were classified as having hearing impairments in the earlier study and conducting several follow-up auditory tests. Significant abnormalities were reported for the children in the acoustic reflex test, which suggested effects to the lower brainstem auditory pathway with the large effect measure, the OR, was reported for the high-cumulative-exposure group. Strength of analyses was its adjustment for potential confounding effects of age, sex, medical history, and other chemical contaminants in drinking water supplies. The ATSDR findings were important in that the results supported Burg et al. (1999; 1995). Rasmussen et al. (1993c) also evaluated auditory function in metal workers with inhalation exposure to either TCE or CFC113. Results from tasks, including an auditory element, suggested that these workers may have some auditory impairment. However, the tasks did not directly measure auditory function.

Animals studies strongly indicated that TCE produces deficits in hearing and provides biological context to the epidemiological study observations. Although there is a strong association between TCE and ototoxicity in the animal studies, most of the effects began to occur at higher inhalation exposures. NOAELs for ototoxicity ranged from 800 to 1,600 ppm for exposure durations of at least 12 weeks (Albee et al., 2006; Boyes et al., 2000; Crofton and Zhao, 1997; Rebert et al., 1991). Inhalation exposure to TCE was the route of administration in all of the animal studies. These studies either used reflex modification audiometry (Muijser et al., 2000; Crofton and Zhao, 1997; Crofton et al., 1994; Jaspers et al., 1993) procedures or measured BAERs (Rebert et al., 1995; Rebert et al., 1993; Rebert et al., 1991) to evaluate hearing in rats. Collectively, the animal database demonstrates that TCE produces ototoxicity at midfrequency tones (4–24 kHz), and no changes in auditory function were observed at either the low (<4 kHz) or high (>24 kHz) frequency tones. Additionally, deficits in auditory effects were found to persist for at least 7 weeks after the cessation of TCE exposure (Boyes et al., 2000; Fechter et al., 1998; Crofton and Zhao, 1997; Jaspers et al., 1993; Rebert et al., 1991). Decreased amplitude and latency were noted in the BAERs (Rebert et al., 1995; Rebert et al., 1993; Rebert et al., 1991), suggesting that TCE exposure affects central auditory processes. Decrements in auditory function following reflex modification audiometry (Muijser et al., 2000; Crofton and Zhao, 1997; Crofton et al., 1994; Jaspers et al., 1993) combined with changes observed in cochlear histopathology (Albee et al., 2006; Fechter et al., 1998) suggest that ototoxicity is occurring at the level of the cochlea and/or brainstem.

Changes in auditory function are noteworthy considering that TCE exposure is also associated with immunotoxicity and inflammatory-based diseases (discussed in Section 4.6). Autoimmune sensorineural hearing loss is a rare condition, sometimes seen with systemic

autoimmune diseases ([Bovo et al., 2006](#); [Ruckenstein, 2004](#)). The potential role of immunotoxicity in the observed auditory impairment seen with TCE is an area that requires additional research.

### **4.3.3. Vestibular Function**

#### **4.3.3.1. Vestibular Function: Human Studies**

The earliest reports of neurological effects resulting from TCE exposures focused on subjective vestibular system symptoms, such as headaches, dizziness, and nausea. These symptoms are subjective and self-reported. However, there is little doubt that these effects can be caused by exposures to TCE, as they have been reported extensively in the literature, resulting from occupational exposures ([Liu et al., 1988](#); [Rasmussen and Sabroe, 1986](#); [Smith, 1970](#); [Grandjean et al., 1955](#)), environmental exposures ([Hirsch et al., 1996](#)), and chamber studies ([Smith, 1970](#); [Stewart et al., 1970](#)).

Kylin et al. ([1967](#)) exposed 12 volunteers to 1,000 ppm (5,500 mg/m<sup>3</sup>) TCE for 2 hours in a 1.5 × 2 × 2 chamber. Volunteers served as their own controls since 7 of the 12 were pretested prior to exposure and the remaining 5 were post-tested days after exposure. Subjects were tested for optokinetic nystagmus, which was recorded by electronystagmography, that is, “the potential difference produced by eye movements between electrodes placed in lateral angles between the eyes.” Venous blood was also taken from the volunteers to measure blood TCE levels during the vestibular task. The authors concluded that there was an overall reduction in the limit (“fusion limit”) to reach optokinetic nystagmus when individuals were exposed to TCE. Reduction of the “fusion limit” persisted for up to 2 hours after the TCE exposure was stopped and the blood TCE concentration was 0.2 mg/100 mL.

#### **4.3.3.2. Vestibular Function: Laboratory Animal Data**

The effect of TCE on vestibular function was evaluated by either: (1) promoting nystagmus (vestibular system dysfunction) and comparing the level of effort required to achieve nystagmus in the presence and absence of TCE or (2) using an elevated beam apparatus and measuring the balance. Overall, it was found that TCE disrupts vestibular function as presented below and summarized in Table 4-24.

**Table 4-24. Summary of vestibular system studies**

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
<b>Vestibular system studies—humans</b>					
Kylin et al. (1967)	Inhalation	Humans, male and female, 12	1,000 ppm; 2 hrs	LOAEL: 1,000 ppm	Reduction in potential to reach nystagmus following TCE exposure.
<b>Vestibular system studies—animals</b>					
Tham et al. (1979)	i.v.	Rabbit, strain unknown, sex unspecified, 19	1–5 mg/kg/min	–	Positional nystagmus developed once blood levels reached 30 ppm.
Tham et al. (1984)	i.v.	Rat, Sprague-Dawley, female, 11	80 µg/kg/min	–	Excitatory effects on the vestibule-oculomotor reflex. Threshold effect at blood (TCE) of 120 ppm or 0.9 mM/L.
Niklasson et al. (1993)	Inhalation	Rat, strain unknown, male and female, 28	0, 2,700, 4,200, 6,000, or 7,200 ppm; 1 hr	LOAEL: 2,700 ppm	Increased ability to produce nystagmus.
Umezu et al. (1997)	i.p.	Mouse, ICR, male, 116	0, 250, 500, or 1,000 mg/kg, single dose and evaluated 30 mins postadministration	NOAEL: 250 mg/kg LOAEL: 500 mg/kg	Decreased equilibrium and coordination as measured by the Bridge test (staying time on an elevated balance beam).

Niklasson et al. (1993) showed acute impairment of vestibular function in male- and female-pigmented rats during acute inhalation exposure to TCE (2,700–7,200 ppm) and to trichloroethane (500–2,000 ppm). Both of these agents were able to promote nystagmus during optokinetic stimulation in a dose-related manner. While there were no tests performed to assess persistence of these effects, Tham et al. (1984; 1979) did find complete recovery of vestibular function in rabbits (n = 19) and female Sprague-Dawley rats (n = 11) within minutes of terminating a direct arterial infusion with TCE solution.

The finding that TCE can yield transient abnormalities in vestibular function is not unique. Similar impairments have also been shown for toluene, styrene, and trichloroethane (Niklasson et al., 1993) and for a broad range of aromatic hydrocarbons (Tham et al., 1984). The concentration of TCE in blood at which effects were observed for TCE (0.9 mM/L) was quite close to that observed for most of these other vestibulo-active solvents.

#### 4.3.3.3. Summary and Conclusions for the Vestibular Function Studies

Studies of TCE exposure in both humans and animals reported abnormalities in vestibular function. Headaches, dizziness, nausea, and motor incoordination, among other subjective symptoms, are reported in occupational epidemiological studies of TCE exposure (Hirsch et al., 1996; Liu et al., 1988; Rasmussen and Sabroe, 1986; Smith, 1970; Stewart et al., 1970;

[Grandjean et al., 1955](#)). One human exposure study ([Kylin et al., 1967](#)) found that vestibular function was affected following an acute exposure to 1,000-ppm TCE (LOAEL). Individuals had a decreased threshold to reach nystagmus than when exposed to TCE than to air. Animal studies also evaluated the threshold to reach nystagmus and reported that TCE decreased the threshold to produce nystagmus in rats (LOAEL: 2,700 ppm) ([Niklasson et al., 1993](#); [Tham et al., 1984](#)) and rabbits ([Tham et al., 1984](#)).

#### **4.3.4. Visual Effects**

##### **4.3.4.1. Visual Effects: Human Studies**

Visual impairment in humans has been demonstrated following exposures through groundwater ([Reif et al., 2003](#); [Kilburn, 2002b, a](#)), from occupational exposure through inhalation ([Rasmussen et al., 1993c](#); [Tröster and Ruff, 1990](#)), and from a controlled inhalation exposure study ([Vernon and Ferguson, 1969](#)). Visual functions such as color discrimination and visuospatial learning tasks are impaired in TCE-exposed individuals. Additionally, an acute exposure can impair visual depth perception. Details of the studies are provided below and summarized in Table 4-25.

**Table 4-25. Summary of human visual function studies**

Reference	Subjects	Exposure	Effect
Kilburn et al. (2002b, a) (2002a)	236 residents near a microchip plant in Phoenix, Arizona. Controls: 67 local referents from Phoenix, Arizona and 161 regional referents from Wickenburg, Arizona.	TCE, TCA, 1,1-DCE, 1,2-DCE, perchloroethylene, and vinyl chloride detected in well water up to 260,000 ppm; TCE concentrations in well water were 0.2–10,000 ppb. Exposure duration 2–37 yrs.	Color discrimination errors were increased among residents compared to regional referents ( $p < 0.01$ ). No adjustment for possible confounding factors.
Reif et al. (2003)	143 residents of the Rocky Mountain Arsenal community of Denver. Referent group at lowest concentration (<5 ppb).	Exposure modeling of TCE concentrations in groundwater and in distribution system to estimate mean TCE concentration by census block of residence. High exposure group >15 ppb. Medium exposure group $\geq 5$ – $\leq 15$ ppb. Low exposure referent group <5 ppb.	Contrast sensitivity test performances (C and D) was marginally statistically significant ( $p = 0.06$ and $0.07$ , respectively). No significant effects reported for the Benton visual retention test. Significant decrements ( $p = 0.02$ ) were reported in the Benton visual retention test when stratified with alcohol consumption.
Rasmussen et al. (1993c)	96 Danish metal degreasers. Age range: 19–68; no unexposed controls; low exposure group was referent.	Average exposure duration: 7.1 yrs; range of full-time degreasing: 1 mo to 36 yrs. Exposure to TCE or CFC113: (1) Low exposure: $n = 19$ , average full-time exposure 0.5 yr. (2) Medium exposure: $n = 36$ , average full-time exposure 2.1 yrs. (3) high exposure: $n = 41$ , average full-time exposure 11 yrs. TCA in high exposure group = 7.7 mg/L (maximum = 26.1 mg/L).	Statistically significant relationship of exposure was found with the Visual Gestalts learning and retention test (cognitive test) indicating deficits in visual performance.
Troster and Ruff (1990)	Two occupationally TCE-exposed workers. Controls: two groups of $n = 30$ matched controls; (all age and education matched).	Exposure concentration unknown. Exposure duration, 3–8 mo.	Both workers experienced impaired visuospatial learning.
Vernon and Ferguson (1969)	8 male volunteers age range 21–30; self controls.	0, 100, 300, and 1,000 ppm of TCE for 2 hrs.	Statistically significant effects on visual depth perception as measured by the Howard-Dolman test. NOAEL: 300 ppm; LOAEL: 1,000 ppm. No significant changes in any of the other visual test measurements.

Geographical-based studies utilized color discrimination and contrast sensitivity tests to determine the effect of TCE exposure on vision. In these studies, it was reported that TCE exposure significantly increased color discrimination errors (Kilburn, 2002b, a) or that decreased contrast sensitivity tests approached statistical significance after adjustments for several possible confounders ( $p = 0.06$  or  $0.07$ ) (Reif et al., 2003). Exposure in Kilburn (2002b, a) is poorly

characterized, and for both studies, TCE is one of several contaminants in drinking water supplies; neither study provided an estimate of an individual's exposure to TCE.

Rasmussen et al. ([1993c](#)) evaluated visual function in 96 metal workers, working in degreasing at various factories and with exposure to TCE or CFC113. Visual function was tested through the visual gestalts test (visual perception) and a visual recall test. In the visual gestalts test, the number of total errors significantly increased from the low-exposure group (3.4 errors) to the high-exposure group (6.5 errors;  $p = 0.01$ ). No significant changes were observed in the visual recall task. Troster and Ruff ([1990](#)) presented case studies conducted on two occupationally exposed workers to TCE. Both patients presented with a visual-spatial task and neither could complete the task within the number of trials allowed, suggesting visual function deficits as a measure of impaired visuospatial learning.

In a chamber exposure study ([Vernon and Ferguson, 1969](#)), eight male volunteers (ages 21–30) were exposed to 0, 100, 300, and 1,000-ppm TCE for 2 hours. Each individual was exposed to all TCE concentrations and a span of at least 3 days was given between exposures. When the individuals were exposed to 1,000-ppm TCE (5,500 mg/m<sup>3</sup>), significant abnormalities were noted in depth perception as measured by the Howard-Dolman test ( $p < 0.01$ ). There were no effects on the flicker fusion frequency test (threshold frequency at which the individual sees a flicker as a single beam of light) or on the form perception illusion test (volunteers presented with an illusion diagram).

#### **4.3.4.2. Visual Effects: Laboratory Animal Data**

Changes in visual function have been demonstrated in animal studies during acute ([Boyes et al., 2005b](#); [Boyes et al., 2003](#)) and subchronic exposure ([Blain et al., 1994](#); [Rebert et al., 1991](#)). In these studies, the effect of TCE on visual evoked responses to patterns ([Boyes et al., 2005b](#); [Boyes et al., 2003](#); [Rebert et al., 1991](#)) or a flash stimulus ([Blain et al., 1994](#); [Rebert et al., 1991](#)) were evaluated. Overall, the studies demonstrated that exposure to TCE results in significant changes in the visual evoked response, which is reversible once TCE exposure is stopped. Details of the studies are provided below and are summarized in Table 4-26.

**Table 4-26. Summary of animal visual system studies**

Reference	Exposure route	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL	Effects
Rebert et al. (1991)	Inhalation	Rat, Long-Evans, male, 10/group	0, 1,600, and 3,200 ppm; 12 hrs/d, 12 wks	NOAEL: 1,600 ppm	Significant amplitude decreases in pattern reversal evoked potentials (N1P1 amplitude) at 6, 9, and 12 wks.
Boyes et al. (2003)	Inhalation	Rat, Long-Evans, male, 9–10/group	0 ppm, 4 hrs; 1,000 ppm, 4 hrs; 2,000 ppm, 2 hrs; 3,000 ppm, 1.3 hrs; 4,000 ppm, 1 hr	LOAEL: 1,000 ppm, 4 hrs	Visual function significantly affected as measured by decreased amplitude (F2) in Fourier-transformed visual evoked potentials. Peak brain TCE concentration correlated with dose-response.
Boyes et al. (2005a)	Inhalation	Rat, Long-Evans, male, 8–10/group	0 ppm, 4 hrs; 500 ppm, 4 hrs; 1,000 ppm, 4 hrs; 2,000 ppm, 2 hrs; 3,000 ppm, 1.3 hrs; 4,000 ppm, 1 hr; 5,000 ppm, 0.8 hr	LOAEL: 500 ppm, 4 hrs	Visual function significantly affected as measured by decreased amplitude (F2) in Fourier-transformed visual evoked potentials. Peak brain TCE concentration correlated with dose-response.
Blain et al. (1994)	Inhalation	Rabbit, New Zealand albino, male, 6–8/group	0, 350, and 700 ppm; 4 hrs/d, 4 d/wk, 12 wks	LOAEL: 350 ppm	<b>Significant effects noted in visual function as measured by ERG and OPs immediately after exposure. No differences in ERG or OP measurements were noted at 6 wks post-TCE exposure.</b>

**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

ERG = electroretinogram, OP = oscillatory potential

Boyes et al. (2005a; 2003) exposed adult, male Long-Evans rats to TCE in a head-only exposure chamber while pattern onset/offset visual evoked potentials (VEPs) were recorded. Exposure conditions were designed to provide concentration × time products of 0 ppm/hours (0 ppm for 4 hours) or 4,000 ppm/hours (see Table 4-26 for more details). VEP amplitudes were depressed by TCE exposure during the course of TCE exposure. The degree of VEP depression showed a high correlation with the estimated brain TCE concentration for all levels of atmospheric TCE exposure.

In a subchronic exposure study, Rebert et al. (1991) exposed male Long-Evans rats to 1,600 or 3,200 ppm TCE, for 12 weeks, 12 hours/day. No significant changes in flash evoked potential measurements were reported following this exposure paradigm. Decreases in pattern reversal VEPs (N1P1 amplitude) reached statistical significance following 6, 9, and 12 weeks of

exposure. The drop in response amplitude ranged from approximately 20% after 8 weeks to nearly 50% at week 14, but recovered completely within 1 week postexposure.

This transient effect of TCE on the peripheral visual system has also been reported by Blain (1994) in which New Zealand albino rabbits were exposed by inhalation to 350 and 700 ppm TCE 4 hours/day, 4 days/week for 12 weeks. Electroretinograms (ERG) and oscillatory potentials (OPs) were recorded weekly under mesopic conditions. Recordings from the 350- and 700-ppm exposed groups showed a significant increase in the amplitude of the a- and b-waves (ERG). The amplitude of the OPs was significantly decreased at 350 ppm (57%) and increased at 700 ppm (117%). These electroretinal changes returned to pre-exposure conditions within 6 weeks after the inhalation stopped.

#### 4.3.4.3. Summary and Conclusion of Visual Effects

Changes in visual function are reported in human studies. Although central visual function was not evaluated in the human studies (such as ERGs, evoked potential measurements), clinical tests indicated deficits in color discrimination (Kilburn, 2002b, a) visual depth perception (Vernon and Ferguson, 1969), and contrast sensitivity (Reif et al., 2003). These changes in visual function were observed following both an acute exposure (Vernon and Ferguson, 1969) and residence in areas with groundwater contamination with TCE and other chemicals (Reif et al., 2003; Kilburn, 2002b, a). The exposure assessment approach of Reif et al., (2003) who adopted exposure modeling and information on water distribution patterns, is considered superior to that of Kilburn (Kilburn, 2002b, a), who used residence location as a surrogate for exposure. In the one acute inhalation study (Vernon and Ferguson, 1969), a NOAEL of 300 ppm and a LOAEL of 1,000 ppm for 2 hours was reported for visual effects. A NOAEL is not available from the drinking water studies since well water TCE concentration is a poor surrogate for an individual's TCE ingestion (Kilburn, 2002b, a) and there was limited statistical analysis comparing the high-exposure group to the low-exposure group (Reif et al., 2003).

Animal studies have also demonstrated changes in visual function. All of the studies evaluated central visual function by measuring changes in evoked potential response following a visual stimulus that was presented to the animal. Two acute exposure inhalation studies (Boyes et al., 2005a; Boyes et al., 2003) exposed Long-Evans rats to TCE based on a concentration  $\times$  time schedule (Haber's law) and reported decreases in VEP amplitude. All of the exposures from these two studies resulted in decreased visual function with a LOAEL of 500 ppm for 4 hours. Another important finding that was noted is the selection of the appropriate dose-metric for visual function changes following an acute exposure. Boyes et al. (2005a; 2003) found that among other potential dose-metrics, brain TCE concentration was best correlated with changes in visual function as measured by evoked potentials under acute exposure conditions. Two subchronic exposure studies (Blain et al., 1994; Rebert et al., 1991) demonstrated visual function changes as measured by pattern reversal evoked potentials (Rebert et al., 1991) or ERGs/OPs (Blain et al., 1994). Unlike

the other three visual function studies conducted with rats, Blain et al. (1994) demonstrated these changes in rabbits. Significant changes in ERGs and OPs were noted following a 12-week exposure at 350 ppm (LOAEL) in rabbits (Blain et al., 1994), and in rats exposed to 3,200-ppm TCE for 12 weeks, there were significant decreases in pattern reversal evoked potentials, but no effect was noted in the 1,600-ppm exposure group (Rebert et al., 1991). Both subchronic studies examined visual function following an exposure-free period of either 2 weeks (Rebert et al., 1991) or 6 weeks (Blain et al., 1994) and found that visual function returned to pre-exposure levels and the changes are reversible.

#### **4.3.5. Cognitive Function**

##### **4.3.5.1. Cognitive Effects: Human Studies**

Effects of TCE on learning and memory have been evaluated in populations environmentally exposed to TCE through well water, in workers occupationally exposed through inhalation and under controlled exposure scenarios. Details of the studies are provided in Table 4-27 and discussed briefly below. In the geographical-based studies (Kilburn, 2002b, a; Kilburn and Warshaw, 1993a) cognitive function was impaired in both studies and was evaluated by testing verbal recall and digit span memory among other measures. In Arizona residents involved in a lawsuit (Kilburn and Warshaw, 1993a), significant impairments in all three cognitive measures were reported; verbal recall ( $p = 0.001$ ), visual recall ( $p = 0.03$ ), and digit span test ( $p = 0.07$ ), although a question exists whether the referent group was comparable to exposed subjects and the study had a lack of consideration of possible confounding exposures in statistical analyses. Significant decreases in verbal recall ability was also reported in another environmental exposure study where 236 residents near a microchip plant with TCE concentration in well water ranging from 0.2 to 10,000 ppb (Kilburn, 2002b, a).

**Table 4-27. Summary of human cognition effect studies**

Reference	Subjects	Exposure	Effect
Kilburn and Warshaw (1993a)	170 residents living in Southwest Tucson with TCE, other solvents, and chromium in groundwater. Control: 68 residential referents matched to subjects from two previous studies of waste oil and oil refinery exposures.	>500 ppb of TCE in well water before 1981 and 25–100 ppb afterwards. Exposure duration ranged from 1 to 25 yrs.	Decreased performance in the digit span memory test and story recall ability.
Kilburn (2002b, a)	236 residents near a microchip plant. Controls: 67 local referents from Phoenix, Arizona and 161 regional referents from Wickenburg, Arizona.	<0.2–10,000 ppb TCE, <0.2–260,000 ppb TCA, <0.2–6,900 ppb 1,1-DCE, <0.2–1,600 1,2-DCE, <0.2–23,000 ppb perchloroethylene, <0.02–330 ppb vinyl chloride in well water. Exposure duration 2 to 37 yrs.	Cognitive effects decreased as measured by lower scores on Culture Fair 2A, vocabulary, grooved pegboard (dominant hand), trail making test, and verbal recall (i.e., memory).
Rasmussen (1993c, d)	96 Danish metal degreasers. Age range: 19–68; no external controls.	Average exposure duration: 7.1 yrs); range of full-time degreasing: 1 mo to 36 yrs. 1) Low exposure: n = 19, average full-time exposure 0.5 yr. 2) Medium exposure: n = 36, average full-time exposure 2.1 yrs. 3) High exposure: n = 41, average full-time exposure 11 yrs. TCA in high exposure group = 7.7 mg/L (maximum = 26.1 mg/L).	Cognitive impairment (psycho-organic syndrome) prevalent in exposed individuals. The incidence of this syndrome was 10.5% for low exposure, 39.5% for medium exposure, and 63.4% for high exposure. Age is a confounder. Dose-response with 9 of 15 tests; Controlling for confounds, significant relationship of exposure was found with acoustic-motor function ( $p < 0.001$ ), Paced Auditory Serial Addition Test ( $p < 0.001$ ), Rey Auditory Verbal-Learning Test ( $p < 0.001$ ), vocabulary ( $p < 0.001$ ), and visual gestalts ( $p < 0.001$ ); significant age effects. Age is a confounder.
Troster and Ruff (1990)	Two occupationally TCE-exposed workers. Controls: two groups of n = 30 matched controls; (all age and education matched).	Exposure concentration unknown; exposure duration, 3–8 mo.	Both TCE cases exhibited significant deficits in verbal recall and visuospatial learning.
Triebig (1976)	Controlled exposure study four females, three males. Controls: four females, three males.	0, 100 ppm (550 mg/m <sup>3</sup> ), 6 hrs/d, 5 d.	There was no correlation seen between exposed and unexposed subjects for any measured psychological test results. No methods description was provided.
Triebig (1977c)	Seven men and one woman occupationally exposed with an age range from 23 to 38 yrs. No control group.	50 ppm (260 mg/m <sup>3</sup> ). Exposure duration not reported.	The psychological tests showed no statistically significant difference in the results before or after the exposure-free time period. No methods description was provided.

**Table 4-27. Summary of human cognition effect studies (continued)**

Reference	Subjects	Exposure	Effect
Salvini et al. (1971)	Controlled exposure study six students, male. Self used as control.	TCE concentration was 110 ppm for 4-hr intervals, twice per d. 0 ppm control exposure for all as self controls	Statistically significant results were observed for perception tests learning ( $p < 0.001$ ) and CRT learning ( $p < 0.01$ ).
Gamberale et al. (1976)	15 healthy men aged 20–31 yrs old. Controls: Within Subjects (15 self-controls).	0 mg/m <sup>3</sup> , 540 mg/m <sup>3</sup> (97 ppm), 1,080 mg/m <sup>3</sup> (194 ppm), 70 min.	Repetition of the testing led to a pronounced improvement in performance as a result of the training effect; no interaction effects between exposure to TCE and training.
Stewart et al. (1970)	130 (108 males, 22 females). Controls: 63 unexposed men.	TCA metabolite levels in urine were measured: 60.8% had levels up to 20 mg/L, and 82.1% had levels up to 60 mg/L.	No significant effect on cognitive tests noted, but more effort required to perform the test in exposed group.
Chalupa (1960)	Case study—6 subjects. Average age 38.	No exposure data were reported.	80% of those with pathological EEG displayed memory loss; 30% of those with normal EEGs displayed memory loss.

EEG = electroencephalogram

Cognitive impairments are assessed in the occupational exposure and case studies (Rasmussen et al., 1993c, d; Tröster and Ruff, 1990). In metal degreasers occupationally exposed to TCE and CFC113, significant cognitive performance decreases were noted in verbal recall testing ( $p = 0.03$ ) and verbal learning ( $p = 0.04$ ) (Rasmussen et al., 1993d). No significant effects were found in the visual recall or digit span test for these workers. Troster and Ruff (1990) reported decrements (no statistical analysis performed) in cognitive performance as measured in verbal and visual recall tests that were conducted immediately after presentation (learning phase) and 1 hour after original presentation (retention/memory phase) for two case studies.

Several controlled (chamber) exposure studies were conducted to cognitive ability during TCE exposure and most did not find any significant decrements in the neurobehavioral measurement. Only Salvini et al. (1971) found significant decrements in cognitive function. Six males were exposed to 110 ppm (550 mg/m<sup>3</sup>) TCE for 4-hour intervals, twice per day. Statistically significant results were observed for perception tests learning ( $p < 0.001$ ) and choice reaction time (CRT) learning ( $p < 0.01$ ). Triebig et al. (1977a; 1977b; 1977c; 1976) exposed seven total subjects (male and female) to 100 ppm TCE for 6 hours/day, 5 days/week and did not report any decreases in cognition, but details on the experimental procedures were not provided. Additionally, Gamberale et al. (1976) found that subjects exposed to TCE as high as 194 ppm for 70 minutes did not exhibit any impairments on a short-term memory test in comparison to an air exposure.

#### 4.3.5.2. Cognitive Effects: Laboratory Animal Studies

Many reports have demonstrated significant differences in performance of learning tasks such as the speed to complete the task. However, there is little evidence that learning and memory function are themselves impaired by exposure. There are also limited data that suggest alterations in the hippocampus of laboratory animals exposed to TCE. Given the important role that this structure plays in memory formation, such data may be relevant to the question of whether TCE impairs memory. The studies are briefly discussed below and details are provided in Table 4-28.

**Table 4-28. Summary of animal cognition effect studies**

Reference <sup>a</sup>	Exposure route	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL	Effects
Kjellstrand et al. (1980)	Inhalation	Gerbil, Mongolian, males and females, 12/sex/dose	0, 320 ppm; 9 mo, continuous (24 hrs/d) except 1–2 hrs/wk for cage cleaning	NOAEL: 320 ppm	No significant effect on spatial memory (radial arm maze).
Isaacson et al. (1990)	Oral, drinking water	Rat, Sprague-Dawley, male weanlings, 12/dose	(1) 0 mg/kg-d, 8 wks. (2) 5.5 mg-d (47 mg/kg-d <sup>b</sup> ), 4 wks + 0 mg/kg-d, 4 wks. (3) 5.5 mg/d, 4 wks (47 mg/kg-d) + 0 mg/kg-d, 2 wks + 8.5 mg/d (24 mg/kg-d), 2 wks	NOAEL: 5.5 mg/d, 4 wks—spatial learning	Decreased latency to find platform in the Morris water maze (Group # 3); Hippocampal demyelination observed in all TCE-treated groups.
				LOAEL: 5.5 mg/d—hippocampal demyelination	
Kishi et al. (1993)	Inhalation	Rats, Wistar, male, number not specified	0, 250,500, 1,000, 2,000, and 4,000 ppm, 4 hrs	LOAEL: 250 ppm	Decreased lever presses and avoidance responses in a shock avoidance task.
Umezu et al. (1997)	i.p.	Mouse, ICR, male, six exposed to all treatments (repeated exposure)	0, 125, 250, 500, and 1,000 mg/kg, single dose and evaluated 30 min postadministration	NOAEL: 500 mg/kg LOAEL: 1,000 mg/kg	Decreased response rate in an operant response—condition avoidance task.
Oshiro et al. (2004)	Inhalation	Rat, Long-Evans, male, 24	0, 1,600, and 2,400 ppm; 6 hrs/d, 5 d/wk, 4 wks	NOAEL: 2,400 ppm	No change in reaction time in signal detection task and when challenged with amphetamine, no change in response from control.

<sup>a</sup>**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

<sup>b</sup>mg/kg-day conversion estimated from average male Sprague-Dawley rat body weight from ages 21–49 days (118 g) for the 5.5 mg dosing period and ages 63–78 d (354 g) for the 8.5 mg dosing period.

Two studies (Umezu et al., 1997; Kulig, 1987) reported decreased performance in operant-conditioning cognitive tasks for rodents. Kishi et al. (1993) acutely exposed Wistar rats to TCE at concentrations of 250, 500, 1,000, 2,000, and 4,000 ppm for 4 hours. Rats exposed to  $\geq 250$  ppm TCE showed a significant decrease both in the total number of lever presses and in

avoidance responses compared with controls. The rats did not recover their pre-exposure performance until about 2 hours after exposure. Likewise, Umezu et al. (1997) reported a depressed rate of operant responding in male ICR strain mice (n = 6, exposed to all TCE doses, see Table 4-28) in a conditioned avoidance task that reached significance with i.p. injections of 1,000 mg/kg. Increased responding during the signaled avoidance period at lower doses (250 and 500 mg/kg) suggests an impairment in ability to inhibit responding or failure to attend to the signal.

Although cognitive impairments are noted, two additional studies indicate no change in cognition with continuous TCE exposure or improvements in cognitive tasks. No decrements in cognitive function as measured by the radial arm maze were observed in Mongolian gerbils exposed continuously by inhalation to 320 ppm TCE for 9 months (Kjellstrand et al., 1980). Improved performance was noted in a Morris swim test for weanling rats orally dosed with 5.5 mg/day for 4 weeks followed by 2 weeks of no exposure and an additional 2 weeks of 8.5 mg/day (Isaacson et al., 1990). This improved performance occurred despite a loss in hippocampal myelination.

#### **4.3.5.3. Summary and Conclusions of Cognitive Function Studies**

Human environmental and occupational exposure studies suggest impairments in cognitive function. Kilburn and Warshaw (1993a) and Kilburn (2002b, a) reported memory deficits in individuals, although a question exists whether the referent group was comparable to exposed subjects and these studies lack of consideration of possible confounding exposures in statistical analyses. Significant impairments were found in visual and verbal recall and with the digit span test. Similarly, in occupational exposure studies (Rasmussen et al., 1993c, d; Tröster and Ruff, 1990), short-term memory tests indicated that immediate memory and learning were impaired in the absence of an effect on digit span performance. In controlled exposure and/or chamber studies, two studies did not report any cognitive impairment (Gamberale et al., 1976; Stewart et al., 1970) and one study (Salvini et al., 1971) reported significant impairments in learning memory and complex choice reaction tasks. All of the controlled exposure studies were acute and/or short-term exposure studies and the sensitivity of test procedures is unknown due to the lack of methodologic information provided in the reports. Despite identified study deficiencies, these studies collectively suggest cognitive function impairment.

The animal studies measured cognitive function through spatial memory and operant responding tasks. In the two studies where spatial memory was evaluated, there was either no effect at 320 ppm TCE (Kjellstrand et al., 1980) or improved cognitive performance in weanling rats at a dose of 5.5 mg/day for 4 weeks (Isaacson et al., 1990). Improved cognitive performance was observed in weanling rats (Isaacson et al., 1990) and could be due to continuing neurodevelopment as well as compensation from other possible areas in the brain since there was a significant loss in hippocampal myelination. Significant decreases in operant responding

(avoidance/punished responding) during TCE exposure were reported in two studies ([Umezu et al., 1997](#); [Kishi et al., 1993](#)). When TCE exposure was discontinued, operant responding return to control levels; it is unclear if the significant effects are due to decreased motor function or decreased cognitive ability.

#### **4.3.6. Psychomotor Effects**

There is considerable evidence in the literature for both animals and humans on psychomotor testing, although human and laboratory animal studies utilize very different measures of motor behavior. Generally, the human literature employs a wide variety of psychomotor tasks and assesses error rates and reaction time (RT) in the performance of the task. The laboratory animal data, by contrast, tend to include unlearned naturalistic behaviors such as locomotor activity, gait changes, and foot splay to assess neuromuscular ability.

##### **4.3.6.1. Psychomotor Effects: Human Studies**

The effects of TCE exposure on psychomotor response have been studied primarily as a change in RT with studies on motor dyscoordination resulting from TCE exposure providing subjective reporting.

##### **4.3.6.1.1. Reaction time**

Several studies have evaluated the effects of TCE on RT using simple and CRT tasks (simple reaction time [SRT] and CRT tasks). The studies are presented below and summarized in more detail in Table 4-29.

**Table 4-29. Summary of human CRT studies**

Reference	Subjects	Exposure	Effect
Kilburn (2002b, a)	236 residents near a microchip plant in Phoenix, Arizona. Controls: 161 regional referents from Wickenburg, Arizona. 67 referents from Phoenix, Arizona not residing near a plant.	0.2–10,000 ppb of TCE, chronic exposure.	SRT and CRT were increased in the exposed group ( $p < 0.05$ ).
Kilburn and Warshaw (1993a)	160 residents living in Southwest Tucson with TCE and other solvents in groundwater. Control: 68 residential referents matched to subjects from two previous studies of waste oil and oil refinery exposures.	>500 ppb of TCE in well water before 1981 and 25–100 ppb afterwards. Exposure duration 1 to 25 yrs.	Mean SRT was 67 milliseconds (msec) longer than the referent group ( $p < 0.0001$ ). CRT of the exposed subjects was between 93 and 100 msec longer in three different trials ( $p < 0.0001$ ) compared to referents.
Reif et al. (2003)	143 residents of the Rocky Mountain Arsenal community of Denver. Referent group at lowest concentration (<5 ppb).	High exposure group >15 ppb. Medium exposure group $\geq 5$ – $\leq 15$ ppb. Low exposure referent group <5 ppb.	Significant increase in RT as measured by the SRT test ( $p < 0.04$ ) in only among subjects who reported alcohol use (defined as having at least one drink per mo).
Kilburn and Thornton (1996)	Group A: Registered voters from Arizona and Louisiana with no exposure to TCE: $n = 264$ , aged 18–83. Group B volunteers from California $n = 29$ (17 males and 12 females). Group C: exposed to TCE and other chemicals for $\geq 5$ yrs $n = 217$ .	No exposure or groundwater analyses reported.	Significant increase in SRT and CRT in exposed group compared to the unexposed populations.
Gamberale et al. (1976)	15 healthy men aged 20–31 yrs old. Controls: Within subjects (15 self-controls).	0, 540 $\text{mg}/\text{m}^3$ (97 ppm), 1,080 $\text{mg}/\text{m}^3$ (194 ppm), 70 min.	No change in CRT or SRT. Increase in time required to perform the RT-Addition Test (task for adding numbers) ( $p < 0.05$ ).
Gun et al. (1978)	Four female workers from one plant exposed to TCE and four female workers from another plant exposed to TCE + nonhalogenated hydrocarbon solvent. Control: ( $n = 8$ ) four unexposed female workers from each plant.	3–419 ppm, duration not specified.	TCE-only exposure increased RT in comparison to controls. In TCE + solvent group, ambient TCE was lower and mean RT shortened in Session 2, then rose subsequently to be greater than at the start.

Increases in RT were observed in environmental exposure studies by Kilburn (2002b, a), Kilburn and Warshaw (1993a), and Kilburn and Thornton (1996) as well as in an occupational exposure study by Gun et al. (1978). All populations except that of Gun et al. (1978) were exposed through groundwater contaminated as the result of environmental spills; the exposure duration was for at least 1 year and exposure levels ranged from 0.2 to 10,000 ppb for the three studies. Kilburn and Warshaw (1993a) reported that SRT significantly increased from

281 ± 55 msec to 348 ± 96 msec in individuals ( $p < 0.0001$ ). CRT of the exposed subjects was 93 msec longer ( $p < 0.0001$ ) than referents. Kilburn and Thornton (1996) evaluated SRT and CRT function and also found similar increases in RT. The average SRT and CRT for the combined control groups were 276 and 532 msec, respectively. These RTs increased in the TCE exposure group where the average SRT was 334 msec and CRT was 619 msec. Similarly, Kilburn (2002b, a) compared RTs between 236 TCE-exposed persons and the 161 unexposed regional controls. SRTs significantly increased from 283 ± 63 msec in controls to 334 ± 118 msec in TCE-exposed individuals ( $p < 0.0001$ ). Similarly, CRTs also increased from 510 ± 87 to 619 ± 153 msec with exposure to TCE ( $p < 0.0001$ ).

No effect on SRT was reported in a geographical-based study by Reif et al. (2003). SRTs were 301 msec for the lowest exposure group and 316 msec for the highest exposure group ( $p = 0.42$ ). When the SRT data were analyzed for individuals who consumed at least one alcoholic drink per month ( $n = 80$ ), a significant increase (18%,  $p < 0.04$ ) in SRT times was observed between the lowest exposure and the highest exposure groups. In TCE-exposed individuals who did not consume alcohol ( $n = 55$ ), SRTs decreased from 321 msec in the lowest exposed group to 296 msec in the highest exposed group, but this effect was not statistically significantly different. A controlled exposure (chamber study) of 15 healthy men aged 20–31 years old, were exposed to 0, 540, and 1,080 mg/m<sup>3</sup> TCE for 70 minutes or served as his own control, reported no statistically significant differences with the SRT or CRT tasks. However, in the RT-addition test, the level of performance varied between the different exposure conditions ( $F(2,24) = 4.35$ ;  $p < 0.05$ ) and between successive measurement occasions ( $F(2,24) = 19.25$ ;  $p < 0.001$ ).

#### **4.3.6.1.2. Muscular dyscoordination**

Three studies examined motor dyscoordination effects from TCE exposure using subjective and self-reported individual assessment. Rasmussen et al. (1993a) presented findings on muscular dyscoordination for 96 metal degreasers exposed to either TCE or CFC113. A statistically significant increasing trend of dyscoordination with TCE exposure was observed ( $p = 0.01$ ) in multivariate regression analyses, which adjusted for the effects of age, neurological disease, arteriosclerotic disease, and alcohol abuse. Furthermore, a greater number of abnormal coordination tests were observed in the higher-exposure group compared to the low-exposure group ( $p = 0.003$ ).

Gash et al. (2008) reported fine motor hand movement times in subjects who had filed workman compensation claims were significantly slower ( $p < 0.0001$ ) than age-matched nonexposed controls. Exposures were based on self-reported information, and no information on the control group was presented. Troster and Ruff (1990) reported a case study conducted on two occupationally exposed workers to TCE. Mild deficits in motor speed were reported for both cases. In the first case, manual dexterity was impaired in a male exposed to TCE (unknown

concentration) for 8 months. In the second case study where a female was exposed to TCE (low concentration; exact level not specified) for 3 months, there was weakness in the quadriceps muscle as evaluated in a neurological exam and a decreased sensation to touch on one hand. Both Gash et al. (2008) and Troster and Ruff (1990) provide very limited information given their deficiencies related to lack of exposure data, self-reported information, and limited reporting of referents and statistical analysis.

#### 4.3.6.2. Psychomotor Effects: Laboratory Animal Data

Several animal studies have demonstrated that TCE exposure produces changes in psychomotor function. At high doses ( $\geq 2,000$  mg/kg), TCE causes mice to lose their righting reflex when the compound is injected i.p. (Shih et al., 2001; Umezu et al., 1997). At lower exposures (inhalation and oral), TCE produces alterations in neurobehavioral measures including locomotor activity, gait, operant responding, and reactivity. The studies are described in Sections 4.3.6.2.1–4.3.6.2.3 and summarized in Tables 4-30 and 4-31.

**Table 4-30. Summary of animal psychomotor function and RT studies**

Reference <sup>a</sup>	Exposure route	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL	Effects
Savolainen et al. (1977)	Inhalation	Rat, Sprague-Dawley, male, 10	0 and 200 ppm; 6 hrs/d, 4 d	LOAEL: 200 ppm	Increased frequency of preening, rearing, and ambulation. Increased preening time.
Kishi et al. (1993)	Inhalation	Rats, Wistar, male, number not specified	0, 250, 500, 1,000, 2,000, and 4,000 ppm, 4 hrs	LOAEL: 250 ppm	Decreased lever presses and increased responding when lever press coupled with a 10-s electric shock (decreased avoidance response).
Kulig et al. (1987)	Inhalation	Rat, Wistar, male, 8/dose	0, 500, 1,000, and 1,500 ppm; 16 hrs/d, 5 d/wk, 18 wks	NOAEL: 1,500 ppm	No change in spontaneous activity, grip strength, or hindlimb movement.
Moser et al. (1995)	Oral	Rat, F344, female, 8/dose	0, 150, 500, 1,500, and 5,000 mg/kg, one dose	NOAEL: 500 mg/kg LOAEL: 1,500 mg/kg	Decreased motor activity; Neuro-muscular and sensorimotor impairment.
			0, 50, 150, 500, and 1,500 mg/kg-d, 14 d	NOAEL: 150 mg/kg-d LOAEL: 500 mg/kg-d	Increased rearing activity and decreased forelimb grip strength.
Bushnell (1997)	Inhalation	Rat, Long-Evans, male, 12	0, 400, 800, 1,200, 1,600, 2,000, or 2,400 ppm, 1 hr/test d, 4 consecutive test d, 2 wks	NOAEL: 800 ppm LOAEL: 1,200 ppm	Decreased sensitivity and increased response time in the signal detection task.
Shih et al. (2001)	i.p.	Mouse, MF1, male, 6	0 and 5,000 mg/kg, acute	LOAEL: 5,000 mg/kg	Impairment of righting reflex.

**Table 4-30. Summary of animal psychomotor function and RT studies (continued)**

Reference <sup>a</sup>	Exposure route	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL	Effects
Umezu et al. (1997)	i.p.	Mouse, ICR, male, 10/group	0, 2,000, 4,000, and 5,000 mg/kg—loss of righting reflex measure	LOAEL: 2,000 mg/kg—loss of righting reflex	Loss of righting reflex.
		Mouse, ICR, male, 6–10/group	0, 62.5, 125, 250, 500, and 1,000 mg/kg, single dose and evaluated 30 min postadministration	NOAEL: 500 mg/kg LOAEL: 1,000 mg/kg—operant behavior NOAEL: 125 mg/kg LOAEL: 250 mg/kg—punished responding	Decreased responses (lever presses) in an operant response task for food reward. Increased responding when lever press coupled with a 20-V electric shock (punished responding).
Bushnell and Oshiro (2000)	Inhalation	Rat, Long-Evans, male, 32	0, 2,000, 2,400 and ppm; 70 min/d, 9 d	LOAEL: 2,000 ppm	Decreased performance on the signal detection task. Increased response time and decreased response rate.
Nunes et al. (2001)	Oral	Rat, Sprague-Dawley, male, 10/group	<b>0 and 2,000 mg/kg-d, 7 d</b>	<b>LOAEL: 2,000 mg/kg-d</b>	<b>Increased foot splay. No change in any other FOB parameter (e.g., piloerection, activity, reactivity to handling).</b>
Moser et al. (2003)	Oral	Rat, F344, female, 10/group	0, 40, 200, 800, and 1,200 mg/kg-d, 10 d	–	Decreased motor activity; Decreased sensitivity to tail pinch; Increased abnormality in gait; Decreased grip strength; Adverse changes in several FOB parameters.
Albee et al. (2006)	Inhalation	Rat, F344, male and female, 10/sex/group	0, 250, 800, and 2,500 ppm; 6 hrs/d, 5 d/wk, 13 wks	NOAEL: 2,500 ppm	No change in any FOB measured parameter.

<sup>a</sup>**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

FOB = functional observational battery

**Table 4-31. Summary of animal locomotor activity studies**

Reference <sup>a</sup>	Exposure route	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL	Effects
Wolff and Siegmund (1978)	i.p.	Mouse, AB, male, 18	0 and 182 mg/kg, tested 30 min after injection	LOAEL: 182 mg/kg	Decreased spontaneous motor activity.
<b>Kulig et al. (1987)</b>	<b>Inhalation</b>	<b>Rat, Wistar, male, 8/dose</b>	<b>0, 500, 1,000, and 1,500 ppm; 16 hrs/d, 5 d/wk, 18 wks</b>	<b>NOAEL: 500 ppm LOAEL: 1,000 ppm</b>	<b>No change in spontaneous activity, grip strength, or hindlimb movement. Increased latency time in the 2-choice visual discrimination task (cognitive disruption and/or motor activity related effect).</b>
Moser et al. (1995)	Oral	Rat, F344, female, 8/dose	0, 150, 500, 1,500, and 5,000 mg/kg, one dose	NOAEL: 500 mg/kg LOAEL: 1,500 mg/kg	Decreased motor activity; neuro-muscular and sensorimotor impairment.
			0, 50, 150, 500, and 1,500 mg/kg-d, 14 d	NOAEL: 150 mg/kg-d LOAEL: 500 mg/kg-d	Increased rearing activity.
<b>Waseem et al. (2001)</b>	Oral	Rat, Wistar, male, 8/group	0, 350, 700, and 1,400 ppm in drinking water for 90 d	NOAEL: 1,400 ppm	No significant effect on spontaneous locomotor activity.
	<b>Inhalation</b>	<b>Rat, Wistar, male, 8/group</b>	<b>0 and 376 ppm for up to 180 d; 4 hrs/d, 5 d/wk</b>	<b>LOAEL: 376 ppm</b>	<b>Changes in locomotor activity and vary by timepoint when measured over the 180-d period.</b>
Moser et al. (2003)	Oral	Rat, F344, female, 10/group	0, 40, 200, 800, and 1,200 mg/kg-d, 10 d	–	Decreased motor activity; Decreased sensitivity; Increased abnormality in gait; Adverse changes in several FOB parameters.

<sup>a</sup>**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

#### 4.3.6.2.1. Loss of righting reflex

Umezumi et al. (1997) studied disruption of the righting reflex following acute injection (i.p.) of 2,000, 4,000, and 5,000 mg/kg TCE in male ICR mice. TCE disrupted the righting reflex at doses of 2,000 mg/kg and higher. At 2,000 mg/kg, loss of righting reflex (LORR) was observed in only 2/10 animals injected. At 4,000 mg/kg, 9/10 animals experienced LORR and 100% of the animals experienced LORR at 5,000 mg/kg.

Shih et al. (2001) reported impaired righting reflexes at exposure doses of 5,000 mg/kg (i.p.) in male MF1 mice. Mice pretreated with dimethyl sulfoxide or disulfiram (CYP2E1 inhibitor) delayed LORR in a dose related manner. By contrast, the alcohol dehydrogenase inhibitor, 4-methylpyridine, did not delay LORR that resulted from 5,000 mg/kg TCE. These

data suggest that the anesthetic properties of TCE involve its oxidation via CYP2E1 to an active metabolite.

#### **4.3.6.2.2. Activity, sensory-motor and neuromuscular function**

Changes in sensory-motor and neuromuscular activity were reported in three studies ([Moser et al., 2003](#); [Moser et al., 1995](#); [Kishi et al., 1993](#)). Kishi et al. (1993) exposed male Wistar rats to 250, 500, 1,000, 2,000, and 4,000 ppm TCE for 4 hours. Rats exposed to 250 ppm TCE showed a significant decrease both in the total number of lever presses and in avoidance responses at 140 minutes of exposure compared with controls. Moser et al. (1995) evaluated the effects of acute and short-term (14 day) administration of TCE in adult female F344 rats (n = 8–10/dose) on activity level, neuromuscular function, and sensorimotor function as part of a larger functional observational battery (FOB) testing. The NOAEL levels identified by the authors are 500 mg/kg (10% of the limit dose) for the acute treatment and 150 mg/kg (3% of the limit dose) for the 14-day study. In the acute study, TCE produced the most significant effects in motor activity (activity domain), gait (neuromuscular domain), and click response (sensorimotor domain). In the 14-day study, only the activity domain (rearing) and neuromuscular domain (forelimb grip strength) were significantly different ( $p < 0.05$ ) from control animals. In a separate 10-day study ([Moser et al., 2003](#)), TCE administration significantly ( $p < 0.05$ ) reduced motor activity, tail pinch responsiveness, reactivity to handling, hind limb grip strength and body weight. Significant increases ( $p < 0.05$ ) in piloerection, gait scores, lethality, body weight loss, and lacrimation were also reported in comparison to controls.

There are also two negative studies that used adequate numbers of subjects in their experimental design but used lower doses than did Moser et al. (2003). Albee et al. (2006) exposed male and female F344 rats (n = 10/sex) to TCE by inhalation at exposure doses of 250, 800, and 2,500 ppm, for 6 hours/day, 5 days/week, for 13 weeks. The FOB was performed monthly, although it is not certain how much time elapsed from the end of exposure until the FOB test was conducted. No treatment-related differences in grip strength or landing foot splay were demonstrated in this study. Kulig et al. (1987) also failed to show significant effects of TCE inhalation exposure on markers of motor behavior. Wistar rats (n = 8) exposed to 500, 1,000, and 1,500 ppm, for 16 hours/day, 5 days/week, for 18 weeks failed to show changes in spontaneous activity, grip strength, or coordinated hind limb movement. Measurements were made every 3 weeks during the exposure period and occurred between 45 and 180 minutes following the previous TCE inhalation exposure.

#### **4.3.6.2.3. Locomotor activity**

The data, with regard to locomotor activity, are inconsistent. Several studies showed that TCE exposure can decrease locomotor activity including Wolff and Siegmund (1978) where AB mice (n = 18) were treated acutely with a dose of 182 mg/kg, i.p. at one of four time points

during a 24-hour day. Moser et al. (2003; 1995) reported reduced locomotor activity in female F344 rats (n = 8–10) gavaged with TCE over an acute (LOAEL = 5,000 mg/kg TCE) or subacute period (LOAEL = 500 mg/kg, but no effect at 5,000 mg/kg). In the Moser et al. (2003) study, it appears that 200 mg/kg TCE yielded a significant reduction in locomotor activity and that the degree of impairment at this dose represented a maximal effect on this measure. That is, higher doses of TCE appear to have produced equivalent or slightly less of an effect on this behavior. While this study identifies a LOAEL of 200 mg/kg TCE by gavage over a 10-day period, this is a much lower dose effect than that reported in Moser et al. (1995). Both studies (Moser et al., 2003; Moser et al., 1995) demonstrate a depression in motor activity that occurs acutely following TCE administration. Kulig et al. (1987) demonstrated that rats had increased response latency to a two choice visual discrimination following 1,000- and 1,500-ppm TCE exposures for 18 weeks. However, no significant changes in grip strength, hindlimb movement, or any other motor activity measurements were noted.

There are also a few studies (Waseem et al., 2001; Fredriksson et al., 1993) generally conducted using lower exposure doses that failed to demonstrate impairment of motor activity or ability following TCE exposure. Waseem et al. (2001) failed to demonstrate changes in locomotor activity in male Wistar rats (n = 8) dosed with TCE (350, 700, and 1,400 ppm) in drinking water for 90 days. Wistar rats (n = 8) exposed to 500, 1,000, and 1,500 ppm for 16 hours/day, 5 days/week, for 18 weeks failed to show changes in spontaneous activity. No changes in locomotor activity were observed for 17-day-old male NMRI mice that were dosed postnatally with 50 or 290 mg/kg-day from day 10 to 16 (Fredriksson et al., 1993). However, rearing activity was significantly decreased in the NMRI mice at day 60.

#### **4.3.6.3. Summary and Conclusions for Psychomotor Effects**

In human studies, psychomotor effects such as RT and muscular dyscoordination have been examined following TCE exposure. In the RT studies, statistically significant increases in CRT and SRT were reported in the Kilburn studies (2002b, a; 1996; 1993a). All of these studies were geographically based and it was suggested that the results were used for litigation and the differences between exposed and referent groups on other factors influencing reaction speed time may introduce a bias to the findings. Additionally, in these studies exposure to TCE and other chemicals occurred through drinking water for at least 1 year and TCE concentrations in well water ranged from 0.2 to 10,000 ppb. Reif et al. (2003) whose exposure assessment approach included exposure modeling of water distribution system to estimate TCE concentrations in tap water at census track of residence found that residents with drinking water containing TCE (up to >15 ppb—the highest level not specified) and other chemicals did not significantly increase CRTs or SRTs. Inhalation studies also demonstrated increased RTs. An acute exposure chamber study (Gamberale et al., 1976) tested for CRT, SRT, and RT-addition following a 70-minute exposure to TCE. A concentration-dependent significant decrease in performance was

observed with the RT-addition test and not for CRT or SRT tasks. An occupational exposure study on eight female workers exposed to TCE ([Gun et al., 1978](#)) also reported increased RT in the females exposed to TCE-only. Muscular dyscoordination for humans following TCE exposure has been reported in a few studies as a subjective observation. The studies indicated that exposure resulted in decreased motor speed and dexterity ([Rasmussen et al., 1993a](#); [Tröster and Ruff, 1990](#)) and self-reported faster asymptomatic fine motor hand movements ([Gash et al., 2008](#)).

Animal studies evaluated psychomotor function by examining locomotor activity, operant responding, changes in gait, loss of righting reflex, and general motor behavior (see Tables 4-30 and 4-31 for references). Overall, the studies demonstrated that TCE causes loss of righting reflex at injection doses of  $\geq 2,000$  mg/kg ([Shih et al., 2001](#); [Umezue et al., 1997](#)). Regarding general psychomotor testing, significant decreases in lever presses and avoidance were observed at inhalation exposures as low as 250 ppm for 4 hours (LOAEL; [Kishi et al., 1993](#)). Following subchronic inhalation exposures, no significant changes in psychomotor activity were noted at up to 2,500 ppm for 13 weeks ([Albee et al., 2006](#)) or at 1,500 ppm for 18 weeks ([Kulig, 1987](#)). In the oral administration studies ([Moser et al., 2003](#); [Moser et al., 1995](#)), psychomotor effects were evaluated using an FOB. More psychomotor domains were significantly affected by TCE treatment in the acute study in comparison to the 14-day study, but a lower NOAEL (150 mg/kg-day) was reported for the 14-day study in comparison to the acute study ([500 mg/kg; Moser et al., 1995](#)). Upon closer examination of the data, a biphasic effect in one measure of the FOB (rearing) was resulting in the lower NOAEL for the 14-day study and doses that were higher and lower than the NOAEL did not produce a statistically significant increase in the number of rears. Therefore, it can be surmised that acute exposure to TCE results in significant changes in psychomotor function. However, there may be some tolerance to these psychomotor changes in increased exposure duration to TCE as evidenced by the results noted in the short-term and subchronic exposure studies.

#### **4.3.7. Mood Effects and Sleep Disorders**

##### **4.3.7.1. Effects on Mood: Human Studies**

Reports of mood disturbance (depression, anxiety) resulting from TCE exposure are numerous in the human literature. These symptoms are subjective and difficult to quantify. Studies by Gash et al. ([2008](#)), Kilburn and Warshaw ([1993a](#)), Kilburn ([2002b, a](#)), McCunney et al. ([1988](#)), Mitchell et al. ([1969](#)), Rasmussen and Sabroe ([1986](#)), and Troster and Ruff ([1990](#)) reported mood disturbances in humans. Reif et al. ([2003](#)) and Triebig et al. ([1976](#)) reported no effect on mood following TCE exposures.

#### 4.3.7.2. Effects on Mood: Laboratory Animal Findings

It is difficult to obtain comparable data of emotionality in laboratory studies. However, Moser et al. (2003) and Albee et al. (2006) both report increases in handling reactivity among rats exposed to TCE. In the Moser study, female F344 rats received TCE by gavage for periods of 10 days at doses of 0, 40, 200, 800, and 1,200 while Albee et al. (2006) exposed F344 rats to TCE by inhalation at exposure doses of 250, 800, and 2,500 ppm for 6 hours/day, 5 days/week, for 13 weeks. These studies are summarized and described in Table 4-32.

**Table 4-32. Summary of animal mood effect and sleep disorder studies**

Reference <sup>a</sup>	Exposure route	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL	Effects
<b>Mood effects</b>					
Albee et al. (2006)	Inhalation	Rat, F344, male and female, 10/sex/group	0, 250, 800, and 2,500 ppm; 6 hrs/d, 5 d/wk, 13 wks	NOAEL: 800 ppm	<b>Increased handling reactivity.</b>
Moser et al. (2003)	Oral	Rat, F344, female, 10/group	0, 40, 200, 800, and 1,200 mg/kg-d, 10 d	–	Decreased handling reactivity score.
<b>Sleep disorder</b>					
Arito et al. (1994)	Inhalation	Rat, Wistar, male, 5/group	0,50, 100, and 300 ppm; 8 hrs/d, 5 d/wk, 6 wks	LOAEL: 50 ppm	<b>Significant changes in sleep cycle as measured through EEG changes; significant decreases in wakefulness.</b>

<sup>a</sup>**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

#### 4.3.7.3. Sleep Disturbances

Arito et al. (1994) exposed male Wistar rats to 50, 100, and 300 ppm TCE for 8 hour/day, 5 days/week, for 6 weeks and measured electroencephalographic (EEG) responses (see Table 4-32). EEG responses were used as a measure to determine the number of awake (wakefulness hours) and sleep hours. Exposure to all of the TCE levels significantly decreased amount of time spent in wakefulness during the exposure period. Some carry over was observed in the 22 hours post exposure period, with significant decreases in wakefulness seen at 100 ppm TCE. Significant changes in wakefulness-sleep elicited by the long-term exposure appeared at lower exposure levels. These data seem to identify a low dose effect of TCE and established a LOAEL of 50 ppm for sleep changes.

#### 4.3.8. Developmental Neurotoxicity

##### 4.3.8.1. Human Studies

In humans, CNS birth defects were observed in a few studies (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986). Postnatally, observed adverse effects in humans include delayed newborn reflexes following exposure to TCE during childbirth (Beppu, 1968),

impaired learning or memory ([White et al., 1997](#); [Bernad et al., 1987, abstract](#)); aggressive behavior ([Bernad et al., 1987, abstract](#)); hearing impairment ([Burg and Gist, 1999](#)); speech impairment ([Burg and Gist, 1999](#); [White et al., 1997](#)); encephalopathy ([White et al., 1997](#)); impaired executive and motor function ([White et al., 1997](#)); attention deficit ([White et al., 1997](#); [Bernad et al., 1987, abstract](#)); and autism spectrum disorder (ASD) ([Windham et al., 2006](#)). The human developmental neurotoxicity studies are discussed in more detail in Section 4.8.3.1.2.1, and summarized in Table 4-33.

**Table 4-33. Summary of human developmental neurotoxicity associated with TCE exposures**

Finding	Species	References
CNS defects, neural tube defects	Human	ATSDR ( <a href="#">2001</a> )
		Bove ( <a href="#">1996</a> ); Bove et al. ( <a href="#">1995</a> )
		Lagakos et al. ( <a href="#">1986</a> )
Delayed newborn reflexes	Human	Beppu ( <a href="#">1968</a> )
Impaired learning or memory	Human	Bernad et al. ( <a href="#">1987, abstract</a> )
		White et al. ( <a href="#">1997</a> )
Aggressive behavior	Human	Bernad et al., ( <a href="#">1987, abstract</a> )
Hearing impairment	Human	Burg and Gist ( <a href="#">1999</a> )
Speech impairment	Human	Burg and Gist ( <a href="#">1999</a> )
		White et al. ( <a href="#">1997</a> )
Encephalopathy	Human	White et al. ( <a href="#">1997</a> )
Impaired executive function	Human	White et al. ( <a href="#">1997</a> )
Impaired motor function	Human	White et al. ( <a href="#">1997</a> )
Attention deficit	Human	White et al. ( <a href="#">1997</a> )
	Human	Bernad et al. ( <a href="#">1987, abstract</a> )
ASD	Human	Windham et al. ( <a href="#">2006</a> )

#### 4.3.8.2. Animal Studies

There are a few studies demonstrating developmental neurotoxicity following TCE exposure (range of exposures) to experimental animals. These studies collectively suggest that developmental neurotoxicity result from TCE exposure; however, some types of effects such as learning and memory measures have not been evaluated. Most of the studies demonstrate either spontaneous motor activity changes ([Taylor et al., 1985](#)) or neurochemical changes such as decreased glucose uptake and changes in the specific gravity of the cortex and cerebellum ([Isaacson and Taylor, 1989](#); [Noland-Gerbec et al., 1986](#); [Westergren et al., 1984](#)). In addition, in most of these studies, there is no assessment of the exposure to TCE or metabolites in the pups/offspring. Details of the studies are presented below and summarized in Table 4-34.

**Table 4-34. Summary of mammalian in vivo developmental neurotoxicity studies—oral exposures**

Reference <sup>a</sup>	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL <sup>b</sup>	Effects
Fredriksson et al. (1993)	Mouse, NMRI, male pups, 12 pups from 3–4 different litters/group	0, 50, or 290 mg/kg-d PNDs 10–16	LOAEL: 50 mg/kg-d	Rearing activity significant ↓ at both dose levels on PND 60.
George et al. (1986)	Rat, F334, male and female, 20 pairs/treatment group, 40 controls/sex	0, 0.15, 0.30, or 0.60% microencapsulated TCE in diet. Breeders exposed 1 wk pre mating, then for 13 wks; pregnant females throughout pregnancy (i.e., 18 wks total).	LOAEL: 0.15%	Open field testing in pups: a significant dose-related trend toward ↑ time required for male and female pups to cross the first grid in the test device.
Isaacson and Taylor (1989)	Rat, Sprague-Dawley, females, six dams/group	0, 312, or 625 mg/L (0, 4.0, or 8.1 mg/d) <sup>c</sup> . Dams (and pups) exposed from 14 d prior to mating until end of lactation.	LOAEL: 312 mg/L	Significant ↓ myelinated fibers in the stratum lacunosum-moleculare of pups. Reduction in myelin in the CA1 region of the hippocampus.
Noland-Gerbec et al. (1986)	Rat, Sprague-Dawley, females, 9–11 dams/group	0, 312 mg/L. Average total intake of dams: 825 mg TCE over 61 d. Dams (and pups) exposed from 14 d prior to mating until end of lactation.	LOAEL: 312 mg/L	Significant ↓ uptake of [ <sup>3</sup> H]-2-DG in whole brains and cerebella (no effect in hippocampus) of exposed pups at 7, 11, and 16 d, but returned to control levels by 21 d.
Taylor et al. (1985)	Rat, Sprague-Dawley, females, no. dams/group not reported	0, 312, 625, and 1,250 mg/L in drinking water. Dams (and pups) exposed from 14 d prior to mating until end of lactation.	LOAEL: 312 mg/L	<b>Exploratory behavior significant ↑ in 60- and 90-d-old male rats at all treatment levels.</b> Locomotor activity (measured through the wheel-running tasks) was higher in rats from dams exposed to 1,250 mg/L TCE.
Blossom et al. (2008)	Mouse, MRL +/+, dams and both sexes offspring, eight litters/group; three–eight pups/group	Drinking water, from GD 0 to PND 42; 0 or 0.1 mg/mL; maternal dose = 25.7 mg/kg-d; offspring PNDs 24–42 dose = 31.0 mg/kg-d.	LOAEL: 31 mg/kg-d for offspring	Righting reflex, bar holding, and negative geotaxis were not impaired. Significant association between impaired nest quality and TCE exposure. Lower GSH levels and GSH:GSSG ratios with TCE exposure.

<sup>a</sup>**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

<sup>b</sup>LOAELs are based upon reported study findings.

<sup>c</sup>Dose conversions provided by study author(s).

GSSG = oxidized GSH

Taylor et al. (1985) administered TCE to female Sprague-Dawley rats in their drinking water from 14 days before breeding throughout gestation and until pups were weaned at 21 days. Measured TCE concentrations in the dams were 312–646, 625–1,102, and 1,250–1,991 mg/L in

the low-, mid-, and high-dose groups as measured from the drinking water. Pups were evaluated for exploratory activity at 28, 60, or 90 days. No significant differences were noted between control and treated pups at 28 days. At 60 days, all TCE-treated animals had significantly increased exploratory activity in comparison to age-matched controls, but only the high-dose group had increased activity at 90 days. A significant increase in spontaneous motor activity (as measured by a wheel-running task) was noted in only the high dose TCE (1,250–1,991 mg/L) group during the onset of the darkness period. This study demonstrated that both spontaneous and open field activities are significantly affected by developmental TCE exposure.

Spontaneous behavioral changes were also investigated in another study by Fredriksson et al. (1993). Male and female NMRI pups (mice) were orally administered 50 or 290 mg/kg-day for 7 days starting at PND 10. Spontaneous motor activity was investigated in male mice at ages 17 and 60 days. TCE-treated animals tested at day 17 did not demonstrate changes in any spontaneous activity measurements in comparison to control animals. Both doses of TCE (50 and 290 mg/kg-day) significantly decreased rearing in 60-day-old male mice.

Westergren et al. (1984) examined the brain specific gravity of litters from mice exposed to TCE. NMRI mice (male and female) were exposed to 150 ppm TCE (806.1 mg/m<sup>3</sup>) for 30 days prior to mating. Exposure in males continued until the end of mating and females were exposed until the litters were born. Brains were removed from the offspring at either PNDs 1, 10, 20–22, or 29–31. At PNDs 1 and 10, significant decreases were noted in the specific gravity of the cortex. Significant decreases in the specific gravity of the cerebellum were observed at PND 10 (decrease from  $1.0429 \pm 0.00046$  to  $1.0405 \pm 0.00030$ ) and 20–22 (decrease from  $1.0496 \pm 0.00014$  to  $1.0487 \pm 0.00060$ ). Cerebellum measurements were not reported for PND 29–31 animals. Neurobehavioral assessments were not conducted in this study. Additionally, decreased brain specific gravity is suggestive of either decreased brain weight or increased brain volume (probably from edema) or a combination of the two factors and is highly suggestive of an adverse neurological effect. The effects of TCE on the cortical specific gravity were not persistent since cortices from PNDs 29–31 animals did not exhibit any significant changes. It is unclear if the effects on the cerebellum were persistent since results were not reported for the PND 29–31 animals. However, the magnitude of the change in the specific gravity of the cerebellum is decreased from PNDs 10 to 20–22, suggesting that the effect may be reversible given a longer recovery period from TCE.

The effect of TCE on glucose uptake in the brain was evaluated in rat pups exposed to TCE during gestation and through weaning. The primary source of energy utilized in the CNS is glucose. Changes in glucose uptake in the brain are a good indicator for neuronal activity modification. Noland-Grebec et al. (1986) administered 312 mg/L TCE through drinking water to female Sprague-Dawley rats from 2 weeks before breeding and up until pups reached 21 days of age. To measure glucose uptake, 2-deoxyglucose was administered intraperitoneally to male pups at either PND 7, 11, 16, or 21. Significant decreases in glucose uptake were noted in whole

brain and cerebellum at all PNDs tested. Significant decreases in glucose uptake were also observed in the hippocampus except for animals tested at PND 21. The observed decrease in glucose uptake suggests decreased neuronal activity.

Female Sprague-Dawley rats (70 days old) were administered TCE in drinking water at a level of either 4.0 or 8.1 mg/day for 14 days prior to mating and continuing up through lactation ([Isaacson and Taylor, 1989](#)). Only the male pups were evaluated in the studies. At PND 21, brains were removed from the pups, sectioned, and stained to evaluate the changes in myelin. There was a significant decrease (40% decrease) in myelinated fibers in the CA1 region of the hippocampus of the male pups. This effect appeared to be limited to the CA1 region of the hippocampus since other areas such as the optic tract, fornix, and cerebral peduncles did not have decreases in myelinated fibers.

Neurological changes were found in pups exposed to TCE in a study conducted by the National Toxicology Program (NTP) in F344 rats ([George et al., 1986](#)). TCE was administered to rats at dietary levels of 0, 0.15, 0.30, or 0.60%. No intake calculations were presented for the rat study and therefore, a dose rate is unavailable for this study. Open field testing revealed a significant ( $p < 0.05$ ) dose-related trend toward an increase in the time required for male and female F1 weanling pups (PND 21) to cross the first grid in the testing device, suggesting an effect on the ability to react to a novel environment.

Blossom et al. ([2008](#)) treated male and female MRL +/+ mice with 0 or 0.1 mg/mL TCE in the drinking water. Treatment was initiated at the time of mating, and continued in the females (8/group) throughout gestation and lactation. Behavioral testing consisted of righting reflex on PNDs 6, 8, and 10; bar-holding ability on PNDs 15 and 17; and negative geotaxis on PNDs 15 and 17. Nest building was assessed and scored on PND 35, the ability of the mice to detect and distinguish social odors was examined with an olfactory habituation/dishabituation method at PND 29, and a resident intruder test was performed at PND 40 to evaluate social behaviors. Righting reflex, bar holding, and negative geotaxis were not impaired by treatment. There was a significant association between impaired nest quality and TCE exposure in tests of nest-building behavior; however, TCE exposure did not have an effect on the ability of the mice to detect social and nonsocial odors using habituation and dishabituation methods. Resident intruder testing identified significantly more aggressive activities (i.e., wrestling and biting) in TCE-exposed juvenile male mice as compared to controls, and the cerebellar tissue from the male TCE-treated mice had significantly lower GSH levels and GSH:oxidized GSH (GSH:GSSG) ratios, indicating increased oxidative stress and impaired thiol status, which have been previously reported to be associated with aggressive behaviors ([Franco et al., 2006](#)). Histopathological examination of the brain did not identify alterations indicative of neuronal damage or inflammation.

### **4.3.8.3. Summary and Conclusions for the Developmental Neurotoxicity Studies**

Gestational exposure to TCE in humans has resulted in several developmental abnormalities. These changes include neuroanatomical changes such as neural tube defects ([ATSDR, 2001](#); [Bove, 1996](#); [Bove et al., 1995](#); [Lagakos et al., 1986](#)) and encephalopathy ([White et al., 1997](#)). Clinical neurological changes such as impaired cognition ([White et al., 1997](#); [Bernad et al., 1987](#)), aggressive behavior ([Bernad et al., 1987](#)), and speech and hearing impairment ([Burg and Gist, 1999](#); [White et al., 1997](#)) are also observed when TCE exposure occurs in utero.

In animal studies, anatomical and clinical developmental neurotoxicity is also observed. Following inhalation exposures of 150 ppm to mice during mating and gestation, the specific gravity of offspring brains was significantly decreased at postnatal time points through the age of weaning; this effect did not persist to 1 month of age ([Westergren et al., 1984](#)). In studies reported by Taylor et al. ([1985](#)), Isaacson and Taylor ([1989](#)), and Noland-Gerbec et al. ([1986](#)), 312 mg/L exposures in drinking water that were initiated 2 weeks prior to mating and continued to the end of lactation resulted in: (1) significant increase in exploratory behavior at PNDs 60 and 90; (2) reductions in myelination in the CA1 hippocampal region of offspring at weaning; and (3) significantly decreased uptake of 2-deoxyglucose in the rat brain at PND 21. Gestational exposures to mice ([Fredriksson et al., 1993](#)) resulted in significantly decreased rearing activity on PND 60, and dietary exposures during the course of a continuous breeding study in rats ([George et al., 1986](#)) found a significant trend toward increased time to cross the first grid in open field testing. In a study by Blossom et al. ([2008](#)), male mice exposed gestationally to TCE exhibited lower GSH levels and lower GSH:GSSG ratios, which are also observed in mice that have more aggressive behaviors ([Franco et al., 2006](#)).

## **4.3.9. Mechanistic Studies of TCE Neurotoxicity**

### **4.3.9.1. Dopamine Neuron Disruption**

There are very recent laboratory animal findings resulting from short-term TCE exposures that demonstrate vulnerability of dopamine neurons in the brain to this chlorinated hydrocarbon. The key limitation of these laboratory animal studies is that only one dosing regimen was included in each study. Moreover, there has been no systematic body of data to show that other chlorinated hydrocarbons such as tetrachloroethylene or aromatic solvents similarly target this cell type. Confidence in the limited data regarding dopamine neuron death and in vivo TCE exposure would be greatly enhanced by identifying a dose-response relationship. If indeed TCE can target dopamine neurons, it would be anticipated that human exposure to this agent would result in elevated rates of parkinsonism. There are no systematic studies of this potential relationship in humans, although one limited report attempted to address this possibility. Difficulties in subject recruitment into that study limit the weight that can be given to the results.

Endogenously formed chlorinated tetrahydro-beta-carbolines (TaClo) have been suggested to contribute to the development of Parkinson-like symptoms ([Kochen et al., 2003](#); [Riederer et al., 2002](#); [Bringmann et al., 1995](#); [Bringmann et al., 1992](#)). TaClo can be formed endogenously from metabolites of TCE such as trichloroacetaldehyde. TaClo has been characterized as a potent neurotoxicant to the dopaminergic system. Some research groups have hypothesized that Parkinson-like symptoms resulting from TCE exposure may occur through the formation of TaClo, but not enough evidence is available to determine if this mechanism occurs.

#### **4.3.9.1.1. Dopamine neuron disruption: human studies**

There are no human studies that present evidence that TCE exposure results in dopamine neuron disruption. Nagaya et al. ([1990](#)) examined serum dopamine  $\beta$ -hydroxylase activity without differences observed in mean activities between control and exposed subjects. In the study, 84 male workers exposed to TCE were compared to 83 male age-matched controls. The workers had constantly used TCE in their jobs and their length of employment ranged from 0.1 to 34 years.

#### **4.3.9.1.2. Dopamine neuron disruption: animal studies**

There are limited data from mice and rats that suggest the potential for TCE to disrupt dopamine neurons in the basal ganglia (see Table 4-35). Gash et al. ([2008](#)) showed that TCE administered by gavage in F344 rats (n = 9) at an exposure level of 1,000 mg/kg-day, 5 days/week, for 6 weeks yielded degeneration of dopamine neurons in the substantia nigra and alterations in dopamine turnover as reflected in a shift in dopamine metabolite to parent compound ratios. Guehl et al. ([1999](#)) reported similar findings in OF1 mice (n = 10) that were injected i.p. with 400 mg/kg-day TCE 5 days/week for 4 weeks. Each of these studies evaluated only a single dose level of TCE, so establishing a dose-response relationship is not possible. Consequently, these data are of limited utility in risk assessment because they do not establish the potency of TCE to damage dopamine neurons. They are important, however, in identifying a potential permanent impairment that might occur following TCE exposure at relatively high exposure doses. They also identify a potential mechanism by which TCE could produce CNS injury.

**Table 4-35. Summary of animal dopamine neuronal studies**

Reference <sup>a</sup>	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Guehl et al. (1999)	i.p. administration	Mouse, OF1, male, 10	0 and 400 mg/kg-d; 5 d/wk, 4 wks	LOAEL: 400 mg/kg-d	Significant dopaminergic neuronal death in substantia nigra.
<b>Gash et al. (2008)</b>	<b>Gavage</b>	<b>Rat, F344, male, 9/group</b>	<b>0 and 1,000 mg/kg-d; 5 d/wk, 6 wks</b>	<b>LOAEL: 1,000 mg/kg-d</b>	<b>Degeneration of dopamine-containing neurons in substantia nigra. Change in dopamine metabolism.</b>

<sup>a</sup>**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

#### 4.3.9.1.3. Summary and conclusions of dopamine neuron studies

Only two animal studies have reported changes in dopamine neuron effects from TCE exposure (Gash et al., 2008; Guehl et al., 1999). Both studies demonstrated toxicity to dopaminergic neurons in the substantia nigra in rats or mice. LOAELs of 400 mg/kg-day (mice; Guehl et al., 1999) and 1,000 mg/kg-day (rats; Gash et al., 2008) were reported for this effect. Dopaminergic neuronal degeneration following TCE exposure has not been studied in humans. However, there were no changes in serum dopamine β-hydroxylase activity in TCE-exposed or control individuals (Nagaya et al., 1990). Loss of dopaminergic neurons in the substantia nigra also occurs in patients with Parkinson’s disease and the substantia nigra is an important region in helping to control movements. As a result, loss of dopaminergic neurons in the substantia nigra may be one of the potential mechanisms involved in the clinical psychomotor effects that is observed following TCE exposure.

#### 4.3.9.2. Neurochemical and Molecular Changes

There are limited data obtained only from laboratory animals that TCE exposure may have consequences on GABAergic (gamma-amino butyric acid [GABA]) and glutamatergic neurons (Shih et al., 2001; see Table 4-36; Briving et al., 1986). However, the data obtained are limited with respect to brain region examined, persistence of effect, and whether there might be functional consequences to these changes. The data of Briving et al. (1986) demonstrating changes in cerebellar high affinity uptake for GABA and glutamate following chronic, low-level (50 and 150 ppm) TCE exposure do not appear to be reflected in the only other brain region evaluated (hippocampus). However, glutamate levels were increased in the hippocampus. The data of Shih et al. (2001) are indirect in that it shows an altered response to GABAergic antagonist drugs in mice treated by acute injection with 250, 500, 1,000, and 2,000 mg/kg TCE. However, these data do show some dose dependency with significant findings observed with TCE exposure as low as 250 mg/kg.

**Table 4-36. Summary of neurophysiological, neurochemical, and neuropathological effects with TCE exposure**

Reference <sup>a</sup>	Exposure route	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL	Effects
<b>Neurophysiological studies</b>					
Shih et al. (2001)	i.p.	Mouse, MF1, male, 6/group	0, 250 500, 1,000, or 2,000 mg/kg, 15 min; followed by tail infusion of PTZ (5 mg/mL), picrotoxin (0.8 mg/mL), bicuculline (0.06 mg/mL), strychnine (0.05 mg/mL), 4-AP (2 mg/mL), or NMDA (8 mg/mL)	–	Increased threshold for seizure appearance with TCE pretreatment for all convulsants. Effects strongest on the GABA <sub>A</sub> antagonists, PTZ, picrotoxin, and bicuculline, suggesting GABA <sub>A</sub> receptor involvement. NMDA and glycine Rc involvement also suggested.
Ohta et al. (2001)	i.p.	Mouse, ddY, male, 5/group	0, 300, or 1,000 mg/kg, sacrificed 24 hrs after injection	LOAEL: 300 mg/kg	Decreased response (long-term potentiation response) to tetanic stimulation in the hippocampus.
<b>Neurochemical studies</b>					
Briving et al. (1986)	Inhalation	Gerbils, Mongolian, male and female, 6/group	0, 50, or 150 ppm, continuous, 24 hrs/d, 12 mo	NOAEL: 50 ppm; LOAEL: 150 ppm for glutamate levels in hippocampus. NOAEL: 150 ppm for glutamate and GABA uptake in hippocampus. LOAEL: 50 ppm for glutamate and GABA uptake in cerebellar vermis.	Increased glutamate levels in the hippocampus. Increased glutamate and GABA uptake in the cerebellar vermis.
Subramoniam et al. (1989)	Oral	Rat, Wistar, female	0 or 1,000 mg/kg, 2 or 20 hrs. 0 or 1,000 mg/kg-d, 5 d/wk, 1 yr	–	PI and PIP decreased by 24 and 17% at 2 hrs. PIP and PIP2 increased by 22 and 38% at 20 hrs. PI, PIP, and PIP2 reduced by 52, 23, and 45% in 1 yr study.

**Table 4-36. Summary of neurophysiological, neurochemical, and neuropathological effects with TCE exposure (continued)**

Reference <sup>a</sup>	Exposure route	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL	Effects
Haglid et al. (1981)	Inhalation	Gerbil, Mongolian, male and female, 6–7/group	0, 60, or 320 ppm, 24 hrs/d, 7 d/wk, 3 mo	LOAEL: 60 ppm, brain protein changes. NOAEL: 60 ppm; LOAEL: 320 ppm, brain DNA changes	(1) Decreases in total brain soluble protein whereas increase in S100 protein. (2) Elevated DNA in cerebellar vermis and sensory motor cortex.
<b>Neuropathological studies</b>					
Kjellstrand et al. (1987)	Inhalation	Mouse, NMRI, male	0, 150, or 300 ppm, 24 hrs/d, 4 or 24 d	LOAEL: 150 ppm, 4 and 24 d	<b>Sciatic nerve regeneration was inhibited in both mice and rats.</b>
		<b>Rat, Sprague-Dawley, female</b>	<b>0, 300 ppm, 24 hrs/d, 4 or 24 d</b>	<b>NOAEL: 300 ppm, 4 d. LOAEL: 300 ppm, 24 d.</b>	
Isaacson and Taylor (1989)	Oral	Rat, Sprague-Dawley, females, six dams/group	0, 312, or 625 mg/L. (0, 4.0, or 8.1 mg/d); dams (and pups) exposed from 14 d prior to mating until end of lactation	LOAEL: 312 mg/L	Significant ↓ myelinated fibers in the stratum lacunosum-moleculare of pups. Reduction in myelin in the hippocampus.

<sup>a</sup>**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

NMDA = N-nitrosodimethylamine; PI = phosphatidyl inositol; PIP = phosphatidyl inositol-4-phosphate; PIP2 = phosphatidylinositol-4,5-bisphosphate; PTZ = pentylenetetrazole

The development and physiology of the hippocampus have also been evaluated in two different studies (Ohta et al., 2001; Isaacson and Taylor, 1989). Isaacson and Taylor (1989) found a 40% decrease in myelinated fibers from hippocampi dissected from neonatal Sprague-Dawley rats (n = 2–3) that were exposed to TCE (4 and 8.1 mg/day) in utero and during the preweaning period. Ohta et al. (2001) injected male ddY mice with 300 mg/kg TCE and found a significant reduction in response to titanic stimuli in excised hippocampal slices. Both of these studies demonstrated that there is some interaction with TCE and the hippocampal area in the brain.

Impairment of sciatic nerve regeneration was demonstrated in mice and rats exposed to TCE (Kjellstrand et al., 1987). Under heavy anesthesia, the sciatic nerve of the animals was artificially crushed to create a lesion. Prior to the lesion, some animals were pre-exposed to TCE for 20 days and then for an additional 4 days after the lesion. Another set of animals was only exposed to TCE for 4 days following the sciatic nerve lesion. For mice, regeneration of the sciatic nerve in comparison to air-exposed animals was 20 and 33% shorter in groups exposed to 150 and 300 ppm TCE for 4 days, respectively. This effect did not significantly increase in mice pre-exposed to TCE for 20 days, and the regeneration was 30% shorter in the 150-ppm group

and 22% shorter in the 300-ppm group. Comparatively, a 10% reduction in sciatic nerve regeneration length was observed in rats exposed to TCE for 20 days prior to the lesion plus the 4 days after the sciatic nerve lesion.

There are also a few in vitro studies (summarized in Table 4-37) that have demonstrated that TCE exposure alters the function of inhibitory ion channels such as GABA<sub>A</sub> and glycine receptors (Beckstead et al., 2000; Krasowski and Harrison, 2000), and serotonin receptors (Lopreato et al., 2003). Krasowski and Harrison (2000) and Beckstead et al. (2000) were able to demonstrate that human GABA<sub>A</sub> and glycine receptors could be potentiated by TCE when a receptor agonist was coapplied. Krasowski and Harrison (2000) conducted an additional experiment in order to determine if TCE was interacting with the receptor or perturbing the cellular membrane (bilipid layer). Specific amino acids on the GABA<sub>A</sub> and glycine receptors were mutated and in the presence of a receptor agonist (GABA for GABA<sub>A</sub> and glycine for glycine receptors) and in these mutated receptors, TCE-mediated potentiation was significantly decreased or abolished, suggesting that there was an interaction between TCE and these receptors. Lopreato et al. (2003) conducted a similar study with the 5HT<sub>3A</sub> serotonin receptor and found that when TCE was coapplied with serotonin, there was a potentiation in receptor response. Additionally, TCE has been demonstrated to alter the function of voltage sensitive calcium channels (VSCCs) by inhibiting the calcium mediated-current at a holding potential of -70 mV and shifting the activation of the channels to a more hyperpolarizing potential (Shafer et al., 2005).

**Table 4-37. Summary of in vitro ion channel effects with TCE exposure**

Reference	Cellular system	Neuronal channel/receptor	Concentrations	Effects
<b>In vitro studies</b>				
Shafer et al. (2005)	PC12 cells	VSCC	0, 500, 1,000, 1,500, or 2,000 μM	Shift of VSCC activation to a more hyperpolarizing potential. Inhibition of VSCCs at a holding potential of -70 mV.
Beckstead et al. (2000)	<i>Xenopus</i> oocytes	Human recombinant: glycine receptor α1, GABA <sub>A</sub> receptors, α1β1, α1β2γ2L	0 or 390 μM	50% potentiation of the GABA <sub>A</sub> receptors; 100% potentiation of the glycine receptor.
Lopreato et al. (2003)	<i>Xenopus</i> oocytes	Human recombinant serotonin 3A receptor	0 or 390 μM	Potentiation of serotonin receptor function.
Krasowski and Harrison (2000)	Human embryonic kidney 293 cells	Human recombinant Glycine receptor α1, GABA <sub>A</sub> receptors α2β1	Not provided	Potentiation of glycine receptor function with an EC <sub>50</sub> of 0.65 ± 0.05 mM <sup>a</sup> . Potentiation of GABA <sub>A</sub> receptor function with an EC <sub>50</sub> of 0.85 ± 0.2 mM.

<sup>a</sup>EC<sub>50</sub> = concentration of the chemical at which 50% of the maximal effect is produced

#### 4.3.10. Potential Mechanisms for TCE-Mediated Neurotoxicity

The mechanisms of TCE neurotoxicity have not been established despite a significant level of research on the outcomes of TCE exposure. Results from several mechanistic studies can be used to help elucidate the mechanism(s) involved in TCE-mediated neurological effects.

The disruption of the trigeminal nerve appears to be a highly idiosyncratic outcome of TCE exposure. There are limited data to suggest that it might entail a demyelination phenomenon, but similar demyelination does not appear to occur in other nerve tracts. In this regard, then, TCE is unlike a variety of hydrocarbons that have more global demyelinating action. There are some data from CNS data that focus on shifts in lipid profiles as well as data showing loss of myelinated fibers in the hippocampus. However, the changes in lipid profiles are both quite small and, also, inconsistent. And the limited data from hippocampus are not sufficient to conclude that TCE has significant demyelinating effects in this key brain region. Indeed, the bulk of the evidence from studies of learning and memory function (which would be tied to hippocampal function) suggests no clear impairments due to TCE.

Some researchers ([Albee et al., 2006](#); [Albee et al., 1997](#); [Laureno, 1993](#); [Barret et al., 1992](#); [Barret et al., 1991](#); [Laureno, 1988](#)) have indicated that changes in trigeminal nerve function may be due to dichloroacetylene, which is formed under nonbiological conditions of high alkalinity or temperature during volatilization of TCE. In experimental settings, trigeminal nerve function ([Albee et al., 1997](#)) and trigeminal nerve morphology ([Barret et al., 1992](#); [Barret et al., 1991](#)) were found to be more altered following a low exposure to dichloroacetylene in comparison to the higher TCE exposure. Barret et al. ([1992](#); [1991](#)) also demonstrated that TCE administration results in morphological changes in the trigeminal nerve. Thus, dichloroacetylene may contribute to trigeminal nerve impairment may be plausible following an inhalation exposure under conditions favoring its formation. Examples of such conditions include passing through a carbon dioxide scrubber containing alkaline materials, application to remove a wax coating from a concrete-lined stone floor, or mixture with alkaline solutions or caustic ([Bingham et al., 2001](#); [Greim et al., 1984](#); [Saunders, 1967](#)). However, dichloroacetylene exposures have not been identified or measured in human epidemiologic studies with TCE exposure, and thus, do not appear to be common to occupational or residential settings ([Lash and Green, 1993](#)). Moreover, changes in trigeminal nerve function have also been consistently reported in humans exposed to TCE following an oral exposure ([Kilburn, 2002b, a](#)), across many human studies of occupational and drinking water exposures under conditions with highly varying potentials for dichloroacetylene formation individuals ([Feldman et al., 1988](#); [Barret et al., 1987](#); [Barret et al., 1984](#); [Barret et al., 1982](#)). As a result, the mechanism(s) for trigeminal nerve function impairment following TCE exposure is unknown ([Campo et al., 2007](#); [Mhiri et al., 2004](#); [Kilburn, 2002a](#); [Kilburn and Warshaw, 1993a](#); [Ruijten et al., 1991](#)). The varying dichloroacetylene exposure potential across these studies suggests TCE exposure, which is common to all of them, as the most likely etiologic agent for the observed effects.

The clearest consequences of TCE are permanent impairment of hearing in animal models and disruption of trigeminal nerve function in humans with animal models showing comparable changes following administration of a TCE metabolite. With regard to hearing loss, the effect of TCE has much in common with the effects of several aromatic hydrocarbons including ethylbenzene, toluene, and *p*-xylene. Many studies have attempted to determine how these solvents damage the cochlea. Of the hypotheses that have been advanced, there is little evidence to suggest oxidative stress, changes in membrane fluidity, or impairment of central efferent nerves whose endings innervate receptor cells in the cochlea. Rather, for reasons that are still uncertain, these solvents seem to preferentially target supporting cells in the cochlea whose death then alters key structural elements of the cochlea resulting ultimately in hair cell displacement and death. Recently, potential modes of action resulting in ototoxicity have been speculated to be due to blockade of neuronal nicotinic receptors present on the auditory cells ([Campo et al., 2007](#)) and potentially changes in calcium transmission ([Maguin et al., 2009](#)) from toluene exposure. Although these findings were reported following an acute toluene exposure, it is speculated that this mechanism may be a viable mechanism for TCE-mediated ototoxicity.

A few studies have tried to relate TCE exposure with selective impairments of dopamine neurons. Two studies ([Gash et al., 2008](#); [Guehl et al., 1999](#)) demonstrated dopaminergic neuronal death and/or degeneration following an acute TCE administration. However, the only human TCE exposure study examining dopamine neuronal activity found no changes in serum dopamine  $\beta$ -hydroxylase activity in comparison to nonexposed individuals ([Nagaya et al., 1990](#)). It is thought that TaClo, which can be formed from TCE metabolites such as trichloroacetaldehyde, may be the potent neurotoxicant that selectively targets the dopaminergic system. More studies are needed to confirm the dopamine neuronal function disruption and if this disruption is mediated through TaClo.

There is good evidence that TCE and certain metabolites such as choral hydrate have CNS depressant properties and may account for some of the behavioral effects (such as vestibular effects, psychomotor activity changes, central visual changes, sleep and mood changes) that have been observed with TCE. Specifically, *in vitro* studies have demonstrated that TCE exposure results in changes in neuronal receptor function for the GABA<sub>A</sub>, glycine, and serotonin receptors ([Lopreato et al., 2003](#); [Beckstead et al., 2000](#); [Krasowski and Harrison, 2000](#)). All of these inhibitory receptors that are present in the CNS are potentiated when a receptor-specific agonist and TCE are applied. These results are similar to other anesthetics and suggest that some of the behavioral functions are mediated by modifications in ion channel function. However, it is quite uncertain whether there are persistent consequences to such high dose TCE exposure. Additionally, with respect to the GABAergic system, acute administration of TCE increased the seizure threshold appearance and this effect was the strongest with convulsants that were GABA receptor antagonists ([Shih et al., 2001](#)). Therefore, this result

suggests that TCE interacts with the GABA receptor; this was also verified in vitro ([Beckstead et al., 2000](#); [Krasowski and Harrison, 2000](#)).

TCE exposure has also been linked to decreased sensitivity to titanic stimulation in the hippocampus ([Ohta et al., 2001](#)) as well as to a significant reduction in myelin in the hippocampus in a developmental exposure study ([Isaacson et al., 1990](#)). These effects are notable since the hippocampus is highly involved in memory and learning functions. Changes in the hippocampal physiology may correlate with the cognitive changes that were reported following TCE exposure.

#### **4.3.11. Overall Summary and Conclusions—Weight of Evidence**

Both human and animal studies have associated TCE exposure with effects on several neurological domains. The strongest neurological evidence of hazard in humans is for changes in trigeminal nerve function or morphology and impairment of vestibular function. Fewer and more limited evidence exists in humans on delayed motor function and changes in auditory, visual, and cognitive function or performance. Acute and subchronic animal studies show morphological changes in the trigeminal nerve, disruption of the peripheral auditory system leading to permanent function impairments and histopathology, changes in visual evoked responses to patterns or flash stimulus, and neurochemical and molecular changes. Additional acute studies reported structural or functional changes in hippocampus, such as decreased myelination or decreased excitability of hippocampal CA1 neurons, although the relationship of these effects to overall cognitive function is not established. Some evidence exists for motor-related changes in rats/mice exposed acutely/subchronically to TCE, but these effects have not been reported consistently across all studies.

Epidemiologic evidence supports a relationship between TCE exposure and trigeminal nerve function changes, with multiple studies in different populations reporting abnormalities in trigeminal nerve function in association with TCE exposure ([Mhiri et al., 2004](#); [Kilburn, 2002b, a](#); [Kilburn and Warshaw, 1993a](#); [Feldman et al., 1992](#); [Ruijten et al., 1991](#); [Feldman et al., 1988](#); [Barret et al., 1987](#); [Barret et al., 1984](#); [Barret et al., 1982](#)). Of these, two well-conducted occupational cohort studies, each including >100 TCE-exposed workers without apparent confounding from multiple solvent exposures, additionally reported statistically significant dose-response trends based on ambient TCE concentrations, duration of exposure, and/or urinary concentrations of the TCE metabolite TCA ([Barret et al., 1987](#); [Barret et al., 1984](#)). Limited additional support is provided by a positive relationship between prevalence of abnormal trigeminal nerve or sensory function and cumulative exposure to TCE (most subjects) or CFC113 (<25% of subjects) ([Rasmussen et al., 1993a](#)). Test for linear trend in this study was not statistically significant and may reflect exposure misclassification since some subjects included in this study did not have TCE exposure. The lack of association between TCE exposure and overall nerve function in three small studies ([ulnar and medial: Triebig et al., 1983](#);

[Triebig et al., 1982](#); [trigeminal: El Ghawabi et al., 1973](#)) does not provide substantial evidence against a causal relationship between TCE exposure and trigeminal nerve impairment because of limitations in statistical power, the possibility of exposure misclassification, and differences in measurement methods. Laboratory animal studies have also shown TCE-induced changes in the trigeminal nerve. Although one study reported no significant changes in TSEP in rats exposed to TCE for 13 weeks ([Albee et al., 2006](#)), there is evidence of morphological changes in the trigeminal nerve following short-term exposures in rats ([Barret et al., 1992](#); [Barret et al., 1991](#)).

Human chamber, occupational, geographic-based/drinking water, and laboratory animal studies clearly established TCE exposure causes transient impairment of vestibular function. Subjective symptoms such as headaches, dizziness, and nausea resulting from occupational ([Liu et al., 1988](#); [Rasmussen and Sabroe, 1986](#); [Smith, 1970](#); [Grandjean et al., 1955](#)), environmental ([Hirsch et al., 1996](#)), or chamber exposures ([Smith, 1970](#); [Stewart et al., 1970](#)) have been reported extensively. A few laboratory animal studies have investigated vestibular function, either by promoting nystagmus or by evaluating balance ([Umezu et al., 1997](#); [Niklasson et al., 1993](#); [Tham et al., 1984](#); [Tham et al., 1979](#)).

In addition, mood disturbances have been reported in a number of studies, although these effects also tend to be subjective and difficult to quantify ([Gash et al., 2008](#); [Kilburn, 2002b, a](#); [Kilburn and Warshaw, 1993a](#); [Tröster and Ruff, 1990](#); [McCunney, 1988](#); [Rasmussen and Sabroe, 1986](#); [Mitchell and Parsons-Smith, 1969](#)), and a few studies have reported no effects from TCE on mood ([Reif et al., 2003](#); [Triebig et al., 1977a](#); [Triebig et al., 1976](#)). Few comparable mood studies are available in laboratory animals, although both Moser et al. (2003) and Albee et al. (2006) reported increases in handling reactivity among rats exposed to TCE. Finally, a significantly increased number of sleep hours was reported by Arito et al. (1994) in rats exposed via inhalation to 50–300 ppm TCE for 8 hours/day for 6 weeks.

Four epidemiologic studies of chronic exposure to TCE observed disruption of auditory function. One large occupational cohort study showed a statistically significant difference in auditory function with cumulative exposure to TCE or CFC113 as compared to control groups after adjustment for possible confounders, as well as a positive relationship between auditory function and increasing cumulative exposure ([Rasmussen et al., 1993c](#)). Of the three studies based on populations from ATSDR's TCE Subregistry from the National Exposure Registry, more limited than Rasmussen et al. (1993c) due to inferior exposure assessment, Burg et al. (1995) and Burg and Gist (1999) reported a higher prevalence of self-reported hearing impairments. The third study reported that auditory screening revealed abnormal middle ear function in children <10-years-old, although a dose-response relationship could not be established and other tests did not reveal differences in auditory function ([ATSDR, 2002](#)). Further evidence for these effects is provided by numerous laboratory animal studies demonstrating that high-dose subacute and subchronic TCE exposures in rats disrupt the auditory system, leading to permanent functional impairments and histopathology.

Studies in humans exposed under a variety of conditions, both acutely and chronically, report impaired visual functions such as color discrimination, visuospatial learning tasks, and visual depth perception in subjects with TCE exposure. Abnormalities in visual depth perception were observed with a high acute exposure to TCE under controlled conditions ([Vernon and Ferguson, 1969](#)). Studies of lower TCE exposure concentrations also observed visuofunction effects. One occupational study ([Rasmussen et al., 1993c](#)) reported a statistically significant positive relationship between cumulative exposure to TCE or CFC113 and visual gestalts learning and retention among Danish degreasers. Two studies of populations living in a community with drinking water containing TCE and other solvents further suggested changes in visual function ([Reif et al., 2003](#); [Kilburn, 2002b, a](#)). These studies used more direct measures of visual function as compared to Rasmussen et al. ([1993c](#)), but their exposure assessment is more limited because TCE exposure is not assigned to individual subjects ([Kilburn, 2002b, a](#)) or because there are questions regarding control selection ([Kilburn, 2002b, a](#)) and exposure to several solvents ([Reif et al., 2003](#); [Kilburn, 2002b, a](#)).

Additional evidence of effects of TCE exposure on visual function is provided by a number of laboratory animal studies demonstrating that acute or subchronic TCE exposure causes changes in visual evoked responses to patterns or flash stimulus ([Boyes et al., 2005a](#); [Boyes et al., 2003](#); [Blain et al., 1994](#)). Animal studies have also reported that the degree of some effects is correlated with simultaneous brain TCE concentrations ([Boyes et al., 2005a](#); [Boyes et al., 2003](#)) and that, after a recovery period, visual effects return to control levels ([Blain et al., 1994](#); [Rebert et al., 1991](#)). Overall, the human and laboratory animal data together suggest that TCE exposure can cause impairment of visual function, and some animal studies suggest that some of these effects may be reversible with termination of exposure.

Studies of human subjects exposed to TCE either acutely in chamber studies or chronically in occupational settings have observed deficits in cognition. Five chamber studies reported statistically significant deficits in cognitive performance measures or outcome measures suggestive of cognitive effects ([Triebig et al., 1977a](#); [Triebig et al., 1977b](#); [Gamberale et al., 1976](#); [Triebig et al., 1976](#); [Stewart et al., 1970](#)). Danish degreasers with high cumulative exposure to TCE or CFC113 had a high risk (OR: 13.7, 95% CI: 2.0–92.0) for psychoorganic syndrome characterized by cognitive impairment, personality changes, and reduced motivation, vigilance, and initiative compared to workers with low cumulative exposure. Studies of populations living in a community with contaminated groundwater also reported cognitive impairments ([Kilburn, 2002b, a](#); [Kilburn and Warshaw, 1993a](#)), although these studies carry less weight in the analysis because TCE exposure is not assigned to individual subjects and their methodological design is weaker.

Laboratory studies provide some additional evidence for the potential for TCE to affect cognition, though the predominant effect reported has been a change in the time needed to complete a task, rather than impairment of actual learning and memory function ([Umezu et al.,](#)

[1997](#); [Kishi et al., 1993](#); [Kulig, 1987](#)). In addition, in laboratory animals, it can be difficult to distinguish cognitive changes from motor-related changes. However, several studies have reported structural or functional changes in the hippocampus, such as decreased myelination ([Isaacson et al., 1990](#); [Isaacson and Taylor, 1989](#)) or decreased excitability of hippocampal CA1 neurons ([Ohta et al., 2001](#)), although the relationship of these effects to overall cognitive function has not been established.

Two studies of TCE exposure, one chamber study of acute exposure duration and one occupational study of chronic duration, reported changes in psychomotor responses. The chamber study of Gamberale et al. ([1976](#)) reported a dose-related decrease in performance in a CRT test in healthy volunteers exposed to 100 and 200 ppm TCE for 70 minutes as compared to the same subjects without exposure. Rasmussen et al. ([1993a](#)) reported a statistically significant association with cumulative exposure to TCE or CFC113 and dyscoordination trend among Danish degreasers. Observations in a third study ([Gun et al., 1978](#)) are difficult to judge given the author's lack of statistical treatment of data. In addition, Gash et al. ([2008](#)) reported that 14/30 TCE-exposed workers exhibited significantly slower fine motor hand movements as measured through a movement analysis panel test. Studies of population living in communities with TCE and other solvents detected in groundwater supplies reported significant delays in SRTs and CRTs in individuals exposed to TCE in contaminated groundwater as compared to referent groups ([Kilburn, 2002b, a](#); [Kilburn and Thornton, 1996](#); [Kilburn and Warshaw, 1993a](#)). Observations in these studies are more uncertain given questions of the representativeness of the referent population, lack of exposure assessment to individual study subjects, and inability to control for possible confounders including alcohol consumption and motivation. Finally, in a presentation of two case reports, decrements in motor skills as measured by the grooved pegboard and finger tapping tests were observed ([Tröster and Ruff, 1990](#)).

Laboratory animal studies of acute or subchronic exposure to TCE observed psychomotor effects, such as loss of righting reflex ([Shih et al., 2001](#); [Umezue et al., 1997](#)) and decrements in activity, sensory-motor function, and neuromuscular function ([Moser et al., 2003](#); [Moser et al., 1995](#); [Kishi et al., 1993](#)). However, two studies also noted an absence of significant changes in some measures of psychomotor function ([Albee et al., 2006](#); [Kulig, 1987](#)). In addition, less consistent results have been reported with respect to locomotor activity in rodents. Some studies have reported increased locomotor activity after an acute i.p. dosage ([Wolff and Siegmund, 1978](#)) or decreased activity after acute- or short-term gavage dosing (Moser et al., 1995, 2003). No change in activity was observed following exposure through drinking water ([Waseem et al., 2001](#)), inhalation ([Kulig, 1987](#)), or orally during the neurodevelopment period ([Fredriksson et al., 1993](#)).

Several neurochemical and molecular changes have been reported in laboratory investigations of TCE toxicity. Kjellstrand et al. ([1987](#)) reported inhibition of sciatic nerve regeneration in mice and rats exposed continuously to 150 ppm TCE via inhalation for 24 days.

Two studies reported changes in GABAergic and glutamatergic neurons in terms of GABA or glutamate uptake ([Briving et al., 1986](#)) or response to GABAergic antagonistic drugs ([Shih et al., 2001](#)) as a result of TCE exposure, with the Briving et al. (1986) study conducted at 50 ppm for 12 months. Although the functional consequences of these changes is unclear, Tham et al. (1984; 1979) described central vestibular system impairments as a result of TCE exposure that may be related to altered GABAergic function. In addition, several in vitro studies have demonstrated that TCE exposure alters the function of inhibitory ion channels such as receptors for GABA<sub>A</sub> glycine, and serotonin ([Lopreato et al., 2003](#); [Beckstead et al., 2000](#); [Krasowski and Harrison, 2000](#)) or of voltage-sensitive calcium channels ([Shafer et al., 2005](#)).

#### **4.4. KIDNEY TOXICITY AND CANCER**

##### **4.4.1. Human Studies of Kidney**

###### **4.4.1.1. Nonspecific Markers of Nephrotoxicity**

Investigations of nephrotoxicity in human populations show that workers highly exposed to TCE exhibit evidence of damage to the proximal tubule ([NRC, 2006](#)). The magnitude of exposure needed to produce kidney damage is not clear. Several kidney early biological effect markers, or biomarkers, are examined in these studies, as are less sensitive clinical kidney outcomes such as glomerular filtration rate and end-stage disease. Observation of elevated excretion of urinary proteins in the four studies of TCE exposure ([Bolt et al., 2004](#); [Green et al., 2004](#); [Brüning et al., 1999a](#); [Brüning et al., 1999b](#)) indicates the occurrence of a toxic insult among TCE-exposed subjects compared to unexposed controls. Two studies are of subjects with previously diagnosed kidney cancer ([Bolt et al., 2004](#); [Brüning et al., 1999a](#)), with limited interpretation of whether effects are associated with exposure or to the disease process. Subjects in Brüning et al. (1999b) and Green et al. (2004) were disease-free. Urinary proteins are considered nonspecific markers of nephrotoxicity and include  $\alpha$ 1-microglobulin, albumin, and *N*-acetyl- $\beta$ -D-glucosaminidase (NAG; [Lybarger et al., 1999](#); [Price et al., 1999](#); [Price et al., 1996](#)). Four studies measure  $\alpha$ 1-microglobulin with elevated excretion observed in the German studies ([Bolt et al., 2004](#); [Brüning et al., 1999a](#); [Brüning et al., 1999b](#)) but not Green et al. (2004). However, Green et al. (2004) found statistically significant group mean differences in NAG, another nonspecific marker of tubular toxicity, in disease-free subjects. Observations in Green et al. (2004) provide evidence of tubular damage among workers exposed to TCE at 32 ppm (mean) (range, 0.5–252 ppm). Elevated excretion of NAG as a nonspecific marker of tubular damage has also been observed with acute TCE poisoning ([Carrieri et al., 2007](#)). These and other studies relevant to evaluating TCE nephrotoxicity are discussed in more detail below.

Biological monitoring of persons who previously experienced “high” exposures to TCE (100–500 ppm) in the workplace show altered kidney function evidenced by urinary excretion of proteins suggestive of renal tubule damage. Similar results were observed in the only study available of subjects with TCE exposure at current occupational limits ([NRC, 2006](#)). Table 4-38

provides details and results from these studies. Brüning et al. (1999a) reported that a statistically significantly higher prevalence of elevated proteinuria suggestive of severe tubular damage ( $n = 24$ , 58.5%,  $p < 0.01$ ) and an elevated excretion of  $\alpha 1$ -microglobulin, another urinary biomarker of renal tubular function, were observed in 41 RCC cases with prior TCE exposure and with pending workman's compensation claims compared with the nonexposed renal cell cancer patients ( $n = 14$ , 28%) and to hospitalized surgical patients ( $n = 2$ , 2%). Statistical analyses did not adjust for differences in median systolic and diastolic blood pressure that appeared higher in exposed RCC cases compared to nonexposed controls. Similarly, severe tubular proteinuria is seen in 14/39 workers (35%) exposed to TCE in the electrical department, fitters shop, and through general degreasing operations of felts and sieves in a cardboard manufacturing factory compared to no subjects of 46 nonexposed males office and administrative workers from the same factory ( $p < 0.01$ ) (Brüning et al., 1999b). Furthermore, slight tubular proteinuria was seen in 20% of exposed workers and 2% of nonexposed workers (Brüning et al., 1999b). Exposed subjects also had statistically significantly elevated levels of  $\alpha 1$ -microglobulin compared to unexposed controls. Subjects with tubular damage, as indicated by urinary protein patterns, had higher GST-alpha concentrations than nonexposed subjects ( $p < 0.001$ ). Both sex and use of spot or 24-hour urine samples were shown to influence  $\alpha 1$ -microglobulin (Andersson et al., 2008); however, these factors are not considered to greatly influence observations given that only males were subjects and  $\alpha 1$ -microglobulin levels in spot urine sample were adjusted for creatinine concentration.

**Table 4-38. Summary of human kidney toxicity studies**

Subjects	Effect	Exposure	Reference
206 subjects— 104 male workers exposed to TCE; 102 male controls (source not identified)	Increased $\beta$ 2-microglobulin and total protein in spot urine specimen. $\beta$ 2-microglobulin: Exposed, $129.0 \pm 113.3$ mg/g creatinine. Controls, $113.6 \pm 110.6$ mg/g creatinine. Total protein: Exposed, $83.4 \pm 113.2$ mg/g creatinine. Controls, $54.0 \pm 18.6$ mg/g creatinine.	TCE exposure was through degreasing activities in metal parts factory or semiconductor industry. U-TTCs: Exposed, 83.4 mg/g Creatinine (range, 2–66.2 mg/g creatinine). Controls, ND. 8.4 $\pm$ 7.9 yrs mean employment duration.	Nagaya et al. (1989a)
29 metal workers	NAG in morning urine specimen, $0.17 \pm 0.11$ U/mmol creatinine.	Breathing zone monitoring, 3 ppm (median) and 5 ppm (mean).	Seldén et al. (1993)
191 subjects— 41 RCC cases pending cases involving compensation with TCE exposure; 50 unexposed RCC cases from the same area as TCE-exposed cases; 100 nondiseased control and hospitalized surgical patients	Increased urinary proteins patterns, $\alpha$ 1-microglobulin, and total protein in spot urine specimen. Slight/severe tubular damage: TCE RCC cases, 93%. Nonexposed RCC cases, 46%. Surgical controls, 11%. $p < 0.01$ . $\alpha$ 1-microglobulin (mg/g creatinine): Exposed RCC cases, $24.6 \pm [SD] 13.9$ Unexposed RCC cases, $11.3 \pm [SD] 9.8$ . Surgical controls, $5.5 \pm [SD] 6.8$ .	All exposed RCC cases exposed to “high” and “very high” TCE intensity. 18 yrs mean exposure duration.	Brüning et al. (1999a)
85 male workers employed in cardboard manufacturing factory (39 TCE exposed, 46) nonexposed office and administrative controls)	Increased urinary protein patterns and excretion of proteins in spot urine specimen. Slight/severe tubular damage: TCE exposed, 67% Nonexposed, RCC cases, 9% $p < 0.001$ . $\alpha$ 1-microglobulin (mg/g creatinine): Exposed, $16.2 \pm [SD] 10.3$ Unexposed, $7.8 \pm [SD] 6.9$ $p < 0.001$ . GST-alpha ( $\mu$ g/g creatinine): Exposed $6.0 \pm [SD] 3.3$ Unexposed, $2.0 \pm [SD] 0.57$ $p < 0.001$ . No group differences in total protein or GST-pi.	“High” TCE exposure to workers in the fitters shop and electrical department. “Very high” TCE exposure to workers through general degreasing operations in carton machinery section.	Brüning et al. (1999b)

**Table 4-38. Summary of human kidney toxicity studies (continued)**

Subjects	Effect	Exposure	Reference
99 RCC cases and 298 hospital controls (from Brüning et al. [(2003)] and alive at the time of interview)	<p>Increased excretion of <math>\alpha</math>1-microglobulin in spot urine specimen.</p> <p>Proportion of subjects with <math>\alpha</math>1-microglobulin &lt;5.0 mg/L:</p> <p>Exposed cases, 15%</p> <p>Unexposed cases, 51%</p> <p>Exposed controls, 55%</p> <p>Unexposed controls, 55%</p> <p><math>p &lt; 0.05</math>, prevalence of exposed cases compared to prevalences of either exposed controls or unexposed controls.</p> <p>Mean <math>\alpha</math>1-microglobulin:</p> <p>Exposed cases, 18.1 mg/L</p> <p>Unexposed cases, &lt;5.0 mg/L</p> <p><math>p &lt; 0.05</math>.</p>	All exposed RCC cases exposed to ‘high’ and ‘‘very high’’ TCE intensity.	Bolt et al. (2004)
124 subjects (70 workers currently exposed to TCE and 54 hospital and administrative staff controls)	<p>Analysis of urinary proteins in spot urine sample obtained 4 d after exposure.</p> <p>Increased excretion of albumin, NAG, and formate in spot urine specimen.</p> <p>Albumin (mg/g creatinine):<sup>a</sup></p> <p>Exposed, 9.71 <math>\pm</math> [SD] 11.6</p> <p>Unexposed, 5.50 <math>\pm</math> [SD] 4.27</p> <p><math>p &lt; 0.05</math>.</p> <p>Total NAG (U/g creatinine):</p> <p>Exposed, 5.27 <math>\pm</math> [SD] 3.78</p> <p>Unexposed, 2.41 <math>\pm</math> [SD] 1.91</p> <p><math>p &lt; 0.01</math>.</p> <p>Format (mg/g creatinine):</p> <p>Exposed, 9.45 <math>\pm</math> [SD] 4.78</p> <p>Unexposed, 5.55 <math>\pm</math> [SD] 3.00</p> <p><math>p &lt; 0.01</math>.</p> <p>No group mean differences in GST-alpha, retinol binding protein, <math>\alpha</math>1-microglobulin, <math>\beta</math>2-microglobulin, total protein, and methylmalonic acid.</p>	<p>Mean U-TCA of exposed workers was 64 <math>\pm</math> [SD] 102 (Range, 1–505).</p> <p>Mean U-TCOH of exposed workers was 122 <math>\pm</math> [SD] 119 (Range, 1–639).</p> <p>Mean TCE concentration to exposed subjects was estimated as 32 ppm (range, 0.5–252 ppm) and was estimated by applying the German occupational exposure limit (maximale arbeitsplatz konzentration, MAK) standard to U-TCA and assuming that the linear relationship holds for exposures &gt;100 ppm.</p> <p>86% of subjects with exposure to &lt;50 ppm TCE.</p>	Green et al. (2004)

**Table 4-38. Summary of human kidney toxicity studies (continued)**

Subjects	Effect	Exposure	Reference
101 cases or deaths from ESDR among male and female subjects in Hill Air Force Base aircraft maintenance worker cohort of Blair et al. (1998)	TCE exposure: Cox Proportional Hazard Analysis: Ever exposed to TCE, <sup>b</sup> 1.86 (1.02, 3.39). Logistic regression: <sup>b</sup> No chemical exposure (referent group): 1.0 <5 unit-yr, 1.73 (0.86, 3.48) 5–25 unit-yr, 1.65 (0.82, 3.35) >25 unit-yr, 1.65 (0.82, 3.35) Monotonic trend test, <i>p</i> > 0.05. Indirect low-intermittent TCE exposure, 2.47 (1.17, 5.19) Indirect peak/infrequent TCE exposure 3.55 (1.25, 10.74) Direct TCE exposure, “not statistically significant” but hazard ratio and CIs were not presented in paper.	Cumulative TCE exposure (intensity × duration) identified using three categories, <5 unit-yr, 5–25 unit-yr, >25 unit-yr per JEM of Stewart et al. (1991).	Radican et al. (2006)
269 cases of IgA nephropathy or membranous nephropathy glomerulonephritis followed 5 yrs (mean) for progression to ESRD	TCE exposure: Cox Proportional Hazard Analysis: Ever exposed to TCE, <sup>b</sup> 2.5 (0.9, 6.5) High exposure level to TCE, <sup>b</sup> 2.7 (0.7, 10.1).	Exposure to TCE assigned using job title and JEM; two dose surrogates, ever exposed and high exposure level.	Jacob et al. (2007)

<sup>a</sup>For a urine sample, 10–17 mg of albumin per g of creatinine is considered to be suspected albuminuria in males (15–25 in females) (de Jong and Brenner, 2004).

<sup>b</sup>Hazard ratio and 95% CI.

ESRD = end-stage renal disease; ND = not detectable

Bolt et al. (2004) measured  $\alpha$ 1-microglobulin excretion in living subjects from the RCC case-control study by Brüning et al. (2003). Some subjects in this study were highly exposed. Of the 134 with renal cell cancer, 19 reported past exposures that led to narcotic effects and 18 of the 401 controls experienced similar effects (OR: 3.71, 95% CI: 1.80–7.54) (Brüning et al., 2003). Bolt et al. (2004) found that  $\alpha$ 1-microglobulin excretion increased in exposed renal cancer patients compared with nonexposed patients controls. A lower proportion of exposed cancer patients had normal  $\alpha$ 1-microglobulin excretion, <5 mg/L, the detection level for the assay and the level considered by these investigators as associated with no clinical or subclinical tubule damage, and a higher proportion of high values, defined as  $\geq$ 45 mg/L, compared to cases who did not report TCE occupational exposure and to nonexposed controls (*p* < 0.05). Exposed cases, additionally, had statistically significantly higher median concentrations of  $\alpha$ 1-microglobulin compared to unexposed cases in creatinine-unadjusted spot urine specimens (*p* < 0.05). Reduced clearance of creatinine attributable to renal cancer does not explain the lower

percentage of normal values among exposed cases given findings of similar prevalence of normal excretion among unexposed renal cell cases and controls.

In their study of 70 current employees (58 males, 12 females) of an electronic factory with TCE exposure and 54 (50 males, 4 females) age-matched subjects drawn from hospital or administrative staff, Green et al. (2004) found that urinary excretion of albumin, total NAG and formate were increased in the exposed group compared with the unexposed group.<sup>4</sup> No differences between exposed and unexposed subjects were observed in other urinary proteins, including  $\alpha$ 1-microglobulin,  $\beta$ 2-microglobulin, and GST-alpha. Green et al. (2004) stated that NAG is not an indicator of nephropathy, or kidney damage, but rather is an indicator of functional change in the kidney. Green et al. (2004) further concluded that increased urinary albumin or NAG was not related to TCE exposure; analyses to examine the exposure-response relationship found neither NAG nor albumin concentration correlated to U-TCA or employment duration (years). The National Research Council (NRC, 2006) did not consider U-TCA as sufficiently reliable to use as a quantitative measure of TCE exposure, concluding that the data reported by Green et al. (2004) were inadequate to establish exposure-response information because the relationship between U-TCA and ambient TCE intensity is highly variable and nonlinear, and conclusions about the absence of association between TCE and nephrotoxicity cannot be made based on U-TCA. Moreover, use of employment duration does not consider exposure intensity differences between subjects with the same employment duration, and bias introduced through misclassification of exposure may explain the Green et al. (2004) findings.

Seldén et al. (1993), in their study of 29 metal workers (no controls), reported a correlation between NAG and U-TCA ( $r = 0.48$ ,  $p < 0.01$ ) but not with other exposure metrics of recent or long-term exposure. Personal monitoring of worker breath indicated median and mean TWA TCE exposures of 3 and 5 ppm, respectively. Individual NAG concentrations were within normal reference values. Rasmussen et al. (1993b) also reported a positive relationship ( $p = 0.05$ ) between increasing urinary NAG concentration (adjusted for creatinine clearance) and increasing duration in their study of 95 metal degreasers (no controls) exposed to either TCE (70 subjects) or CFC113 (25 subjects). Multivariate regression analyses that adjusted for age were suggestive of an association between NAG and exposure duration ( $p = 0.011$ ). Mean urinary NAG concentration was higher among subjects with annual exposure of >30 hours/week, defined as peak exposure, compared to subjects with annual exposure of <30 hours/week ( $72.4 \pm 44.1$  compared to  $45.9 \pm 30.0$   $\mu\text{g/g}$  creatinine,  $p < 0.01$ ).

Nagaya et al. (1989a) did not observe statistically significant group differences in urinary  $\beta$ 2-microglobulin and total protein in spot urine specimens of male degreasers and their controls, nor were these proteins correlated with urinary total trichloro-compounds (U-TTCs). The paper

---

<sup>4</sup>Elevation of NAG in urine is a sign of proteinuria, and proteinuria is both a sign and a cause of kidney malfunction (Zandi-Nejad et al., 2004). For a urine sample, 10–17 mg of albumin per g of creatinine is considered to be suspected albuminuria in males (15–25 in females) (de Jong and Brenner, 2004).

lacks details on subject selection, whether urine collection was at the start of work week or after sufficient exposure, and presentation of *p*-values and correlation coefficients. The presentation of urinary protein concentrations stratified by broad age groups is less statistically powerful than examination of this confounder using logistic regression. Furthermore, although valid for pharmacokinetic studies, examination of renal function using U-TTC as a surrogate for TCE exposure is uncertain, as discussed above for Green et al. (2004).

#### 4.4.1.2. End-Stage Renal Disease (ESRD)

ESRD is associated with hydrocarbon or organic solvent exposures in two studies examining this endpoint (Jacob et al., 2007; Radican et al., 2006). Table 4-38 provides details and results from Radican et al. (2006) and Jacob et al. (2007). Radican et al. (2006) assessed ESRD in a cohort of aircraft maintenance workers at Hill Air Force Base (Blair et al., 1998) with strong exposure assessment to TCE (NRC, 2006) and reported a twofold risk with overall TCE exposure and ESRD (1.86, 95% CI: 1.02, 3.39). A second study, the GN-PROGRESS retrospective cohort study, observed a twofold elevated risk for progression of glomerulonephritis to ESRD from TCE (overall exposure: 2.5, 95% CI: 0.9–6.5; high-level TCE exposure: 2.7, 95% CI: 0.7, 10.1) (Jacob et al., 2007). Statistical power was more limited in Jacob et al. (2007) because of its smaller number of exposed cases, 21 with overall exposure, compared to 56 exposed cases in Radican et al. (2006). Other occupational studies do not examine ESRD specifically, instead reporting RRs associated with deaths due to nephritis and nephrosis (Boice et al., 2006b; ATSDR, 2004a; Boice et al., 1999), all genitourinary system deaths (Ritz, 1999a; Costa et al., 1989; Garabrant et al., 1988), or providing no information on renal disease mortality in the published paper (Chang et al., 2003; Blair et al., 1998; Morgan et al., 1998).

#### 4.4.2. Human Studies of Kidney Cancer

Cancer of the kidney and renal pelvis is the 6<sup>th</sup> leading cause of cancer in the United States with an estimated 54,390 (33,130 men and 21,260 women) newly diagnosed cases and 13,010 deaths (Jemal et al., 2008; Ries et al., 2008). Age-adjusted incidence rates based on cases diagnosed in 2001–2005 from 17 Surveillance, Epidemiology, and End Results (SEER) geographic areas are 18.3 per 100,000 for men and 9.2 per 100,000 for women. Age-adjusted mortality rates are much lower; 6.0 per 100,000 for men and 2.7 for women.

Cohort, case-control, and geographical studies have examined TCE and kidney cancer, defined either as cancer of kidney and renal pelvis in cohort and geographic-based studies or as RCC, the most common type of kidney cancer, in case-control studies. Appendix C identifies these studies' design and exposure assessment characteristics. Observations in these studies are presented below in Table 4-39. Rate ratios for incidence studies in Table 4-39 are, generally, larger than for mortality studies.

**Table 4-39. Summary of human studies on TCE exposure and kidney cancer**

Exposure group		RR (95% CI)	Number of observable events	Reference
<b>Cohort and PMR studies—incidence</b>				
Aerospace workers (Rocketdyne)				Zhao et al. (2005)
	Any exposure to TCE	Not reported		
	Low cumulative TCE score	1.00 <sup>a</sup>	6	
	Medium cumulative TCE score	1.87 (0.56, 6.20)	6	
	High TCE score	4.90 (1.23, 19.6)	4	
	<i>p</i> for trend	<i>p</i> = 0.023		
TCE, 20 yr exposure lag <sup>b</sup>				
	Low cumulative TCE score	1.00 <sup>a</sup>	6	
	Medium cumulative TCE score	1.19 (0.22, 6.40)	7	
	High TCE score	7.40 (0.47, 116)	3	
	<i>p</i> for trend	<i>p</i> = 0.120		
All employees at electronics factory (Taiwan)				Chang et al. (2005)
	Males	1.06 (0.45, 2.08) <sup>c</sup>	8	
	Females	1.09 (0.56, 1.91) <sup>c</sup>	12	Sung et al. (2008)
	Females	1.10 (0.62, 1.82) <sup>c</sup>	15	
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al. (2003)
	Any exposure, all subjects	1.2 (0.98, 1.46)	103	
	Any exposure, males	1.2 (0.97, 1.48)	93	
	Any exposure, females	1.2 (0.55, 2.11)	10	
Exposure lag time				
	20 yrs	1.3 (0.86, 1.88)	28	
Employment duration				
	<1 yr	0.8 (0.5, 1.4)	16	
	1–4.9 yrs	1.2 (0.8, 1.7)	28	
	≥5 yrs	1.6 (1.1, 2.3)	32	
Subcohort with higher exposure				
	Any TCE exposure	1.4 (1.0, 1.8)	53	
Employment duration				
	1–4.9 yrs	1.1 (0.7, 1.7) <sup>d</sup>	23	
	≥5 yrs	1.7 (1.1, 2.4) <sup>d</sup>	30	

**Table 4-39. Summary of human studies on TCE exposure and kidney cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
Biologically monitored Danish workers		1.1 (0.3, 2.8)	4	Hansen et al. ( <a href="#">2001</a> )
	Any TCE exposure, males	0.9 (0.2, 2.6)	3	
	Any TCE exposure, females	2.4 (0.03, 14)	1	
	Cumulative exposure (Ikeda)	Not reported		
	<17 ppm-yr			
	≥17 ppm-yr			
	Mean concentration (Ikeda)	Not reported		
	<4 ppm			
	4+ ppm			
	Employment duration	Not reported		
	<6.25 yrs			
	≥6.25			
Aircraft maintenance workers from Hill Air Force Base				Blair et al. ( <a href="#">1998</a> )
	TCE subcohort	Not reported		
	Males, cumulative exposure			
	0	1.0 <sup>a</sup>		
	<5 ppm-yr	1.4 (0.4, 4.7)	9	
	5–25 ppm-yr	1.3 (0.3, 4.7)	5	
	>25 ppm-yr	0.4 (0.1, 2.3)	2	
	Females, cumulative exposure			
	0	1.0 <sup>a</sup>		
	<5 ppm-yr		0	
	5–25 ppm-yr		0	
	>25 ppm-yr	3.6 (0.5, 25.6)	2	
Biologically-monitored Finnish workers				Anttila et al. ( <a href="#">1995</a> )
	All subjects	0.87 (0.32, 1.89)	6	
	Mean air-TCE (Ikeda extrapolation)			
	<6 ppm	Not reported		
	6+ ppm	Not reported		
Cardboard manufacturing workers in Arnsberg, Germany				Henschler et al. ( <a href="#">1995</a> )
	Exposed workers	7.97 (2.59, 8.59) <sup>e</sup>	5	
Biologically-monitored Swedish workers				Axelson et al. ( <a href="#">1994</a> )
	Any TCE exposure, males	1.16 (0.42, 2.52)	6	
	Any TCE exposure, females	Not reported		

**Table 4-39. Summary of human studies on TCE exposure and kidney cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
Cardboard manufacturing workers, Atlanta area, Georgia				Sinks et al. ( <a href="#">1992</a> )
	All subjects	3.7 (1.4, 8.1)	6	
	All departments	$\infty$ (3.0, $\infty$ ) <sup>f</sup>	5	
	Finishing department	16.6 (1.7, 453.1) <sup>f</sup>	3	
<b>Cohort and PMR studies—mortality</b>				
Computer manufacturing workers (IBM), New York				
Males		1.64 (0.45, 4.21) <sup>g</sup>	4	Clapp and Hoffman ( <a href="#">2008</a> )
Females			0	
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	2.22 (0.89, 4.57)	7	Boice et al. ( <a href="#">2006b</a> )
	Any exposure to TCE	Not reported		Zhao et al. ( <a href="#">2005</a> )
	Low cumulative TCE score	1.00 <sup>a</sup>	7	
	Medium cumulative TCE score	1.43 (0.49, 4.16)	7	
	High TCE score	2.13 (0.50, 8.32)	3	
	<i>p</i> for trend	<i>p</i> = 0.31		
	TCE, 20 yr exposure lag <sup>b</sup>			
	Low cumulative TCE score	1.00 <sup>a</sup>	10	
	Medium cumulative TCE score	1.69 (0.29, 9.70)	6	
	High TCE score	1.82 (0.09, 38.6)	1	
	<i>p</i> for trend	<i>p</i> = 0.635		
View-Master employees				ATSDR ( <a href="#">2004a</a> )
	Males	2.76 (0.34, 9.96) <sup>g</sup>	2	
	Females	6.21 (2.68, 12.23) <sup>g</sup>	8	
United States Uranium-processing workers (Fernald)				Ritz ( <a href="#">1999a</a> ) (as reported in NRC, <a href="#">2006</a> )
	Any TCE exposure	Not reported		
	Light TCE exposure, 2–10 yrs duration	1.94 (0.59, 6.44)	5	
	Light TCE exposure, >10 yrs duration	0.76 (0.14, 400.0)	2	
	Mod TCE exposure, >2 yrs duration		0	

**Table 4-39. Summary of human studies on TCE exposure and kidney cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
Aerospace workers (Lockheed)				Boice et al. (1999)
Routine exposure	0.99 (0.40, 2.04)	7		
Routine-Intermittent <sup>a</sup>	Not presented	11		
Duration of exposure				
0 yr	1.0	22		
<1 yr	0.97 (0.37, 2.50)	6		
1–4 yrs	0.19 (0.02, 1.42)	1		
≥5 yrs	0.69 (0.22, 2.12)	4		
<i>p</i> for trend				
Aerospace workers (Hughes)				Morgan et al. (1998)
TCE subcohort	1.32 (0.57, 2.60)	8		
Low intensity (<50 ppm) <sup>e</sup>	0.47 (0.01, 2.62)	1		
High intensity (>50 ppm) <sup>e</sup>	1.78 (0.72, 3.66)	7		
TCE subcohort (Cox analysis)				
Never exposed	1.00 <sup>a</sup>	24		
Ever exposed	1.14 (0.51, 2.58) <sup>h</sup>	8		
Peak				
No/Low	1.00 <sup>a</sup>	24		
Med/Hi	1.89 (0.85, 4.23) <sup>h</sup>	8		
Cumulative				
Referent	1.00 <sup>a</sup>	24		
Low	0.31 (0.04, 2.36) <sup>h</sup>	1		
High	1.59 (0.68, 3.71) <sup>h</sup>	7		
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. (1998)
TCE subcohort	1.6 (0.5, 5.1) <sup>a</sup>	15		
Males, cumulative exposure				
0	1.0 <sup>a</sup>			
<5 ppm-yr	2.0 (0.5, 7.6)	8		
5–25 ppm-yr	0.4 (0.1, 4.0)	1		
>25 ppm-yr	1.2 (0.3, 4.8)	4		
Females, cumulative exposure				
0	1.0 <sup>a</sup>			
<5 ppm-yr		0		
5–25 ppm-yr	9.8 (0.6, 157)	1		
>25 ppm-yr	3.5 (0.2, 56.4)	1		
TCE subcohort	1.18 (0.47, 2.94) <sup>i</sup>	18	Radican et al. (2008)	
Males, cumulative exposure	1.24 (0.41, 3.71) <sup>i</sup>	16		
0	1.0 <sup>l</sup>			

**Table 4-39. Summary of human studies on TCE exposure and kidney cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
Aircraft maintenance workers (Hill Air Force Base, Utah) (continued)				Blair et al. ( <a href="#">1998</a> )
	<5 ppm-yr	1.87 (0.59, 5.97) <sup>i</sup>	10	
	5–25 ppm-yr	0.31 (0.03, 2.75) <sup>i</sup>	1	
	>25 ppm-yr	1.16 (0.31, 4.32) <sup>i</sup>	5	
Females, cumulative exposure		0.93 (0.15, 5.76) <sup>i</sup>	2	
	0	1.0 <sup>a</sup>		
	<5 ppm-yr		0	
	5–25 ppm-yr	2.86 (0.27, 29.85) <sup>i</sup>	1	
	>25 ppm-yr	0.97 (0.10, 9.50) <sup>i</sup>	1	
Cardboard manufacturing workers in Arnsberg, Germany				Henschler et al. ( <a href="#">1995</a> )
	TCE exposed workers	3.28 (0.40, 11.84)	2	
	Unexposed workers	- (0.00, 5.00)	0	
Deaths reported to among GE pension fund (Pittsfield, Massachusetts)		0.99 (0.30, 3.32) <sup>f</sup>	12	Greenland et al. ( <a href="#">1994</a> )
Cardboard manufacturing workers, Atlanta area, Georgia				Sinks et al. ( <a href="#">1992</a> )
		1.4 (0.0, 7.7)	1	
U.S. Coast Guard employees				Blair et al. ( <a href="#">1989</a> )
	Marine inspectors	1.06 (0.22, 3.10)	3	
	Noninspectors	1.03 (0.21, 3.01)	3	
Aircraft manufacturing plant employees (Italy)				Costa et al. ( <a href="#">1989</a> )
	All subjects	Not reported		
Aircraft manufacturing plant employees (San Diego, California)				Garabrant et al. ( <a href="#">1988</a> )
	All subjects	0.93 (0.48, 1.64)	12	
<b>Case-control studies</b>				
Population of four countries in central and eastern Europe				Moore et al. ( <a href="#">2010</a> )
	Any TCE exposure	1.63 (1.04, 2.54)	48	
	Any TCE exposure (High confidence exposure)	2.05 (1.13, 3.73)	29	
Cumulative TCE exposure				
	<1.58 ppm-yr	1.19 (0.61, 2.35)	17	
	>1.58 ppm-yr	2.02 (1.14, 3.59) <sup>j</sup>	31	
	<i>p</i> for trend	<i>p</i> = 0.02		
Average intensity				
	<0.076 ppm	1.38 (0.81, 2.35)	31	
	>0.076 ppm	2.34 (1.05, 5.21)	17	
	<i>p</i> for trend	<i>p</i> = 0.02		

**Table 4-39. Summary of human studies on TCE exposure and kidney cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
Population of Arve Valley, France				Charbotel et al. ( <a href="#">2009</a> ; <a href="#">2007</a> ; <a href="#">2006</a> )
Any TCE exposure		1.64 (0.95, 2.84)	37	
Any TCE exposure (High confidence exposure)		1.88 (0.89, 3.98)	16	
Cumulative TCE exposure				
Referent/nonexposed		1.00 <sup>a</sup>	49	
Low, 62.4 ppm-yr <sup>k</sup>		1.62 (0.75, 3.47)	12	
Medium, 253.2 ppm-yr <sup>k</sup>		1.15 (0.47, 2.77)	9	
High, 925 ppm-yr <sup>k</sup>		2.16 (1.02, 4.60) <sup>l</sup>	16	
Test for trend		$p = 0.04$		
Cumulative TCE exposure + peak				
Referent/nonexposed		1.00 <sup>a</sup>	49	
Low/medium, no peaks		1.35 (0.69, 2.63)	18	
Low/medium + peaks		1.61 (0.36, 7.30)	3	
High, no peaks		1.76 (0.65, 4.73)	8	
High + peaks		2.73 (1.06, 7.07) <sup>l</sup>	8	
Cumulative TCE exposure, 10-yr lag				
Referent/nonexposed		1.00 <sup>a</sup>	49	
Low/medium, no peaks		1.44 (0.69, 2.80)	19	
Low/medium + peaks		1.38 (0.32, 6.02)	3	
High, no peaks		1.50 (0.53, 4.21)	7	
High + peaks		3.15 (1.19, 8.38)	8	
TWA TCE exposure <sup>m</sup>				
Referent/nonexposed		1.00 <sup>a</sup>	46	
Any TCE without cutting fluid		1.62 (0.76, 3.44)	15	
Any cutting fluid without TCE		2.39 (0.52, 11.03)	3	
<50 ppm TCE + cutting fluid		1.14 (0.49, 2.66)	12	
50 + ppm TCE + cutting fluid		2.70 (1.02, 7.17)	10	
Population of Arnsberg Region, Germany				Brüning et al. ( <a href="#">2003</a> )
Longest job held—TCE/PERC (CAREX)		1.80 (1.01, 3.20)	117	
Self-assessed exposure to TCE		2.47 (1.36, 4.49)	25	
Duration of self-assessed TCE exposure				
0		1.00 <sup>a</sup>	109	
<10 yrs		3.78 (1.54, 9.28)	11	
10–20 yrs		1.80 (0.67, 4.79)	7	
>20 yrs		2.69 (0.84, 8.66)	8	

**Table 4-39. Summary of human studies on TCE exposure and kidney cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
Population in five German Regions				Pesch et al., (2000b)
Any TCE exposure		Not reported		
Males		Not reported		
Females		Not reported		
TCE exposure (JTEM)				
Males				
Medium	1.3 (1.0, 1.8)	68		
High	1.1 (0.8, 1.5)	59		
Substantial	1.3 (0.8, 2.1)	22		
Females				
Medium	1.3 (0.7, 2.6)	11		
High	0.8 (0.4, 1.9)	7		
Substantial	1.8 (0.6, 5.0)	5		
Population of Minnesota				Dosemeci et al. (1999)
Ever exposed to TCE, NCI JEM				
Males	1.04 (0.6, 1.7)	33		
Females	1.96 (1.0, 4.0)	22		
Males + Females	1.30 (0.9, 1.9)	55		
Population of Arnsberg Region, Germany				Vamvakas et al. (1998)
Self-assessed exposure to TCE	10.80 (3.36, 34.75)	19		
Population of Montreal, Canada				Siemiatycki et al. (1991)
Any TCE exposure		0.8 (0.4, 2.0) <sup>n</sup>	4	
Substantial TCE exposure		0.8 (0.2, 2.6) <sup>n</sup>	2	
<b>Geographic-based studies</b>				
Residents in two study areas in Endicott, New York		1.90 (1.06, 3.13)	15	ATSDR (2006a) (2008b)
Residents of 13 census tracts in Redlands, California		0.80 (0.54, 1.12) <sup>o</sup>	54	Morgan and Cassady (2002)
Finnish residents				Vartiainen et al. (1993)
Residents of Hausjarvi		Not reported		
Residents of Huttula		Not reported		

<sup>a</sup>Internal referents, workers not exposed to TCE.

<sup>b</sup>RRs for TCE exposure after adjustment for 1<sup>st</sup> employment, SES status, age at event, and all other carcinogens, including hydrazine.

<sup>c</sup>Chang et al. (2005)—urinary organs combined.

<sup>d</sup>SIR for RCC.

<sup>e</sup>Henschler et al. (1995) Expected number of incident cases calculated using incidence rates from the Danish Cancer Registry.

<sup>f</sup>OR from nested case-control analysis.

<sup>g</sup>PMR.

**Table 4-39. Summary of human studies on TCE exposure and kidney cancer (continued)**

<sup>h</sup>Risk ratio from Cox Proportional Hazard Analysis, stratified by age, sex and decade ([EHS, 1997](#)).

<sup>i</sup>In Radican et al. ([2008](#)), kidney cancer defined as RCC (ICDA 8 code 189.0) and estimated RRs from Cox proportional hazard models were adjusted for age and sex.

<sup>j</sup>The OR, adjusted for age, sex, and center, for subjects with high-confidence exposure assessment with cumulative exposure,  $\geq 1.58$  ppm-yr, was 2.23 (95% CI: 1.07, 4.64) and *p*-value for trend = 0.02.

<sup>k</sup>Mean cumulative exposure score in Charbotel et al. ([2006](#)) (personal communication from Barbara Charbotel, University of Lyon, to Cheryl Scott, U.S. EPA, 11 April 2008).

<sup>l</sup>In Charbotel et al. ([2006](#)) analyses adjusted for age, sex, smoking, and BMI. The OR, adjusted for age, sex, smoking, BMI, and exposure to cutting fluids and other petroleum oils, for high cumulative TCE exposure was 1.96 (95% CI: 0.71, 5.37) and for high cumulative + peak TCE exposure was 2.63 (95% CI: 0.79, 8.83). The OR for, considering only job periods with high confidence TCE exposure assessment, adjusted for age, sex, smoking, and BMI, for high cumulative dose plus peaks was 3.80 (95% CI: 1.27, 11.40).

<sup>m</sup>The exposure surrogate is calculated for one occupational period only and is not the average exposure concentration over the entire employment period.

<sup>n</sup>90% CI.

<sup>o</sup>99% CI.

PERC = perchloroethylene

Additionally, a large body of evidence exists on kidney cancer risk and either job or industry titles where TCE usage has been documented. TCE has been used as a degreasing solvent in a number of jobs, task, and industries, some of which include metal, electronic, paper and printing, leather manufacturing, and aerospace/aircraft manufacturing or maintenance industries and job title of degreaser, metal workers, electrical worker, and machinist ([Purdue et al., 2011](#); [IARC, 1995b](#)). NRC ([2006](#)) identifies characteristics for kidney cancer case-control studies that assess job title or occupation in their Table 3-8. RRs and 95% CIs reported in these studies are found in Table 4-40.

**Table 4-40. Summary of case-control studies on kidney cancer and occupation or job title**

Case ascertainment area/exposure group		RR (95% CI)	No. exposed cases	Reference
Swedish Cancer Registry Cases				Wilson et al. (2008)
Machine/electronics industry		1.30 (1.08, 1.55) <sup>a</sup> [M]	120	
		1.75 (1.04, 2.76) <sup>a</sup> [F]	18	
Shop and construction metal work		1.19 (1.00, 1.40) <sup>a</sup> [M]	143	
Machine assembly		1.62 (0.94, 2.59) <sup>a</sup> [M]		
Metal plating work		2.70 (0.73, 6.92) <sup>a</sup> [M]	4	
Shop and construction metal work		1.66 (0.71, 3.26) <sup>a</sup> [F]	8	
Arve Valley, France				Charbotel et al. (2006)
Metal industry		1.02 (0.59, 1.76)	28	
Metal workers, job title		1.00 (0.56, 1.77)	25	
Metal industry, screw-cutting workshops		1.39 (0.75, 2.58)	22	
Machinery, electrical and transportation equipment manufacture		1.19 (0.61, 2.33)	15	
Iowa Cancer Registry Cases				Zhang et al. (2004)
Assemblers		2.5 (0.8, 7.6)	5	
>10 yr employment		4.2 (1.2, 15.3)	4	
Arnsberg Region, Germany				Brüning et al. (2003)
Iron/steel		1.15 (0.29, 4.54)	3	
Occupations with contact to metals		1.53 (0.97, 2.43)	46	
Longest job held		1.14 (0.66, 1.96)	24	
Metal greasing/degreasing		5.57 (2.33, 13.32)	15	
Degreasing agents				
Low exposure		2.11 (0.86, 5.18)	9	
High exposure		1.01 (0.40, 2.54)	7	
Bologna, Italy				Mattioli et al. (2002)
Metal workers		2.21 (0.99, 5.37)	37	
Printers		1.55 (0.17, 13.46)	7	
Solvents		0.79 (0.31, 1.98) [M]	17	
		1.47 (0.12, 17.46) [F]	3	
Montreal, Canada				Parent et al. (2000a)
Metal fabricating and machining industry		1.0 (0.6, 1.8)	14	
Metal processors		1.2 (0.4, 3.4)	4	
Printing and publishing industry		1.1 (0.4, 3.0)	4	
Printers		3.0 (1.2, 7.5)	6	
Aircraft mechanics		2.8 (1.0, 8.4)	4	

**Table 4-40. Summary of case-control studies on kidney cancer and occupation or job title (continued)**

Case ascertainment area/exposure group		RR (95% CI)	No. exposed cases	Reference
Five Regions in Germany				Pesch et al. ( <a href="#">2000b</a> )
Electrical and electronic equipment assembler	3.2 (1.0, 10.3) [M]	5		
	2.7 (1.3, 5.8) [F]	11		
Printers	3.5 (1.1, 11.2) [M]	5		
	2.1 (0.4, 11.7) [F]	2		
Metal cleaning/degreasing, job task	1.3 (0.7, 2.3) [M]	15		
	1.5 (0.3, 7.7) [F]	2		
New Zealand Cancer Registry				Delahunt et al. ( <a href="#">1995</a> )
Toolmakers and blacksmiths	1.48 (0.72, 3.03)	No information		
Printers	0.67 (0.25, 1.83)			
Minnesota Cancer Surveillance System				Mandel et al. ( <a href="#">1995</a> )
Iron or steel	1.6 (1.2, 2.2)	8		
Rhein-Neckar-Odenwald Area, Germany				Schlehofer et al. ( <a href="#">1995</a> )
Metal				
Industry	1.63 (1.07, 2.48)	71		
Occupation	1.38 (0.89, 2.12)			
Electronic				
Industry	0.51 (0.26, 1.01)	14		
Occupation	0.57 (0.25, 1.33)	9		
Chlorinated solvents	2.52 (1.23, 5.16)	27		
Metal and metal compounds	1.47 (0.94, 2.30)	62		
Danish Cancer Registry				Mellempgaard et al. ( <a href="#">1994</a> )
Iron and steel	1.4 (0.8, 2.4) [M]	31		
	1.0 (0.1, 3.2) [F]	1		
Solvents	1.5 (0.9, 2.4) [M]	50		
	6.4 (1.8, 23) [F]	16		
France				Aupérin et al. ( <a href="#">1994</a> )
Machine fitters, assemblers, and precision instrument makers	0.7 (0.3, 1.9)	16		

**Table 4-40. Summary of case-control studies on kidney cancer and occupation or job title (continued)**

Case ascertainment area/exposure group		RR (95% CI)	No. exposed cases	Reference
New South Wales, Australia				McCredie and Stewart ( <a href="#">1993</a> )
Iron and steel		1.18 (0.75, 1.85) <sup>b</sup>	52	
		2.39 (1.26, 4.52) <sup>c</sup>	19	
Printing or graphics		1.18 (0.87, 2.08) <sup>b</sup>	29	
		0.82 (0.32, 2.11) <sup>d</sup>	6	
Machinist or tool maker		1.15 (0.72, 1.86) <sup>b</sup>	48	
		1.83 (0.92, 3.61) <sup>c</sup>	16	
Solvents		1.54 (1.11, 2.14) <sup>b</sup>	109	
		1.40 (0.82, 2.40) <sup>c</sup>	24	
Finnish Cancer Registry				Partanen et al. ( <a href="#">1991</a> )
Iron and metalware work		1.87 (0.94, 3.76)	22	
	Machinists	2.33 (0.83, 6.51)	10	
Paper and pulp; printing/publishing		2.20 (1.02, 4.72) [M]	18	
		5.95 (1.21, 29.2) [F]	7	
Nonchlorinated solvents		3.46 (0.91, 13.2) [M]	9	
West Midlands U.K. Cancer Registry				Harrington et al. ( <a href="#">1989</a> )
Organic solvents	Ever exposed	1.30 (0.31, 8.50)	3	
	Intermediate exposure	1.54 (0.69, 4.10)	3	
Montreal, Canada				Sharpe et al. ( <a href="#">1989</a> )
Organic solvents		1.68 (0.83, 2.22)	33	
	Degreasing solvents		3.42 (0.92, 12.66)	10
Oklahoma				Asal et al. ( <a href="#">1988a</a> ; <a href="#">1988b</a> )
Metal degreasing		1.7 (0.7, 3.8) [M]	19	
	Machining	1.7 (0.7, 4.3) [M]	13	
	Painter, paint manufacture	1.3 (0.7, 2.6) [M]	22	
Missouri Cancer Registry				Brownson ( <a href="#">1988</a> )
Machinists		2.2 (0.5, 10.3)	3	
Danish Cancer Registry				Jensen et al. ( <a href="#">1988</a> )
Iron and metal, blacksmith		1.4 (0.7, 2.9) <sup>d</sup>	17	
	Painter, paint manufacture		1.8 (0.7, 4.6)	10

<sup>a</sup>Renal pelvis, Wilson et al. ([2008](#)).

<sup>b</sup>RCC, McCredie and Stewart ([1993](#)).

<sup>c</sup>Renal pelvis, McCredie and Stewart ([1993](#)).

<sup>d</sup>Renal pelvis and ureter, Jensen et al. ([1988](#)).

#### 4.4.2.1. Studies of Job Titles and Occupations with Historical TCE Usage

Elevated risks are observed in many of the cohort or case-control studies between kidney cancer and industries or job titles with historical use of TCE ([Wilson et al., 2008](#); [Charbotel et al., 2006](#); [Zhang et al., 2004](#); [Brüning et al., 2003](#); [Mattioli et al., 2002](#); [Parent et al., 2000a](#);

[Pesch et al., 2000b](#); [Mandel et al., 1995](#); [Schlehofer et al., 1995](#); [McCredie and Stewart, 1993](#); [Partanen et al., 1991](#)). Overall, these studies, although indicating association with metal work exposures and kidney cancer, are insensitive for identifying a TCE hazard. The use of job title or industry as a surrogate for exposure to a chemical is subject to substantial misclassification that will attenuate rate ratios due to exposure variation and differences among individuals with the same job title. Several small case-control studies ([Parent et al., 2000a](#); [Vamvakas et al., 1998](#); [Auperin et al., 1994](#); [Harrington et al., 1989](#); [Sharpe et al., 1989](#); [Jensen et al., 1988](#)) have insufficient statistical power to detect modest associations due to their small size and potential exposure misclassification ([NRC, 2006](#)). For these reasons, statistical variation in the risk estimate is large and observation of statistically significantly elevated risks associated with metal work in many of these studies is noteworthy. Some studies also examined broad chemical grouping such as degreasing solvents or chlorinated solvents. Observations in studies that assessed degreasing agents or chlorinated solvents reported statistically significant elevated kidney cancer risk ([Brüning et al., 2003](#); [Pesch et al., 2000b](#); [Schlehofer et al., 1995](#); [Mellempgaard et al., 1994](#); [McCredie and Stewart, 1993](#); [Harrington et al., 1989](#); [Asal et al., 1988a](#); [Asal et al., 1988b](#)). Observations of association with degreasing agents, together with job title or occupations where TCE has been used historically, provide a signal and suggest an etiologic agent common to degreasing activities.

#### **4.4.2.2. Cohort and Case-Controls Studies of TCE Exposure**

Cohort and case-controls studies that include JEMs for assigning TCE exposure potential to individual study subjects show associations with kidney cancer, specifically RCC, and TCE exposure. Support for this conclusion derives from findings of increased risks in cohort studies ([Zhao et al., 2005](#); [Raaschou-Nielsen et al., 2003](#); [Henschler et al., 1995](#)) and in case-control studies from the Arnsberg region of Germany ([Brüning et al., 2003](#); [Pesch et al., 2000b](#); [Vamvakas et al., 1998](#)), the Arve Valley region in France ([Charbotel et al., 2009](#); [Charbotel et al., 2006](#)), the United States ([Dosemeci et al., 1999](#); [Sinks et al., 1992](#)), and the four central and eastern Europe countries of Czech Republic, Poland, Romania, and Russia ([Moore et al., 2010](#)).

A consideration of a study's statistical power and exposure assessment approach is necessary to interpret observations in Table 4-39. Most cohort studies are underpowered to detect a doubling of kidney cancer risks including the essentially null studies by Greenland et al. (1994), Axelson et al. (1994 [incidence]), Anttila et al. (1995 [incidence]), Blair et al. (1998 [incidence and mortality]), Morgan et al. (1998), Boice et al. (1999), and Hansen et al. (2001). Only the exposure duration-response analysis of Raaschou-Nielsen et al. (2003) had over 80% statistical power to detect a doubling of kidney cancer risk ([NRC, 2006](#)), and they observed a statistically significant association between kidney cancer and  $\geq 5$ -year employment duration. Rate ratios estimated in the mortality cohort studies of kidney cancer (e.g., [Boice et al., 2006b](#); [Ritz, 1999a](#); [Blair et al., 1998](#); [Morgan et al., 1998](#); [Axelson et al., 1994](#); [Greenland et al., 1994](#);

[Sinks et al., 1992](#); [Garabrant et al., 1988](#)) are likely underestimated to some extent because their reliance on death certificates and increased potential of nondifferential misclassification of outcome in these studies, although the magnitude is difficult to predict ([NRC, 2006](#)). Cohort or PMR studies with more uncertain exposure assessment approaches, e.g., studies of all subjects working at a factory ([Clapp and Hoffman, 2008](#); [Sung et al., 2007](#); [Chang et al., 2005](#); [ATSDR, 2004a](#); [Chang et al., 2003](#); [Costa et al., 1989](#); [Garabrant et al., 1988](#)), do not show association but are quite limited given their lack of attribution of higher or lower exposure potentials; risks are likely diluted due to their inclusion of no or low exposed subjects.

Two studies were carried out in geographic areas with a high frequency and a high degree of TCE exposure and were designed with a priori hypotheses to test for the effects of TCE exposure on renal cell cancer risk ([Charbotel et al., 2009](#); [Charbotel et al., 2006](#); [Brüning et al., 2003](#)) and a third study carried out in four central and eastern European countries with high RCC rates unexplained by established risk factors ([Moore et al., 2010](#); [Ferlay et al., 2008](#)). For these reasons, their observations have important bearing to the epidemiologic evidence evaluation. These studies found a twofold elevated risk with any TCE exposure after adjustment for several possible confounding factors including smoking (2.47, 95% CI: 1.36, 4.49) for self-assessed exposure to TCE ([Brüning et al., 2003](#)); any confidence job with high cumulative TCE exposure, 925 ppm-years (2.16, 95% CI: 1.02, 4.60) with a positive and statistically significant trend test,  $p = 0.04$ , high confidence jobs with high cumulative TCE exposure (3.34, 95% CI: 1.27, 8.74) ([Charbotel et al., 2006](#)); high confidence assessment of high TCE cumulative exposure  $\geq 1.58$  ppm-years (2.23, 95% CI: 1.07, 4.64) with a positive and statistically significant trend test,  $p = 0.02$  ([Moore et al., 2010](#)). Furthermore, RCC risk in Charbotel et al. (2005) increased to over threefold (95% CI: 1.19, 8.38) in statistical analyses, which considered a 10-year exposure lag period. An exposure lag period is often adopted in analysis of cancer epidemiology to reduce exposure measurement biases ([Salvan et al., 1995](#)). Most exposed cases in this study were exposed to TCE below any current occupational standard (26 of 37 cases [70%]) had held a job with a highest TWA (<50 ppm) ([Charbotel et al., 2009](#)). A subsequent analysis of Charbotel et al. (2009) using an exposure surrogate defined as the highest TWA for any job held, an inferior surrogate given that TCE exposures in other jobs were not considered, reported an almost threefold elevated risk (2.80, 95% CI: 1.12, 7.03) adjusted for age, sex, body mass index (BMI), and smoking with exposure to TCE in any job to  $\geq 50$ -ppm TWA ([Charbotel et al., 2009](#)). Considering all jobs, Moore et al. (2010) reported a risk of 2.34 (95% CI: 1.05, 5.21) for average TCE intensity (>0.76 ppm), an exposure metric similar to a TWA exposure category. Zhao et al. (2005) compared 2,689 TCE-exposed workers at a California aerospace company to nonexposed workers from the same company as the internal referent population, and found a monotonic increase in incidence of kidney cancer by increasing cumulative TCE exposure. In addition, a fivefold increased incidence was associated with high cumulative TCE exposure. This relationship for high cumulative TCE exposure, lagged 20 years, was accentuated with

adjustment for other occupational exposures (RR = 7.40, 95% CI: 0.47, 116), although the CIs were increased. An increased CI with adjustments is not unusual in occupational studies, as exposure is usually highly correlated with them, so that adjustments often inflate SE without removing any bias (NRC, 2006). Observed risks were lower for kidney cancer mortality and because of reliance on cause of death on death certificates are likely underestimated because of nondifferential misclassification of outcome (Percy et al., 1981). Boice et al. (2006b), another study of 1,111 workers with potential TCE exposure at this company and which overlaps with Zhao et al. (2005), found a twofold increase in kidney cancer mortality (standardized mortality ratio [SMR] = 2.22, 95% CI: 0.89, 4.57). This study examined mortality in a cohort whose definition date differs slightly from Zhao et al. (2005), working between 1948 and 1999 with vital status as of 1999 (Boice et al., 2006b) compared to working between 1950 and 1993 with follow-up for mortality as of 2001 (Zhao et al., 2005), and used a qualitative approach for TCE exposure assessment. Boice et al. (2006b) is a study of fewer subjects identified with potential TCE exposure, of fewer kidney cancer deaths [7 deaths; 10 incident cases, 10 deaths in Zhao et al. (2005)], of subjects with more recent exposures, and with a inferior exposure assessment approach compared to Zhao et al. (2005); a finding of a twofold mortality increase (95% CI: 0.89, 4.57) is noteworthy given the insensitivities.

Zhao et al. (2005), Charbotel et al. (2006), and Moore et al. (2010), furthermore, are three of the few studies to conduct a detailed assessment of exposure that allowed for the development of a JEM that provided rank-ordered levels of exposure to TCE and other chemicals. NRC (2006) discussed the inclusion of rank-ordered exposure levels is a strength increasing precision and accuracy of exposure information compared to more inferior exposure assessment approaches in some other studies such as duration of exposure or a grouping of all exposed subjects.

The finding in Raaschou-Nielsen et al. (2003) of an elevated RCC risk with longer employment duration is noteworthy given this study's use of a relatively insensitive exposure assessment approach. One strength of this study is the presentation of incidence ratios for a subcohort of higher exposed subjects, those with at least a 1-year duration of employment and first employment before 1980, as a sensitivity analysis for assessing the effect of possible exposure misclassification bias. RCC risk was higher in this subcohort compared to the larger cohort and indicated some potential for misclassification bias in the grouped analysis. For both the cohort and subcohort analyses, risk appeared to increase with increasing employment duration, although formal statistical tests for trend are not presented in the published paper.

#### **4.4.2.2.1. Discussion of controversies on studies in the Arnsberg region of Germany**

Two previous studies of workers in this region, a case-control study of Vamvakas et al. (1998) and Henschler et al. (1995), a study prompted by a kidney cancer case cluster, observed strong associations between kidney cancer and TCE exposure. A fuller discussion of the studies

from the Arnsberg region and their contribution to the overall weight of evidence on cancer hazard is warranted in this evaluation given the considerable controversy ([Cherrie et al., 2001](#); [Mandel, 2001](#); [Green and Lash, 1999](#); [McLaughlin and Blot, 1997](#); [Bloemen and Tomenson, 1995](#); [Swaen, 1995](#)) surrounding Henschler et al. ([1995](#)) and Vamvakas et al. ([1998](#)).

Criticisms of Henschler et al. ([1995](#)) and Vamvakas et al. ([1998](#)) relate, in part, to possible selection biases that would lead to inflating observed associations and limited inferences of risk to the target population. Specifically, these include: (1) the inclusion of kidney cancer cases first identified from a cluster and the omission of subjects lost to follow-up from Henschler et al. ([1995](#)); (2) use of a Danish population as referent, which may introduce bias due to differences in coding cause of death and background cancer rate differences ([Henschler et al., 1995](#)); (3) follow-up of some subjects outside the stated follow-up period ([Henschler et al., 1995](#)); (4) differences between hospitals in the identification of cases and controls in Vamvakas et al. ([1998](#)); (5) lack of temporality between case and control interviews ([Vamvakas et al., 1998](#)); (6) lack of blinded interviews ([Vamvakas et al., 1998](#)); (7) age differences in Vamvakas et al. ([1998](#)) cases and controls that may lead to a different TCE exposure potential; (8) inherent deficiencies in Vamvakas et al. ([1998](#)) as reflected by its inability to identify other known kidney cancer risk factors; and (9) exposure uncertainty, particularly unclear intensity of TCE exposure. Overall, NRC ([2006](#)) noted that some of the points above may have contributed to an underestimation of the true exposure distribution of the target population (points 5, 6, and 7), other points would underestimate risk (points 3), and that these effects could not have explained the entire excess risk observed in these studies (points 1, 2, and 4). The NRC ([2006](#)) furthermore disagreed with the exposure uncertainty criticism (point 9), and concluded TCE exposures, although of unknown intensity, were substantial and, clearly showed graded differences on several scales in Vamvakas et al. ([1998](#)) consistent with this study's semiquantitative exposure assessment.

Brüning et al. ([2003](#)) was carried out in a broader region in southern Germany, which included the Arnsberg region and a different set of cases and control identified from a later time period than Vamvakas et al. ([1998](#)). The TCE exposure range in this study was similar to that in Vamvakas et al. ([1998](#)), although at a lower exposure prevalence because of the larger and more heterogeneous ascertainment area for cases and controls. For "ever exposed" to TCE, Brüning et al. ([2003](#)) observed a risk ratio of 2.47 (95% CI: 1.36, 4.49) and a fourfold increase in risk (95% CI: 1.80, 7.54) among subjects with any occurrence of narcotic symptom and a sixfold increase in risk (95% CI: 1.46, 23.99) for subjects who had daily occurrences of narcotic symptoms; risks which are lower than observed in Vamvakas et al. ([1998](#)). The lower rate ratio in Brüning et al. ([2003](#)) might indicate bias in the Vamvakas et al. ([1998](#)) study or statistical variation between studies related to the broader base population included in Brüning et al. ([2003](#)).

Observational studies such as epidemiologic studies are subject to biases and confounding, which can be minimized but never completely eliminated through a study's design and statistical analysis methods. While Brüning et al. (2003) overcome many of the deficiencies of Henschler et al. (1995) and Vamvakas et al. (1998), nonetheless, possible biases and measurement errors could be introduced through their use of prevalent cases and residual noncases, use of controls from surgical and geriatric clinics, nonblinding of interviewers, a 2-year difference between cases and controls in median age, use, or proxy or next-of-kin interviews, and self-reported occupational history.

The impact of any one of the above points could either inflate or depress observed associations. Biases related to a longer period for case compared to control ascertainment could go in either direction. Next-of-kin interviewers for deceased cases, all controls being alive at the time of interview, would be expected to underestimate risk if exposures were not fully reported and thus, misclassified. On the other hand, the control subjects who were enrolled when the interviews were conducted might not represent the true exposure distribution of the target population through time and would lead to overestimate of risk. Selection of controls from clinics is not expected to greatly influence observed associations since these clinics specialized in the type of care they provided (NRC, 2006). Brüning et al. (2003) is not the only kidney case-control study where interviewers were not blinded; in fact, only the study of Charbotel et al. (2006) included blinding of interviewers. Blinding of interviewers is preferred to reduce possible bias. The Brüning et al. (2003) study's use of frequency matching using 5-year age groupings is common in epidemiologic studies and any biases introduced by age difference between cases and controls is expected to be minimal because the median age difference was 3 years.

Despite these issues, the three studies of the Arnsberg region, with very high apparent exposure and different base populations showed a significant elevation of risk and all have bearing on kidney cancer hazard evaluations. The emphasis provided by each study for identifying a kidney cancer hazard depends on its strengths and weaknesses. Brüning et al. (2003) overcomes many of the deficiencies in Henschler et al. (1995) and Vamvakas et al. (1998). The finding of a statistically significantly approximately threefold elevated OR with occupational TCE exposure in Brüning et al. (2003) strengthens the signal previously reported by Henschler et al. (1995) and Vamvakas et al. (1998). A previous study of cardboard workers in the United States (Sinks et al., 1992), a study like Henschler et al. (1995), which was prompted by a reported cancer cluster, had observed association with kidney cancer incidence, particularly with work in the finishing department where TCE use was documented. Henschler et al. (1995), Vamvakas et al. (1998), and Sinks et al. (1992) are less likely to provide a precise estimate of the magnitude of the association given greater uncertainty in these studies compared to Brüning et al. (2003). For this reason, Brüning et al. (2003) is preferred for meta-analysis treatment since it is considered to better reflect risk in the target population than the two other studies. Another

study ([Charbotel et al., 2006](#)) of similar exposure conditions of a different base population and of different case and control ascertainment methods as the Arnsberg region studies has become available since the Arnsberg studies. This study shows a statistically significant elevation of risk and high cumulative TCE exposure in addition to a positive trend with rank-order exposure levels. Charbotel et al. ([2006](#)) added evidence to observations from earlier studies on high TCE exposures in Southern Germany and suggested that peak exposure may add to risk associated with cumulative TCE exposure.

#### **4.4.2.3. Examination of Possible Confounding Factors**

Examination of potential confounding factors is an important consideration in the evaluation of observations in the epidemiologic studies on TCE and kidney cancer. A known risk factor for kidney cancer is cigarette smoking. Obesity, diabetes, hypertension and antihypertensive medications, and analgesics are linked to kidney cancer, but causality has not been established ([McLaughlin et al., 2006](#); [Moore et al., 2005](#)). On the other hand, fruit and vegetable consumption is considered protective of kidney cancer risk ([McLaughlin et al., 2006](#)). Studies by Asal et al. ([1988a](#); [1988b](#)), Partanen et al. ([1991](#)), McCredie and Stewart ([1993](#)), Aupérin et al. ([1994](#)), Chow et al. ([1994](#)), Mellemgard et al. ([1994](#)), Mandel et al. ([1995](#)), Vamvakas et al. ([1998](#)), Dosemeci et al. ([1999](#)), Pesch et al. ([2000b](#)), Brüning et al. ([2003](#)), and Charbotel et al. ([2006](#)) controlled for smoking, and all studies except Pesch et al. ([2000b](#)) controlled for BMI. Moore et al. ([2010](#)) examined, but did not find, smoking or BMI as potential confounders because statistical examination of cigarette smoking and BMI altered risk estimates for the association between TCE exposure and kidney cancer by <10%. Vamvakas et al. ([1998](#)) and Dosemeci et al. ([1999](#)) controlled for hypertension and/or diuretic intake in the statistical analysis. Because it is unlikely that exposure to TCE is associated with smoking, BMI, hypertension, or diuretic intake, these possible confounders do not significantly affect the estimates of risk ([NRC, 2006](#)).

Direct examination of possible confounders is less common in cohort studies than in case-control studies where information is obtained from study subjects or their proxies. Use of internal controls, such as for Zhao et al. ([2005](#)), in general, minimizes effects of potential confounding due to smoking or socioeconomic (SES) status since exposed and referent subjects are drawn from the same target population. Information on possible confounding due to BMI (obesity) and to diabetes is lacking in cohort studies; however, any uncertainties are likely small given the generally healthy nature of an employed population and its favorable access to medical care.

The effect of smoking as a possible confounder may be assessed indirectly through: (1) examination of risk ratios for other smoking-related sites; (2) examination of the expected contribution by smoking to cancer risks; and (3) examination of lung cancer in nine TCE cohort studies in which there is a high likelihood of TCE exposure in individual study subjects (and

which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review using meta-analysis methods). Some information on smoking-related lung and kidney cancer risks may be obtained from IARC ([2004a](#)) for indirectly evaluating the expected magnitude by smoking on kidney cancer risks in TCE cohort studies. Five cohort studies of cigarette smoking reported risk estimates for both lung and kidney cancers with an observed ratio of lung:kidney cancer risks of 3.5–10.6 for active smokers, who will have higher smoking-related risks than former smokers (see Table 4-41). The nine cohort studies ([Radican et al., 2008](#); [Zhao et al., 2005](#); [Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#); [Boice et al., 1999](#); [Morgan et al., 1998](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#); [Greenland et al., 1994](#)) present lung cancer risks and reported risks for overall TCE exposure ranging from 0.69 (95% CI: 0.31, 1.30) by Axelson et al. ([1994](#)) to 1.4 (95% CI: 1.32, 1.52) by Raaschou-Nielsen et al. ([2003](#)) (see Table 4-81). Smoking was more prevalent in the Raaschou-Nielsen et al. ([2003](#)) cohort than the background population as suggested by the elevated risks for lung and other smoking-related sites. If smoking fully contributes to the observed 40% excess lung cancer risk in this study and based on observations in the five smoking cohorts, the expected contribution by smoking to RCC risk is estimated as 1–6% and far smaller than the 20 and 40% excess in RCC risk in the cohort and subcohort. The use of internal referents who are unexposed subjects drawn from the occupational settings as TCE exposed subjects in three studies reduces any confounding related to smoking as referents ([Radican et al., 2008](#); [Zhao et al., 2005](#); [Morgan et al., 1998](#)). In the other cohort studies lacking direct adjustment for smoking and internal referents, difference in cigarette smoking between exposed and referent subjects is not sufficient to fully explain observed excess kidney cancer risks associated with TCE, particularly high TCE exposure. Lung cancer risk estimates are lower than or equal to kidney cancer risk estimates and inconsistent with observations in the five smoking cohorts ([Hansen et al., 2001](#); [Boice et al., 1999](#); [Axelson et al., 1994](#)).

**Table 4-41. Summary of lung and kidney cancer risks in active smokers**

Cohort	RR		Ratio lung; kidney	Reference
	Lung	Kidney		
MRFIT (USA) 1975–1985, men	6.7	1.9	3.5	Kuller et al. ( <a href="#">1991</a> )
British Doctor's Study (United Kingdom) 1957–1991, men	14.9 <sup>a</sup>	1.4 <sup>a</sup>	10.6	Doll et al. (1994)
U.S. Veterans Study (United States) 1954–1980, men	11.6	1.5	7.7	McLaughlin et al. ( <a href="#">1995</a> )
Swedish Census Study (Sweden) 1963–1989, women	4.7	1.1	4.3	Nordlund et al. ( <a href="#">1999</a> , <a href="#">1997</a> )
Cancer Prevention Study II (United States) 1982–1986, women	12.4	1.4	9.1	Garfinkel and Stellman ( <a href="#">1988</a> ); Heath et al. ( <a href="#">1997</a> )

<sup>a</sup>Relative mortality rate compared to nonsmokers.

Source: from IARC ([2004a](#))

Meta-analysis methods were adopted, additionally, as a tool for examining risk estimates from the nine cohort studies in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review reporting lung cancer to assess the presence of potential systematic error related to confounding from smoking. Significant heterogeneity was observed across the nine studies of overall exposure ( $I^2 = 90\%$ ) and for six of the nine studies with highest exposure groups ( $I^2 = 80\%$ ). Although the appropriateness of conducting any meta-analysis without attempting to explain the heterogeneity is arguable, the summary estimate from the primary random effects meta-analysis of the nine studies was 0.96 (95% CI: 0.76, 1.21) for overall TCE exposure, and 0.96 (95% CI: 0.72, 1.27) for the highest group exposure reported by six studies. These observations suggest potential confounding by smoking of kidney cancer summary risk estimates can be reasonably excluded in cohort studies of TCE exposure.

Mineral oils such as cutting fluids or hydrazine common to some job titles with potential TCE exposures (such as machinists, metal workers, and test stand mechanics) were included as covariates in statistical analyses of Zhao et al. ([2005](#)), Boice et al. ([2006b](#)) and Charbotel et al. ([2009](#); [2006](#)) or evaluated as a single exposure for cases and controls in Moore et al. ([Karami et al., 2011](#); [2010](#)). A TCE effect on kidney cancer incidence was still evident, although effect estimates were often imprecise due to lowered statistical power ([Charbotel et al., 2009](#); [Charbotel et al., 2006](#); [Zhao et al., 2005](#)). Observed associations were similar in analyses including chemical co-exposures in both Zhao et al. ([2005](#)) and Charbotel et al. ([2009](#); [2006](#)) compared to chemical co-exposure unadjusted risks. The association or OR between high TCE score and kidney cancer incidence in Zhao et al. ([2005](#)) was 7.71 (95% CI: 0.65, 91.4) after

adjustment for other carcinogens including hydrazine and cutting oils, compared to analyses unadjusted for chemical co-exposures (4.90, 95% CI: 1.23, 19.6).

In Charbotel et al. (2006), exposure to TCE was strongly associated with exposure to cutting fluids and petroleum oils (22 of the 37 TCE-exposed cases were exposed to both). Statistical modeling of all factors significant at 10% threshold showed the OR for cutting fluids to be almost equal to one, whereas the OR for the highest level of TCE exposure was close to two (Charbotel et al., 2006). Moreover, when exposure to cutting oils was divided into three levels, a decrease in OR with level of exposure was found. In conditional logistic regression adjusted for cutting oil exposure, the OR for RCC and TCE was similar to ORs unadjusted for cutting fluid exposures (high cumulative TCE exposure: 1.96 [95% CI: 0.71–5.37] compared to 2.16 [95% CI: 1.02–4.60]; high cumulative and peak: 2.63 [95% CI: 0.79–8.83] compared to 2.73 [95% CI: 1.06–7.07] (Charbotel et al., 2006). Charbotel et al. (2009) further examined TCE exposure defined as the highest TWA in any job held, inferior to cumulative exposure given its lack of consideration of TCE exposure potential in other jobs, either as exposure to TCE alone, cutting fluids alone, or to both after adjusting for smoking, BMI, age, sex, and exposure to other oils (TCE alone: 1.62 [95% CI: 0.75, 3.44]); cutting fluids alone: 2.39 (95% CI: 0.52, 11.03); TCE >50-ppm TWA + cutting fluids: 2.70 (95% CI: 1.02, 7.17). There were few cases exposed to cutting fluids alone (n = 3) or to TCE alone (n = 15), all of whom had TCE exposure (in the highest exposed job held) of <35 ppm TWA, and the subgroup analyses were of limited statistical power. A finding of higher risk for both cutting oil and TCE exposure  $\geq$ 50 ppm compared to cutting oil alone supports a TCE effect for kidney cancer. Adjustment for cutting oil exposures, furthermore, did not greatly affect the magnitude of TCE effect measures in the many analyses presented by Charbotel et al. (2009; 2006) suggesting cutting fluid exposure as not greatly confounding TCE effect measures. Two other kidney case-control studies of TCE exposure examined the effect of cutting oil as a single occupational exposure on kidney cancer risk (Karami et al., 2011; Brüning et al., 2003). Although Brüning et al. (2003) reported an OR of 2.11 (95% CI: 0.66, 6.70) for self-reported cutting oil exposure and kidney cancer, cutting oil exposure did not appear highly correlated with TCE exposure as only 5 cases reported exposure to cutting oils compared to 25 cases reporting TCE exposure. Karami et al. (2011), who examined mineral oil or cutting fluid exposure among cases and controls in Moore et al. (2010), reported an OR of 0.8 (95% CI: 0.6, 1.1) and 1.1 (95% CI: 0.8, 1.4), for cutting oil mists or other mineral oil mists respectively, and provides evidence that the reported association with TCE exposure in Moore et al. (2010) is not likely confounded by cutting or mineral oil exposures. Moreover, cutting oils and mineral oils have not been associated with kidney cancer in other cohort or case-control studies (Mirer, 2010; NIOSH, 1998), which provide additional support for potential confounding by cutting oils as of minimal concern.

Boice et al. (2006b) was unable to directly examine hydrazine exposure on TCE effect measures because of a lack of model convergence in statistical analyses. Three of seven TCE-exposed kidney cancer cases were identified with hydrazine exposure of  $\leq 1.5$  years and the absence of exposure to the other four cases suggested confounding related to hydrazine was unlikely to greatly modify observed association between TCE and kidney cancer.

#### 4.4.2.4. Susceptible Populations—Kidney Cancer and TCE Exposure

Two studies of kidney cancer cases from the Arnsberg region in Germany and the study of kidney cancer cases from three Central and Eastern European countries have examined the influence of polymorphisms of the GST metabolic pathway on RCC risk and TCE exposure (Moore et al., 2010; Wiesenhütter et al., 2007; Brüning et al., 1997a). In their study of 45 TCE-exposed male and female RCC cases pending legal compensation and 48 unmatched male TCE-exposed controls, Brüning et al. (1997a) observed a higher prevalence of exposed cases homozygous and heterozygous for GSTM1 positive, 60%, than the prevalence for this genotype among exposed controls, 35%. The frequency of GSTM1 positive was lower among this control series than the frequency found in other European population studies, 50% (Brüning et al., 1997a). The prevalence of the GSTT1 positive genotype was 93% among exposed cases and 77% among exposed controls. The prevalence of GSTT1 positive genotype in the European population is 75% (Brüning et al., 1997a).

Wiesenhütter et al. (2007) compares the frequency of genetic polymorphism among subjects from the renal cancer case-control study of Brüning et al. (2003) and to the frequencies of genetic polymorphisms in the areas of Dortmund and Lutherstadt Wittenberg, Germany. Wiesenhütter et al. (2007) identified the genetic frequencies of GSTM1 and GSTT1 phenotypes for 98 of the original 134 cases (73%) and 324 of the 401 controls (81%). The prevalence of GSTM1 positive genotype was 48% among all RCC cases, 40% among TCE-exposed cases, and 52% among all controls. The prevalence of GSTT1 positive genotypes was 81% among all cases and 81% among all controls. The prevalence of GSTT1 positive genotypes reported in this paper for all TCE-exposed cases was 20%. Wiesenhütter et al. (2007) noted background frequencies in the German population in the expanded control group were 50% for GSTM1 positive and 81% for GSTT1 positive genotypes. The observations are limited as the paper is sparsely reported and numbers of exposed ( $n = 4$ ) and unexposed ( $n = 15$ ) GSTT1 positive cases do not sum to the 79 cases with the GSTT1 positive genotype identified in the table's first row.

Moore et al. (2010) presents associations between TCE exposure and RCC risk stratified by GSTT genotype and for single nucleotide polymorphisms (SNPs) of the renal cysteine conjugate  $\beta$ -lyase gene. Genotyping was available for 925 of the 1,097 cases and 1,192 of the 1,476 controls. The percentage of cases and controls genotyped did not significantly differ among TCE-exposed and unexposed subjects nor was the active GSTT1 genotype association with kidney cancer risk (0.94, 95% CI: 0.75, 1.19). However, adopting statistical analysis

examining TCE exposure and kidney cancer that stratified on GSTT1 polymorphism as null (deleted allele) or active ( $\geq 1$  intact allele), Moore et al. (2010) reported significant associations for GSTT1 active genotype and no association was suggested for subjects with GSTT1 null genotype. The risk estimate for the association for TCE exposure and kidney cancer among subjects with an active GSTT1 genotype was 1.88 (95% CI: 1.06, 3.33), with higher risk estimates for long exposure duration, cumulative exposure, and average exposure intensity ( $\geq 13.5$  years, 2.13 [95% CI: 1.04, 4.39];  $\geq 1.58$  ppm-years, 2.59 [95% CI: 1.25, 5.35];  $\geq 0.076$  ppm, 2.77 [95% CI: 1.01, 7.58]) and a positive trend with increasing exposure duration, cumulative exposure or average intensity categories ( $p \leq 0.03$ ) (Moore et al., 2010). The associations between TCE exposure and kidney cancer was stronger for subjects with a functionally active GSTT1 than those for all subjects (both genotypes combined) (see Table 4-39). Moore et al. (2010) tested but did not find statistical interaction between GSTT1 genotype and TCE exposure ( $p \geq 0.17$ ). Moore et al. (2010) also examined the effect of polymorphisms of the cysteine conjugate  $\beta$ -lyase gene on TCE risk and reported interaction between TCE exposure and four minor alleles (SNPs rs2293968, rs2280841, rs2259043, and rs941960) ( $p < 0.05$ ). Associations with TCE exposure and kidney cancer were threefold higher compared to unexposed subjects with these SNPs.

Observations in Brüning et al. (1997a) and Wiesenhütter et al. (2007) must be interpreted cautiously. Few details were provided in these studies on selection criteria and not all subjects from the Brüning et al. (2003) case-control study were included. For GSTM1 positive, the higher prevalence among exposed cases in Brüning et al. (1997a) compared Wiesenhütter et al. (2007) and the lower prevalence among controls compared to background frequency in the European population may reflect possible selection biases. On the other hand, the broader base population included in Brüning et al. (2003) may explain the observed lower frequency of GSTM1 positive cases in Wiesenhütter et al. (2007). Moreover, Wiesenhütter et al. (2007) does not report genotype frequencies for controls by exposure status and this information is essential to an examination of whether RCC risk and TCE exposure may be modified by polymorphism status. The statistical analyses in both studies was a simple comparison of exposure prevalence between cases and controls and did not include analyses that stratified on exposure status. An examination of exposure prevalence is limited as Moore et al. (2010), too, reported TCE exposure prevalence as similar between exposed cases and controls. Associations between TCE exposure and kidney cancer for GSTT1 active genotype, however, were reported in stratified analyses. The more rigorous study design and statistical methods in Moore et al. (2010) affords more weight to their reported observations than for Brüning et al. (1997a) and Wiesenhütter et al. (2007). Moore et al. (2010) provides evidence of greater susceptibility to TCE exposure and kidney cancer among subjects with a functionally active GSTT polymorphism, particularly among those with certain alleles in single nucleotide polymorphisms of the cysteine conjugation  $\beta$ -lyase gene region.

Of the three larger (in terms of number of cases) studies that did provide results separately by sex, Dosemeci et al. (1999) suggested that there may be a sex difference for TCE exposure and RCC (OR: 1.04, [95% CI: 0.6, 1.7]) in males and 1.96 (95% CI: 1.0, 4.0 in females), while Raaschou-Nielsen et al. (2003) report the same standardized incidence ratio (SIR = 1.2) for both sexes and crude ORs calculated from data from the Pesch et al. (2000b) study (provided in a personal communication from Beate Pesch, Forschungsinstitut für Arbeitsmedizin, to Cheryl Scott, EPA, 21 February 2008) are 1.28 for males and 1.23 for females. Whether the Dosemeci et al. (1999) observations are due to susceptibility differences or to exposure differences between males and females cannot be evaluated. Blair et al. (1998) and Hansen et al. (2001) also present some results by sex, but these two studies have too few cases to be informative about a sex difference for kidney cancer.

#### 4.4.2.5. Meta-Analysis for Kidney Cancer

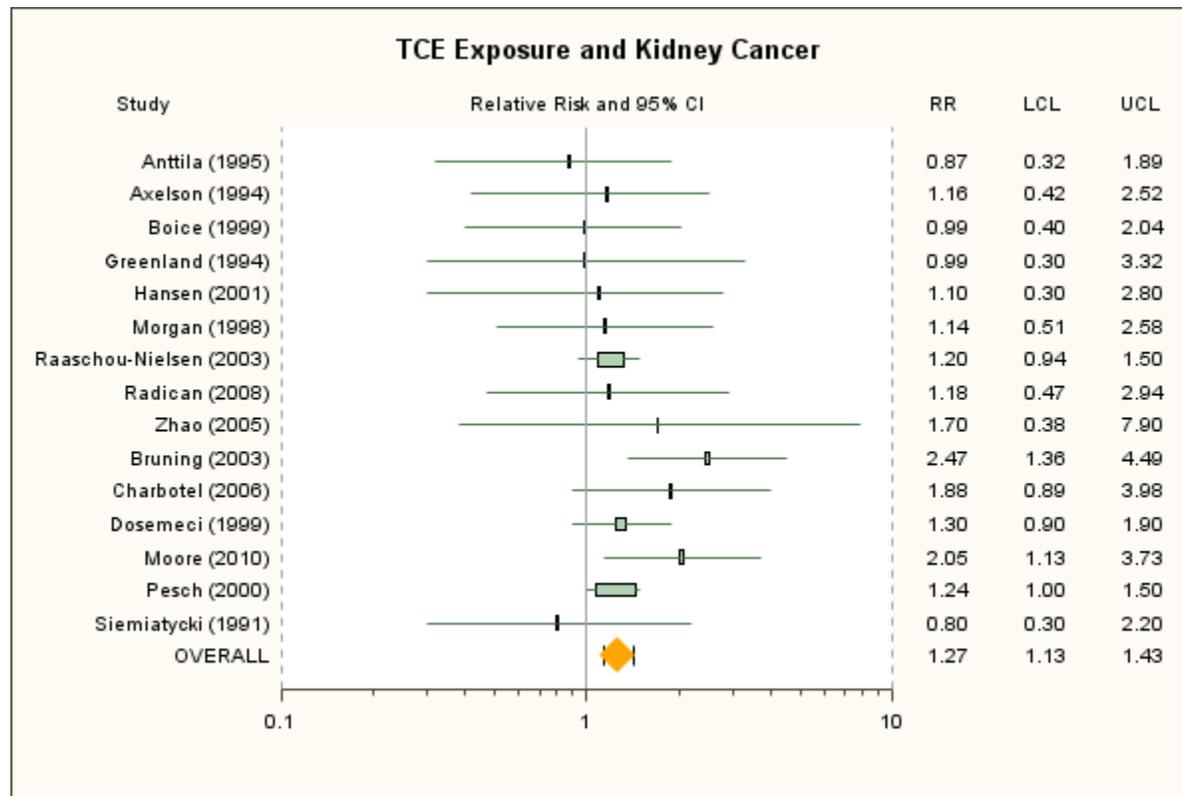
Meta-analysis (detailed methodology in Appendix C) was adopted as a tool for examining the body of epidemiologic evidence on kidney cancer and TCE exposure and to identify possible sources of heterogeneity. The meta-analyses of the overall effect of TCE exposure on kidney cancer suggest a small, statistically significant increase in risk that was stronger in a meta-analysis of the highest exposure group. There was no observable heterogeneity for any of the meta-analyses of the 15 studies and no indication of publication bias. Thus, these findings of increased risks of kidney cancer associated with TCE exposure are robust.

The meta-analysis of kidney cancer examines 15 cohort and case-control studies identified through a systematic review and evaluation of the epidemiologic literature on TCE exposure (Moore et al., 2010; Charbotel et al., 2006; Zhao et al., 2005; Brüning et al., 2003; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Pesch et al., 2000b; Boice et al., 1999; Dosemeci et al., 1999; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994; Siemiatycki, 1991). Details of the systematic review and meta-analysis of the TCE studies are fully discussed in Appendix B and C.

The summary relative risk (RR<sub>m</sub>) estimate from the primary random effects meta-analysis of the 15 studies was 1.27 (95% CI: 1.13, 1.43). The analysis was dominated by two (contributing almost 70% of the weight) or three (almost 80% of the weight) large studies (Raaschou-Nielsen et al., 2003; Pesch et al., 2000b; Dosemeci et al., 1999). Figure 4-1 arrays individual studies by their weight. No single study was overly influential; removal of individual studies resulted in RR<sub>m</sub> estimates that were all statistically significant ( $p < 0.005$ ) and ranged from 1.24 (with the removal of Brüning et al., (2003)) to 1.30 (with the removal of Raaschou-Nielsen et al., (2003)). Similarly, the overall RR<sub>m</sub> estimate was not highly sensitive to alternate RR estimate selections nor was publication bias apparent. There was no apparent heterogeneity across the 15 studies, i.e., the random effects model and the fixed effect model gave the same

results ( $p_{hetero} = 0.67$ ;  $I = 0\%$ ). Nonetheless, subgroup analyses were done examining the cohort and case-control studies separately with the random effects model; the resulting RRM estimates were 1.16 (95% CI: 0.96, 1.40) for the cohort studies and 1.48 (1.15, 1.91) for the case-control studies. There was no heterogeneity in the cohort subgroup ( $p = 0.998$ ;  $I^2 = 0\%$ ). There was heterogeneity in the case-control subgroup, but it was not statistically significant ( $p = 0.14$ ) and the  $I^2$  value of 41% suggests that the extent of the heterogeneity in this subgroup was low-to-moderate.

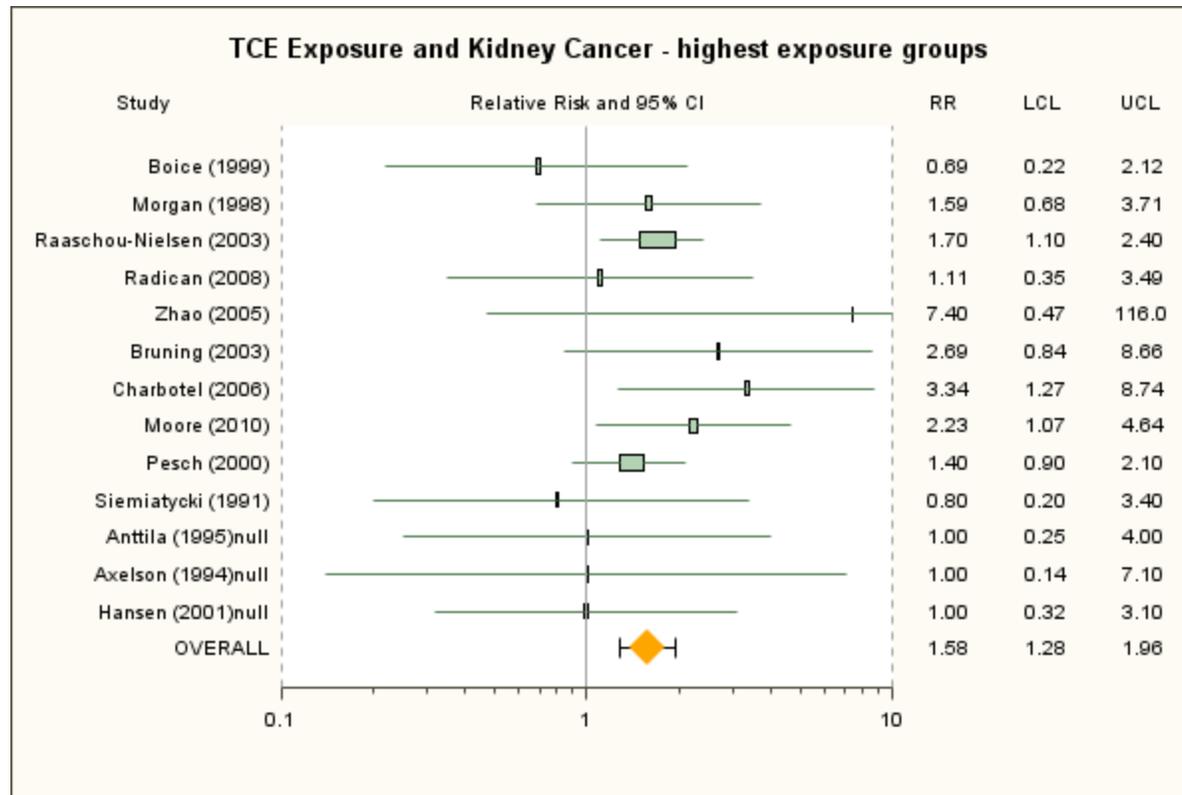
Ten studies reported risks for higher exposure groups ([Moore et al., 2010](#); [Charbotel et al., 2006](#); [Zhao et al., 2005](#); [Brüning et al., 2003](#); [Raaschou-Nielsen et al., 2003](#); [Pesch et al., 2000b](#); [Boice et al., 1999](#); [Dosemeci et al., 1999](#); [Morgan et al., 1998](#); [Siemiatycki, 1991](#)). Different exposure metrics were used in the various studies, and the purpose of combining results across the different highest exposure groups was not to estimate an RRM associated with some level of exposure. Instead, the focus on the highest exposure category was meant to result in an estimate less affected by exposure misclassification. In other words, it is more likely to represent a greater differential TCE exposure compared to people in the referent group than the exposure differential for the overall (typically any vs. none) exposure comparison. Thus, if TCE exposure increases the risk of kidney cancer, the effects should be more apparent in the highest exposure groups.



Random effects model; fixed effect model same. The summary estimate is in the bottom row, represented by the diamond. Symbol sizes reflect relative weights of the studies.

**Figure 4-1. Meta-analysis of kidney cancer and overall TCE exposure.**

The RRM estimate from the random effects meta-analysis of the studies with results presented for higher exposure groups was 1.64 (95% CI: 1.31, 2.04), higher than the RRM from the overall kidney cancer meta-analysis. As with the overall analyses, the meta-analyses of the highest-exposure groups were dominated by Pesch et al. (2000b) and Raaschou-Nielsen et al. (2003), which provided about 60% of the weight. Axelson et al. (1994), Anttila et al. (1995), and Hansen et al. (2001) do not report risk ratios for kidney cancer by higher exposure and a sensitivity analysis was carried out to address reporting bias. The RRM estimate from the primary random effects meta-analysis with null RR estimates (i.e., RR = 1.0) included for Axelson et al. (1994), Anttila et al. (1995), and Hansen et al. (2001) to address reporting bias associated with ever exposed was 1.58 (95% CI: 1.28, 1.96). Figure 4-2 arrays individual studies by their weight. The inclusion of these three additional studies contributed <7% of the total weight. No single study was overly influential; removal of individual studies resulted in RRM estimates that were all statistically significant ( $p < 0.005$ ) and that ranged from 1.52 [with the removal of Raaschou-Nielsen et al., (2003)] to 1.64 [with the removal of Pesch et al. (2000b)]. Similarly, the RRM estimate was not highly sensitive to alternate RR estimate selections (all with  $p < 0.0005$ ) and other than a negligible amount of heterogeneity observed in the sensitivity analysis with the Pesch JEM alternate ( $I^2 = 0.64\%$ ), there was no observable heterogeneity across the studies for any of the meta-analyses conducted with the highest-exposure groups, including those in which RR values for Anttila, Axelson, and Hansen were assumed ( $I^2 = 0\%$ ). For Pesch, the job-task exposure matrix (JTEM) approach is preferred because it seemed to be a more comprehensive and discriminating approach, taking actual job tasks into account, rather than just larger job categories. No subgroup analyses (e.g., cohort vs. case-control studies) were done with the highest exposure group results.



With assumed null RR estimates for Antilla, Axelson, and Hansen (see Appendix C text). Random effects model; fixed effect model same. The summary estimate is in the bottom row, represented by the diamond. Symbol sizes reflect relative weights of the studies.

**Figure 4-2. Meta-analysis of kidney cancer and TCE exposure—highest exposure groups.**

NRC (2006) deliberations on TCE commented on two prominent evaluations of the then-current TCE epidemiologic literature using meta-analysis techniques, Wartenberg et al. (2000) and Kelsh et al. (2005), submitted by Exponent-Health Sciences to NRC during their deliberations and who updated their analysis by including subsequently published studies of Boice et al. (2006b) and Charbotel et al. (2006) but not Radican et al. (2008), and presented summary relative risk (RRm) estimates for cohort and (Kelsh et al., 2005) case-control studies, separately, and combined (Kelsh et al., 2010). Wartenberg et al. (2000) reported an RRm of 1.7 (95% CI: 1.1, 2.7) for kidney cancer incidence in the TCE subcohorts (Blair et al., 1998; Anttila et al., 1995; Henschler et al., 1995; Axelson et al., 1994). For kidney cancer mortality in TCE subcohorts (Boice et al., 1999; Ritz, 1999a; Blair et al., 1998; Morgan et al., 1998; Henschler et al., 1995), Wartenberg et al. (2000) reported an RRm of 1.2 (95% CI: 0.8, 1.7). Kelsh et al. (2010) examined a slightly different grouping of cohort studies as did Wartenberg et al. (2000), presenting an RRm estimate for kidney cancer incidence and mortality combined. The RRm for kidney cancer in Group I cohort studies (Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994) was 1.34 (95% CI: 1.07–1.67) with no evidence of heterogeneity and, in Group II cohort studies, studies lacking documented TCE exposure (Chang et al., 2003; Henschler et al., 1995; Sinks et al., 1992; Seldén and Ahlborg, 1991; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988), was 1.58 (95% CI: 0.75, 3.32) with evidence of heterogeneity. Removing both Henschler et al. (1995) and Sinks et al. (1992), considered by Kelsh et al. (2010) as outliers, eliminated observed heterogeneity and the summary risk estimate was 0.88 (95% CI: 0.8, 1.33). Kelsh et al. (2010), also, presented separately a RRm for renal cancer case-control studies and TCE. For case-control studies (Charbotel et al., 2005; Brüning et al., 2003; Pesch et al., 2000b; Dosemeci et al., 1999; Vamvakas et al., 1998; Greenland et al., 1994; Siemiatycki, 1991), the RRm for RCC was 1.57 (95% CI: 1.06, 2.30) with evidence of heterogeneity, and 1.33 (95% CI: 1.02, 1.73) with no evidence of heterogeneity in a sensitivity analysis removing Vamvakas et al. (1998), a study Kelsh et al. (2010) considered as an outlier. Last, Kelsh et al. (2010) presented three RRm estimates for renal cell cancer Groups I and II cohort and case-control studies combined: 1.30 (95% CI: 1.04, 1.61) with evidence of heterogeneity and included 23 studies with kidney cancer risk estimates for all subjects, those with documented TCE exposure and those unexposed to TCE, and Ritz (1999a) in Group I studies; 1.42 (95% CI 1.13, 1.77) with evidence of heterogeneity and included 23 studies, with TCE subcohort kidney cancer risk estimates replacing the total cohort estimate for Group I studies; and 1.24 (95% CI; 1.06, 1.45) with no evidence of heterogeneity and included 20 studies, counting TCE subcohort kidney cancer risk estimates in Group I studies and removing the three studies Kelsh et al. (2010) considered as outliers.

The present analysis was conducted according to NRC (2006) suggestions for transparency, systematic review criteria, and examination of both cohort and case-control

studies. EPA's meta-analysis has several advantages to previous ones of TCE exposure and cancer. The selection criteria adopted in this meta-analysis were intended to identify informative studies for the evaluation of TCE exposure and cancer, studies with reduced systematic errors. Neither Henschler et al. (1995) nor Vamvakas et al. (1998), two studies with incomplete cohort identification or potential selection bias of study controls, met our inclusion criteria, and their inclusion in other meta-analysis may have contributed to the observed heterogeneity in kidney cancer RRm (Kelsh et al., 2010). Studies with background or low TCE exposure potential also did not meet another selection criterion as our analysis focused on TCE exposure potential inferred to each subject by reference to industrial hygiene records, individual biomarkers, JEMs, water distribution models, or questionnaire responses that likely had fewer biases associated with exposure misclassification, although this bias would not have been completely minimized. Inclusion of studies of lower exposure potential in meta-analyses can have important implications for identifying a cancer hazard (Vlaanderen et al., 2011; Zhang et al., 2009; Steinmaus et al., 2008). The present analysis includes the recently published studies of Charbotel et al. (2006), Moore et al. (2010), and updated mortality of the Blair et al. (1998) cohort by Radican et al. (2008). As discussed above, the summary estimate from the primary random effects meta-analysis of the 15 studies was 1.27 (95% CI: 1.13, 1.43). Additionally, EPA examined kidney cancer risk for higher exposure group. The RRm estimate from the random effects meta-analysis of the studies with results presented for higher exposure groups was 1.64 (95% CI: 1.31, 2.04), higher than the RRm from the overall kidney cancer meta-analysis, and 1.58 (95% CI: 1.28, 1.96) in the meta-analysis with null RR estimates (i.e., RR = 1.0) to address possible reporting bias for three studies.

#### 4.4.3. Human Studies of Somatic Mutation of *VHL* Gene

Studies have been conducted to identify mutations in the *VHL* gene in RCC patients, with and without TCE exposures (Wells et al., 2009; Toma et al., 2008; Charbotel et al., 2007; Furge et al., 2007; Brauch et al., 1999; Schraml et al., 1999; Kenck et al., 1996). Inactivation of the *VHL* gene through mutations, LOH, and imprinting has been observed in about 70% of sporadic renal clear cell carcinomas, the most common RCC subtype (Kenck et al., 1996). Other genes or pathways, including c-Myc activation and VEGF, have also been examined as to their role in various RCC subtypes (Toma et al., 2008; Furge et al., 2007). Furge et al. (2007) reported that there are molecularly distinct forms of RCC and possibly molecular differences between clear-cell RCC subtypes. This study was performed using tissues obtained from paraffin blocks. These results are supported by a more recent study that examined the genetic abnormalities of clear cell RCC using frozen tissues from 22 clear cell-RCC patients and paired normal tissues (Toma et al., 2008). This study found that 20 (91%) of the 22 cases had LOH on chromosome 3p (harboring the *VHL* gene). Alterations in copy number were also found on chromosome 9 (32% of cases), chromosome arm 14q (36% of cases), chromosome arm 5q (45% of cases), and

chromosome 7 (32% of cases), suggesting roles for multiple genetic changes in RCC, and is also supported by genomes-wide single-nucleotide polymorphism analysis ([Toma et al., 2008](#)).

Several papers link mutation of the *VHL* gene in RCC patients to TCE exposure. These reports are based on comparisons of *VHL* mutation frequencies in TCE-exposed cases from RCC case-control studies or from comparison to background mutation rates among RCC case series (see Table 4-42). Brüning et al. ([1997b](#)) first reported a high somatic mutation frequency (100%) in a series of 23 RCC cases with medium to high intensity TCE exposure as determined by an abnormal single strand conformation polymorphism (SSCP) pattern, with most variations found in exon two. Only four samples were sequenced at the time of publication and showed mutations in exon one, two and three (see Table 4-42). Some of the cases in this study were from the case-control study of Vamvakas et al. ([1998](#)) (see Section 4.4.3 and Appendix B).

Brauch et al. ([2004](#); [1999](#)) analyzed renal cancer cell tissues for mutations of the *VHL* gene and reported increased occurrence of mutations in patients exposed to high concentrations of TCE. In the first study ([Brauch et al., 1999](#)), an employer's liability or worker's compensation registry was used to identify 44 RCC cases, 18 of whom were also included in Brüning et al. ([1997b](#)). Brauch et al. ([1999](#)) found multiple mutations in 42% of the exposed patients who experienced any mutation and 57% showed loss of heterozygosity. A hot spot mutation of cytosine to thymine at nucleotide 454 (C454T) was found in 39% of samples that had a *VHL* mutation and was not found in renal cell cancers from nonexposed patients or in lymphocyte DNA from either exposed or nonexposed cases or controls. As discussed above, little information was given on how subjects were selected and whether there was blinding of from the RCC case-control study of Vamvakas et al. ([1998](#)). Brauch et al. ([2004](#)) compared age at diagnosis and histopathologic parameters of tumors as well as somatic mutation characteristics in the *VHL* tumor suppressor gene between the TCE-exposed and non-TCE-exposed RCC patient groups (TCE-exposed from their previous 1999 publication to the non TCE-exposed cases newly sequenced in this study). RCC did not differ with respect to histopathologic characteristics in either patient group. Comparing results from TCE-exposed and nonexposed patients revealed clear differences with respect to: (1) frequency of somatic *VHL* mutations; (2) incidence of C454T transition; and (3) incidence of multiple mutations. The C454T hot spot mutation at codon 81 was exclusively detected in tumors from TCE-exposed patients, as were multiple mutations. Also, the incidence of *VHL* mutations in the TCE-exposed group was at least twofold higher than in the nonexposed group. Overall, these findings support the view that the effect of TCE is not limited to clonal expansion of cells mutated spontaneously or by some other agent.

**Table 4-42. Summary of human studies on somatic mutations of the *VHL* gene<sup>a</sup>**

TCE exposure status	Brüning et al. (1997b)	Brauch et al. (1999)		Schraml et al. (1999)		Brauch et al. (2004)		Charbotel et al. (2007)	
	Exposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed
Number of subjects/ number with mutations (%)	23/23 (100%)	44/33 (75%)	73/42 (58%)	9/3 (33%)	113/38 (34%)	17/14 (82%)	21/2 (10%)	25/2 (9%)	23/2 (8%)
RCC subtype	Unknown	Unknown		Clear cell 9 (75%) Papillary 2 (18%) Oncocytomas 1 (8%)	Unknown	Clear cell 37 (%) Oncocytic adenoma 1 (%) Bilateral metachronous 1 (%)	Clear cell 51 (75%) Papillary 10 (10–15%) Chromophobe 4 (5%) Oncocytomas 4 (5%)		
Tissue type analyzed	Paraffin	Paraffin, fresh (lymphocyte)		Paraffin		Paraffin		Paraffin, frozen tissues, Bouin's fixative	
Assay	SSCP <sup>b</sup> , sequencing <sup>b</sup>	SSCP, sequencing, restriction enzyme digestion		CGH, sequencing		Sequencing		Sequencing	
Number of mutations	23	50	42	4	50	24	2	2	2
Type of mutation									
Missense	1	27	NA	1	Unknown	17	2	1	1
Nonmissense <sup>c</sup>	3	23	NA	3	Unknown	7	0	1	1

<sup>a</sup>Adapted from NRC (2006) with addition of Schraml et al. (1999) and Charbotel et al. (2007).

<sup>b</sup>By SSCP. Four (4) sequences confirmed by comparative genomic hybridization.

<sup>c</sup>Includes insertions, frameshifts, and deletions.

CGH = comparative genomic hybridization

Brauch et al. (2004) were not able to analyze all RCCs from the Vamvakas study (Vamvakas et al., 1998), in part because samples were no longer available. Using the data described by Brauch et al. (2004) (*VHL* mutation found in 15 exposed and 2 nonexposed individuals, and *VHL* mutation not found in 2 exposed and 19 unexposed individuals), the calculated OR is 71.3. The lower bound of the OR including the excluded RCCs is derived from the assumption that all 20 cases that were excluded were exposed but did not have mutations in *VHL* (*VHL* mutations were found in 15 exposed and 2 unexposed individuals and *VHL* was not found in 22 exposed and 18 unexposed individuals), leading to an OR of 6.5 that remains statistically significant.

Charbotel et al. (2007) examines somatic mutations in the three *VHL* coding exons in RCC cases from their case-control study (Charbotel et al., 2006). Of the 87 RCCs in the case-control study, tissue specimens were available for 69 cases (79%) of which 48 were clear cell-RCC. *VHL* sequencing was carried out for only the clear cell-RCC cases, 66% of the 73 clear cell-RCC cases in Charbotel et al. (2006). Of the 48 clear cell-RCC cases available for *VHL* sequencing, 15 subjects were identified with TCE exposure (31%), an exposure prevalence lower than 43% observed in the case-control study. Partial to full sequencing of the *VHL* gene was carried out using polymerase chain reaction (PCR) amplification and *VHL* mutation pattern recognition software of Bérout et al. (1998). Full sequencing of the *VHL* gene was possible for only 26 RCC cases (36% of all RCC cases). Single point mutations were identified in four cases (8% prevalence): two unexposed cases, a G > C mutation in exon 2 splice site and a G > A in exon 1; one case identified with low/medium exposure, T > C mutation in exon 2, and, one case identified with high TCE exposure, T > C in exon 3. It should be noted that the two cases with T > C mutations were smokers unlike the cases with G > A or G > C mutations. The prevalence of somatic *VHL* mutation in this study is quite low compared to that observed in other RCC case series from this region; around 50% (Gallou et al., 2001; Bailly et al., 1995). To address possible bias from misclassification of TCE exposure, Charbotel et al. (2006) examined renal cancer risk for jobs associated with a high level of confidence for TCE exposure. As would be expected if bias was a result of misclassification, they observed a stronger association between higher confidence TCE exposure and RCC, suggesting that some degree of misclassification bias is associated with their broader exposure assessment approach. Charbotel et al. (2007) do not present findings on *VHL* mutations for those subjects with higher level of confidence TCE exposure assignment.

Schraml et al. (1999) did not observe statistically significant differences in DNA sequence or mutation type in a series of 12 RCCs from subjects exposed to solvents including varying TCE intensity and a parallel series of 113 clear cell carcinomas from non-TCE exposed patients. Only nine of the RCC were clear cell-RCC and were sequenced for mutations. *VHL* mutations were observed in clear cell tumors only; 4 mutations in 3 TCE-exposed subjects compared to 50 mutations in tumors of 38 nonexposed cases. Details as to exposure conditions

are limited to a statement that subjects had been exposed to high doses of solvents, potential for mixed solvent exposures, and that exposure included a range of TCE concentrations. Limitations of this study include having a wider range of TCE exposure intensities as compared to the studies described above ([Brauch et al., 1999](#); [Brüning et al., 1997b](#)), which focused on patients exposed to higher levels of TCE, and the limited number of TCE-exposed subjects analyzed, being the smallest of all available studies on RCC, TCE, and *VHL* mutation. For these reasons, Schraml et al. ([1999](#)) is quite limited for examining the question of *VHL* mutations and TCE exposure.

Szymańska et al. ([2010](#)) examined somatic mutations in three *VHL* coding exons in 359 RCC cases, 334 with clear-cell carcinomas, from the case-control study of Moore et al. ([2010](#)) as part of a pilot examination of mutation in three other genes, TP53, EGFR, and KRAS. The prevalence of *VHL* mutations was high in the RCC series, 72% of the tumors carried at least one function mutation. Although occupational exposures were not examined and data were not presented, Szymańska et al. ([2010](#)) reported that *VHL* mutations were not associated with TCE exposure.

A number of additional methodological issues need to be considered in interpreting these studies. Isolation of DNA for mutation detection has been performed using various tissue preparations, including frozen tissues, formalin fixed tissues and tissue sections fixed in Bouin's solution. Ideally, studies would be performed using fresh or freshly frozen tissue samples to limit technical issues with the DNA extraction. When derived from other sources, the quality and quantity of the DNA isolated can vary, as the formic acid contained in the formalin solution, fixation time and period of storage of the tissue blocks often affect the quality of DNA. Picric acid contained in Bouin's solution is also known to degrade nucleic acids resulting in either low yield or poor quality of DNA. In addition, during collection of tumor tissues, contamination of neighboring normal tissue can easily occur if proper care is not exercised. This could lead to the 'dilution effect' of the results—i.e., because of the presence of some normal tissue, frequency of mutations detected in the tumor tissue can be lower than expected. These technical difficulties are discussed in these papers, and should be considered when interpreting the results.

Additionally, selection bias is possible given tissue specimens were not available for all RCC cases in Vamvakas et al. ([1998](#)) or in Charbotel et al. ([2006](#)). Some uncertainty associated with misclassification bias is possible given the lack of TCE exposure information to individual subjects in Schraml et al. ([1999](#)) and in Charbotel et al. ([2007](#)) from their use of broader exposure assessment approach compared to that associated with the higher confident exposure assignment approach. A recent study by Nickerson et al. ([2008](#)) addresses many of these concerns by utilizing more sensitive methods to look at both the genetic and epigenetic issues related to *VHL* inactivation. This study was performed on DNA from frozen tissue samples and used a more sensitive technique for analysis for mutations (endonuclease scanning) as well as analyzing for methylation changes that may lead to inactivation of the *VHL* gene. This method of analysis was validated on tissue samples with known mutations. Of the 205 clear cell-RCC

samples analyzed, 169 showed mutations in the *VHL* gene (82.4%). Of those 36 without mutation, 11 were hypermethylated in the promoter region, which will also lead to inactivation of the *VHL* gene. Therefore, this study showed inactivating alterations in the *VHL* gene (either by mutation or hypermethylation) in 91% tumor samples analyzed.

The limited animal studies examining the role of *VHL* mutation following exposure to chemicals including TCE are described below in Section 4.4.6.1.1. Conclusions as to the role of *VHL* mutation in TCE-induced kidney cancer, taking into account both human and experimental data, are presented below in Section 4.4.7.

#### **4.4.4. Kidney Noncancer Toxicity in Laboratory Animals**

Acute, subchronic, and chronic exposures to TCE cause toxicity to the renal tubules in rats and mice of both sexes, via both inhalation (see Table 4-43) and oral (see Table 4-44) exposures. Nephrotoxicity from acute exposures to TCE has only been reported at relatively high doses, although histopathological changes have not been investigated in these experiments. Information about specific location of lesions is presented where available. TCE exposure for 13-weeks (corn oil gavage) led to increased nephrotoxicity but no significant increases in preneoplastic or neoplastic lesions as compared to controls ([Mally et al., 2006](#)). Chronic nephropathy was also observed in both sexes of Osborne-Mendel rats following exposure to TCE (549 and 1,097 mg/kg-day, 78 week). Chakrabarti and Tuchweber ([1988](#)) found that TCE administered to male F344 rats by i.p. injection (723–2,890 mg/kg) or by inhalation (1,000–2,000 ppm for 6 hours) produced elevated urinary NAG, GGT, glucose excretion, blood urea nitrogen (BUN), and high molecular weight protein excretion, characteristic signs of proximal tubular, and possibly glomerular injury, as soon as 24 hours postexposure. In the i.p. injection experiments, inflammation was observed, although some inflammation is expected due to the route of exposure, and nephrotoxicity effects were only statistically significantly elevated at the highest dose (2,890 mg/kg). In the inhalation experiments, the majority of the effects were statistically significant at both 1,000 and 2,000 ppm. Similarly, at these exposures, renal cortical slice uptake of *p*-aminohippurate was inhibited, indicating reduced proximal tubular function. Cojocel et al. ([1989](#)) found similar effects in mice administered TCE by i.p. injection (120–1,000 mg/kg) at 6 hours postexposure, such as the dose-dependent increase in plasma BUN concentrations and decrease in *p*-aminohippurate accumulation in renal cortical slices. In addition, malondialdehyde (MDA) and ethane production were increased, indicating lipid peroxidation.

**Table 4-43. Inhalation studies of kidney noncancer toxicity in laboratory animals**

Reference <sup>a</sup>	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Kidney effect(s) discussed in Section 4.4.4
Chakrabarti and Tuchweber (1988)	F344 rats (M)	Inhalation	0–20,00 ppm, 6 hrs	6/group	Increased signs of proximal tubular damage.
Green et al. (1998)	F344 rats (M)	Inhalation	0, 250, and 500 ppm, 6 hrs/d for 1, 7, 15, 21, 28 d	3–5/group	Increased formic acid excretion; plasma and urinary markers of nephrotoxicity unchanged.
<b>Kjellstrand et al. (1983a)</b>	NMRI mice (M and F)	<b>Inhalation</b>	<b>0–3,600 ppm, variable time periods of 1–24 hrs/d, for 30 or 120 d</b>	<b>10–20/group</b>	<b>Increased kidney weight.</b>
<b>Maltoni et al. (1986)</b>	<b>Sprague-Dawley rats, (M and F) B6C3F<sub>1</sub> mice (M and F)</b>	<b>Inhalation</b>	<b>0, 100, 300, and 600 ppm, 7 hrs/d, 5 d/wk, 104 wks exposure, observed for lifespan</b>	<b>116–141/group</b>	<b>Meganeucleocytosis in male rats (Details in Table 4-49).</b>
Mensing et al. (2002)	Long-Evans rats (M)	Inhalation	0–500 ppm, 6 hrs/5 d/wk, 6 mo	5/group	Increased signs of nephrotoxicity.
<b>Woolhiser et al. (2006)</b>	<b>Sprague-Dawley rats (F)</b>	<b>Inhalation</b>	<b>0, 100, 300, and 1,000 ppm, 6 hrs/d, 5 d/wk, 4 wks</b>	<b>16/group</b>	<b>Increased kidney weight.</b>

<sup>a</sup>**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

**Table 4-44. Oral and i.p. studies of kidney noncancer toxicity in laboratory animals**

Reference <sup>a</sup>	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Kidney effect(s) discussed in Section 4.4.4
Chakrabarti and Tuchweber (1988)	F344 rats (M)	i.p. injection	0–2,890 mg/kg-d	6/group	Increased signs of proximal tubular damage.
Cojocel et al. (1989)	NMRI mice (M)	i.p. injection (sesame oil)	0–1,000 mg/kg	4/group	Increased signs of nephrotoxicity.
Green et al. (1997a)	F344 rats (M) B6C3F <sub>1</sub> mice (M)	Gavage (corn oil)	0, 500, and 2,000 mg/kg-d, 1 or 10 d	5 or 10/group	Increases in biochemical markers of kidney damage.
Green et al. (2003)	F344 rats (M)	Drinking water	0–54.3 mg/kg-d, 52 wks	60/group	Increased kidney weights and tubular degeneration.
Mally et al. (2006)	Eker rat (M)	Gavage (corn oil)	0–1,000 mg/kg-d body weight, 5 d/wk, 13 wks	10/group	Increased nephrotoxicity.
Maltoni et al. (1986)	Sprague-Dawley rats (M and F)	Gavage (olive oil)	0, 50, and 250 mg/kg-d 4–5 d/wk, 52 wks	30/group	Megakaryocytosis in male rats (details in Table 4.47).
<b>NCI (1976)</b>	<b>Osborne-Mendel rats (M and F) B6C3F<sub>1</sub> mice (M and F)</b>	<b>Gavage (corn oil)</b>	<b>0–2,339 mg/kg-d, variable doses, 5 d/wk, 78 wks</b>	<b>50/group</b>	<b>Toxic nephrosis in all exposed animals (details in Table 4.46).</b>
<b>NTP (1988)</b>	<b>ACI, August, Marshall, and Osborne-Mendel rats (M and F)</b>	<b>Gavage (corn oil)</b>	<b>0, 500, and 1,000 mg/kg-d, 5 d/wk, 103 wks</b>	<b>50/group</b>	<b>Cytomegaly and toxic nephropathy observed in all exposed rats (details in Table 4-48).</b>
<b>NTP (1990)</b>	<b>F344 rats (M and F) B6C3F<sub>1</sub> mice (M and F)</b>	<b>Gavage (corn oil)</b>	<b>Rats: 0–2,000 mg/kg-d, Mice: 0–6,000 mg/kg-d, 5d/wk, 13 wks</b>	<b>10/group</b>	<b>Cytomegaly and karyomegaly of renal tubular epithelium in mice and rats (details in Table 4-45).</b>
Peden-Adams et al. (2008)	MRL mice (M and F)	Drinking water	0; 1,400; and 14,000 ppb; lifetime	6/group	Increased kidney weight in male mice.

<sup>a</sup>**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

Kidney weight increases have been observed following inhalation exposure to TCE in both mice (Kjellstrand et al. (1983a) and rats (Woolhiser et al., 2006) and following lifetime drinking water exposure in a genetically-prone murine model (Peden-Adams et al., 2008). Kjellstrand et al. (1983a) demonstrated an increase in kidney weights in both male (20% compared to control) and female (10% compared to control) mice following intermittent and continuous TCE whole-body inhalation exposure (up to 120 days). This increase was significant in males as low as 75 ppm exposure and in females starting at 150 ppm. The latter inhalation study, an unpublished report by Woolhiser et al. (2006), was designed to examine immunotoxicity of TCE but also contains information regarding kidney weight increases in female Sprague-Dawley rats exposed to 0-, 100-, 300-, and 1,000-ppm TCE for 6 hours/day, 5 days/week, for 4 weeks. Relative kidney weights were significantly elevated (17.4% relative to controls) at 1,000 ppm. However, the small number of animals and the variation in initial animal weight limit the ability of this study to determine statistically significant increases. The Peden-Adams et al. (2008) study was designed to assess the effects of TCE exposure in a genetically-prone murine lupus model. Although the study did not demonstrate an increase in the development of autoimmune disease markers (for details, see Section 4.6.2), changes in body weight and organ weights in males were observed. Following lifetime exposure to TCE (14,000 ppb) in drinking water, males exhibited a decreasing trend in body mass of 12% from controls (female body weights not altered). Spleen, thymic, and kidney mass in females were not altered following exposure to TCE, while an 18% increase in kidney mass was observed in the high-dose treatment group (14,000 ppb) in males.

Similarly, overt signs of subchronic nephrotoxicity, such as changes in blood or urinary biomarkers, are also primarily a high-dose phenomenon, although histopathological changes are evident at lower exposures. Green et al. (1997a) reported administration of 2,000 mg/kg-day TCE by corn oil gavage for 42 days in F344 rats caused increases of around twofold of control results in urinary markers of nephrotoxicity such as urine volume and protein (both 1.8 ×), NAG (1.6 ×), glucose (2.2 ×) and alkaline phosphatase (ALP; 2.0 ×), similar to the results of the acute study of Chakrabarti and Tuchweber (1988), above. No morphological changes were observed in kidneys from any animals (Green et al., 1997a). At lower dose levels, Green et al. (1998) reported that plasma and urinary markers of nephrotoxicity were unchanged. In particular, after 1–28 day exposures to 250 or 500 ppm TCE for 6 hours/day, there were no statistically significant differences in plasma levels of BUN or in urinary levels of creatinine, protein, ALP, NAG, or GGT. However, increased urinary excretion of formic acid, accompanied by changes in urinary pH and increased ammonia, was found at these exposures. Interestingly, at the same exposure level of 500 ppm (6 hours/day, 5 days/week, for 6 months), Mensing et al. (2002) reported elevated excretion of low molecular weight proteins and NAG, biomarkers of nephrotoxicity, but after the longer exposure duration of 6 months.

Numerous studies have reported histological changes from TCE exposure for subchronic and chronic durations ([Mensing et al., 2002](#); [NTP, 1990](#); [Maltoni et al., 1988](#); [NTP, 1988](#); [Maltoni et al., 1986](#)). As summarized in Table 4-45, in 13-week studies in F344 rats and B6C3F<sub>1</sub> mice, NTP ([1990](#)) reported relatively mild cytomegaly and karyomegaly of the renal tubular epithelial cells at the doses 1,000–6,000 mg/kg-day (at the other doses, tissues were not examined). The NTP report noted that “these renal effects were so minimal that they were diagnosed only during a reevaluation of the tissues... prompted by the production of definite renal toxicity in the 2-year study.” In the 6-month, 500-ppm inhalation exposure experiments of Mensing et al. ([2002](#)), some histological changes were noted in the glomeruli and tubuli of exposed rats, but they provided no detailed descriptions beyond the statement that “perivascular, interstitial infections and glomerulonephritis could well be detected in kidneys of exposed rats.”

**Table 4-45. Summary of renal toxicity and tumor findings in gavage studies of TCE by NTP ([1990](#))<sup>a</sup>**

Sex	Dose (mg/kg) <sup>b</sup>	Cytomegaly and karyomegaly incidence (severity <sup>c</sup> )	Adenoma (overall; terminal)	Adenocarcinoma (overall; terminal)
1/d, 5 d/wk, 13-wk study, F344/N rats				
Male	0, 125, 250, 500, 100	Tissues not evaluated	None reported	
	2,000	8/9 (minimal/mild)		
Female	0, 62.5, 125, 250, 500	Tissues not evaluated		
	1,000	5/10 (equivocal/minimal)		
1/d, 5 d/wk, 13-wk study, B6C3F <sub>1</sub> mice				
Male	0, 375, 750, 1,500	Tissues not evaluated	None reported	
	3,000	7/10 <sup>d</sup> (mild/moderate)		
	6,000	<sup>e</sup>		
Female	0, 375, 750, 1,500	Tissues not evaluated		
	3,000	9/10 (mild/moderate)		
	6,000	1/10 (mild/moderate)		
1/d, 5 d/wk, 103-wk study, F344/N rats				
Male	0	0% (0)	0/48; 0/33	0/48; 0/33
	500	98% (2.8)	2/49; 0/20	0/49; 0/20
	1,000	98% (3.1)	0/49; 0/16	3/49; 3/16 <sup>f</sup>
Female	0	0% (0)	0/50; 0/37	0/50; 0/37
	500	100% (1.9)	0/49; 0/33	0/49; 0/33
	1,000	100% (2.7)	0/48; 0/26	1/48; 1/26
1/d, 5 d/wk, 103-wk study, B6C3F <sub>1</sub> mice				
Male	0	0% (0)	1/49; 1/33	0/49; 0/33
	1,000	90% (1.5)	0/50; 0/16	1/50; 0/16
Female	0	0% (0)	0/48; 0/32	0/48; 0/32
	1,000	98% (1.8)	0/49; 0/23	0/49; 0/23

<sup>a</sup>Study carried forward for consideration in dose-response assessment (see Chapter 5).

<sup>b</sup>Corn oil vehicle.

<sup>c</sup>Numerical scores reflect the average grade of the lesion in each group (1, slight; 2, moderate; 3, well marked; and 4, severe).

<sup>d</sup>Observed in four mice that died after 7–13 weeks and in three that survived the study.

<sup>e</sup>All mice died during the first week.

<sup>f</sup>*p* = 0.028.

After 1–2 years of chronic TCE exposure by gavage (NTP, 1990, 1988; NCI, 1976) or inhalation (Maltoni et al., 1988; Maltoni et al., 1986) (see Tables 4-45 to 4-49), both the incidence and severity of these effects increases, with mice and rats exhibiting lesions in the tubular epithelial cells of the inner renal cortex that are characterized by cytomegaly, karyomegaly, and toxic nephrosis. As with the studies at shorter duration, these chronic studies reported cytomegaly and karyomegaly of tubular cells. NTP (1990) specified the area of damage as the pars recta, located in the corticomedullary region. It is important to note that these effects are distinct from the chronic nephropathy and inflammation observed in control mice and rats (Lash et al., 2000b; Maltoni et al., 1988; Maltoni et al., 1986; NCI, 1976).

**Table 4-46. Summary of renal toxicity and tumor findings in gavage studies of TCE by NCI (1976)<sup>a</sup>**

Sex	Dose (mg/kg) <sup>b</sup>	Toxic nephrosis (overall; terminal)	Adenoma or adenocarcinoma (overall; terminal) <sup>c</sup>
<b>1/d, 5 d/wk, 2-yr study, Osborne-Mendel rats</b>			
Males	0	0/20; 0/2	0/20; 0/2
	549	46/50; 7/7	1/50; <sup>d</sup> 0/7
	1,097	46/50; 3/3	0/50; 0/3
Females	0	0/20; 0/8	0/20; 0/8
	549	39/48; 12/12	0/48; 0/12
	1,097	48/50; 13/13	0/50; 0/13
<b>1/d, 5 d/wk, 2-yr study, B6C3F<sub>1</sub> mice</b>			
Males	0	0/20; 0/8	0/20; 0/8
	1,169	48/50; 35/35	0/50; 0/35
	2,339	45/50; 20/20	1/50; <sup>e</sup> 1/20
Females	0	0/20; 0/17	0/20; 0/17
	869	46/50; 40/40	0/50; 0/40
	1,739	46/47; <sup>f</sup> 39/39	0/47; 0/39

<sup>a</sup>Study carried forward for consideration in dose-response assessment (see Chapter 5).

<sup>b</sup>Treatment period was 48 weeks for rats, 66 weeks for mice. Doses were changed several times during the study based on monitoring of body weight changes and survival. Dose listed here is the TWA dose over the days on which animals received a dose.

<sup>c</sup>A few malignant mixed tumors and hamartomas of the kidney were observed in control and low-dose male rats, but are not counted here.

<sup>d</sup>Tubular adenocarcinoma.

<sup>e</sup>Tubular adenoma.

<sup>f</sup>One mouse was reported with “nephrosis,” but not “nephrosis toxic,” and so was not counted here.

**Table 4-47. Summary of renal toxicity findings in gavage studies of TCE by Maltoni et al. (1988; 1986)**

Sex	Dose (mg/kg) <sup>a</sup>	Megalonucleocytosis <sup>b</sup> (overall; corrected <sup>c</sup> )
1/d, 4–5 d/wk, 52-wk exposure, observed for lifespan, Sprague-Dawley rats		
Males	0	0/20; 0/22
	50	0/30; 0/24
	250	14/30; 14/21
Females	0	0/30; 0/30
	50	0/30; 0/29
	250	0/30; 0/26

<sup>a</sup>Olive oil vehicle.

<sup>b</sup>Renal tubuli megalonucleocytosis is the same as cytomegaly and karyomegaly of renal tubuli cells (Maltoni et al., 1988; Maltoni et al., 1986).

<sup>c</sup>Denominator for “corrected” incidences is the number of animals alive at the time of the first kidney lesion in this experiment (39 weeks).

**Table 4-48. Summary of renal toxicity and tumor incidence in gavage studies of TCE by NTP (1988)<sup>a</sup>**

Sex	Dose (mg/kg) <sup>b</sup>	Cytomegaly	Toxic nephropathy	Adenoma (overall; terminal)	Adenocarcinoma (overall; terminal)
1/d, 5 d/wk, 2-yr study, ACI rats					
Male	0	0/50	0/50	0/50; 0/38	0/50; 0/38
	500	40/49	18/49	0/49; 0/19	1/49; 0/19
	1,000	48/49	18/49	0/49; 0/11	0/49; 0/11
Female	0	0/48	0/48	0/48; 0/34	0/48; 0/34
	500	43/47	21/47	2/47; 1/20	1/47; 1/20
	1,000	42/43	19/43	0/43; 0/19	1/43; 0/19
1/d, 5 d/wk, 2-yr study, August rats					
Male	0	0/50	0/50	0/50; 0/21	0/50; 0/21
	500	46/50	10/50	1/50; 0/13	1/50; 1/13
	1,000	46/49	31/49	1/49; 1/16	0/49; 0/16
Female	0	0/49	0/49	1/49; 1/23	0/49; 0/23
	500	46/48	8/48	2/48; 1/26	2/48; 2/26
	1,000	50/50	29/50	0/50; 0/25	0/50; 0/25
1/d, 5 d/wk, 2-yr study, Marshall rats					
Male	0	0/49	0/49	0/49; 0/26	0/49; 0/26
	500	48/50	18/50	1/50; 0/12	0/50; 0/12
	1,000	47/47	23/47	0/47; 0/6	1/47; 0/6
Female	0	0/50	0/50	1/50; 0/30	0/50; 0/30
	500	46/48	30/48	1/48; 1/12	1/48; 0/12
	1,000	43/44	30/44	0/44; 0/10	1/44; 1/10
1/d, 5 d/wk, 2-yr study, Osborne-Mendel rats					
Male	0	0/50	0/50	0/50; 0/22	0/50; 0/22
	500	48/50	39/50	6/50; 5/17	0/50; 0/17
	1,000	49/50	35/50	1/50; 1/15	1/50; 0/15
Female	0	0/50	0/50	0/50; 0/20	0/50; 0/20
	500	48/50	30/50	0/50; 0/11	0/50; 0/11
	1,000	49/49	39/49	1/49; 0/7	0/49; 0/7

<sup>a</sup>Study carried forward for consideration in dose-response assessment (see Chapter 5).

<sup>b</sup>Corn oil vehicle.

**Table 4-49. Summary of renal toxicity and tumor findings in inhalation studies of TCE by Maltoni et al. (1988; 1986)<sup>a</sup>**

Sex	Concentration (ppm)	Meganeucleocytosis <sup>b</sup> (overall; corrected)	Adenoma (overall; corrected)	Adenocarcinoma (overall; corrected)
7 hrs/d, 5 d/wk, 2-yr exposure, observed for lifespan, Sprague-Dawley rats <sup>c</sup>				
Male	0	0/135; 0/122	0/135; 0/122	0/135; 0/122
	100	0/130; 0/121	1/130; 1/121	0/130; 0/121
	300	22/130; 22/116	0/130; 0/116	0/130; 0/116
	600	101/130; 101/124	1/130; 1/124	4/130; 4/124
Female	0	0/145; 0/141	0/145; 0/141	0/145; 0/141
	100	0/130; 0/128	1/130; 1/128	0/130; 0/128
	300	0/130; 0/127	0/130; 0/127	0/130; 0/127
	600	0/130; 0/127	0/130; 0/127	1/130; 1/127
7 hrs/d, 5 d/wk, 78-wk exposure, observed for lifespan, B6C3F <sub>1</sub> mice <sup>d</sup>				
Male	0	0/90	0/90	0/90
	100	0/90	0/90	1/90
	300	0/90	0/90	0/90
	600	0/90	0/90	0/90
Female	0	0/90	0/90	1/90
	100	0/90	0/90	0/90
	300	0/90	0/90	0/90
	600	0/90	0/90	0/90

<sup>a</sup>Study carried forward for consideration in dose-response assessment (see Chapter 5); three inhalation experiments in this study found no renal megalonucleocytosis, adenomas, or adenocarcinomas: BT302 (8-week exposure to 0, 100, or 600 ppm in Sprague-Dawley rats); BT303 (8-week exposure to 0, 100, or 600 ppm in Swiss mice); and BT305 (78-week exposure to 0, 100, 300, or 600 ppm in Swiss mice).

<sup>b</sup>Renal tubuli meganeucleocytosis is the same as cytomegaly and karyomegaly of renal tubuli cells (Maltoni et al., 1988; Maltoni et al., 1986).

<sup>c</sup>Combined incidences from experiments BT304 and BT304bis. Corrected incidences reflect number of rats alive at 47 weeks, when the first renal tubular megalonucleocytosis in these experiments appeared.

<sup>d</sup>Female incidences are from experiment BT306, while male incidences are from experiment BT306bis, which was added to the study because of high, early mortality due to aggressiveness and fighting in males in experiment BT306. Corrected incidences not show, because only the renal adenocarcinomas appeared at 107 weeks in the male and 136 weeks in the female, when the most of the mice were already deceased.

These effects of TCE on the kidney appear to be progressive. Maltoni et al. (1988; 1986) noted that the incidence and degree of renal toxicity increased with increased exposure time and increased time from the start of treatment. As mentioned above, signs of toxicity were present in the 13 week study (NTP, 1988), and NTP (1990) noted cytomegaly at 26 weeks. NTP (1990) noted that as “exposure time increased, affected tubular cells continued to enlarge and additional tubules and tubular cells were affected,” with toxicity extending to the cortical area as kidneys became more extensively damaged. NTP (1990, 1988) noted additional lesions that increased in frequency and severity with longer exposure, such as dilation of tubules and loss of tubular cells lining the basement membrane (“stripped appearance” (NTP, 1988) or flattening of these cells

([NTP, 1990](#))). NTP ([1990](#)) also commented on the intratubular material and noted that the tubules were empty or “contained wisps of eosinophilic material.”

With gavage exposure, these lesions were present in both mice and rats of both sexes, but were on average more severe in rats than in mice, and in male rats than in female rats ([NTP, 1990](#)). Thus, it appears that male rats are most sensitive to these effects, followed by female rats and then mice. This is consistent with the experiments of Maltoni et al. ([1988](#); [1986](#)), which only reported these effects in male rats. The limited response in female rats or mice of either sex in these experiments may be related to dose or strain. The lowest chronic gavage doses in the National Cancer Institute ([NCI, 1976](#)) and NTP ([1990, 1988](#)) F344 rat experiments was 500 mg/kg-day, and in all of these cases, at least 80% (and frequently 100%) of the animals showed cytomegaly or related toxicity. By comparison, the highest gavage dose in the Maltoni et al. ([1988](#); [1986](#)) experiments (250 mg/kg-day) showed lower incidences of renal cytomegaly and karyomegaly in male Sprague-Dawley rats (47 and 67%, overall and corrected incidences) and none in female rats. The B6C3F<sub>1</sub> mouse strain was used in the NCI ([1976](#)), NTP ([1990](#)), and Maltoni et al. ([1988](#); [1986](#)) studies (see Tables 4-45–4-49). While the two gavage studies ([NTP, 1990](#); [NCI, 1976](#)) were consistent, reporting at least 90% incidence of cytomegaly and karyomegaly at all studied doses, whether dose accounts for the lack of kidney effects in Maltoni et al. ([1988](#); [1986](#)) requires comparing inhalation and gavage dosing. Such comparisons depend substantially on the internal dose-metric, so conclusions as to whether dose can explain differences across studies cannot be addressed without dose-response analysis using PBPK modeling. Some minor differences were found in the multistrain NTP study ([1988](#)), but the high rate of response makes distinguishing among them difficult. Soffritti (personal communication with JC Caldwell, February 14, 2006) did note that the colony from which the rats in Maltoni et al. ([1988](#); [1986](#)) experiments were derived had historically low incidences of chronic progressive nephropathy and renal cancer.

#### **4.4.5. Kidney Cancer in Laboratory Animals**

##### **4.4.5.1. Inhalation Studies of TCE**

A limited number of inhalation studies examined the carcinogenicity of TCE, with no statistically-significantly increases in kidney tumor incidence reported in mice or hamsters ([Maltoni et al., 1988](#); [1986](#); [Fukuda et al., 1983](#); [Henschler et al., 1980](#)). The cancer bioassay by Maltoni et al. ([1988](#); [1986](#)) reported no statistically significant increase in kidney tumors in mice or hamsters, but renal adenocarcinomas were found in male (4/130) and female (1/130) rats at the high dose (600 ppm) after 2 years of exposure and observation at natural death. In males, these tumors seemed to have originated in the tubular cells, and were reported to have never been observed in over 50,000 Sprague-Dawley rats (untreated, vehicle-treated, or treated with different chemicals) examined in previous experiments in the same laboratory ([Maltoni et al., 1986](#)). The renal adenocarcinoma in the female rat was cortical and reported to be similar to that

seen infrequently in historical controls. This study also demonstrated the appearance of increased cytokaryomegaly or megalonucleocytosis in the tubular cells, a lesion that was significantly and dose-dependently increased in male rats only (see Table 4-49). Maltoni et al. (1986) noted that some considerations supported either the hypothesis that these were precursor lesions of renal adenocarcinomas cancer or the hypothesis that these are not precursors but rather the morphological expression of TCE-induced regressive changes. The inhalation studies by Fukuda et al. (1983) in Sprague-Dawley rats and female ICR mice reported one clear cell carcinoma in rats exposed to the highest concentration (450 ppm) but saw no increase in kidney tumors in mice. This result was not statistically significant (see Table 4-50) and no details are given about the specific location of the tumors. One negative study (Henschler et al., 1980) tested NMRI mice, Wistar rats, and Syrian hamsters of both sexes (60 animals per strain), and observed no significant increase in renal tubule tumors any of the species tested. Benign adenomas were observed in male mice and rats, a single adenocarcinoma was reported in male rats at the highest dose, and no renal adenocarcinomas were reported in females of either species (see Table 4-50). RCCs appear to be very rare in Wistar rats, with historical control rates reported to be about 0.4% in males and 0.2% in females (Poteracki and Walsh, 1998), so these data are very limited in power to detect small increases in their incidence.

**Table 4-50. Summary of renal tumor findings in inhalation studies of TCE by Henschler et al. (1980)<sup>a</sup> and Fukuda et al. (1983)<sup>b</sup>**

Sex	Concentration (ppm)	Adenomas	Adenocarcinomas
6 hrs/d, 5 d/wk, 18-mo exposure, 30-mo observation, Han:NMRI mice (Henschler et al., 1980)			
Males	0	4/30	1/30
	100	1/29	0/30
	500	1/29	0/30
Females	0	0/29	0/29
	100	0/30	0/30
	500	0/28	0/28
6 hrs/d, 5 d/wk, 18-mo exposure, 36-mo observation, Han:WIST rats (Henschler et al., 1980)			
Males	0	2/29	0/29
	100	1/30	0/30
	500	2/30	1/30
Females	0	0/28	0/28
	100	0/30	0/30
	500	1/30	0/30
7 hrs/d, 5 d/wk, 2-yr study, Crj:CD (Sprague-Dawley) rats (Fukuda et al., 1983)			
Females	0	0/50	0/50
	50	0/50	0/50
	150	0/47	0/47
	450	0/51	1/50

<sup>a</sup>Henschler et al. (1980) observed no renal tumors in control or exposed Syrian hamsters.

<sup>b</sup>Fukuda et al. (1983) observed no renal tumors in control or exposed Crj:CD-1 (ICR) mice.

#### 4.4.5.2. Gavage and Drinking Water Studies of TCE

Several chronic gavage studies exposing multiple strains of rats and mice to 0–3,000 mg/kg TCE for at least 52 weeks have been conducted (see Tables 4-45 to 4-48, 4-51) ([Maltoni et al., 1988](#); [Maltoni et al., 1986](#), NCI, [1976](#); NTP, [1988](#), [1990](#); Henschler et al., [1984](#); Van Duuren et al., [1979](#)). Van Duuren et al. ([1979](#)) examined TCE and 14 other halogenated compounds for carcinogenicity in both sexes of Swiss mice. While no excess tumors were observed, the dose rate (0.5 mg once per week, or an average dose rate of approximately 2.4 mg/kg-day for a 30 g mouse) is about 400-fold lower than that in the other gavage studies. Inadequate design and reporting of this study limit the ability to use the results as an indicator of TCE carcinogenicity. In the NCI ([1976](#)) study, the results for Osborne-Mendel rats were considered by the authors to be inconclusive due to significant early mortality. Two male rats demonstrated kidney lesions (dilated renal pelvis and dark red renal medulla), but in rats of both sexes, no increase was seen in primary tumor induction over that observed in controls. While both sexes of B6C3F<sub>1</sub> mice showed a compound-related increase in nephropathy, no increase in tumors over controls was observed. The NCI study ([1976](#)) used technical-grade TCE that contained two known carcinogenic compounds as stabilizers (epichlorohydrin and 1,2-epoxybutane). However, a subsequent study by Henschler et al. ([1984](#)) in mice reported no significant differences in systemic tumorigenesis between pure, industrial, and stabilized TCE, suggesting that concentrations of these stabilizers are too low to be the cause of tumors. A later gavage study by NTP ([1988](#)), using TCE stabilized with diisopropylamine, observed an increased incidence of renal tumors in all four strains of rats (ACI, August, Marshall, and Osborne-Mendel). All animals exposed for up to 2 years (rats and mice) had non-neoplastic kidney lesions (tubular cell cytomegaly), even if they did not later develop kidney cancer (see Table 4-48). This study was also considered inadequate by the authors because of chemically induced toxicity, reduced survival, and incomplete documentation of experimental data. The final NTP study ([1990](#)) in male and female F344 rats and B6C3F<sub>1</sub> mice used epichlorohydrin-free TCE. Only in the highest-dose group (1,000 mg/kg) of male F344 rats was renal carcinoma statistically significant increased. The results for detecting a carcinogenic response in rats were considered by the authors to be equivocal because both groups receiving TCE showed significantly reduced survival compared to vehicle controls and because of a high rate (e.g., 20% of the animals in the high-dose group) of death by gavage error. However, historical control incidences at NTP of kidney tumors in F344 rats is very low,<sup>5</sup> lending biological significance to their occurrence in this study, despite the study's limitations. Cytomegaly and karyomegaly were also increased, particularly in male rats. The toxic nephropathy (specific location in kidney

---

<sup>5</sup>NTP ([1990](#)) reported a historical control incidence of 0.4% in males. The NTP web site reports historical control rates of renal carcinomas for rats dosed via corn oil gavage on the NIH-07 diet (used before 1995, when the TCE studies were conducted) to be 0.5% (2/400) for males and 0% (0/400) for females ([http://ntp-server.niehs.nih.gov/ntp/research/database\\_searches/historical\\_controls/path/r\\_gavco.txt](http://ntp-server.niehs.nih.gov/ntp/research/database_searches/historical_controls/path/r_gavco.txt)). In addition, the two occurrences in males came from the same study, with all other studies reporting 0/50 carcinomas.

not stated) observed in both rats and mice and contributed to the poor survival rate (see Table 4-45). As discussed previously, this toxic nephropathy was clearly distinguishable from the spontaneous chronic progression nephropathy commonly observed in aged rats.

**Table 4-51. Summary of renal tumor findings in gavage studies of TCE by Henschler et al. (1984)<sup>a</sup> and Van Duuren et al. (1979)<sup>b</sup>**

Sex (TCE dose)	Control or TCE exposed (stabilizers if present)	Adenomas	Adenocarcinomas
5 d/wk, 18-mo exposure, 24-mo observation, Swiss mice (Henschler et al., 1984)			
Males (2.4g/kg body weight)	Control (none)	1/50	1/50
	TCE (triethanolamine)	1/50	1/50
	TCE (industrial)	0/50	0/50
	TCE (epichlorohydrin (0.8%))	0/50	0/50
	TCE (1,2-epoxybutane (0.8%))	2/50	2/50
	TCE (both epichlorohydrin (0.25%) and 1,2-epoxybutane (0.25%))	0/50	0/50
Females (1.8 g/kg body weight)	Control (none)	0/50	1/50
	TCE (triethanolamine)	4/50	0/50
	TCE (industrial)	0/50	0/50
	TCE (epichlorohydrin (0.8%))	0/50	0/50
	TCE (1,2-epoxybutane (0.8%))	0/50	0/50
	TCE (both epichlorohydrin (0.25%) and 1,2-epoxybutane (0.25%))	0/50	0/50
1 d/wk, 89-wk exposure, Swiss rats (Van Duuren et al., 1979)			
Males (0.5mg)	Control	0/30	0/30
	TCE (unknown)	0/30	0/30
Females (0.5mg)	Control	0/30	0/30
	TCE(unknown)	0/30	0/30

<sup>a</sup>Henschler et al. (1984). Due to poor condition of the animals resulting from the nonspecific toxicity of high doses of TCE and/or the additives, gavage was stopped for all groups during weeks 35–40, 65, and 69–78, and all doses were reduced by a factor of 2 from the 40<sup>th</sup> week on.

<sup>b</sup>Van Duuren et al. (1979) observed no renal tumors in control or exposed Swiss mice.

#### 4.4.5.3. Conclusions: Kidney Cancer in Laboratory Animals

Chronic TCE carcinogenicity bioassays have shown evidence of neoplastic lesions in the kidney in rats (mainly in males, with less evidence in females), treated via inhalation and gavage. As discussed above, individual studies have a number of limitations and have shown limited increases in kidney tumors. However, given the rarity of these tumors as assessed by historical controls and the repeatability of this result, these are considered biologically significant.

#### 4.4.6. Role of Metabolism in TCE Kidney Toxicity

It is generally thought that one or more TCE metabolites rather than the parent compound are the active moieties for TCE nephrotoxicity. As reviewed in Section 3.3, oxidation by CYPs,

of which CYP2E1 is thought to be the most active isoform, results in the production of CH, TCA, DCA, and TCOH. The GSH conjugation pathway produces metabolites such as DCVG, DCVC, dichlorovinylthiol, and NAcDCVC, although, as discussed in Section 3.3.3.2, the quantitative estimates of the amount systemically produced following TCE exposure remains uncertain. Because several of the steps for generating these reactive metabolites occur in the kidney, the GSH conjugation pathway has been thought to be responsible for producing the active moiety or moieties of TCE nephrotoxicity. A comparison of TCE's nephrotoxic effects with the effects of TCE metabolites, both in vivo and in vitro, thus provides a basis for assessing the relative roles of different metabolites. While most of the available data have been on metabolites from GSH conjugation, such as DCVC, limited information is also available on the major oxidative metabolites, TCOH and TCA.

#### **4.4.6.1. In Vivo Studies of the Kidney Toxicity of TCE Metabolites**

Studies of kidney toxicity of TCE metabolites discussed in this section are shown in Table 4-52.

##### **4.4.6.1.1. Role of GSH conjugation metabolites of TCE**

In numerous studies, DCVC has been shown to be acutely nephrotoxic in rats and mice. Mice receiving a single dose of 1 mg/kg DCVC (the lowest dose tested in this species) exhibited karyolytic proximal tubular cells in the outer stripe of the outer medulla, with some sloughing of cells into the lumen and moderate desquamation of the tubular epithelium ([Eyre et al., 1995a](#)). Higher doses in mice were associated with more severe histological changes similar to those induced by TCE, such as desquamation and necrosis of the tubular epithelium ([Vaidya et al., 2003a, b](#); [Darnerud et al., 1989](#); [Terracini and Parker, 1965](#)). In rats, no histological changes in the kidney were reported after single doses of 1, 5, and 10 mg/kg DCVC ([Green et al., 1997a](#); [Eyre et al., 1995b, a](#)), but cellular debris in the tubular lumen was reported at 25 mg/kg ([Eyre et al., 1995a](#)) and slight degeneration and necrosis were seen at 50 mg/kg ([Green et al., 1997a](#)). [Green et al. \(1997a\)](#) reported no histological changes were noted in rats after 10 doses of 0.1–5.0 mg/kg DCVC (although increases in urinary protein and GGT were found), but some karyomegaly was noted in mice after 10 daily doses of 1 mg/kg. Therefore, mice appear more sensitive than rats to the nephrotoxic effects of acute exposure to DCVC, although the number of animals used at each dose in these studies was limited (10 or less). Although the data are not sufficient to assess the relative sensitivity of other species, it is clear that multiple species, including rabbits, guinea pigs, cats, and dogs, are responsive to DCVC's acute nephrotoxic effects ([Krejci et al., 1991](#); [Wolfgang et al., 1989a](#); [Jaffe et al., 1984](#); [Terracini and Parker, 1965](#)).

**Table 4-52. Laboratory animal studies of kidney noncancer toxicity of TCE metabolites**

<b>Reference</b>	<b>Animals (sex)</b>	<b>Exposure route</b>	<b>Dose/exposure concentration</b>	<b>Exposed</b>	<b>Kidney effect(s) discussed in Section 4.4.4</b>
Dow and Green (2000)	F344 rats (M)	Drinking water	0, 0.5, 1 g/L TCOH, 12 wks	3/group	Increased formic acid in urine.
Jaffe et al. (1984)	Swiss-Webster mice (M)	Drinking water	0–22 mg/kg-d DCVC, 37 wks	5/group	Cytomegaly and tubular degeneration.
Mather et al. (1990)	Sprague-Dawley rats (M)	Drinking water	0–355 mg/kg-d TCA, 90 d	10/group	Increased kidney weight.
Terracini and Parker (1965)	Wistar rats (Gender not specified) Grey mice (Gender not specified)	Drinking water	0, 0.01% DCVC, 12 wks	35/group	Necrosis of tubular epithelium in mice and rats.

Very few studies are available at longer durations. Terracini and Parker (1965) gave DCVC in drinking water to rats at a concentration of 0.01% for 12 weeks (approximately 10 mg/kg-day), and reported consistent pathological and histological changes in the kidney. The progression of these effects was as follows: (1) during the first few days, completely necrotic tubules, with isolated pyknotic cells being shed into the lumen; (2) after 1 week, dilated tubules in the inner part of the cortex, lined with flat epithelial cells that showed thick basal membranes, some with big hyperchromatic nuclei; and (3) in the following weeks, increased prominence of tubular cells exhibiting karyomegaly, seen in almost all animals, less pronounced tubular dilation, and cytomegaly in the same cells showing karyomegaly. In addition, increased mitotic activity was reported the first few days, but was not evident for the rest of the experiment. Terracini and Parker (1965) also reported the results of a small experiment (13 male and 5 female rats) given the same concentration of DCVC in drinking water for 46 weeks, and observed for 87 weeks. They noted renal tubular cells exhibiting karyomegaly and cytomegaly consistently throughout the experiment. Moreover, a further group of eight female rats given DCVC in drinking water at a concentration of 0.001% (approximately 1 mg/kg-day) also exhibited similar, though less severe, changes in the renal tubules. In mice, Jaffe et al. (1984) gave DCVC in drinking water at concentrations of 0.001, 0.005, and 0.01% (estimated daily doses of 1–2, 7–13, and 17–22 mg/kg-day), and reported similar effects in all dose groups, including cytomegaly, nuclear hyperchromatism, and multiple nucleoli, particularly in the pars recta section of the kidney. Thus, effects were noted in both mice and rats under chronic exposures at doses as low as 1–2 mg/kg-day (the lowest dose tested). Therefore, while limited, the available data do not suggest differences between mice and rats to the nephrotoxic effects of DCVC under chronic exposure conditions, in contrast to the greater sensitivity of mice to acute and subchronic DCVC-induced nephrotoxicity.

Importantly, as summarized in Table 4-53, the histological changes and their location in these subchronic and chronic experiments with DCVC are quite similar to those reported in chronic studies of TCE, described above, particularly the prominence of karyomegaly and cytomegaly in the pars recta section of the kidney. Moreover, the morphological changes in the tubular cells, such as flattening and dilation, are quite similar. Similar pathology is not observed with the oxidative metabolites alone (see Section 4.4.6.1.2).

**Table 4-53. Summary of histological changes in renal proximal tubular cells induced by chronic exposure to TCE, DCVC, and TCOH**

Effects	TCE	DCVC	TCOH
Karyomegaly	Enlarged, hyperchromatic nuclei, irregular to oblong in shape. Vesicular nuclei containing prominent nucleoli.	Enlarged, hyperchromatic nuclei with and multiple nucleoli. Nuclear pyknosis and karyorrhexis.	None reported.
Cytomegaly	Epithelial cells were large, elongated, and flattened.	Epithelial cells were large, elongated, and flattened cells.	No report of enlarged cells.
Cell necrosis/hyperplasia	Stratified epithelium that partially or completely filled the tubular lumens. Cells in mitosis were variable in number or absent. Cells had abundant eosinophilic or basophilic cytoplasm.	Thinning of tubular epithelium, frank tubular necrosis, re-epitheliation. Tubular atrophy, interstitial fibrosis and destruction of renal parenchyma. More basophilic and finely vacuolated.	No flattening or loss of epithelium reported. Increased tubular cell basophilia, followed by increased cellular eosinophilia, tubular cell vacuolation.
Morphology/content of tubules	Some tubules enlarged/dilated to the extent that they were difficult to identify. Portions of basement membrane had a stripped appearance. Tubules were empty or contained “wisps of eosinophilic material.”	Tubular dilation, denuded tubules. Thick basal membrane. Focal areas of dysplasia, intraluminal casts.	No tubular dilation reported. Intratubular cast formation.

Sources: NCI ([1976](#)); NTP ([1990](#), [1988](#)); Maltoni et al. ([1988](#); [Maltoni et al., 1986](#)); Terracini and Parker ([1965](#)); Jaffe et al. ([1985](#)); Green et al. ([2003](#)).

Additionally, it is important to consider whether sufficient DCVC may be formed from TCE exposure to account for TCE nephrotoxicity. While direct pharmacokinetic measurements, such as the excretion of NAcDCVC, have been used to argue that insufficient DCVC would be formed to be the active moiety for nephrotoxicity ([Green et al., 1997a](#)), as discussed in Chapter 3, urinary NAcDCVC is a poor marker of the flux through the GSH conjugation pathway because of the many other possible fates of metabolites in that pathway. In another approach, Eyre et al. ([Eyre et al., 1995b](#)), using acid-labile adducts as a common internal dosimeter between TCE and DCVC, reported that a single TCE dose of 400 mg/kg in rats (similar to the lowest daily doses in the NCI and NTP rat bioassays) and 1,000 mg/kg (similar to the lowest daily doses in the NCI and NTP mouse bioassays) corresponded to a single equivalent DCVC dose of 6 and 1 mg/kg-day in rats and mice, respectively. These equivalent doses of DCVC are greater or equal to those in which nephrotoxicity has been reported in these species under chronic conditions. Therefore, assuming that this dose correspondence is accurate under chronic conditions, sufficient DCVC would be formed from TCE exposure to explain the observed histological changes in the renal tubules. Nevertheless, direct estimates of how much DCVC is formed after TCE exposure are lacking.

The Eker rat model (*Tsc-2<sup>±</sup>*) is at increased risk for the development of spontaneous RCC and as such, has been used to understand the mechanisms of renal carcinogenesis ([Stemmer et al., 2007](#); [Wolf et al., 2000](#)). One study has demonstrated similar pathway activation in Eker rats as that seen in humans with *VHL* mutations leading to RCC, suggesting that *Tsc-2* inactivation is analogous to inactivation of *VHL* in human RCC ([Liu et al., 2003](#)). Although the Eker rat model is a useful tool for analyzing the progression of renal carcinogenesis, it has some limitations in analysis of specific genetic changes, particularly given the potential for different genetic changes depending on type of exposure and tumor. The results of short-term assays to genotoxic carcinogens in the Eker rat model ([Stemmer et al., 2007](#); [Morton et al., 2002](#)) reported limited preneoplastic and neoplastic lesions, which may be related to the increased background rate of renal carcinomas in this animal model.

Recently, Mally et al. ([2006](#)) exposed male rats carrying the Eker mutation to TCE (0–1,000 mg/kg body weight) by corn oil gavage and demonstrated no increase in renal preneoplastic lesions or tumors. Primary Eker rat kidney cells exposed to DCVC in this study did induce an increase in transformants in vitro but no DCVC-induced *VHL* or *Tsc-2* mutations were observed. In vivo exposure to TCE (5 days/week for 13 weeks), decreased body weight gain and increased urinary excretion at the two highest TCE concentrations analyzed (500 and 1,000 mg/kg body weight) but did not change standard nephrotoxicity markers (GGT, creatinine, and urinary protein). Renal tubular epithelial cellular proliferation as measured by BrdU incorporation was demonstrated at the three highest concentrations of TCE (250, 500 and 1,000 mg/kg-day). A minority of these cells also showed karyomegaly at the two higher TCE concentrations. Although renal cortical tumors were demonstrated in all TCE exposed groups,

these were not significantly different from controls (13 weeks). These studies were complemented with in vitro studies of DCVC (10–50  $\mu$ M) in rat kidney epithelial (RKE) cells examining proliferation at 8, 24, and 72 hours and cellular transformation at 6–7 weeks. Treatment of RKE cells from susceptible rats with DCVC gave rise to morphologically transformed colonies consistently higher than background ([Mally et al., 2006](#)). Analyzing 10 of the renal tumors from the TCE-exposed rats and 9 of the DCVC transformants from these studies for alterations to the *VHL* gene that might lead to inactivation found no alterations to *VHL* gene expression or mutations.

One paper has linked the *VHL* gene to chemical-induced carcinogenesis. Shiao et al. ([1998](#)) demonstrated *VHL* gene somatic mutations in *N*-nitrosodimethylamine-induced rat kidney cancers that were of the clear cell type. The clear cell phenotype is rare in rat kidney cancers, but it was only the clear cell cancers that showed *VHL* somatic mutation (three of eight tumors analyzed). This provided an additional link between *VHL* inactivation and clear cell kidney cancer. However, this study examined archived formalin-fixed, paraffin-embedded tissues from previous experiments. As described previously (see Section 4.4.3), DNA extraction from this type of preparation creates some technical issues. Similarly, archived formalin-fixed, paraffin-embedded tissues from rats exposed to potassium bromide were analyzed in a later study by Shiao et al. ([2002](#)). This later study examined the *VHL* gene mutations following exposure to potassium bromide, a rat renal carcinogen known to induce clear cell renal tumors. Clear cell renal tumors are the most common form of human renal epithelial neoplasms, but are extremely rare in animals. Although F344 rats exposed to potassium bromide in this study did develop renal clear cell carcinomas, only two of nine carried the same C to T mutation at the core region of the Sp1 transcription-factor binding motif in the *VHL* promoter region, and one of four untreated animals had a C to T mutation outside the conserved core region. Mutation in the *VHL* coding region was only detected in one tumor, so although the tumors developed following exposure to potassium bromide were morphologically similar to those found in humans, no similarities were found in the genetic changes.

Elfarra et al. ([1984](#)) found that both DCVG and DCVC administered to male F344 rats by i.p. injections in isotonic saline resulted in elevations in BUN and urinary glucose excretion. Furthermore, inhibition of renal GGT activity with acivicin-protected rats from DCVG-induced nephrotoxicity. In addition, both the  $\beta$ -lyase inhibitor, AOAA, and the renal organic anion transport inhibitor, probenecid, provided protection from DCVC, demonstrating a requirement for metabolism of DCVG to the cysteine conjugate by the action of renal GGT and dipeptidase, uptake into the renal cell by the organic anion transporter, and subsequent activation by the  $\beta$ -lyase. This conclusion was supported further by showing that the methyl analog of DCVC, which cannot undergo a  $\beta$ -elimination reaction due to the presence of the methyl group, was not nephrotoxic.

Korrapati et al. (2005) built upon a series of investigations of hetero- (by mercuric chloride [HgCl<sub>2</sub>]) and homo-(by DCVC, 15 mg/kg) protection against a lethal dose of DCVC (75 mg/kg). Priming, or preconditioning, with pre-exposure to either HgCl<sub>2</sub> or DCVC of male Swiss-Webster mice was said to augment and sustain cell division and tissue repair, hence protecting against the subsequent lethal DCVC dose (Vaidya et al., 2003b, a; 2003c). Korrapati et al. (2005) showed that a lethal dose of DCVC downregulates phosphorylation of endogenous retinoblastoma protein (pRb), which is considered critical in renal proximal tubular and mesangial cells for the passage of cells from G1 to S-phase, thereby leading to a block of renal tubule repair. Priming, in contrast, upregulated P-pRB which was sustained even after the administration of a lethal dose of DCVC, thereby stimulating S-phase DNA synthesis, which was concluded to result in tissue repair and recovery from acute renal failure and death. These studies are more informative about the mechanism of autoprotection than on the mechanism of initial injury caused by DCVC. In addition, the priming injury (not innocuous, as it caused 25–50% necrosis and elevated BUN) may have influenced the toxicokinetics of the second DCVC injection.

#### 4.4.6.1.2. Role of oxidative metabolites of TCE

Some investigators (Green et al., 2003; Dow and Green, 2000; Green et al., 1998) have proposed that TCE nephrotoxicity is related to formic acid formation. They demonstrated that exposure to either TCOH or TCA causes increased formation and urinary excretion of formic acid (Green et al., 1998). The formic acid does not come from TCE. Rather, TCE (or a metabolite) has been proposed to cause a functional depletion of vitamin B<sub>12</sub>, which is required for the methionine salvage pathway of folate metabolism. Vitamin B<sub>12</sub> depletion results in folate depletion. Folate is a cofactor in one-carbon metabolism and depletion of folate allows formic acid to accumulate, and then to be excreted in the urine (Dow and Green, 2000).

TCE (1 and 5 g/L), TCA (0.25, 0.5, and 1 g/L), and TCOH (0.5 and 1.0 g/L) exposure in male Fischer rats substantially increased excretion of formic acid in urine, an effect suggested as a possible explanation for TCE-induced renal toxicity in rats (Green et al., 1998). Green et al. (2003) reported tubular toxicity as a result of chronic (1 year) exposure to TCOH (0, 0.5, and 1.0 g/L). Although TCOH causes tubular degeneration in a similar region of the kidney as TCE, there are several dissimilarities between the characteristics of nephrotoxicity between the two compounds, as summarized in Table 4-53. In particular, Green et al. (1998) did not observe TCOH causing karyomegaly and cytomegaly. These effects were seen as early as 13 weeks after the commencement of TCE exposure (NTP, 1990), with 300 ppm inhalation exposures to TCE (Maltoni et al., 1988; Maltoni et al., 1986), as well as at very low chronic exposures to DCVC (Jaffe et al., 1984; Terracini and Parker, 1965). In addition, Green et al. (2003) reported neither flattening nor loss of the tubular epithelium nor hyperplasia, but suggested that the increased early basophilia was due to newly divided cells, and therefore, represented tubular regeneration

in response to damage. Furthermore, they noted that such changes were seen with the spontaneous damage that occurs in aging rats. However, several of the chronic studies of TCE noted that the TCE-induced damage observed was distinct from the spontaneous nephropathy observed in rats. A recent *in vitro* study of rat hepatocytes and primary human renal proximal tubule cells from two donors measured formic acid production following exposure to CH (0.3–3 mM, 3–10 days) ([Lock et al., 2007](#)). This study observed increased formic acid production at day 10 in both human renal proximal tubule cell strains, but a similar level of formic acid was measured when CH was added to media alone. The results of this study are limited by the use of only two primary human cell strains, but suggest that exposure to CH does not lead to significant increases in formic acid production *in vivo*.

Interestingly, it appears that the amount of formic acid excreted reaches a plateau at a relatively low dose. Green et al. ([2003](#)) added folic acid to the drinking water of the group of rats receiving the lower dose of TCOH (18.3 mg/kg-day) in order to modulate the excretion of formic acid in that dose group, and retain the dose-response in formic acid excretion relative to the higher-dose group (54.3 mg/kg-day). These doses of TCOH are much lower than what would be expected to be formed *in vivo* at chronic gavage doses. For instance, after a single 500-mg/kg dose of TCE (the lower daily dose in the NTP rat chronic bioassays), Green and Prout ([1985](#)) reported excretion of about 41% of the TCE gavage dose in urine as TCOH or TCOG in 24 hours. Thus, using the measure of additional excretion after 24 hours and the TCOH converted to TCA as a lower bound as to the amount of TCOH formed by a single 500 mg/kg dose of TCE, the amount of TCOH would be about 205 mg/kg, almost fourfold greater than the high dose in the Green et al. ([2003](#)) study. By contrast, these TCOH doses are somewhat smaller than those expected from the inhalation exposures of TCE. For instance, after 6-hour exposures to 100 and 500 ppm TCE (similar to the daily inhalation exposures in Maltoni et al. ([1988](#); [1986](#))), male rats excreted 1.5 and 4.4 mg of TCOH over 48 hours, corresponding to 5 and 15 mg/kg for a rat weighing 0.3 kg ([Kaneko et al., 1994](#)). The higher equivalent TCOH dose is similar to the lower TCOH dose used in Green et al. ([2003](#)), so it is notable that while Maltoni et al. ([1988](#); [Maltoni et al., 1986](#)) reported a substantial incidence of cytomegaly and karyomegaly after TCE exposure (300 and 600 ppm), none was reported in Green et al. ([2003](#)).

TCOH alone does not appear sufficient to explain the range of renal effects observed after TCE exposure, particularly cytomegaly, karyomegaly, and flattening and dilation of the tubular epithelium. However, given the studies described above, it is reasonable to conclude that TCOH may contribute to the nephrotoxicity of TCE, possibly due to excess formic acid production, because: (1) there are some similarities between the effects observed with TCE and TCOH and (2) the dose at which effects with TCOH are observed overlap with the approximate equivalent TCOH dose from TCE exposure in the chronic studies.

Dow and Green ([2000](#)) noted that TCA also induced formic acid accumulation in rats, and suggested that TCA may therefore, contribute to TCE-induced nephrotoxicity. However,

TCA has not been reported to cause any similar histologic changes in the kidney. Mather et al. (1990) reported an increase of kidney-weight to body-weight ratio in rats after 90 days of exposure to TCA in drinking water at 5,000 ppm (5 g/L) but reported no histopathologic changes in the kidney. DeAngelo et al. (1997) reported no effects of TCA on kidney weight or histopathology in rats in a 2-year cancer bioassay. Dow and Green (2000) administered TCA at quite high doses (1 and 5 g/L in drinking water), greater than the subsequent experiments of Green et al. (2003) with TCOH (0.5 and 1 g/L in drinking water), and reported similar amounts of formic acid produced (about 20 mg/day for each compound). However, cytotoxicity or karyomegaly did not appear to be analyzed. Furthermore, much more TCOH is formed from TCE exposure than TCA. Therefore, if TCA contributes substantially to the nephrotoxicity of TCE, its contribution would be substantially less than that of TCOH. Lock et al. (2007) also measured formic acid production in human renal proximal tubule cells exposed to 0.3–3 mM CH for 10 days CH. This study measured metabolism of CH to TCOH and TCA as well as formic acid production and subsequent cytotoxicity. Increased formic acid was not observed in this study, and limited cytotoxicity was observed. However, this study was performed in human renal proximal tubular cells from only two donors, and there is potential for large interindividual variability in response, particularly with CYP enzymes.

In order to determine the ability of various chlorinated hydrocarbons to induce peroxisomal enzymes, Goldsworthy and Popp (1987) exposed male F344 rats and male B6C3F<sub>1</sub> mice to TCE (1,000 mg/kg body weight) and TCA (500 mg/kg body weight) by corn oil gavage for 10 consecutive days. Peroxisomal activation was measured by palmitoyl coenzyme A (CoA) oxidase activity levels. TCE led to increased peroxisomal activation in the kidneys of both rats (300% of control) and mice (625% of control), while TCA led to an increase only in mice (280% of control). A study by Zanelli et al. (1996) exposed Sprague-Dawley rats to TCA for 4 days and measured both renal and hepatic peroxisomal and CYP enzyme activities. TCA-treated rats had increased activity in CYP 4A subfamily enzymes and peroxisomal palmitoyl-CoA oxidase. Both of these acute studies focused on enzyme activities and did not further analyze resulting histopathology.

#### **4.4.6.2. In Vitro Studies of Kidney Toxicity of TCE and Metabolites**

Generally, it is believed that TCE metabolites are responsible for the bulk of kidney toxicity observed following exposure. In particular, studies have demonstrated a role for DCVG and DCVC in kidney toxicity, though, as discussed in Section 3.3.3.2, the precise metabolic yield of these metabolites following TCE exposure remains uncertain. The work by Lash and colleagues (Cummings and Lash, 2000; Cummings et al., 2000a; Cummings et al., 2000b; Lash et al., 2000b) examined the effect of TCE and its metabolites in vitro. TCE and DCVC are toxic to primary cultures of rat proximal and distal tubular cells (Cummings et al., 2000c), while the TCE metabolites, DCVG and DCVC, have been demonstrated to be cytotoxic to rat and rabbit

kidney cells in vitro ([Lash et al., 2001b](#); [Lash et al., 2000b](#); [Groves et al., 1993](#); [Wolfgang et al., 1989b](#); [Hassall et al., 1983](#)). GSH-related enzyme activities were well maintained in the cells, whereas CYP activities were not. The enzyme activity response to DCVC was greater than the response to TCE; however, the proximal and distal tubule cells had similar responses even though the proximal tubule is the target in vivo. The authors attributed this to the fact that the proximal tubule is exposed before the distal tubule in vivo and to possible differences in uptake transporters. They did not address the extent to which transporters were maintained in the cultured cells.

In further studies, Lash et al. ([2001b](#)) assessed the toxicity of TCE and its metabolites, DCVC and DCVG, using in vitro techniques as compared to in vivo studies. Experiments using isolated cells were performed only with tissues from F344 rats, and lactate dehydrogenase (LDH) release was used as the measure of cellular toxicity. The effects were greater in males. DCVC and TCE had similar effects, but DCVG exhibited increased efficacy compared with TCE and DCVC.

In vitro mitochondrial toxicity was assessed in renal cells from both F344 rats and B6C3F<sub>1</sub> mice following exposure to both DCVC and DCVG ([Lash et al., 2001b](#)). Renal mitochondria from male rats and mice responded similarly; a greater effect was seen in cells from the female mice. These studies show DCVC to be slightly more toxic than TCE and DCVG, but species differences are not consistent with the effects observed in long-term bioassays. This suggests that in vitro data should be used with caution in risk assessment, being mindful that in vitro experiments do not account for in vivo pharmacokinetic and metabolic processes.

In LLC-PK1 cells, DCVC causes loss of mitochondrial membrane potential, mitochondrial swelling, release of cytochrome c, caspase activation, and apoptosis ([Chen et al., 2001](#)). Thus, DCVC is toxic to mitochondria, resulting in either apoptosis or necrosis. DCVC-induced apoptosis also has been reported in primary cultures of human proximal tubule cells ([Lash et al., 2001a](#)).

DCVC was further studied in human renal proximal tubule cells for alterations in gene expression patterns related to proposed modes of action in nephrotoxicity ([Lock et al., 2006](#)). In cells exposed to subtoxic levels of DCVC to better mimic workplace exposures, the expression of genes involved with apoptosis (caspase 8, FADD-like regulator) was increased at the higher dose (1  $\mu$ M) but not at the lower dose (0.1  $\mu$ M) of DCVC exposure. Genes related to oxidative stress response (SOD, NF- $\kappa$ B, p53, c-Jun) were altered at both subtoxic doses, with genes generally upregulated at 0.1  $\mu$ M DCVC being downregulated at 1  $\mu$ M DCVC. The results of this study support the need for further study, and highlight the involvement of multiple pathways and variability of response based on different concentrations.

Lash et al. ([2007](#)) examined the effect of modulation of renal metabolism on toxicity of TCE in isolated rat cells and microsomes from kidney and liver. Following exposure to

modulating chemicals, LDH was measured as a marker of cytotoxicity, and the presence of specific metabolites was documented (DCVG, TCA, TCOH, and CH). Inhibition of the CYP stimulated an increase of GSH conjugation of TCE and increased cytotoxicity in kidney cells. This modulation of CYP had a greater effect on TCE-induced cytotoxicity in liver cells than in kidney cells. Increases in GSH concentrations in the kidney cells led to increased cytotoxicity following exposure to TCE. Depletion of GSH in hepatocytes exposed to TCE, however, led to an increase in hepatic cytotoxicity. The results of this study highlight the role of different bioactivation pathways needed in both the kidney and the liver, with the kidney effects being more affected by the GSH conjugation pathways metabolic products.

In addition to the higher susceptibility of male rats to TCE-induced nephrocarcinogenicity and nephrotoxicity, isolated renal cortical cells from male F344 rats are more susceptible to acute cytotoxicity from TCE than cells from female rats. TCE caused a modest increase in LDH release from male rat kidney cells but had no significant effect on LDH release from female rat kidney cells. These results on male susceptibility to TCE agree with the *in vivo* data.

#### **4.4.6.3. Conclusions as to the Active Agents of TCE-Induced Nephrotoxicity**

In summary, the TCE metabolites, DCVC, TCOH, and TCA, have all been proposed as possible contributors to the nephrotoxicity of TCE. Both *in vivo* and *in vitro* data strongly support the conclusion that DCVC and related GSH conjugation metabolites are the active agents of TCE-induced nephrotoxicity. Of these, DCVC induces effects in renal tissues, both *in vivo* and *in vitro*, that are most similar to those of TCE, and formed in sufficient amounts after TCE exposure to account for those effects. A role for formic acid due to TCOH or TCA formation from TCE cannot be ruled out, as it is known that substantial TCOH and TCA are formed from TCE exposure, that formic acid is produced from all three compounds, and that TCOH exposure leads to toxicity in the renal tubules. However, the characteristics of TCOH-induced nephrotoxicity do not account for the range of effects observed after TCE exposure, while those of DCVC-induced nephrotoxicity do. Also, TCOH does not induce the same pathology as TCE or DCVC. TCA has also been demonstrated to induce peroxisomal proliferation in the kidney ([Goldsworthy and Popp, 1987](#)), but this has not been associated with kidney cancer. Therefore, although TCOH and possibly TCA may contribute to TCE-induced nephrotoxicity, their contribution is likely to be small compared to that of DCVC. However, as discussed in Section 3.3.3.2, the precise metabolic yield of these DCVC following TCE exposure remains uncertain.

#### **4.4.7. Mode(s) of Action for Kidney Carcinogenicity**

This section will discuss the evidentiary support for several hypothesized modes of action for kidney carcinogenicity, including mutagenicity, cytotoxicity and regenerative proliferation,

peroxisome proliferation,  $\alpha$ 2 $\mu$ -related nephropathy, and formic acid-related nephropathy, following the framework outlined in the *Cancer Guidelines* (U.S. EPA, 2005b).<sup>6</sup> The data and conclusions for the modes of action with the greatest experimental support are summarized in Table 4-54.

#### 4.4.7.1. Hypothesized Mode of Action: Mutagenicity

One hypothesis is that a mutagenic mode of action is operative in TCE-induced renal carcinogenesis. According to this hypothesis, the key events leading to TCE-induced kidney tumor formation constitute the following: TCE GSH conjugation metabolites (e.g., DCVG, DCVC, NAcDCVC, and/or other reactive metabolites derived from subsequent beta-lyase, flavin monooxygenases [FMO], or CYP metabolism) derived from the GSH-conjugation pathway, after being either produced in situ in or delivered systemically to the kidney, cause direct alterations to DNA (e.g., mutation, DNA damage, and/or micronuclei induction). Mutagenicity is a well-established cause of carcinogenicity.

Toxicokinetic data are consistent with these genotoxic metabolites either being delivered to or produced in the kidney. As discussed in Section 3, following in vivo exposure to TCE, the metabolites DCVG, DCVC, and NAcDCVC have all been detected in the blood, kidney, or urine of rats, and DCVG in blood and NAcDCVC in urine have been detected in humans (2006; Lash et al., 1999b; Bernauer et al., 1996; Birner et al., 1993). In addition, in vitro data have shown DCVG formation from TCE in cellular and subcellular fractions from the liver, from which it would be delivered to the kidney via systemic circulation, and from the kidney (see Tables 3-23–3-24, and references therein). Furthermore, in vitro data in both humans and rodents support the conclusion that DCVC is primarily formed from DCVG in the kidney itself, with subsequent in situ transformation to NAcDCVC by *N*-Acetyl transferase or to reactive metabolites by beta-lyase, FMO, or CYPs (see Sections 3.3.3.2.2 to 3.3.3.2.5). Therefore, it is highly likely that both human and rodent kidneys are exposed to these TCE metabolites.

---

<sup>6</sup>As recently reviewed (Guyton et al., 2008), the approach to evaluating mode of action information described in EPA's *Cancer Guidelines* (2005b) considers the issue of human relevance of a hypothesized mode of action in the context of hazard evaluation. This excludes, for example, consideration of toxicokinetic differences across species; specifically, the *Cancer Guidelines* state, "the toxicokinetic processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the mode of action." In addition, information suggesting quantitative differences in the occurrence of a key event between test species and humans are noted for consideration in the dose-response assessment, but is not considered in human relevance determination. In keeping with these principles, a formal analysis of the dose-response of key events in the hypothesized modes of action is not presented unless it would aid in the overall weight of evidence analysis for carcinogenicity, as presented in Section 4.1.

**Table 4-54. Summary of major mode-of-action conclusions for TCE kidney carcinogenesis**

<i>Hypothesized MOA postulated key events</i>	<i>Experimental support</i>	Human relevance	Weight-of-evidence conclusion
<b>Mutagenicity</b>			
<i>GSH conjugation-derived metabolites produced in situ or delivered systemically to kidney.</i>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>Multiple in vitro and in vivo studies demonstrate GSH conjugation of TCE, and availability to the kidney (see Section 3.3.3).</li> <li>Uncertainties are quantitative (precise amount of flux), not qualitative.</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>Active GSTT1 alleles are associated with higher kidney cancer risk in humans following TCE exposure as compared to null genotypes (Moore et al. 2010).</li> </ul>	Yes: demonstrated in humans in vivo and in human cells in vitro.	Highly likely that both human and rodent kidneys are exposed to the GSH-conjugation derived metabolites.
<i>Mutagenicity induced by GSH-derived metabolites advances acquisition of the multiple critical traits contributing to carcinogenesis.</i>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>GSH conjugation derived metabolites (DCVG, DCVC, NAcDCVC) demonstrated to be genotoxic in most in vitro assays in which they have been tested, including Ames test (see Section 4.2.1.4.1).</li> <li>Kidney-specific genotoxicity in rats and rabbits after in vivo administration of TCE or DCVC. Not seen in mice, but may be due to species differences in metabolism and in sensitivity to renal carcinogenesis.</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>Inconsistent results with respect to <i>VHL</i> mutation status, with some studies providing suggestive evidence of a TCE-induced kidney tumor genotype; no data regarding other specific mutations.</li> </ul>	Yes: no basis for discounting in vitro or in vivo genotoxicity results.	Predominance of positive genotoxicity data consistent with GSH-conjugation derived metabolites causing mutations in the kidney.
<i>Overall Conclusion</i>	<p><i>Sufficiency of MOA for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>Mutagenicity is assumed to cause cancer, as a sufficient cause.</li> </ul>	Yes: well established.	Data are sufficient to conclude that a mutagenic MOA is operative in TCE-induced kidney tumors. (Section 4.4.7.1).

**Table 4-54. Summary of major mode-of-action conclusions for TCE kidney carcinogenesis (continued)**

<i>Hypothesized MOA postulated key events</i>	<i>Experimental support</i>	Human relevance	Weight-of-evidence conclusion
<b>Cytotoxicity and regenerative proliferation</b>			
<i>GSH conjugation-derived metabolites produced in situ or delivered systemically to kidney.</i>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>Multiple in vitro and in vivo studies demonstrate GSH conjugation of TCE, and availability to the kidney (see Section 3.3.3).</li> <li>Uncertainties are quantitative (precise amount of flux), not qualitative.</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>Active GSTT1 alleles are associated with higher kidney cancer risk in humans following TCE exposure as compared to null genotypes (Moore et al. 2010).</li> </ul>	Yes: demonstrated in humans in vivo and in human cells in vitro.	Highly likely that both human and rodent kidneys are exposed to the GSH-conjugation derived metabolites.
<ul style="list-style-type: none"> <li><i>Cytotoxicity.</i></li> <li><i>Compensatory cell proliferation.</i></li> <li><i>Clonal expansion of initiated cells.</i></li> </ul>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>Multiple human and laboratory animal studies demonstrating TCE to be nephrotoxic, including chronic studies (see Sections 4.4.1 and 4.4.4).</li> <li>Multiple laboratory animal studies and in vitro studies in rat and human kidney cells demonstrating DCVC to be nephrotoxic (see Sections 4.4.6.1.1 and 4.4.6.2).</li> <li>Some evidence that TCOH is nephrotoxic, but histological changes caused by TCE more similar to those caused by DCVC (see Section 4.4.6.1 and 4.4.6.3).</li> <li>Increased DNA synthesis as measured by BrdU in Eker rats.</li> <li>No increase in preneoplastic or neoplastic lesions in Eker rats exposed to TCE for 13 wk, but no data on longer durations or from other rat strains sensitive to TCE renal carcinogenesis.</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>No TCE-specific studies to establish the necessity of TCE-induced proliferation resulting from nephrotoxicity to clonal expansion and cancer.</li> </ul>	Yes: demonstrated human nephrotoxicity of TCE in vivo and DCVC in vitro.	<ul style="list-style-type: none"> <li>TCE is nephrotoxic in humans, and DCVC is likely the predominant moiety responsible.</li> <li>TCE increases cell proliferation.</li> <li>Data linking TCE-induced proliferation to clonal expansion are lacking.</li> </ul>
<i>Overall Conclusion</i>	<p><i>Sufficiency of MOA for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>Maximal levels of cytotoxicity are reached at doses below which the incidence of tumors is elevated, suggesting cytotoxicity is not sufficient for carcinogenesis.</li> <li>While cytotoxicity and regenerative cell proliferation occur and are assumed to contribute to carcinogenesis, a more plausible MOA may involve combination of cytotoxicity with mutagenicity.</li> </ul>	Yes: well established.	Data are consistent with hypothesis that cytotoxicity and regenerative proliferation contribute to TCE-induced kidney tumors, but data linking TCE-induced proliferation to clonal expansion are lacking. (Section 4.4.7.2)

**Table 4-54. Summary of major mode-of-action conclusions for TCE kidney carcinogenesis (continued)**

<i>Hypothesized MOA postulated key events</i>	<i>Experimental support</i>	Human relevance	Weight-of-evidence conclusion
<b>Peroxisome proliferation activated receptor alpha activation</b>			
<ul style="list-style-type: none"> <li>• <i>TCE oxidative metabolites (e.g., TCA), after being produced in the liver, activate PPARα in the kidney.</i></li> <li>• <i>Alterations in cell proliferation and apoptosis.</i></li> <li>• <i>Clonal expansion of initiated cells.</i></li> </ul>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>• Increased PCO activity (marker for PPARα activation) observed in rats and mice treated with TCE or TCA.</li> <li>• No increases in kidney/body weight ratios (potential marker for changes in cell proliferation/apoptosis) due to oxidative metabolites.</li> <li>• No data on altered cell proliferation/apoptosis or clonal expansion in the kidney due to PPARα activation.</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• No PCE-specific studies. No data from other chemicals on PPARα involvement in kidney tumors.</li> </ul>	Yes. Humans produce oxidative metabolites of TCE, PPARα is present in the human kidney.	Highly likely that PPARα is activated in the kidney, but little evidence for other key events.
<i>Overall</i>	<p><i>Sufficiency of MOA for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• Inadequate data to support a role for PPARα activation in renal carcinogenesis, in general, or for TCE specifically.</li> </ul>	N/A	Little evidence that PPARα activation contributes to renal carcinogenesis.

**Table 4-54. Summary of major mode-of-action conclusions for TCE kidney carcinogenesis (continued)**

<i>Hypothesized MOA postulated key events</i>	<i>Experimental support</i>	Human relevance	Weight-of-evidence conclusion
<b><math>\alpha</math>2<math>\mu</math>-Globulin-related nephropathy</b>			
<ul style="list-style-type: none"> <li>• <i>TCE oxidative metabolites (e.g., TCOH), cause hyaline droplet accumulation and an increase in <math>\alpha</math>2<math>\mu</math>-globulin, resulting in nephrotoxicity.</i></li> <li>• <i>Subsequent cytotoxicity and necrosis.</i></li> <li>• <i>Sustained regenerative tubule cell proliferation.</i></li> <li>• <i>Development of intraluminal granular casts from sloughed cellular debris associated with tubule dilatation and papillary mineralization.</i></li> <li>• <i>Foci of tubule hyperplasia in the convoluted proximal tubules.</i></li> <li>• <i>Renal tubule tumors.</i></li> </ul>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>• TCOH caused hyaline droplet accumulation and an increase in <math>\alpha</math>2<math>\mu</math>-globulin, but at levels insufficient to account for the observed nephropathy.</li> <li>• TCE is associated with small increases in kidney cancer in female rats (not consistent with <math>\alpha</math>2<math>\mu</math>-globulin hypothesis).</li> <li>• TCE is associated with kidney cancer in humans (not consistent with <math>\alpha</math>2<math>\mu</math>-globulin hypothesis).</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• Inadequate support that it is necessary for TCE-induced renal carcinogenesis.</li> </ul>	No.	Unlikely that $\alpha$ 2 $\mu$ -globulin is the major cause of TCE-induced nephrotoxicity.
<i>Overall</i>	<p><i>Sufficiency of MOA for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• Inadequate data to support a role in TCE-induced renal carcinogenesis.</li> </ul>	No.	Little evidence that increases in $\alpha$ 2 $\mu$ -globulin contribute to TCE-induced renal carcinogenesis.

**Table 4-54. Summary of major mode-of-action conclusions for TCE kidney carcinogenesis (continued)**

<i>Hypothesized MOA postulated key events</i>	<i>Experimental support</i>	Human relevance	Weight-of-evidence conclusion
<b>Formic acid-related nephrotoxicity</b>			
<ul style="list-style-type: none"> <li>• <i>TCE oxidative metabolites (e.g., TCA or TCOH), after being produced in the liver, lead to increased formation and urinary excretion of formic acid, which causes cytotoxicity in the kidney.</i></li> <li>• <i>Compensatory cell proliferation.</i></li> <li>• <i>Clonal expansion of initiated.</i></li> </ul>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>• TCOH causes histological changes in the kidney, along with increased formic acid.</li> <li>• TCOH-induced kidney effects do not account for most of the kidney effects observed after TCE exposure (not consistent with formic acid hypothesis).</li> <li>• No data as to oxidative metabolites causing regenerative proliferation, or other key events in the kidney.</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• Inadequate data to support the necessity of formic acid formation in renal carcinogenesis.</li> </ul>	Yes.	Unlikely that formic acid is a major contributor to TCE-induced nephrotoxicity.
<i>Overall</i>	<p><i>Sufficiency of MOA for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• Inadequate data supporting a sufficient role for formic acid in renal carcinogenesis, whether generally or for TCE specifically.</li> </ul>	N/A	Unlikely that formic acid formation and its sequelae contribute to TCE-induced renal carcinogenesis.

#### 4.4.7.1.1. Experimental support for the hypothesized mode of action

Evidence for the hypothesized mode of action for TCE includes: (1) the formation of GSH-conjugation pathway metabolites in the kidney demonstrated in TCE toxicokinetics studies and (2) the genotoxicity of these GSH-conjugation pathway metabolites demonstrated in most existing *in vitro* and *in vivo* assays of gene mutations (i.e., Ames test) and in assays of unscheduled DNA synthesis, DNA strand breaks, and micronuclei using both “standard” systems and renal cells/tissues.<sup>7</sup> Additional relevant data come from analyses of *VHL* mutations in human kidney tumors and studies using the Eker rat model. These lines of evidence are elaborated below.

Toxicokinetic data are consistent with these genotoxic metabolites either being delivered to or produced in the kidney. As discussed in Chapter 3, following *in vivo* exposure to TCE, the metabolites DCVG, DCVC, and NAcDCVC have all been detected in the blood, kidney, or urine of rats, and DCVG in blood and NAcDCVC in urine have been detected in humans ([2006](#); [Lash et al., 1999b](#); [Bernauer et al., 1996](#); [Birner et al., 1993](#)). In addition, *in vitro* data have shown DCVG formation from TCE in cellular and subcellular fractions from the liver, from which it would be delivered to the kidney via systemic circulation, and from the kidney (see Tables 3-23–3-24, and references therein). Furthermore, *in vitro* data in both humans and rodents support the conclusion that DCVC is primarily formed from DCVG in the kidney itself, with subsequent *in situ* transformation to NAcDCVC by *N*-Acetyl transferase or to reactive metabolites by beta-lyase, FMO, or CYPs (see Sections 3.3.3.2.2 to 3.3.3.2.5). Therefore, it is highly likely that both human and rodent kidneys are exposed to these TCE metabolites.

As discussed in Section 4.2.5, DCVG, DCVC, and NAcDCVC have been demonstrated to be genotoxic in most available *in vitro* assays.<sup>8</sup> In particular, DCVC was mutagenic in the Ames test in three of the tested strains of *S. typhimurium* (TA100, TA2638, TA98) ([Vamvakas et](#)

---

<sup>7</sup>The EPA *Cancer Guidelines* ([2005b, e](#)) note reliance on “evaluation of *in vivo* or *in vitro* short-term testing results for genetic endpoints” and evidence that “the carcinogen or a metabolite is DNA-reactive and/or has the ability to bind to DNA” as part of this weight of evidence supporting a mutagenic mode of action. While evidence from hypothesis-testing experiments that mutation is an early step in the carcinogenic process is considered if available, it is not required for determination of a mutagenic mode of action; rather, reliance on short-term genotoxicity tests is emphasized. Thus, such tests are the focus of this analysis, which also includes an analysis of other available data from humans and animals. In keeping with these principles, a formal analysis of the temporal concordance of key events in the hypothesized modes of action is not presented unless it would aid in the overall weight of evidence analysis for carcinogenicity, as presented in Section 4.11.

<sup>8</sup>Evaluation of genotoxicity data entails a weight of evidence approach that includes consideration of the various types of genetic damage that can occur. In acknowledging that genotoxicity tests are by design complementary evaluations of different mechanisms of genotoxicity, a recent IPCS publication ([Eastmond et al., 2009](#)) notes that “multiple negative results may not be sufficient to remove concern for mutagenicity raised by a clear positive result in a single mutagenicity assay.” These considerations inform the present approach. In addition, consistent with EPA’s *Cancer Guidelines* ([2005b, e](#)), the approach does not address relative potency (e.g., among TCE metabolites, or of such metabolites with other known genotoxic carcinogens) *per se*, nor does it consider quantitative issues related to the probable production of these metabolites *in vivo*. Instead, the analysis of genetic toxicity data presented in Section 4.2 and summarized here focuses on the identification of a genotoxic hazard of these metabolites; a quantitative analysis of TCE metabolism to reactive intermediates, via PBPK modeling, is presented in Section 3.5.

[al., 1988b](#); [Dekant et al., 1986c](#)) and caused dose-dependent increases in UDS in the two available assays: porcine kidney tubular epithelial cell line ([Vamvakas et al., 1996](#)) and Syrian hamster embryo fibroblasts ([Vamvakas et al., 1988a](#)). DCVC has also been shown to induce DNA strand breaks in both available studies ([Robbiano et al., 2004](#); [Jaffe et al., 1985](#)), and induce micronucleus formation in primary kidney cells from rats and humans ([Robbiano et al., 2004](#)) but not in Syrian hamster embryo fibroblasts ([Vamvakas et al., 1988a](#)). Only one study each is available for DCVG and *N*-AcDCVC, but notably, both were positive in the Ames test ([1988b](#); [Vamvakas et al., 1987](#)). Although the number of test systems was limited, these results are consistent.

These *in vitro* results are further supported by studies reporting kidney-specific genotoxicity after *in vivo* administration of TCE or DCVC. In particular, [Robbiano et al. \(1998\)](#) reported increased numbers of micronucleated cells in the rat kidney following oral TCE exposure. Oral exposure to DCVC in both rabbits ([Jaffe et al., 1985](#)) and rats ([Clay, 2008](#)) increased DNA strand breaks in the kidney. However, in one inhalation exposure study in rats, TCE did not increase DNA breakage in the rat kidney, possibly due to study limitations (limited exposure time [6 hours/day for only 5 days] and small number of animals exposed [ $n = 5$ ]; [Clay, \(2008\)](#)). One study of TCE exposure in the Eker rat, a rat model heterozygous for the tumor suppressor gene *Tsc-2*, reported no significant increase in kidney tumors as compared to controls ([Mally et al., 2006](#)). Inactivation of *Tsc-2* in this rat model is associated with spontaneous RCC with activation of pathways similar to that of *VHL* inactivation in humans ([Liu et al., 2003](#)). TCE exposure for 13 weeks (corn oil gavage) led to increased nephrotoxicity but no significant increases in preneoplastic or neoplastic lesions as compared to controls ([Mally et al., 2006](#)). This lack of increased incidence of neoplastic or preneoplastic lesions reported by [Mally et al. \(2006\)](#) in the tumor-prone Eker rat is similar to lack of significant short-term response exhibited by other genotoxic carcinogens in the Eker rat ([Stemmer et al., 2007](#); [Morton et al., 2002](#)) and may be related to the increased background rate of renal carcinomas in this animal model. [Mally et al. \(2006\)](#) also exposed primary kidney epithelial cells from the Eker rat to DCVC *in vitro* and demonstrated increased transformation similar to that of other renal carcinogens ([Horesovsky et al., 1994](#)).

As discussed in Section 4.2.1.4.1, although [Douglas et al. \(1999\)](#) did not detect increased mutations in the kidney of *lacZ* transgenic mice exposed to TCE for 12 days, these results are not highly informative as to the role of mutagenicity in TCE-induced kidney tumors, given the uncertainties in the production in genotoxic GSH conjugation metabolites in mice and the low carcinogenic potency of TCE for kidney tumors in rodents relative to what is detectable in experimental bioassays. Limited, mostly *in vitro*, toxicokinetic data do not suggest that mice have less GSH conjugation or subsequent renal metabolism/bioactivation (see Section 3.3.3.2.7), but quantitatively, the uncertainties in the flux through these pathways remain significant (see Section 3.5). In addition, similar to other genotoxic renal carcinogens analyzed by NTP, there

is limited evidence of mouse kidney tumors following TCE exposure. However, given the already low incidences of kidney tumors observed in rats, a relatively small difference in potency in mice would be undetectable in available chronic bioassays. Notably, of seven chemicals categorized as direct-acting genotoxic carcinogens that induced rat renal tumors in NTP studies, only two also led to renal tumors in the mouse (tris[2,3-dibromopropyl]phosphate and ochratoxin A) ([Reznik et al., 1979](#); [Kanisawa and Suzuki, 1978](#)), so the lack of detectable response in mouse bioassays does not preclude a genotoxic mode of action.

*VHL* inactivation (via mechanisms such as deletion, silencing, or mutation) observed in human renal clear cell carcinomas is the basis of a hereditary syndrome of kidney cancer predisposition and is hypothesized to be an early and causative event in this disease ([e.g., 2008](#)). Therefore, specific actions of TCE metabolites that produce or select for mutations of the *VHL* suppressor gene could lead to kidney tumorigenesis. Several studies have compared *VHL* mutation frequencies in cases with TCE exposures with those from control or background populations. [Brüning et al. \(1997b\)](#) and [Brauch et al. \(2004; 1999\)](#) reported differences between TCE-exposed and nonexposed RCC patients in the frequency of somatic *VHL* mutations, the incidence of a hot spot mutation of cytosine to thymine at nucleotide 454, and the incidence of multiple mutations. These data suggest that kidney tumor genotype data in the form of a specific mutation pattern may potentially serve to discriminate TCE-induced tumors from other types of kidney tumors in humans. If validated, this would also suggest that TCE-induced kidney tumors are dissimilar from those occurring in unexposed individuals. Thus, while not confirming a mutation mode of action, these data suggest that TCE-induced tumors may be distinct from those induced spontaneously in humans. However, it has not been examined whether a possible linkage exists between *VHL* loss or silencing and mutagenic TCE metabolites.

By contrast, [Schraml et al. \(1999\)](#) and [Charbotel et al. \(2007\)](#) reported that TCE-exposed RCC patients did not have significantly higher incidences of *VHL* mutations compared to nonexposed patients. However, details as to the exposure conditions were lacking in [Schraml et al. \(1999\)](#). In addition, the sample preparation methodology employed by [Charbotel et al. \(2007\)](#) and others ([Brauch et al., 1999](#); [Brüning et al., 1997b](#)) often results in poor quality and/or low quantity DNA, leading to study limitations (<100% of samples were able to be analyzed). Therefore, further investigations are necessary to either confirm or contradict the validity of the genetic biomarkers for TCE-related renal tumors reported by [Brüning et al. \(1997b\)](#) and [Brauch et al. \(2004; 1999\)](#).

In addition, while exposure to mutagens is certainly associated with cancer induction (as discussed with respect to the liver in Appendix E, Sections E.3.1 and E.3.2), examination of end-stage tumor phenotype or genotype has limitations concerning determination of early key events. The mutations that are observed with the progression of neoplasia are associated with increased genetic instability and an increase in mutation rate. Further, inactivation of the *VHL* gene also occurs through other mechanisms in addition to point mutations, such as loss of heterozygosity

or hypermethylation ([Nickerson et al., 2008](#); [Kenck et al., 1996](#)) not addressed in these studies. Recent studies examining the role of other genes or pathways suggest roles for multiple genes in RCC development ([Toma et al., 2008](#); [Furge et al., 2007](#)). Therefore, the inconsistent results with respect to *VHL* mutation status do not constitute negative evidence for a mutational mode of action and the positive studies are suggestive of a TCE-induced kidney tumor genotype.

In sum, the predominance of positive genotoxicity data in the database of available studies of TCE metabolites derived from GSH conjugation (in particular the evidence of kidney-specific genotoxicity following in vivo exposure to TCE or DCVC), coupled with the toxicokinetic data consistent with the in situ formation of these GSH-conjugation metabolites of TCE in the kidney, is consistent with the hypothesis that a mutagenic mode of action is operative in TCE-induced kidney tumors. Mutagenicity is a well-established cause of carcinogenicity. Available data on the *VHL* gene in humans add biological plausibility to these conclusions. Quantitatively, however, as discussed in Section 3.3.3.2, the precise metabolic yield of the GSH conjugation metabolites following TCE exposure remains uncertain.

#### **4.4.7.2. Hypothesized Mode of Action: Cytotoxicity and Regenerative Proliferation**

Another hypothesis is that TCE acts by a cytotoxicity mode of action in TCE-induced renal carcinogenesis. According to this hypothesis, the key events leading to TCE-induced kidney tumor formation comprise the following: the TCE GSH-conjugation metabolite, DCVC, after being either produced in situ in or delivered systemically to the kidney, causes cytotoxicity, leading to compensatory cellular proliferation and subsequently increased mutations and clonal expansion of initiated cells.

##### **4.4.7.2.1. Experimental support for the hypothesized mode of action**

Evidence for the hypothesized mode of action consist primarily of (1) the demonstration of nephrotoxicity following TCE exposure at current occupational limits in human studies and chronic TCE exposure in animal studies; (2) the relatively high potential of the TCE metabolite DCVC to cause nephrotoxicity; and (3) toxicokinetic data demonstrating that DCVC is formed in the kidney following TCE exposure. Data on nephrotoxicity of TCE and DCVC are discussed in more detail below, while the toxicokinetic data were summarized previously in the discussion of mutagenicity. Thus, the data are consistent with the hypothesized mode of action, and therefore, do not rule out a contribution from cytotoxicity and regenerative proliferation to TCE-induced kidney carcinogenesis. However, there is a lack of experimental data supporting a causal link between TCE nephrotoxicity combined with sustained cellular proliferation and TCE-induced nephrocarcinogenicity.

There is substantial evidence that TCE is nephrotoxic in humans and laboratory animals and that its metabolite, DCVC, is nephrotoxic in laboratory animals. Epidemiological studies have consistently demonstrated increased excretion of nephrotoxicity markers (NAG, protein,

albumin) at occupational ([Green et al., 2004](#)) and higher ([Bolt et al., 2004](#); [Brüning et al., 1999a](#); [1999b](#)) levels of TCE exposure. However, direct evidence of tubular toxicity, particularly in RCC cases, is not available. These studies are supported by the results of multiple laboratory animal studies. Chronic bioassays have reported very high (nearly 100%) incidences of nephrotoxicity of the proximal tubule in rats ([NTP, 1990, 1988](#)) and mice ([NTP, 1990](#); [NCI, 1976](#)) at the highest doses tested. In vivo studies examining the effect of TCE exposure on nephrotoxicity showed increased proximal tubule damage following i.p. injection and inhalation of TCE in rats ([Chakrabarti and Tuchweber, 1988](#)) and i.p. injection in mice ([Cojocel et al., 1989](#)). Studies examining DCVC exposure in rats ([Elfarrar et al., 1986](#); [Terracini and Parker, 1965](#)) and mice ([Darnerud et al., 1989](#); [Jaffe et al., 1984](#)) have also shown increases in kidney toxicity. The greater potency for kidney cytotoxicity for DCVC compared to TCE was shown by in vitro studies ([Lash et al., 1986, 1995](#); [Stevens et al., 1986](#)). These studies also further confirmed the higher susceptibility of male rats or mice to DCVC-induced cytotoxicity. Cytokaryomegaly (an effect specific to TCE and not part of the chronic progressive nephropathy or the pathology that occurs in aging rat kidneys) was observed in the majority of rodent studies and may or may not progress to carcinogenesis. Finally, as discussed extensively in Section 4.4.6.1, a detailed comparison of the histological changes in the kidney caused by TCE and its metabolites supports the conclusion that DCVC is the predominant moiety responsible for TCE-induced nephrotoxicity.

Because it is known that not all cytotoxins are carcinogens (i.e., cytotoxicity is not a specific predictor of carcinogenicity), additional experimental support is required to causally link nephrotoxicity to nephrocarcinogenicity. For chemicals that bind to  $\alpha_2\mu$ -globulin, a mode of action involving cell necrosis followed by subsequent regenerative proliferation has been hypothesized to cause kidney tumors in the absence of genotoxicity ([Short, 1993](#)). However, for other chemicals, toxicity and increased cell proliferation have been observed in the kidney without inducing tumors even after chronic exposure ([Tennant et al., 1991](#)). Similarly, in the liver, partial hepatectomy leading to regenerative hyperplasia does not by itself lead to increased hepatocarcinogenicity, and requires administration of a mutagen to exhibit enhanced carcinogenic effects. By analogy, a biologically plausible mode of action may involve a combination of mutagenicity and cytotoxicity, with mutagenicity increasing the rate of mutation and regenerative proliferation induced by cytotoxicity enhancing the selection, survival, or clonal expansion of mutated cells.

For TCE and kidney cancer, clearly, cytotoxicity occurs at doses below those causing carcinogenicity, as the incidence of nephrotoxicity in chronic bioassays is an order of magnitude higher than that of renal tumors. Thus, these data are consistent with cytotoxicity being a precursor to carcinogenicity (i.e., if the opposite were the case—carcinogenicity without cytotoxicity—then the hypothesis would be falsified). While chronic nephrotoxicity was reported in the same bioassays showing increased kidney tumor incidences, the use of such data to inform

mode of action is indirect and associative, and do not offer a test of the hypothesis ([Short, 1993](#)). Nephrotoxicity is observed in both mice and rats, in some cases with nearly 100% incidence in all dose groups, but kidney tumors are only observed at low incidences in rats at the highest tested doses ([NTP, 1990](#); [NCI, 1976](#)). Therefore, nephrotoxicity alone appears to be insufficient, or at least not rate-limiting, for rodent renal carcinogenesis, since maximal levels of toxicity are reached before the onset of tumors. Furthermore, there are multiple mechanisms by which TCE has been hypothesized to induce cytotoxicity, including oxidative stress, disturbances in calcium ion homeostasis, mitochondrial dysfunction, and protein alkylation ([Lash et al., 2000a](#)). Some of these effects may therefore, have ancillary consequences related to tumor induction which are independent of cytotoxicity per se. Therefore, data currently cannot distinguish as to whether cytotoxicity is causally related to tumorigenesis or merely associated by virtue of being a marker for a different, key causal event.

Under the hypothesized mode of action, cytotoxicity leads to the induction of repair processes and compensatory proliferation that could lead to an increased production or clonal expansion of cells previously initiated by mutations occurred spontaneously, from co-exposures, or from TCE or its metabolites. Data on compensatory cellular proliferation and the subsequent hypothesized key events in the kidney are few, with no data from rat strains used in chronic bioassays. In rats carrying the Eker mutation, Mally et al. ([2006](#)) reported increased DNA synthesis as measured by BrdU incorporation in animals exposed to the high dose of TCE (1,000 mg/kg-day) for 13 weeks, but there was no evidence of clonal expansion or tumorigenesis in the form of increased preneoplastic or neoplastic lesions as compared to controls. Therefore, in both rodent and human studies of TCE, data demonstrating a causal link between compensatory proliferation and the induction of kidney tumors are lacking.

In sum, the predominance of positive nephrotoxicity data in the database of available studies of TCE metabolites derived from GSH conjugation (in particular the evidence of kidney-specific cytotoxicity following in vivo exposure to TCE or DCVC), coupled with the toxicokinetic data consistent with the in situ formation of these GSH-conjugation metabolites of TCE in the kidney, is consistent with the hypothesis that a mode of action involving cytotoxicity and regenerative proliferation contributes to TCE-induced kidney tumors, either independently or in combination with a mutagenic mode of action. However, nephrotoxicity is not in itself predictive of tumorigenesis, and experimental data supporting for a causal link between TCE nephrotoxicity combined with sustained cellular proliferation and TCE-induced nephrocarcinogenicity are lacking. A more biologically plausible mode of action may involve a combination of mutagenicity and cytotoxicity, with mutagenicity increasing the rate of mutation and regenerative proliferation enhancing the selection, survival or clonal expansion of mutated cells. However, this hypothesis has yet to be tested experimentally.

#### **4.4.7.3. Additional Hypothesized Modes of Action with Limited Evidence or Inadequate Experimental Support**

Along with metabolites derived from GSH conjugation of TCE, oxidative metabolites are also present and could induce toxicity in the kidney. After TCE exposure, the oxidative metabolite and peroxisome proliferator, TCA, is present in the kidney and excreted in the urine as a biomarker of exposure. Hypotheses have also been generated regarding the roles of  $\alpha_2\mu$ -globulin or formic acid in nephrotoxicity induced by TCE oxidative metabolites TCA or TCOH. However, the available data are limited or inadequate for supporting these hypothesized modes of action.

##### **4.4.7.3.1. Peroxisome proliferation**

Although not as well studied as the effects of GSH metabolites in the kidney, there is evidence that oxidative metabolites affect the kidney after TCE exposure. Both TCA and DCA are peroxisome proliferator activated receptor alpha (PPAR $\alpha$ ) agonists although most activity has been associated with TCA production after TCE exposure. Exposure to TCE has been found to induce peroxisome proliferation not only in the liver, but also in the kidney. Peroxisome proliferation in the kidney has been evaluated by only one study of TCE ([Goldsworthy and Popp, 1987](#)), using increases in cyanide-insensitive palmitoyl-CoA oxidation (PCO) activity as a marker. Increases in renal PCO activity were observed in rats (3.0-fold) and mice (3.6-fold) treated with TCE at 1,000 mg/kg-day for 10 days, with smaller increases in both species from TCA treatment at 500 mg/kg-day for 10 days. However, no significant increases in kidney/body weight ratios were observed in either species. There was no relationship between induction of renal peroxisome proliferation and renal tumors (i.e., a similar extent of peroxisome proliferation-associated enzyme activity occurred in species with and without TCE-induced renal tumors). However, the increased peroxisomal enzyme activities due to TCE exposure are indicative of oxidative metabolites being present and affecting the kidney. Such metabolites have been associated with other tumor types, especially liver, and whether co-exposures to oxidative metabolites and GSH metabolites contribute to kidney tumorigenicity has not been examined.

##### **4.4.7.3.2. $\alpha_2\mu$ -Globulin-related nephropathy**

Induction of  $\alpha_2\mu$ -globulin nephropathy by TCE has been investigated by Goldsworthy et al. ([1988](#)), who reported that TCE did not induce increases in this urinary protein, nor did it stimulate cellular proliferation in rats. In addition, whereas kidney tumors associated with  $\alpha_2\mu$ -globulin nephropathy are specific to the male rat, as discussed above, nephrotoxicity is observed in both rats and mice and kidney tumor incidence is elevated (though not always statistically significant) in both male and female rats. TCOH was recently reported to cause hyaline droplet accumulation and an increase in  $\alpha_2\mu$ -globulin, but these levels were insufficient

to account for the observed nephropathy as compared to other exposures ([Green et al., 2003](#)). Therefore, it is unlikely that  $\alpha_2\mu$ -globulin nephropathy contributes significantly to TCE-induced renal carcinogenesis.

#### **4.4.7.3.3. Formic acid-related nephrotoxicity**

Another mode-of-action hypothesis proposes that TCE nephrotoxicity is mediated by increased formation and urinary excretion of formic acid mediated by the oxidative metabolites TCA or TCOH ([Green et al., 2003](#); [Dow and Green, 2000](#); [1998](#)). The subsequent hypothesized key events are the same as those for DCVC-induced cytotoxicity, discussed above (see Section 4.4.7.2). As discussed extensively in Section 4.4.6.1.2, these oxidative metabolites do not appear sufficient to explain the range of renal effects observed after TCE exposure, particularly cytomegaly, karyomegaly, and flattening and dilation of the tubular epithelium. Although TCOH and possibly TCA may contribute to the nephrotoxicity of TCE, perhaps due to excess formic acid production, these metabolites do not show the same range of cytotoxic effects observed following TCE exposure (see Table 4-53). Therefore, without specific evidence linking the specific nephrotoxic effects caused by TCOH or TCA to carcinogenesis, and in light of the substantial evidence that DCVC itself can adequately account for the nephrotoxic effects of TCE, the weight of evidence supports a conclusion that cytotoxicity mediated by increased formic acid production induced by oxidative metabolites TCOH and possibly TCA is not responsible for the majority of the TCE-induced cytotoxicity in the kidneys, and therefore, would not be the major contributor to the other hypothesized key events in this mode of action, such as subsequent regenerative proliferation.

#### **4.4.7.4. Conclusions About the Hypothesized Modes of Action**

##### **4.4.7.4.1. Is the hypothesized mode of action sufficiently supported in the test animals**

###### **4.4.7.4.1.1. Mutagenicity**

The predominance of positive genotoxicity data in the database of available studies of TCE metabolites derived from GSH conjugation (in particular the evidence of kidney-specific genotoxicity following in vivo exposure to TCE or DCVC), coupled with the toxicokinetic data consistent with the in situ formation of these GSH-conjugation metabolites of TCE in the kidney, supports the conclusion that a mutagenic mode of action is operative in TCE-induced kidney tumors.

###### **4.4.7.4.1.2. Cytotoxicity**

As reviewed above, in vivo and in vitro studies have shown a consistent nephrotoxic response to TCE and its metabolites in proximal tubule cells from male rats. Therefore, it has been proposed that cytotoxicity seen in this region of the kidney is a precursor to carcinogenicity. Available data are consistent with the hypothesis that a mode of action involving cytotoxicity

and regenerative proliferation contributes to TCE-induced kidney tumors, either independently or in combination with a mutagenic mode of action. However, it has not been determined whether tubular toxicity is a necessary precursor of carcinogenesis, and there is a lack of experimental support for causal links, such as compensatory cellular proliferation or clonal expansion of initiated cells, between nephrotoxicity and kidney tumors induced by TCE. Nephrotoxicity alone appears to be insufficient, or at least not rate-limiting, for rodent renal carcinogenesis, since maximal levels of toxicity are reached before the onset of tumors. A more biologically plausible mode of action may involve a combination of mutagenicity and cytotoxicity, with mutagenicity increasing the rate of mutation and regenerative proliferation enhancing the survival or clonal expansion of mutated cells. However, this hypothesis has yet to be tested experimentally.

#### **4.4.7.4.1.3. Additional hypothesis**

The kidney is also exposed to oxidative metabolites that have been shown to be carcinogenic in other target organs. TCA is excreted in kidney after its metabolism from TCE and also can cause peroxisome proliferation in the kidney, but there are inadequate data to define a mode of action for kidney tumor induction based on peroxisome proliferation. TCE induced little or no  $\alpha_2\mu$ -globulin and hyaline droplet accumulation to account for the observed nephropathy, so available data do not support this hypothesized mode of action. The production of formic acid following exposure to TCE and its oxidative metabolites TCOH and TCA may also contribute to nephrotoxicity; however, the available data indicate that TCOH and TCA are minor contributors to TCE-induced nephrotoxicity, and therefore, do not support this hypothesized mode of action. Because these additional mode-of-action hypotheses are either inadequately defined or are not supported by the available data, they are not considered further in the conclusions below.

#### **4.4.7.4.2. Is the hypothesized mode of action relevant to humans**

##### **4.4.7.4.2.1. Mutagenicity**

The evidence discussed above demonstrates that TCE GSH-conjugation metabolites are mutagens in microbial as well as test animal species. Therefore, the presumption that they would be mutagenic in humans. Available data on the *VHL* gene in humans add biological plausibility to this hypothesis. The few available data from human studies concerning the mutagenicity of TCE and its metabolites suggest consistency with this mode of action, but are not sufficiently conclusive to provide direct supporting evidence for a mutagenic mode of action. Therefore, this mode of action is considered relevant to humans.

#### **4.4.7.4.2.2. Cytotoxicity**

Although data are inadequate to determine that the mode of action is operative, none of the available data suggest that this mode of action is biologically precluded in humans. Furthermore, both animal and human studies suggest that TCE causes nephrotoxicity at exposures that also induce renal cancer, constituting positive evidence of the human relevance of this hypothesized mode of action.

#### **4.4.7.4.3. Which populations or lifestages can be particularly susceptible to the hypothesized mode of action**

##### **4.4.7.4.3.1. Mutagenicity**

The mutagenic mode of action is considered relevant to all populations and lifestages. According to EPA's *Cancer Guidelines* ([U.S. EPA, 2005b](#)) and *Supplemental Guidance* ([U.S. EPA, 2005e](#)), there may be increased susceptibility to early-life exposures for carcinogens with a mutagenic mode of action. Therefore, because the weight of evidence supports a mutagenic mode of action for TCE carcinogenicity and in the absence of chemical-specific data to evaluate differences in susceptibility, early-life susceptibility should be assumed and the age-dependent adjustment factors (ADAFs) should be applied, in accordance with the *Supplemental Guidance*.

In addition, because the mode of action begins with GSH-conjugation metabolites being delivered systemically or produced in situ in the kidney, toxicokinetic differences (i.e., increased production or bioactivation of these metabolites) may render some individuals more susceptible to this mode of action. However, as discussed in Section 3.3.3.2, quantitative estimates of the amount of GSH conjugation following TCE exposure remain uncertain. Toxicokinetic-based susceptibility is discussed further in Section 4.10.

In rat chronic bioassays, TCE-treated males have higher incidence of kidney tumors than similarly treated females. However, the basis for this sex difference is unknown, and whether it is indicative of a sex difference in human susceptibility to TCE-induced kidney tumors is likewise unknown. The epidemiologic studies generally do not show sex differences in kidney cancer risk. Lacking exposure-response information, it is not known if the sex-difference in one RCC case-control study ([Dosemeci et al., 1999](#)) may reflect exposure differences or susceptibility differences.

##### **4.4.7.4.3.2. Cytotoxicity**

Populations that may be more susceptible based on the toxicokinetics of the production of GSH conjugation metabolites and the sex differences observed in rat chronic bioassays are the same as for a mutagenic mode of action. No data are available as to whether other factors may lead to different populations or lifestages being more susceptible to a cytotoxic mode of action for TCE-induced kidney tumors. For instance, it is not known how the hypothesized key events in this mode of action interact with known risk factors for human RCC.

The weight of evidence sufficiently supports a mutagenic mode of action for TCE in the kidney, based on supporting data that GSH-metabolites are genotoxic and produced in sufficient quantities in the kidney to lead to tumorigenesis. Cytotoxicity and regenerative proliferation were considered as an alternate mode of action; however, there are inadequate data to support a causal association between cytotoxicity and kidney tumors. Further, hypothesized modes of action relating to peroxisomal proliferation,  $\alpha_2\mu$ -globulin nephropathy and formic acid-related nephrotoxicity were considered and rejected due to limited evidence and/or inadequate experimental support.

#### **4.4.8. Summary: TCE Kidney Toxicity, Carcinogenicity, and Mode of Action**

Human studies have shown increased levels of proximal tubule damage in workers exposed to high levels of TCE ([NRC, 2006](#)). These studies analyzed workers exposed to TCE alone or in mixtures and reported increases in various urinary biomarkers of kidney toxicity or ESRD ( $\beta_2$ -microglobulin, total protein, NAG,  $\alpha_1$ -microglobulin) ([Jacob et al., 2007](#); [Radican et al., 2006](#); [Bolt et al., 2004](#); [Green et al., 2004](#); [Brüning et al., 1999a](#); [1999b](#); [Selden et al., 1993](#); [Nagaya et al., 1989a](#)). Laboratory animal studies examining TCE exposure provide additional support, as multiple studies by both gavage and inhalation exposure show that TCE causes renal toxicity in the form of cytomegaly and karyomegaly of the renal tubules in male and female rats and mice. By gavage, incidences of these effects under chronic bioassay conditions approach 100%, with male rats appearing to be more sensitive than either female rats or mice of either sex based on the severity of effects. Under chronic inhalation exposures, only male rats exhibited these effects. Further studies with TCE metabolites have demonstrated a potential role for DCVC, TCOH, and TCA in TCE-induced nephrotoxicity. Of these, DCVC induces the renal effects that are most like TCE, and it is formed in sufficient amounts following TCE exposure to account for these effects.

Kidney cancer risk from TCE exposure has been studied related to TCE exposure in cohort, case-control, and geographical studies. These studies have examined TCE in mixed exposures as well as alone. Elevated risks are observed in many of the cohort and case-control studies examining kidney cancer incidence in industries or job titles with historical use of TCE (see Table 4-39 and 4-40), particularly among subjects ever exposed to TCE ([Moore et al., 2010](#); [Brüning et al., 2003](#); [Raaschou-Nielsen et al., 2003](#); [Dosemeci et al., 1999](#)) or subjects with TCE surrogate for high exposure ([Moore et al., 2010](#); [Charbotel et al., 2006](#); [Zhao et al., 2005](#); [Brüning et al., 2003](#); [Raaschou-Nielsen et al., 2003](#)). Greater susceptibility to TCE exposure and kidney cancer is observed among subjects with a functionally active GSTT polymorphism, particularly among those with certain alleles in single nucleotide polymorphisms of the cysteine conjugation  $\beta$ -lyase gene region ([Moore et al., 2010](#)). Although there are some controversies related to deficiencies of the epidemiological studies ([Vamvakas et al., 1998](#); [Henschler et al., 1995](#)), many of these are overcome in later studies ([Moore et al., 2010](#);

[Charbotel et al., 2006](#); [Brüning et al., 2003](#)). A meta-analysis of the overall effect of TCE exposure on kidney cancer, additionally, suggests a small, statistically significant increase in risk (RR<sub>m</sub> = 1.27 95% CI: 1.13, 1.43) with an RR<sub>m</sub> estimate in the higher exposure group of 1.58, (95% CI: 1.28, 1.96), robust in sensitivity to alternatives and lacking observed statistical heterogeneity among 17 studies meeting explicitly-defined inclusion criteria.

In vivo laboratory animal studies to date suggest a small increase in renal tubule tumors in male rats and, to a lesser extent, in female rats, with no increases seen in mice or hamsters. These results are based on limited studies of both oral and inhalation routes, some of which were deemed insufficient to determine carcinogenicity based on various experimental issues. However, because of the rarity of kidney tumors in rodents, the repeatability of this finding across strains and studies supports their biological significance despite the limitations of individual studies and relatively small increases in reported tumor incidence.

Some, but not all, human studies have suggested a role for *VHL* mutations in TCE-induced kidney cancer ([Charbotel et al., 2007](#); [2004](#); [Brauch et al., 1999](#); [Schraml et al., 1999](#); [Brüning et al., 1997b](#)). Certain aspects of these studies may explain some of these discrepant results. The majority of these studies have examined paraffinized tissue that may lead to technical difficulties in analysis, as paraffin extractions yield small quantities of often low-quality DNA. The chemicals used in the extraction process itself may also interfere with enzymes required for further analysis (PCR, sequencing). Although these studies do not clearly show mutations in all TCE-exposed individuals, or in fact in all kidney tumors examined, this does not take into account other possible means of *VHL* inactivation, including silencing or loss, and other potential targets of TCE mutagenesis were not systematically examined. A recent study by Nickerson et al. ([2008](#)) analyzed both somatic mutation and promoter hypermethylation of the *VHL* gene in clear cell-RCC frozen tissue samples using more sensitive methods. The results of this study support the hypothesis that *VHL* alterations are an early event in clear cell RCC carcinogenesis, but these alterations may not be gene mutations. No experimental animal studies have been performed examining *VHL* inactivation following exposure to TCE, although one in vitro study examined *VHL* mutation status following exposure to the TCE-metabolite DCVC ([Mally et al., 2006](#)). This study found no mutations following DCVC exposure, although this does not rule out a role for DCVC in *VHL* inactivation by some other method or *VHL* alterations caused by other TCE metabolites.

Although not encompassing all of the actions of TCE and its metabolites that may be involved in the formation and progression of neoplasia, available evidence supports the conclusion that a mutagenic mode of action mediated by the TCE GSH-conjugation metabolites (predominantly DCVC) is operative in TCE-induced kidney cancer. This conclusion is based on substantial evidence that these metabolites are genotoxic and are delivered to or produced in the kidney, including evidence of kidney-specific genotoxicity following in vivo exposure to TCE or DCVC. Cytotoxicity caused by DCVC leading to compensatory cellular proliferation is also a

potential mode of action in renal carcinogenesis. A combination of mutagenicity and cytotoxicity, with mutagenicity increasing the rate of mutation and regenerative proliferation enhancing the survival or clonal expansion of mutated cells, while biologically plausible, has yet to be tested experimentally. The additional mode-of-action hypotheses of peroxisome proliferation, accumulation of  $\alpha_2\mu$ -globulin, and cytotoxicity mediated by TCE-induced excess formic acid production are not supported by the available data.

#### **4.5. LIVER TOXICITY AND CANCER**

##### **4.5.1. Liver Noncancer Toxicity in Humans**

The complex of chronic liver disease is a spectrum of effects and comprises nonalcoholic fatty liver disease (nonalcoholic steatohepatitis) and cirrhosis, more rare anomalies ones such as autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis, and hepatocellular and cholangiocarcinoma (intrahepatic bile duct cancer) ([Juran and Lazaridis, 2006](#)). Chronic liver disease and cirrhosis, excluding neoplasia, is the 12<sup>th</sup> leading cause of death in the United States in 2005 with 27,530 deaths ([Kung et al., 2008](#)) with a morality rate of 9.0 per 100,000 ([Jemal et al., 2008](#)).

Eight studies reported on liver outcomes and TCE exposure and are identified in Table 4-55. Three studies are suggestive of effects on liver function tests in metal degreasers occupationally exposed to TCE ([Xu et al., 2009](#); [Nagaya et al., 1993](#); [Rasmussen et al., 1993b](#)). Nagaya et al. ([1993](#)) in their study of 148 degreasers in metal parts factories, semiconductor factors, or other factories, observed total mean serum cholesterol concentration and mean serum high density lipoprotein-cholesterol (HDL-C) concentrations to increase with increasing TCE exposure, as defined by U-TTC), although a statistically significant linear trend was not found. Nagaya et al. ([1993](#)) estimated that TCE exposures were 1 ppm in the low-exposure group, 6 ppm in the moderate-exposure group, and 210 ppm in the high-exposure group. No association was noted between serum liver function tests and U-TTC, a finding not surprising given that individuals with a history of hepatobiliary disease were excluded from this study. Nagaya et al. ([1993](#)) follows 13 workers with higher U-TTC concentrations over a 2-year period; serum HDL-C and two hepatic function enzymes, GGT and aspartate aminotransferase (AST) concentrations were highest during periods of high level exposure, as indicated from U-TTC concentrations. Similarly, in a study of 95 degreasers, 70 exposed to TCE and 25 exposed to CFC113 ([Rasmussen et al., 1993b](#)), mean serum GGT concentration for subjects with the highest TCE exposure duration was above normal reference values and was about threefold higher compared to the lowest exposure group. Rasmussen et al. ([1993b](#)) estimated mean urinary TCE concentration in the highest exposure group as 7.7 mg/L with past exposures estimated as equivalent to 40–60 mg/L. Multivariate regression analysis showed a small statistically nonsignificant association due to age and a larger effect due to alcohol abuse that reduced and changed direction of a TCE exposure affect. The inclusion of CFC113-exposed subjects

introduces a downward bias since liver toxicity is not associated with CFC113 exposure ([U.S. EPA, 2008b](#)) and would underestimate any possible TCE effect. Xu et al. ([2009](#)) reported symptoms and liver function tests of 21 metal degreasers with severe hypersensitivity dermatitis (see last paragraph in this section for discussion of other liver effects in hypersensitivity dermatitis cases). TCE concentration of agent used to clean metal parts ranged from 10.2 to 63.5% with workplace ambient monitoring TWA TCE concentrations of 18–683 mg/m<sup>3</sup> (3–127 ppm). Exposure was further documented by urinary TCA levels in 14 of 21 cases above the recommended occupation level of 50 mg/L. The prevalence of elevated liver enzymes among these subjects was 90% (19 cases) for alanine aminotransferase (ALT), 86% (18 cases) for AST, and 76% (16 cases) for total bilirubin ([Xu et al., 2009](#)). Two studies provide evidence of plasma or serum bile acids changes among TCE-exposed degreasers. Neghab et al. ([1997](#)) in a small prevalence study of 10 healthy workers (5 unexposed controls and 5 exposed) observed statistically significantly elevated total serum bile acids, particularly deoxycholic acid and the subtotal of free bile acids, among TCE subjects at postexposure compared to their pre-exposure concentrations and serum bile acid levels correlated well with TCE exposure ( $r = 0.94$ ). Total serum bile acid concentration did not change in control subjects between pre- and postexposure, nor did enzyme markers of liver function in either unexposed or exposed subjects differ between pre- and postexposure periods. However, the statistical power of this study is quite limited and the prevalence design does not include subjects who may have left employment because of possible liver problems. The paper provides minimal details of subject selection and workplace exposure conditions, except that pre-exposure testing was carried out on the 1<sup>st</sup> work day of the week (pre-exposure), repeated sampling after 2 days (postexposure), and a postexposure 8-hour TWA TCE concentration of 9 ppm for exposed subjects; no exposure information is provided for control subjects. Driscoll et al. ([1992](#)) in a study of 22 subjects (6 unexposed and 16 exposed) employed at a factory manufacturing small appliances reported statistically significant group differences in logistic regression analyses controlling for age and alcohol consumption in mean fasting plasma bile acid concentrations. Other indicators of liver function such as plasma enzyme levels were statistically significant different between exposed and unexposed subjects. Laboratory samples were obtained at the start of subject's work shift. Exposure data are not available on the 22 subjects and assignment of exposed and unexposed was based on work duties. Limited personal monitoring from other nonparticipating workers at this facility indicated TCE exposure as low, <5 ppm, with occasional peaks over 250 ppm, although details are lacking whether these data represent exposures of study subjects.

**Table 4-55. Summary of human liver toxicity studies**

Subjects	Effect	Exposure	Reference
148 male metal degreasers in metal parts, semiconductor and other factories	Serum liver function enzyme (HDL-C, AST, and GGT) concentrations did not correlate with TCE exposure assesses in a prevalence study but did correlate with TCE concentration over a 2-yr follow-up period	U-TTC levels obtained from spot urine sample obtained during working hrs used to assign exposure category included the following: High: 209 ± 99 mg/g Cr Medium: 35 ± 27 mg/g Cr Low: 5 ± 2 mg/g Cr Note: this study does not include an unexposed referent group	Nagaya et al. (1993)
95 workers (70 TCE exposed, 25 CFC113 exposed) selected from a cohort of 240 workers at 72 factors engaged in metal degreasing with chlorinated solvents	Increased serum GGT concentration with increasing cumulative exposure	4 groups (cumulative number of yr exposed over a working life): I: 0.6 (0–0.99) II: 1.9 (1–2.8) III: 4.4 (2.9–6.7) IV: 14.4 (6.8–35.6)	Rasmussen et al. (1993b)
21 metal degreasers with severe hypersensitivity dermatitis	High prevalence of serum liver function enzymes above normal levels: ALT, 19 or 21 cases; AST, 18 of 21 cases, and T-Bili, 16 of 21 cases	TWA mean ambient TCE concentration occupational setting of cases, 18 mg/m <sup>3</sup> –683 mg/m <sup>3</sup> 14 of 21 cases with U-TCE above recommended occupational level of 50 mg/L	Xu et al. (2009)
Five healthy workers engaged in decreasing activities in steel industry and five healthy workers from clerical section of same company	Total serum bile acid concentration increased between pre- and postexposure (2-d period)	8-hr TWA mean personal air: 8.9 ± 3.2 ppm postexposure	Neghab et al. (1997)
22 workers at a factory manufacturing small appliances	Increased in several bile acids	Regular exposure to <5 ppm TCE; peak exposure for two workers to >250 ppm	Driscoll et al. (1992)
4,489 males and female residents from 15 Superfund site and identified from ATSDR TCE Exposure Subregistry	Liver problems diagnosed with past yr	Residency in community with Superfund site identified with TCE and other chemicals	Davis et al. (2005)
Case reports from eight countries of individuals with idiosyncratic generalized skin disorders	Hepatitis in 46–94% of cases; other liver effects includes hepatomegaly and elevated liver function enzymes; and in rare cases, acute liver failure	If reported, TCE, from <50 mg/m <sup>3</sup> to >4,000 mg/m <sup>3</sup> ; symptoms developed within 2–5 wks of initial exposure, with some intervals up to 3 mo	Kamijima et al. (2007)
Deaths in California between 1979 and 1981 due to cirrhosis	SMR of 211 (95% CI: 136, 287) for white male sheet metal workers and SMR = 174 (95% CI: 150–197) for metal workers	Occupational title on death certificate	Leigh and Jiang (1993)

Davis et al. (2005) in their analysis of subjects from the TCE subregistry of ATSDR’s National Exposure Registry examined the prevalence of subjects reporting liver problems (defined as seeking treatment for the problem from a physician within the past year) using rates for the equivalent health condition from the National Health Interview Survey (a nationwide multipurpose health survey conducted by the National Center for Health Statistics, Centers for Disease Control and Prevention). The TCE subregistry is a cohort of exposed persons from 15 sites in 5 states. The shortest time interval from inclusion in the exposure registry and last follow-up was 5 years for one site and 10 years for seven sites. Excess in past-year liver

disorders relative to the general population persisted for much of the lifetime of follow-up. SMRs for liver problems were 3<sup>rd</sup> follow-up, SMR = 2.23 (99% CI: 1.13, 3.92); 4<sup>th</sup> follow-up, SMR = 3.25 (99% CI: 1.82, 5.32); and 5<sup>th</sup> follow-up, SMR = 2.82 (99% CI: 1.46, 4.89). Examination by TCE exposure, duration, or cumulative exposure to multiple organic solvents did not show exposure-response patterns. Overall, these observations are suggestive of liver disorders as associated with potential TCE exposure, but whether TCE caused these conditions is not possible to determine given the study's limitations. These limitations include a potential for misclassification bias, the direction of which could dampen observations in a negative direction, and lack of adjustment in statistical analyses for alcohol consumption, which could bias observations in a positive direction.

Evaluation in epidemiologic studies of risk factors for cirrhosis other than alcohol consumption and Hepatitis A, B, and C is quite limited. NRC (2006) cited a case report of cirrhosis developing in an individual exposed occupationally to TCE for 5 years from a hot-process degreaser and to 1,1,1-trichloroethane for 3 months thereafter (Thiele et al., 1982). One cohort study on cirrhosis deaths in California between 1979 and 1981 and occupational risk factors as assessed using job title observed elevated risks with occupational titles of sheet metal workers and metalworkers and cirrhosis among white males who comprised the majority of deaths (Leigh and Jiang, 1993). This analysis lacks information on alcohol patterns by occupational title in addition to specific chemical exposures. Few deaths attributable to cirrhosis are reported for nonwhite male and for both white and nonwhite female metalworkers with analyses examining these individuals limited by low statistical power. Some, but not all, TCE mortality studies report risk ratios for cirrhosis (see Table 4-56). A statistically significant deficit in cirrhosis mortality was observed in three studies (Boice et al., 2006b; Boice et al., 1999; Morgan et al., 1998) and with risk ratios including a risk of 1.0 in the remaining studies (ATSDR, 2004a; Ritz, 1999a; Blair et al., 1989, 1998; Garabrant et al., 1988). These results do not rule out an effect of TCE on liver cirrhosis since disease misclassification may partly explain observations. Available studies are based on death certificates where a high degree of underreporting, up to 50%, is known to occur (Blake et al., 1988).

**Table 4-56. Selected results from epidemiologic studies of TCE exposure and cirrhosis**

Study population	Exposure group	RR (95% CI)	Number of observable events	Reference
<b>Cohort and PMR-mortality</b>				
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	0.39 (0.16, 0.80)	7	Boice et al. (2006b)
	Low cumulative TCE score	Not reported		Zhao et al. (2005)
	Medium cumulative TCE score			
	High TCE score			
	<i>p</i> for trend			
View-master workers				
	Males	0.76 (0.16, 2.22)	3	ATSDR (2003b)
	Females	1.51 (0.72, 2.78)	10	
Electronic workers (Taiwan)				
	Primary liver, males	Not reported		Chang et al. (2005; 2003)
	Primary liver, females	Not reported		
Uranium-processing workers				
	Any TCE exposure	0.91 (0.63, 1.28)	33	Ritz (1999a)
	Light TCE exposure, >2 yrs duration	Not reported		
	Mod TCE exposure, >2 yrs duration	Not reported		
Aerospace workers (Lockheed)				
	TCE routine exposure	0.61 (0.39, 0.91)	23	Boice et al. (1999)
	TCE routine-intermittent	Not reported	13	
Aerospace workers (Hughes)				
	TCE subcohort	0.55 (0.30, 0.93)	14	Morgan et al. (2000, 1998)
	Low intensity (<50 ppm)	0.95 (0.43, 1.80)	9	
	High intensity (>50 ppm)	0.32 (0.10, 0.74)	5	
Aircraft maintenance workers (Hill Air Force Base, Utah)				
	TCE subcohort	1.1 (0.6, 1.9) <sup>a</sup>	44	Blair et al. (1998)
	Males, cumulative exposure			
	0	1.0 <sup>a</sup>		
	<5 ppm-yr	0.6 (0.2, 1.3)	10	
	5–25 ppm-yr	0.8 (0.3, 1.9)	9	
	>25 ppm-yr	1.2 (0.6, 2.4)	17	

**Table 4-56. Selected results from epidemiologic studies of TCE exposure and cirrhosis (continued)**

Study population	Exposure group	RR (95% CI)	Number of observable events	Reference
Aircraft maintenance workers (continued)	Females, cumulative exposure			Blair et al. (1998) (continued)
	0	1.0 <sup>a</sup>		
	<5 ppm-yr	2.4 (1.4, 13.7)	6	
	5–25 ppm-yr	1.8 (0.2, 15.0)	1	
	>25 ppm-yr	0.6 (0.1, 4.8)	1	Radican et al. (2008)
	TCE subcohort			
	Males, cumulative exposure			
	0	1.0 <sup>a,b</sup>		
	<5 ppm-yr	0.56 (0.23, 1.40)	8	
	5–25 ppm-yr	1.07 (0.45, 2.53)	10	
	>25 ppm-yr	1.06 (0.48, 2.38)	13	
	Females, cumulative exposure			
	0	1.00 <sup>a</sup>		
	<5 ppm-yr	3.30 (0.88, 12.41)	4	
5–25 ppm-yr	2.20 (0.26, 18.89)	1		
>25 ppm-yr	0.59 (0.97, 5.10)	1		
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		Not reported		Greenland et al. (1994)
U.S. Coast Guard employees			Blair et al. (1989)	
	Marine inspectors	1.36 (0.79, 2.17)		17
	Noninspectors	0.53 (0.23, 1.05)		8
Aircraft manufacturing plant employees (Italy)			Costa et al. (1989)	
	All subjects	Not reported		
Aircraft manufacturing plant employees (San Diego, California)			Garabrant et al. (1988)	
	All subjects	0.86 (0.67, 1.11)		63

<sup>a</sup>Referent group are subjects from the same plant or company, or internal referents.

<sup>b</sup>Numbers of cirrhosis deaths in Radican et al. (2008) are fewer than Blair et al. (1989) because Radican et al. (2008) excluded cirrhosis deaths due to alcohol.

A number of case reports exist of liver toxicity including hepatitis accompanying immune-related generalized skin diseases described as a variation of erythema multiforme, Stevens-Johnson syndrome, toxic epiderma necrolysis patients, and hypersensitivity syndrome (Section 4.6.1.2 describes these disorders and evidence on TCE) (Kamijima et al., 2007). Kamijima et al. (2007) reported hepatitis was seen in 92–94% of cases presenting with an immune-related generalized skin diseases of variation of erythema multiforme, Stevens-Johnson syndrome, and toxic epiderma necrolysis patients, but the estimates within the hypersensitivity syndrome group were more variable (46–94%). Many cases developed with a short time after initial exposure and presented with jaundice, hepatomegaly or hepatosplenomegaly, in addition,

to hepatitis. Hepatitis development was of a nonviral etiology, as antibody titers for Hepatitis A, B, and C viruses were not detectable, and not associated with alcohol consumption ([Kamijima et al., 2007](#); [Huang et al., 2002](#)). Liver failure was moreover a leading cause of death among these subjects. Kamijima et al. ([2007](#)) noted the similarities between specific skin manifestations and accompanying hepatic toxicity and case presentations of TCE-related generalized skin diseases and conditions that have been linked to specific medications (e.g., carbamezepine, allupurinol, antibacterial sulfonamides), possibly in conjunction with reactivation of specific latent viruses. However, neither cytomegalovirus nor Epstein-Barr viruses are implicated in the few reports that did include examination of viral antibodies.

#### **4.5.2. Liver Cancer in Humans**

Primary hepatocellular carcinoma (HCC) and cholangiocarcinoma (intrahepatic and extrahepatic bile ducts) are the most common primary hepatic neoplasms ([Blechacz and Gores, 2008](#); [El-Serag, 2007](#)). Primary HCC is the 5<sup>th</sup> most common of cancer deaths in males and 9<sup>th</sup> in females ([Jemal et al., 2008](#)). Age-adjusted incidence rates of HCC and intrahepatic cholangiocarcinoma (ICC) are increasing, with a twofold increase in HCC over the past 20 years. This increase is higher than expected from an expanded definition of liver cancer to include primary or secondary neoplasms since International Classification of Disease (ICD)-9, incorrect classification of hilar cholangiocarcinomas in ICD-O as ICC, or to improved detection methods ([El-Serag, 2007](#)). It is estimated that 21,370 Americans will be diagnosed in 2008 with liver and intrahepatic bile cancer; age-adjusted incidence rates for liver and intrahepatic bile duct cancer for all races are 9.9 per 100,000 for males and 3.5 per 100,000 for females ([Ries et al., 2008](#)). Survival for liver and biliary tract cancers remains poor and age-adjusted mortality rates are just slightly lower than incidence rates. While hepatitis B and C viruses and heavy alcohol consumption are believed major risk factors for HCC and ICC, these risk factors cannot fully account for roughly 10 and 20% of HCC cases ([Kulkarni et al., 2004](#)). Cirrhosis is considered a premalignant condition for HCC; however, cirrhosis is not a sufficient cause for HCC since 10–25% of HCC cases lack evidence of cirrhosis at time of detection ([Kumar et al., 2007](#); [Fattovich et al., 2004](#); [Chiesa et al., 2000](#)). Nonalcoholic steatohepatitis reflecting obesity and metabolic syndrome is recently suggested as contributing to liver cancer risk ([El-Serag, 2007](#)).

All cohort studies, except Zhao et al. ([2005](#)), present risk ratios (SIRs or SMRs) for liver and biliary tract cancer. More rarely reported in cohort studies are risk ratios for primary liver cancer (HCC) or for gallbladder and extrahepatic bile duct cancer. Four community studies also presented risk ratios for liver and biliary tract cancer including a case-control study of primary liver cancer of residents of Taiwanese community with solvent-contaminated drinking water wells (ATSDR, 2006a; [Lee et al., 2003](#); [Morgan and Cassady, 2002](#); [Vartiainen et al., 1993](#)). Several population case-control studies examine liver cancer and organic solvents or occupational job titles with possible TCE usage ([Lindbohm et al., 2009](#); [Ji and Hemminki, 2005](#);

[Kvam et al., 2005](#); [Weiderpass et al., 2003](#); [Porru et al., 2001](#); [Heinemann et al., 2000](#); [Døssing et al., 1997](#); [Hernberg et al., 1988](#); [Austin et al., 1987](#); [Hardell et al., 1984](#); [Hernberg et al., 1984](#); [Stemhagen et al., 1983](#)); however, the lack of detailed exposure assessment to TCE, specifically in the population case-control studies as well as in geographic-based studies, or too few exposed cases and controls in those studies that do present some information limits their usefulness for evaluating hepatobiliary or gall bladder cancer and TCE exposure. Table 4-57 presents observations from cohort, case-control, and community studies on liver and biliary tract cancer, primary liver, and gallbladder and extrahepatic bile duct cancer and TCE.

Excess liver cancer incidence is observed in most studies in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) and which met, to a sufficient degree, the standards of epidemiologic design and analysis were identified ([Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#)) as is mortality ([Radican et al., 2008](#); [Boice et al., 2006b](#); [ATSDR, 2004a](#); [Ritz, 1999a](#); [Blair et al., 1998](#); [Morgan et al., 1998](#)). Risks for primary liver cancer and for gallbladder and biliary tract cancers in females were statistically significantly elevated only in Raaschou-Nielsen et al. (2003), the study with the largest number of observed cases without suggestion of exposure duration-response patterns. Cohort studies with more uncertain exposure assessment approaches, e.g., studies of all subjects working at a factory ([Chang et al., 2005](#); [Chang et al., 2003](#); [Blair et al., 1989](#); [Costa et al., 1989](#); [Garabrant et al., 1988](#)), do not show association but are quite limited given their lacking attribution of who may have higher or lower exposure potentials. Ritz (1999a), the exception, found evidence of an exposure-response relationship; mortality from hepatobiliary cancer was found to increase with degree and duration of exposure and time since first exposure with a statistically significant but imprecise (wide CIs) liver cancer risk for those with the highest exposure and longest time since first exposure. This observation is consistent with association with TCE, but with uncertainty given one TCE exposed case in the highest exposure group and correlation between TCE, cutting fluids, and radiation exposures.

**Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer**

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	Reference
<b>Cohort and PMR studies—incidence</b>								
Aerospace workers (Rocketdyne)								
	Low cumulative TCE score	Not reported						Zhao et al. (2005)
	Medium cumulative TCE score	Not reported						
	High TCE score	Not reported						
	<i>p</i> for trend							
Danish blue-collar workers with TCE exposure								
	Males + females	1.3 (1.0, 1.6) <sup>a</sup>	82					Raaschou-Nielson et al. (2003)
	Males + females	1.4 (1.0, 1.8) <sup>b</sup>	57					
	Males, any exposure	1.1 (0.8, 1.5) <sup>b</sup>	41	1.1 (0.7, 1.6)	27	1.1 (0.6, 1.9)	14	
	<1-yr employment duration	1.2 (0.7, 2.1) <sup>b</sup>	13	1.3 (0.6, 2.5)	9	1.1 (0.3, 2.9)	4	
	1–4.9-yr employment duration	0.9 (0.5, 1.6) <sup>b</sup>	13	1.0 (0.5, 1.9)	9	0.8 (0.2, 2.1)	4	
	≥5-yr employment duration	1.1 (0.6, 1.7) <sup>b</sup>	15	1.1 (0.5, 2.1)	9	1.4 (0.5, 3.1)	6	
	Females, any exposure	2.8 (1.6, 4.6) <sup>b</sup>	16	2.8 (1.1, 5.8)	7	2.8 (1.3, 5.3)	9	
	<1-yr employment duration	2.5 (0.7, 6.5) <sup>b</sup>	4	2.8 (0.3, 10.0)	2	2.3 (0.3, 8.4)	2	
	1–4.9-yr employment duration	4.5 (2.2, 8.3) <sup>b</sup>	10	4.1 (1.1, 10.5)	4	4.8 (1.7, 10.4)	6	
	≥5-yr employment duration	1.1 (0.1, 3.8) <sup>b</sup>	2	1.3 (0.0, 7.1)	1	0.9 (0.0, 5.2)	1	

**Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)**

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Biologically-monitored Danish workers								
	Males + females	2.1 (0.7, 5.0) <sup>b</sup>	5	1.7 (0.2, 6.0)	2	2.5 (0.5, 7.3)	3	Hansen et al. (2001)
	Males	2.6 (0.8, 6.0) <sup>b</sup>	5	1.8 (0.2, 6.6)	2	3.3 (0.7, 9.7)	3	
	Females		0 (0.4 exp)		0 (0.1 exp)		0 (0.3 exp)	
	Cumulative exposure (Ikeda)	Not reported						
	<17 ppm-yr							
	≥17 ppm-yr							
	Mean concentration (Ikeda)	Not reported						
	<4 ppm							
	4+ ppm							
	Employment duration	Not reported						
	<6.25 yrs							
	≥6.25							

**Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)**

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Aircraft maintenance workers from Hill Air Force Base								
	TCE subcohort	Not reported	9	Not reported				Blair et al. (1998)
	Males, cumulative exposure							
	0	1.0 <sup>c</sup>		1.03				
	<5 ppm-yr	0.6 (0.1, 3.1)	3	1.2 (0.1, 2.1)	2			
	5–25 ppm-yr	0.6 (0.1, 3.8)	2	1.0 (0.1, 16.7)	1			
	>25 ppm-yr	1.1 (0.2, 4.8)	4	2.6 (0.3, 25.0)	3			
	Females, cumulative exposure		0		0			
Biologically-monitored Finnish workers								
	All subjects	1.89 (0.86, 3.59) <sup>b</sup>	9	2.27 (0.74, 5.29)	5	1.56 (0.43, 4.00)	4	Anttila et al. (1995)
	Mean air-TCE (Ikeda extrapolation from U-TCA)							
	<6 ppm	Not reported		1.64 (0.20, 5.92)	2			
	6+ ppm			2.74 (0.33, 9.88)	2			
Biologically-monitored Swedish workers								
	Males	1.41 (0.38, 3.60) <sup>b</sup>	4					Axelson et al. (1994)
	Females	Not reported						
<b>Cohort and PMR-mortality</b>								
	Computer manufacturing workers (IBM), New York	Not reported	1					Clapp and Hoffman (2008)

**Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)**

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Aerospace workers (Rocketdyne)								
	Any TCE (utility/eng flush)	1.28 (0.35, 3.27)	4					Boice et al. (2006b)
	Low cumulative TCE score	Not reported						Zhao et al. (2005)
	Med cumulative TCE score							
	High TCE score							
	<i>p</i> for trend							
View-Master workers								
	Males	2.45 (0.50, 7.12) <sup>d</sup>	3	1.01 (0.03, 5.63) <sup>d</sup>	1	8.41 (1.01, 30.4) <sup>d</sup>	2	ATSDR (2003b)
	Females		0 (2.61 exp)		0 (1.66 exp)		0 (0.95 exp)	
Electronic workers (Taiwan)								
	Primary liver, males	Not reported			0 (0.69 exp)			Chang et al. (2005; 2003)
	Primary liver, females	Not reported			0 (0.57 exp)			

**Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)**

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Uranium-processing workers								
	Any TCE exposure	Not reported						Ritz ( <a href="#">1999a</a> )
	Light TCE exposure, >2 yr-duration	0.93 (0.19, 4.53) <sup>e</sup>	3					
	Mod TCE exposure, >2 yr-duration	4.97 (0.48, 51.1) <sup>e</sup>	1					
	Light TCE exposure, >5 yr-duration	2.86 (0.48, 17.3) <sup>f</sup>	3					
	Mod TCE exposure, >5 yr-duration	12.1 (1.03, 144) <sup>f</sup>	1					
Aerospace workers (Lockheed)								
	TCE routine exposure	0.54 (0.15, 1.38)	4					Boice et al. ( <a href="#">1999</a> )
	TCE routine-intermittent							
	0 yr	1.00 <sup>c</sup>	22					
	Any exposure	Not reported	13					
	<1 yr	0.53 (0.18, 1.60)	4					
	1–4 yrs	0.52 (0.15, 1.79)	3					
	≥5 yrs	0.94 (0.36, 2.46)	6					
	<i>p</i> for trend	>0.20						

**Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)**

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference	
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events		
Aerospace workers (Hughes)									
	TCE subcohort	0.98 (0.36, 2.13)	6					Morgan et al. (2000, 1998)	
	Low intensity (<50 ppm) <sup>e</sup>	1.32 (0.27, 3.85)	3						
	High intensity (>50 ppm) <sup>e</sup>	0.78 (0.16, 2.28)	3						
	TCE subcohort (Cox analysis)								
	Never exposed	1.00 <sup>c</sup>	14						
	Ever exposed	1.48 (0.56, 3.91) <sup>g,h</sup>	6						
	Cumulative								
	Low	2.12 (0.59, 7.66) <sup>h</sup>	3						
	High	1.19 (0.34, 4.16) <sup>h</sup>	3						
	Peak								
	No/low	1.00 <sup>c</sup>	17						
	Medium/high	0.98 (0.29, 3.35) <sup>h</sup>	3						

**Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)**

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Aircraft maintenance workers (Hill Air Force Base, Utah)								Blair et al. ( <a href="#">1998</a> )
	TCE subcohort	1.3 (0.5, 3.4) <sup>c</sup>	15	1.7 (0.2, 16.2) <sup>3</sup>	4			
	Males, cumulative exposure							
	0	1.0 <sup>c</sup>						
	<5 ppm-yr	1.1 (0.3, 4.1)	6					
	5–25 ppm-yr	0.9 (0.2, 4.3)	3					
	>25 ppm-yr	0.7 (0.2, 3.2)	3					
	Females, cumulative exposure							
	0	1.0 <sup>c</sup>						
	<5 ppm-yr	1.6 (0.2, 18.2)	1					
	5–25 ppm-yr		0					
	>25 ppm-yr	2.3 (0.3, 16.7)	2					
	TCE subcohort	1.12 (0.57, 2.19) <sup>ci</sup>	31	1.25 (0.31, 4.97) <sup>ci</sup>	8			
	Males, cumulative exposure							
	0	1.0 <sup>c</sup>		1.03				
	<5 ppm-yr	1.17 (0.45, 3.09)	10	3.28 (0.37, 29.45)	4			
	5–25 ppm-yr	1.16 (0.39, 3.46)	6		0			
	>25 ppm-yr	1.72 (0.68, 4.38)	12	4.05 (0.45, 36.41)	4			

**Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)**

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Aircraft maintenance workers (continued)	Females, cumulative exposure	0.74 (0.18, 2.97) <sup>c</sup>	3		0			Radican et al. (2008) (continued)
	0	1.03						
	<5 ppm-yr	0.69 (0.08, 5.74)	1					
	5–25 ppm-yr		0					
	>25 ppm-yr	0.98 (0.20, 4.90)	2					
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		0.54 (0.11, 2.63) <sup>j</sup>	9					Greenland et al. (1994)
U.S. Coast Guard employees								
	Marine inspectors	1.12 (0.23, 3.26)	3					Blair et al. (1989)
	Noninspectors	Not reported	0 (2 exp)					
Aircraft manufacturing plant employees (Italy)								
	All subjects	0.70 (0.23, 1.64)	5					Costa et al. (1989)
Aircraft manufacturing plant employees (San Diego, California)								
	All subjects	0.94 (0.40, 1.86)	8					Garabrant et al. (1988)
<b>Case-control studies</b>								
Residents of community with contaminated drinking water (Taiwan)								
	Village of residency, males							Lee et al. (2003)
	Upstream	1.00						
	Downstream	2.57 (1.21, 5.46)	26					

**Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)**

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	Reference
<b>Geographic studies</b>								
Residents in two study areas in Endicott, New York		0.71 (0.09, 2.56)	<6					ATSDR ( <a href="#">2006a</a> )
Residents in 13 census tracts in Redlands, California		1.29 (0.74, 2.05) <sup>k</sup>	28					Morgan and Cassidy ( <a href="#">2002</a> )
Finnish residents								
	Residents of Hausjarvi	0.76 (0.3, 1.4)	7					Vartiainen et al. ( <a href="#">1993</a> )
	Residents of Huttula	0.6 (0.2, 1.3)	6					

<sup>a</sup>ICD-7, 155 and 156; primary liver (155.0), gallbladder, and biliary passages (155.1), and liver secondary and unspecified (156).

<sup>b</sup>ICD-7, 155; primary liver, gallbladder, and biliary passages.

<sup>c</sup>Internal referents, workers without TCE exposure.

<sup>d</sup>PMR.

<sup>e</sup>Logistic regression analysis with a 0-year lag for TCE exposure.

<sup>f</sup>Logistic regression analysis with a 15-year lag for TCE exposure.

<sup>g</sup>Risk ratio from Cox Proportional Hazard Analysis, stratified by age, sex, and decade in Environmental Health Strategies ([1997](#)).

<sup>h</sup>Morgan et al. ([1998](#)) do not identify if SIR is for liver and biliary passage or primary liver cancer; identified as primary liver in NRC ([2006](#)).

<sup>i</sup>Radican et al. ([2008](#)) provide results for TCE exposure for follow-up through 1990, comparing the Poisson model rate ratios as reported by Blair et al. ([1998](#)) with Cox model hazard ratios. RR from Cox model adjusted for age and gender for liver and intrahepatic bile duct cancer was 1.2 (95% CI: 0.5, 3.4) and for primary liver cancer was 1.3 (95% CI: 0.1, 12.0).

<sup>j</sup>OR.

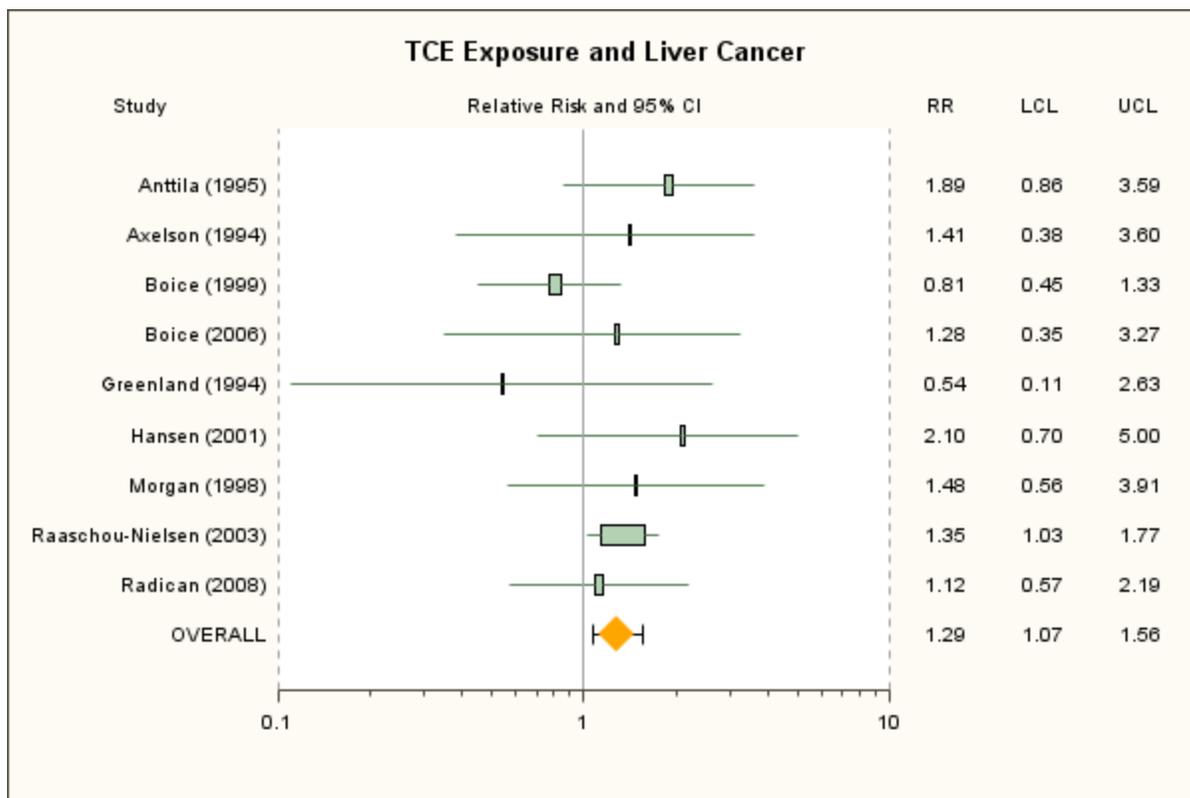
<sup>k</sup>99% CIs.

exp = expected

Observations in these studies provide some evidence of susceptibility of liver, gallbladder, and biliary tract; these observations are consistent with pharmacokinetic processing of TCE and the extensive intra- and extrahepatic recirculation of metabolites. Magnitude of risk of gallbladder and biliary tract cancer is slightly higher than that for primary liver cancer in Raaschou-Nielsen et al. (2003), the study with the most cases. Observations in Blair et al. (1998), Hansen et al. (2001), and Radican et al. (2008), three smaller studies, suggest slightly larger risk ratios for primary liver cancer compared to gallbladder and biliary tract cancer. Overall, these studies are not highly informative for cross-organ comparison of relative magnitude of susceptibility.

The largest geographic studies (Lee et al., 2003; Morgan and Cassady, 2002) are also suggestive of association with the risk ratio (mortality OR) in Lee et al. (2003) as statistically significantly elevated. The geographic studies do not include a characterization of TCE exposure to individual subjects other than residency in a community with groundwater contamination by TCE with potential for exposure misclassification bias dampening observations; these studies lack characterization of TCE concentrations in drinking water and exposure characteristics such as individual consumption patterns. For this reason, observations in Morgan and Cassidy (2002) and Lee et al. (2003) are noteworthy, particularly if positive bias leading to false positive finding is considered minimal, and the lack of association with liver cancer in the two other community studies (ATSDR, 2006a; Vartiainen et al., 1993) does not detract from Morgan and Cassidy (2002) or Lee et al. (2003). Lee et al. (2003), however, do not address possible confounding related to hepatitis viral infection status, a risk factor for liver cancer, or potential misclassification due to the inclusion of secondary liver cancer among the case series, factors which may amplify observed association.

Meta-analysis is adopted as a tool for examining the body of epidemiologic evidence on liver cancer and TCE exposure, to identify possible sources of heterogeneity and as an additional means to identify cancer hazard. The meta-analyses of the overall effect of TCE exposure on liver (and gall bladder/biliary passages) cancer suggest a small, statistically significant increase in risk. The summary estimate from the primary random effects meta-analysis of the 9 (all cohort) studies is 1.29 (95% CI: 1.07, 1.56) (see Figure 4-3). The study of Raaschou-Nielsen et al. (2003) contributes about 57% of the weight; its removal from the analysis decreases somewhat the RR<sub>m</sub> estimate and is no longer statistically significant (RR<sub>m</sub> = 1.22; 95% CI: 0.93, 1.61). The summary estimate was not overly influenced by any other single study, nor was it overly sensitive to individual RR estimate selections. There is no evidence of publication bias in this data set, and no observable heterogeneity ( $I^2 = 0\%$ ) across the study results.



Random effects model; fixed effect model same. The summary estimate is in the bottom row, represented by the diamond. Symbol sizes reflect relative weights of the studies.

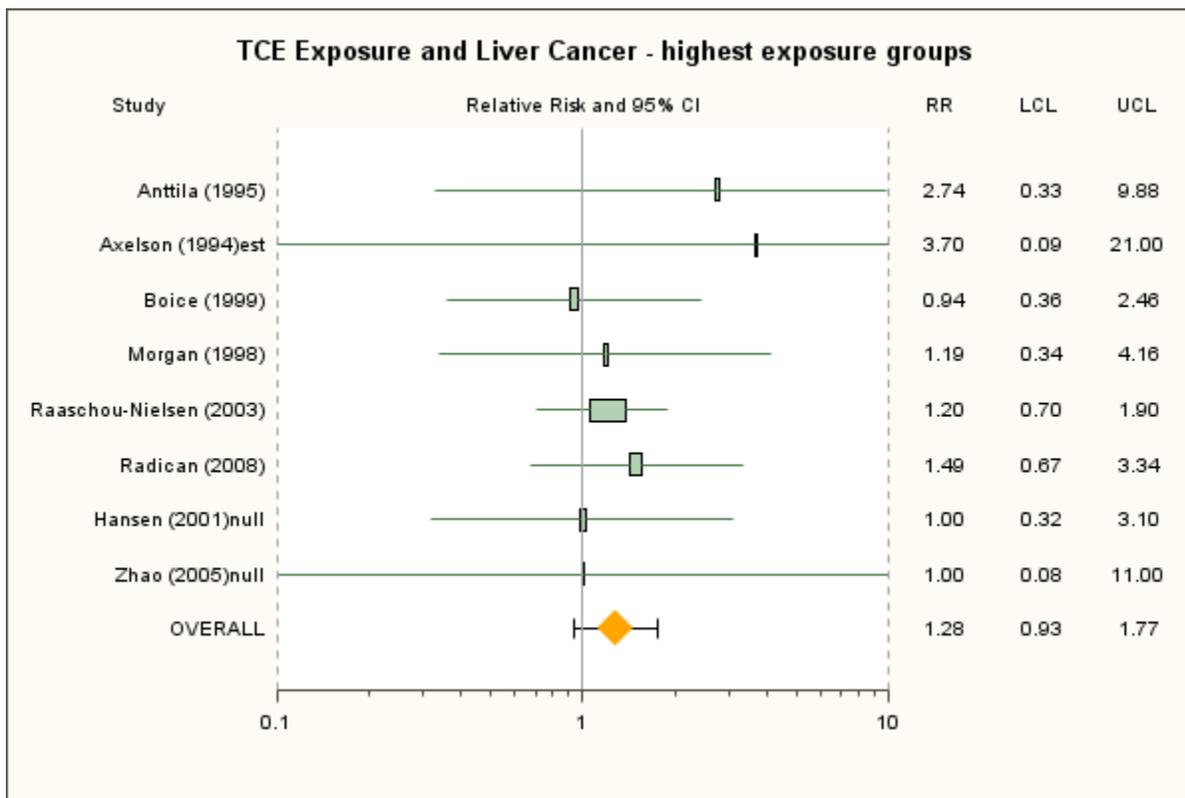
**Figure 4-3. Meta-analysis of liver and biliary tract cancer and overall TCE exposure.**

Examination of sites individually (i.e., primary liver and intrahepatic bile ducts separate from the combined liver and gallbladder/biliary passage grouping) resulted in the RRM estimate for liver cancer alone (for the three studies for which the data are available; for the other studies, results for the combined grouping were used) slightly lower than the one based entirely on results from the combined cancer categories and was just short of statistical significance (1.25; 95% CI: 0.99, 1.57). This result is driven by the fact that the risk ratio estimate from the large Raaschou-Nielsen et al. (2003) study decreased from 1.35 for liver and gall bladder/biliary passage cancers combined to 1.28 for liver cancer alone.

The RRM estimate from the random effects meta-analysis of liver cancer in the highest exposure groups in the six studies that provide risk estimates associated with highest exposure.

Primary liver cancer is 1.32 (95% CI: 0.93, 1.86), slightly lower than the RRM estimate for liver and gallbladder/biliary cancer and any TCE exposure of 1.33 (95% CI: 1.09, 1.64), and not statistically significant (see Figure 4-4). Again, the RRM estimate of the highest-exposure groups is dominated by one study (Raaschou-Nielsen et al., 2003). Two studies lack reporting of liver cancer risk associated with highest exposure, so consideration of reporting bias (considered the primary analysis) lead to a result of 1.28 (95% CI: 0.93, 1.77), similar to that estimated in the

more restricted set of studies presenting risk ratios association with highest exposure groups in published papers.



Random effects model; fixed effect model same. The summary estimate is in the bottom row, represented by the diamond. Symbol sizes reflect relative weights of the studies. Assumed null RR estimates for Hansen and Zhao (see Appendix C text).

**Figure 4-4. Meta-analysis of liver cancer and TCE exposure—highest exposure groups.**

Different exposure metrics are used in the various studies, and the purpose of combining results across the different highest exposure groups is not to estimate an RRm associated with some level of exposure, but rather to examine impacts of combining RR estimates that should be less affected by exposure misclassification. In other words, the highest exposure category is more likely to represent a greater differential TCE exposure compared to people in the referent group than the exposure differential for the overall (typically any vs. none) exposure comparison. Thus, if TCE exposure increases the risk of liver and gallbladder/biliary cancer, the effects should be more apparent in the highest exposure groups. The findings of a lower RRm associated with highest exposure group reflects observations in Radican et al. (2008) and Raaschou-Nielsen et al. (2003), the study contributing greatest weight to the meta-analysis, that

RR estimates for the highest-exposure groups, although  $>1.0$ , are less than the RR estimates with any TCE exposure.

Thus, while the finding of an elevated and statistically significant RRM for liver and gallbladder/biliary cancer and any TCE exposure provides evidence of association, the statistical significance of the summary estimates is dependent on one study, which provides the majority of the weight in the meta-analyses. Furthermore, combining results from the highest-exposure groups yields lower RRM estimates than for an overall effect. These results do not rule out an effect of TCE on liver cancer, because the liver cancer results are relatively underpowered with respect to numbers of studies and number of cases; overall, the meta-analysis provides only minimal support for association between TCE exposure and liver and gallbladder/biliary cancer.

NRC (2006) deliberations on TCE commented on two prominent evaluations of the then-current TCE epidemiologic literature using meta-analysis techniques, Wartenberg et al. (2000) and Kelsh et al. (2005), submitted by Exponent-Health Sciences to NRC during their deliberations and published afterwards in the open literature as Alexander et al. (2007a) adding the then-published study of Boice et al. (2006b). NRC (2006) found weaknesses in the techniques used in Wartenberg et al. (2000) and the Exponent analyses. EPA staff conducted their analysis according to NRC (2006) suggestions for transparency, systematic review criteria, and examination of both cohort and case-control studies. The EPA analysis of liver cancer considered a similar set of studies as Alexander et al. (2007a), although treatment of these studies differs between analyses. Alexander et al. (2007a) in their Table 2, for example, present RRM estimates, grouping of studies with differing exposure potentials, for example, including liver and biliary cancer risk estimates for all subjects, those exposed and unexposed to TCE, in Boice et al. (1999), Blair et al. (1998), Morgan et al. (1998), and Boice et al. (2006b), with biomarker studies (Hansen et al., 2001; Anttila et al., 1995; Axelson et al., 1994). The inclusion of risk estimates for subjects who have little to no TCE exposure over background levels has the potential to introduce misclassification bias and dampen observed risk ratios. Potential bias from exposure misclassification may be substantial in Alexander et al. (2007a) since the percentage of TCE exposed subjects to all cohort subjects in the four studies was 3, 23, 51 and 68% in Boice et al. (1999), Morgan et al. (1998), Blair et al. (1998), and Boice et al. (2006b), respectively, and is a likely alternative explanation for observed inconsistency across occupational groups reported by the authors. Another difference between the EPA and previous meta-analyses is their treatment of Ritz (1999a), included in Wartenberg et al. (2000), Kelsh et al. (2005), and Alexander et al. (2007a), but not in this analysis. For a grouping of studies with subcohorts most similar to those in EPA's analysis, summary liver and gall bladder/biliary tract cancer risk estimates for overall TCE exposure for TCE subcohorts is of a similar magnitude as that observed in EPA's updated and expanded analysis, Wartenberg et al. (2000), 1.1 (95% CI: 0.3, 4.8) for incidence and 1.1 (95% CI: 0.7, 1.7) for mortality, Kelsh et al. (2005), 1.32 (95% CI: 1.05, 1.66) and Alexander et al. (2007a), 1.30 (95% CI: 1.09–1.55).

### 4.5.3. Experimental Studies of TCE in Rodents—Introduction

The previous sections have described available human data for TCE-induced noncancer effects (e.g., disturbances in bile production) and whether an increased risk of liver cancer in humans has been established from analysis of the epidemiological literature. A primary concern for effects on the liver comes from a large database in rodents indicating that, not only TCE, but also a number of its metabolites are capable of inducing hepatocellular adenomas and carcinomas in rodent species. Thus, many of rodent bioassays have focused on the study of liver cancer for TCE and its metabolites and possible early effects specifically that may be related to tumor induction.

This section describes the hazard data for TCE effects in the rodent liver and inferences from studies of its metabolites. For more detailed descriptions of the issues providing context for these data in terms the state of the science of liver physiology (see Section E.1), cancer (see Section E.3), liver cancer (see Section E.3), and the mode of action of liver cancer and other TCE-induced effects (see Section E.3.4), please see Appendix E. A more comprehensive review of individual studies of TCE-induced liver effects in laboratory animals is also provided in Section E.2 that includes detailed analyses of the strengths and the limitations of these studies. Issues have been raised regarding the relevance of mouse liver tumor data to human liver cancer risk that are addressed in Sections E.3.2 and E.3.3. Given that activation of the PPAR $\alpha$  receptor has received great attention as a potential mode of action for TCE-induced liver tumors, the current status of that hypothesis is reviewed in Section E.3.4.1. Finally, comparative studies of TCE metabolites and the similarities and differences of such study results are described in summary sections of Appendix E (i.e., Section E.2.4) as well as discussions of proposed modes of action for TCE-induced liver cancer (i.e., Sections E.2.4 and E.3.4.2).

A number of acute and subchronic studies have been undertaken to describe the early changes in the rodent liver after TCE administration, with the majority using the gavage route of administration. Several key issues affect the interpretation of these data. The few drinking water studies available for TCE have recorded a significant loss of TCE through volatilization in drinking water solutions and thus, this route of administration is generally not used. Some short-term studies of TCE have included detailed examinations, while others have reported primarily liver weight changes as a marker of TCE response. The matching and recording of age, but especially initial and final body weight, for control and treatment groups is of particular importance for studies using liver weight gain as a measure of TCE response as differences in these parameters affect TCE-induced liver weight gain. Most data are for TCE exposures of at least 10–42 days. For many of the subchronic inhalation studies ([Kjellstrand et al., 1983a](#); [Kjellstrand et al., 1983b](#); [Kjellstrand et al., 1981b](#)), issues associated with whole-body exposures make determination of dose levels more difficult. The focus of the long-term studies of TCE is primarily detection and characterization of liver tumor formation.

For gavage experiments, death due to gavage errors and specifically from use of this route of administration, especially at higher TCE exposure concentrations, has been a recurring problem, especially in rats. Unlike inhalation exposures, the effects of vehicle can also be an issue for background liver effects in gavage studies. Concerns regarding effects of oil vehicles, especially corn oil, have been raised ([Charbonneau et al., 1991](#); [Kim et al., 1990a](#)). Several oral studies in particular document that use of corn oil as the vehicle for TCE gavage dosing induces a different pattern of toxicity, especially in male rodents ([see Merrick et al., 1989, Section E.2.2.1](#)). Several studies also report the effects of corn oil on hepatocellular DNA synthesis and indices of lipid peroxidation ([Rusyn et al., 1999](#); [Channel et al., 1998](#)). For example, Rusyn et al. ([1999](#)) report that a single dose of dietary corn oil increases hepatocyte DNA synthesis 24 hours after treatment by ~3.5-fold of control, activates of NF-κB to a similar extent ~2 hours after treatment almost exclusively in Kupffer cells, and induces an approximate three- to fourfold increase of control NF-κB in hepatocytes after 8 hours and an increase in tumor necrosis factor (TNF)-α mRNA between 8 and 24 hours after a single dose in female rats.

In regard to studies that have used the i.p. route of administration, as noted by Kawamoto et al. ([1988b](#)), injection of TCE may result in paralytic ileus and peritonitis and that subcutaneous treatment paradigm will result in TCE not immediately being metabolized but retained in the fatty tissue. Wang and Stacey ([1990](#)) state that “intraperitoneal injection is not particularly relevant to humans” and suggest that intestinal interactions require consideration in responses such as increase serum bile acid.

While studies of TCE metabolites have been almost exclusively conducted via drinking water, and thus, have avoided vehicle effects and gavage error, they have issues of palatability at high doses and decreased drinking water consumption as a result that raises issues not only of the resulting internal dose of the agent, but also of effects of drinking water reduction.

Although there are data for both mice and rats for TCE exposure and studies of its metabolites, the majority of the available information has been conducted in mice. This is especially the case for long-term studies of DCA and TCA in rats. There is currently one study each available for TCA and DCA in rats and both were conducted with such few numbers of animals that the ability to detect and discern whether there was a treatment-related effect are very limited ([DeAngelo et al., 1997, 1996](#); [Richmond et al., 1995](#)).

With regard to the sensitivity of studies used to detect a response, there are issues regarding not only the number of animals used, but also the strain and weight of the animals. For some studies of TCE strains were used that have less background rate of liver tumor development and carcinogenic response. As for the B6C3F<sub>1</sub> mouse, the strain most used in the bioassays of TCE metabolites, the susceptibility of the B6C3F<sub>1</sub> to hepatocarcinogenicity has made the strain a sensitive biomarker for a variety of hepatocarcinogens. Moreover, Leakey et al. ([2003a](#)) demonstrated that increased body weight at 45 weeks of life is an accurate predictor of large background tumor rates. Unfortunately a 2-year study of CH ([George et al.,](#)

2000) and the only available 2-year study of TCA (DeAngelo et al., 2008), which used the same control animals, were both conducted in B6C3F<sub>1</sub> mice that grew very large (~50 g) and prone to liver cancer (64% background incidence of hepatocellular adenomas and carcinomas) and premature mortality. Thus, these bioassays are of limited value for determination of the dose-response for carcinogenicity.

Finally, as discussed below, the administration of TCE to laboratory animals as well as environmental exposure of TCE in humans are effectively co-exposure studies. TCE is metabolized to a number of hepatoactive as well as hepatocarcinogenic agents. A greater variability of response is expected than from exposure to a single agent, making it particularly important to look at the TCE database in a holistic fashion rather than the results of a single study, especially for quantitative inferences. This approach is particularly useful given that the number of animals in treatment groups in a variety of TCE and TCE metabolite studies have been variable and small for control and treatment groups. Thus, their statistical power was limited not only for detection of statistically significant changes, but also, in many cases, to be able to determine whether there is not a treatment related effect (i.e., Type II error for power calculation). Section E.2.4.2 provides detailed analyses of the database for liver weight induction by TCE and its metabolites in mice and the results of those analyses are described below. Specifically, the relationship of liver weight induction, but also other endpoints such as peroxisomal enzyme activation and increases in DNA synthesis to liver tumor responses are also addressed as well.

#### **4.5.4. TCE-Induced Liver Noncancer Effects**

A number of effects have been studied as indicators of TCE effects on the liver but also as proposed events whose sequelae could be associated with resultant liver tumors after chronic TCE exposure in rodents. Similar effects have been studied in rodents exposed to TCE metabolites, which may be useful for determining not only whether such effects are associated with liver tumors induced by these metabolites but also if they are similar to what has been observed for TCE. Summaries of the laboratory animal studies of TCE noncancer effects in the liver are provided in Table 4-58 (oral studies) and Table 4-59 (inhalation studies), along with the types of effects discussed in the subsections below for each study.

**Table 4-58. Oral studies of TCE-induced liver effects in mice and rats**

Reference <sup>a</sup>	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Section(s) where noncancer liver effects are discussed
Berman et al. (1995)	F344 rats (F)	Corn oil gavage	0, 150, 500, 1,500, or 5,000 mg/kg for 1 d 0, 50, 150, 500, or 1,500 mg/kg-d for 14 d	8/group	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology E.2.1.1.1. Berman et al. (1995)
Buben and O'Flaherty (1985)	Swiss-Cox mice (M)	Corn oil gavage	0, 100, 200, 400, 800, 1,600, 2,400, or 3,200 mg/kg-d, 5 d/wk for 6 wks	12–15/group	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology E.2.2.7. Buben and O'Flaherty (Buben and O'Flaherty, 1985)
Channel et al. (1998)	B6C3F <sub>1</sub> /CrIBR mice (M)	Corn oil gavage	0 (water), 0 (corn oil), 400, 800, or 1,200 mg/kg-d, 5 d/wk for up to 8 wks	77/group	4.5.4.2 Cytotoxicity and histopathology 4.5.4.3 Measures of DNA Synthesis, Cellular Proliferation, and Apoptosis 4.5.4.4 Peroxisome proliferation and related effects 4.5.4.5 Oxidative stress E.2.2.8. Channel et al. (1998)
Dees and Travis (1993)	B6C3F <sub>1</sub> mice (M and F)	Corn oil gavage	0, 100, 250, 500, or 1,000 mg/kg-d for 10 d	5/group	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology 4.5.4.3 Measures of DNA Synthesis E.2.1.9. Dees and Travis (1993)
Elcombe et al. (1985)	B6C3F <sub>1</sub> and Alderley Park (Swiss) mice (M) Osborne-Mendel and Alderley Park (Wistar) rats (M)	Corn oil gavage	0, 500, 1,000, or 1,500 mg/kg-d for 10 d	6–10/group	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology 4.5.4.3 Measures of DNA Synthesis, Cellular Proliferation, and Apoptosis 4.5.4.4 Peroxisome proliferation and related effects E.2.1.8. Elcombe et al. (1985)
Goel et al. (1992)	Swiss albino mice (M)	Groundnut oil gavage	0, 500, 1,000, or 2,000 mg/kg-d, 5 d/wk for 28 d	6/group	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology 4.5.4.4 Peroxisome proliferation and related effects E.2.2.2. Goel et al. (1992)

**Table 4-58. Oral studies of TCE-induced liver effects in mice and rats (continued)**

Reference <sup>a</sup>	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Section(s) where noncancer liver effects are discussed
Goldsworthy and Popp (1987)	F344 rats (M) B6C3F <sub>1</sub> mice (M)	Corn oil or methyl cellulose gavage	1,000 mg/kg-d for 10 d	5–7/group	4.5.4.1 Liver weight 4.5.4.4 Peroxisome proliferation and related effects E.2.1.7. Goldsworthy and Popp (1987)
Laughter et al. (2004)	Sv/129 and PPAR $\alpha$ -null mice (M)	Methyl-cellulose gavage	0–1,500 mg/kg-d for 3 d; and 5 d/wk for 3 wks	4–5/group	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology 4.5.4.3 Measures of DNA Synthesis, Cellular Proliferation, and Apoptosis 4.5.4.4 Peroxisome proliferation and related effects E.2.1.13. Laughter et al. (2004)
Melnick et al. (1987)	F344 rats (M)	Micro-encapsulated in feed Corn oil gavage	0, 0.055, 1.10, 2.21, or 4.41% in feed for 14 d, equivalent to 0, 600, 1.300, 2.200, or 4.800 mg/kg-d	10/group	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology 4.5.4.4 Peroxisome proliferation and related effects E.2.1.12. Melnick et al. (1987)
Merrick et al. (1989)	B6C3F <sub>1</sub> mice (M and F)	Corn oil and 20% Emulphor in water gavage	Males: 0, 600, 1,200, or 2,400 mg/kg-d Females: 0, 450, 900, or 1,800 mg/kg-d	12/group	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology E.2.2.1. Merrick et al. (1989)
Mirsalis et al. (1989)	B6C3F <sub>1</sub> mice (M and F) F344 rats (M)	Corn oil gavage	0, 50, 200, or 1,000 mg/kg (single dose)	3/group	4.5.4.3 Measures of DNA Synthesis, Cellular Proliferation, and Apoptosis E.2.4.1. Summary of Results for Short-term Effects of TCE
Nakajima et al. (2000)	Sv/129 and PPAR $\alpha$ -null mice (M and F)	Corn oil gavage	0 or 750 mg/kg-d for 14 d	6/sex/ group	4.5.4.1 Liver weight 4.5.4.4 Peroxisome proliferation and related effects E.2.1.10. Nakajima et al. (2000)
NTP (1990)	B6C3F <sub>1</sub> mice (M and F) F334/N rats (M and F)	Corn oil gavage	Mice: 0, 375–6,000 mg/kg-d, 5 d/wk, 13 wks Rats: 0, 62.5–1,000 mg/kg-d, 5 d/wk, 13 wks	10/group	4.5.4.2 Cytotoxicity and histopathology E.2.2.12.1 13-wk studies
NTP (1990)	B6C3F <sub>1</sub> mice (M and F) F334/N rats (M and F)	Corn oil gavage	Mice: 0, or 1,000 mg/kg-d, 5 d/wk, 103 wks Rats: 0, 500, or 1,000 mg/kg-d, 5 d/wk, 103 wks	50/group	4.5.4.2 Cytotoxicity and histopathology E.2.2.12.2 2-yr studies

**Table 4-58. Oral studies of TCE-induced liver effects in mice and rats (continued)**

<b>Reference<sup>a</sup></b>	<b>Animals (sex)</b>	<b>Exposure route</b>	<b>Dose/exposure concentration</b>	<b>Exposed</b>	<b>Section(s) where noncancer liver effects are discussed</b>
Nunes et al. ( <a href="#">2001</a> )	Sprague-Dawley rats (M)	Corn oil gavage	2,000 mg/kg-d on d 10–16 (with and without lead carbonate pretreatment for 9 d)	10/group	4.5.4.1. Liver weight 4.5.4.2. Cytotoxicity and histopathology E.2.1.4. Nunes et al. ( <a href="#">2001</a> )
Tao et al. ( <a href="#">2000</a> )	B6C3F <sub>1</sub> mice (F)	Corn oil gavage	1,000 mg/kg-d for 5 d	4–6/group	4.5.4.1. Liver weight E.2.1.5. Tao et al., ( <a href="#">2000</a> )
Tucker et al. ( <a href="#">1982</a> )	CD-1 mice (M and F)	Drinking water with 1% Emulphor	0 (untreated), 0 (vehicle), 0.1, 1.0, 2.5, or 5 mg/mL for 4 or 6 mo M: 0, 0, 18.4, 216.7, 393.0, or 660.2 mg/kg-d F: 0, 0, 17.9, 193.0, 437.1, or 793.3 mg/kg-d	140/group untreated and TCE-treated 260/group vehicle-treated	4.5.4.1. Liver weight E.2.1.6. Tucker et al. ( <a href="#">1982</a> )

<sup>a</sup>**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

**Table 4-59. Inhalation and i.p. studies of TCE-induced liver effects in mice and rats**

Reference <sup>a</sup>	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Section(s) where noncancer liver effects are discussed
Hamdan and Stacey (1993)	Sprague-Dawley rats (M)	i.p. in corn oil	0 or 131 mg/kg	6/group	4.5.4.6. Bile production E.2.6. Serum Bile Acid Assays
Kaneko et al. (2000)	MRL-lpr/lpr mice (M)	Inhalation	0, 500, 1,000, or 2,000 ppm, 4 hrs/d, 6 d/wk, for 8 wks	5/group	4.5.4.2. Cytotoxicity and histopathology
Kjellstrand et al. (1981b)	NMRI mice. Sprague-Dawley rats Mongolian gerbils	Inhalation	150 ppm continuous for 2–30 d	4–12/group	4.5.4.1. Liver weight E.2.2.3. Kjellstrand et al., (1981b)
Kjellstrand et al. (1983b)	wild, C57Bl, DBA, B6CBA, A/sn, NZB, and NMRI mice (M and F)	Inhalation	150 ppm continuous for 30 d	6/group	4.5.4.1. Liver weight E.2.2.5. Kjellstrand et al., (1983b)
<b>Kjellstrand et al. (1983a)</b>	<b>NMRI mice (M and F)</b>	<b>Inhalation</b>	<b>0–3,600 ppm, variable time periods of 1–24 hrs/d, for 30 or 120 d.</b>	<b>10–20/group</b>	<b>4.5.4.1. Liver weight 4.5.4.2. Cytotoxicity and histopathology E.2.2.6. Kjellstrand et al., (1983a)</b>
Kumar et al. (2001a)	Wistar rats (M)	Inhalation	376 ppm, 4 hrs/d, 5 d/wk, 8–24 wks	6/group	4.5.4.2. Cytotoxicity and histopathology E.2.2.10. Kumar et al.(2001b)
Okino et al. (1991)	Wistar rats (M)	Inhalation	0, 500 (8 hrs), 2,000 (2 or 8 hrs), or 8,000 ppm (2 hrs) (single exposure)	5/group	4.5.4.2. Cytotoxicity and histopathology E.2.1.3. Okino et al. (1991)
Ramdhan et al. (2008)	SV/129 mice (M) CYP2E1-null mice (M)	Inhalation	0, 1,000, or 2,000 ppm, 8 hrs/d, 7 d	6/group	4.5.4.2. Cytotoxicity and histopathology 4.5.6.2.1. Hepatomegally- qualitative and quantitative comparisons 4.5.6.2.2. Cytotoxicity E.2.1.14. Ramdhan et al. (2008)

**Table 4-59. Inhalation and i.p. studies of TCE-induced liver effects in mice and rats (continued)**

Reference <sup>a</sup>	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Section(s) where noncancer liver effects are discussed
Ramdhan et al. (2010)	Sv/129, PPAR $\alpha$ -null, and hPPAR $\alpha$ mice (M)	Inhalation	0, 1,000, or 2,000 ppm, 8 hrs/d, 7 d	6/group]	4.5.4.1. Liver weight 4.5.4.2. Cytotoxicity and histopathology 4.5.6.2.1. Hepatomegally- qualitative and quantitative comparisons 4.5.6.2.2. Cytotoxicity 4.5.7.2. Peroxisome Proliferator Activated Receptor Alpha (PPAR $\alpha$ ) Receptor Activation E.2.1.15. Ramdhan et al. (2010)
Toraason et al. (1999)	Fischer rats (M)	i.p. in Alkamuls/ water	0, 100, 500, or 1,000 mg/kg	6/group	4.5.4.5. Oxidative stress E.2.4.3. Summary of TCE Subchronic and Chronic Studies E.3.4.2.3. Oxidative Stress
Wang and Stacey (1990)	Sprague-Dawley rats (M)	i.p. in corn oil Inhalation	i.p.: 0, 1.3–1,314 mg/kg-d for 3 d Inhalation: 0, 200, or 1,000 ppm, 6 hrs/d for 28 d	4–6/group	4.5.4.6. Bile production E.2.2. Subchronic and Chronic Studies of TCE
Watanabe and Fukui (2000)	ddY mice (M)	i.p. in corn oil	0, 158 mg/kg (single dose)	4/group	4.5.4.4. Peroxisome proliferation and related effects
<b>Woolhiser et al. (2006)</b>	<b>Sprague-Dawley rats (F)</b>	<b>Inhalation</b>	<b>0, 100, 300, or 1,000 ppm, 6 hrs/d, 5 d/wk, for 4 wks</b>	<b>16/group</b>	4.5.4.1. Liver weight E.2.2.4. Woolhiser et al. (2006)

<sup>a</sup>**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

#### 4.5.4.1. Liver Weight

Increases in liver weight in mice, rats, and gerbils have been reported as a result of acute short-term, and subchronic TCE treatment by inhalation and oral routes of exposure ([Laughter et al., 2004](#); [Nunes et al., 2001](#); [Nakajima et al., 2000](#); [Tao et al., 2000](#); [Berman et al., 1995](#); [Dees and Travis, 1993](#); [Goel et al., 1992](#); [Merrick et al., 1989](#); [Goldsworthy and Popp, 1987](#); [Melnick et al., 1987](#); [Buben and O'Flaherty, 1985](#); [Elcombe et al., 1985](#); [Kjellstrand et al., 1983a](#); [Kjellstrand et al., 1983b](#); [Tucker et al., 1982](#); [Kjellstrand et al., 1981b](#)). The extent of TCE-induced liver weight gain is dependent on species, strain, gender, nutrition status, duration of exposure, route of administration, vehicle used in oral studies, and concentration of TCE administered. Of great importance to the determination of the magnitude of response is whether the dose of TCE administered also affects whole-body weight, and thus, liver weight and the percentage liver/body weight ratio. Therefore, studies that employed high enough doses to induce whole-body weight loss generally showed a corresponding decrease in percentage liver/body weight at such doses and "flattening" of the dose-response curve, while studies that did not show systemic toxicity reported liver/body weight ratios generally proportional to dose. Chronic studies, carried out for longer durations, that examine liver weight are few and often confounded by the presence of preneoplastic foci or tumors that also affect liver weight after an extended period of TCE exposure. The number of studies that examine liver weight changes in the rat are much fewer than for mouse. Overall, the database for mice provides data for examination of the differences in TCE-induced effects from differing exposure levels, durations of exposure, vehicle, strain, and gender. One study provided a limited examination of TCE-induced liver weight changes in gerbils.

TCE-induced increases in liver weight have been reported to occur quickly. [Kjellstrand et al. \(1981b\)](#) reported liver weight increases after 2 days of inhalation exposure in NMRI mice, [Laughter et al. \(2004\)](#) reported increased liver weight in SV129 mice in their 3-day study (see below), and [Tao et al. \(2000\)](#) reported an increased in percentage liver/body weight ratio in female B6C3F<sub>1</sub> mice for after 5 days. [Elcombe et al. \(1985\)](#) and [Dees and Travis \(1993\)](#) reported gavage results in mice and rats after 10 days of exposure to TCE, which showed TCE-induced increases in liver weight. [Tucker et al. \(1982\)](#) reported that 14 days of exposure to 24 and 240 mg/kg TCE via gavage to induce a dose-related increase in liver weight in male CD-1 mice but did not show the data.

For mice, the inhalation studies of [Kjellstrand et al.](#) provided the most information on the affect of duration of exposure, dose of exposure, strain tested, gender, initial weight, and variability in response between experiments on TCE-induced liver weight increases. These experiments also provided results that were independent of vehicle effect. Although the determination of the exact magnitude of response is limited by experimental design, [Kjellstrand et al. \(1981b\)](#) reported that in NMRI mice, continuous TCE inhalation exposure induced increased percentage liver/body weight by 2 days and that by 30 days (the last recorded

data point) the highest percentage liver/body weight ratio was reported (~1.75-fold over controls) in both male and female mice. Kjellstrand et al. (1983b) exposed seven different strains of mice (wild, C57BL, DBA, B6CBA, A/sn, NZB, NMRI) to 150 ppm TCE for 30 days and demonstrated that strain, gender, and toxicity, as reflected by changes in whole-body weight, affected the percentage liver/body weight ratios induced by 30 days of continuous TCE exposure. In general for the seven strains of mice examined, female mice had the less variable increases in TCE-induced liver weight gain across duplicate experiments than male mice. For instance, in strains that did not exhibit changes in body weight (reflecting systemic toxicity) in either gender (wild-type and DBA), 150 ppm TCE exposure for 30 days induced 1.74–1.87-fold of control percentage liver/body weight ratios in female mice and 1.45–2.00-fold of control percentage liver/body weight ratios in male mice. The strain with the largest TCE-induced increase in percentage liver/body weight increase was the NZB strain (~2.08-fold of control for females and 2.34–3.57-fold of control for males). Kjellstrand et al. (1983a) provided dose-response information for the NMRI strain of mice (A Swiss-derived strain) that indicated dose-related increases in percentage liver/body weight ratios between 37 and 300 ppm TCE exposure for 30 days. The 150 ppm dose was reported to induce a 1.66- and 1.69-fold increases in percentage liver/body weight ratios in male and female mice, respectively. Interestingly, they also reported similar liver weight increases among groups with the same cumulative exposure, but with different daily exposure durations (1 hour/day at 3,600 ppm to 24 hours/day at 150 ppm for 30 days).

Not only have most gavage experiments been carried out in male mice, which Kjellstrand et al. (1983b) had demonstrated to have more variability in response than females, but also vehicle effects were noted to occur in experiments that examined them. Merrick et al. (1989) reported that corn oil induced a similar increase in percentage liver/body weight ratios in female mice fed TCE in Emulphor and corn oil for 4 weeks; male mice TCE administered in the corn oil vehicle induced a greater increase in liver weight than Emulphor but less mortality at a high dose.

Buben and O'Flaherty (1985) treated male, outbred Swiss-Cox mice for 6 weeks at doses ranging from 100 to 3,200 mg/kg-day, and reported increased liver/body-weight ratios at all tested doses (1.12–1.75-fold of controls). Given the large strain differences observed by Kjellstrand et al. (1983b), the use of predominantly male mice, and the effects of vehicle in gavage studies, interstudy variability in dose-response relationships is not surprising.

Dependence of PPAR $\alpha$  activation for TCE-liver weight gain has been investigated in PPAR $\alpha$  null mice by Nakajima et al. (2000), Laughter et al. (2004), and Ramdhan et al. (2010), the latter of which also investigated PPAR $\alpha$  null mice with human PPAR $\alpha$  inserted. Nakajima et al. (2000) reported that after 2 weeks of 750 mg/kg TCE exposure to carefully matched SV129 wild-type or PPAR $\alpha$ -null male and female mice (n = 6 group), there was a reported 1.50-fold increase in wild-type and 1.26-fold of control percentage liver/body weight ratio in PPAR $\alpha$ -null

male mice. For female mice, there was ~1.25-fold of control percentage liver/body weight ratios for both wild-type and PPAR $\alpha$ -null mice. Thus, TCE-induced liver weight gain was not dependent on a functional PPAR $\alpha$  receptor in female mice and some portion of it may have been in male mice. Both wild-type male and female mice were reported to have similar increases in the number of peroxisome in the pericentral area of the liver and TCE exposure and, although increased twofold, were still only ~4% of cytoplasmic volume. Female wild-type mice were reported to have less TCE-induced elevation of very long chain acyl-CoA synthetase, D-type peroxisomal bifunctional protein, mitochondrial trifunctional protein  $\alpha$  subunits  $\alpha$  and  $\beta$ , and CYP 4A1 than males mice, even though peroxisomal volume was similarly elevated in male and female mice. The induction of PPAR $\alpha$  protein by TCE treatment was also reported to be slightly less in female than male wild-type mice (2.17- vs. 1.44-fold of control induction, respectively). Thus, differences between genders in this study were for increased liver weight were not associated with differences in peroxisomal volume in the hepatocytes but there was a gender-related difference in induction of enzymes and proteins associated with PPAR $\alpha$ .

The study of Laughter et al. (2004) used SV129 wild-type and PPAR $\alpha$ -null male mice treated with three daily doses of TCE in 0.1% methyl cellulose for either 3 days (1,500 mg/kg TCE) or 3 weeks (0, 10, 50, 125, 500, 1,000, or 1,500 mg/kg TCE 5 days/week). However, the paradigm is not strictly comparable to other gavage paradigms due to the different dose vehicle and the documented impacts of vehicles such as corn oil on TCE-induced effects. In addition, no initial or final body weights of the mice were reported and thus, the influence of differences in initial body weight on percentage liver/body weight determinations could not be ascertained. While control wild-type and PPAR $\alpha$ -null mice were reported to have similar percentage liver/body weight ratios (i.e., ~4.5%) at the end of the 3-day study, at the end of the 3-week experiment, the percentage liver/body weight ratios were reported to be larger in the control PPAR $\alpha$ -null male mice (5.1%). TCE treatment for 3 days was reported for percentage liver/body weight ratio to be 1.4-fold of control in the wild-type mice and 1.07-fold of control in the null mice. After 3 weeks of TCE exposure at varying concentrations, wild-type mice were reported to have percentage liver/body weight ratios that were within ~2% of control values with the exception of the 1,000 and 1,500 mg/kg treatment groups (~1.18- and 1.30-fold of control, respectively). For the PPAR $\alpha$ -null mice, the variability in percentage liver/body weight ratios was reported to be greater than that of the wild-type mice in most of the TCE groups and the baseline levels of percentage liver/body weight ratio for control mice 1.16-fold of that of wild-type mice. TCE exposure was apparently more toxic in the PPAR $\alpha$ -null mice. Decreased survival at the 1,500 mg/kg TCE exposure level resulted in the prevention of recording of percentage liver/body weight ratios for this group. At 1,000 mg/kg TCE exposure level, there was a reported 1.10-fold of control percentage liver/body weight ratio in the PPAR $\alpha$ -null mice. None of the increases in percentage liver/body weight in the null mice were reported to be statistically significant by Laughter et al. (2004). However, the power of the study was limited

due to low numbers of animals and increased variability in the null mice groups. The percentage liver/body weight ratio after TCE treatment reported in this study was actually greater in the PPAR $\alpha$ -null mice than the wild-type male mice at the 1,000 mg/kg TCE exposure level ( $5.6 \pm 0.4$  vs.  $5.2 \pm 0.5\%$ , for PPAR $\alpha$ -null and wild-type mice, respectively) resulting in a 1.18-fold of wild-type and 1.10-fold of PPAR $\alpha$ -null mice. Although the results reported in Laughter et al. (2004) for DCA and TCA were not conducted in experiments that used the same paradigm, the TCE-induced increase in percentage liver/body weight more closely resembled the dose-response pattern for DCA than for DCA wild-type SV129 and PPAR $\alpha$ -null mice.

Ramadhan et al. (2010) examined TCE-induced hepatic steatosis and toxicity using male wild type, PPAR $\alpha$ -null, and human PPAR $\alpha$  inserted (“humanized”) mice exposed to high inhalation concentrations of TCE for 7 days. Significant differences were observed among control mice for each genotype with reduced body weight in untreated humanized mice. Liver/body weight ratios were 11% higher in untreated PPAR $\alpha$ -null mice than wild type mice. Higher levels of liver triglycerides and hepatic steatosis were reported in the untreated humanized mice and PPAR $\alpha$  null mice than wild type mice. Background expression of a number of genes and protein expression levels were significantly different between the untreated strains. In particular, human PPAR $\alpha$  protein levels were >10-fold greater in the humanized mice than mouse PPAR $\alpha$  in untreated wild type mice. Insertion of human PPAR $\alpha$  in the null mice did not return the mice to a normal state. Both PPAR $\alpha$  null and humanized mice were more susceptible to TCE toxicity. Hepatomegaly was induced in all strains to a similar extent after TCE exposure. However, urinary TCA concentrations were reported to be significantly lower and TCOH levels significantly higher in TCE-treated PPAR $\alpha$ -null mice in comparison to treated wild type mice. This difference was not related to changes in expression of metabolic enzymes.

No study examined strain differences among rats, and cross-study comparisons are confounded by heterogeneity in the age of animals, dosing regimen, and other design characteristics that may affect the degree of response. For rats, TCE-induced percentage liver/body weight ratios were reported to range from 1.16- to 1.46-fold of control values depending on the study paradigm. The studies that employed the largest range of exposure concentrations (Berman et al., 1995; Melnick et al., 1987) examined four doses in the rat. In general, there was a dose-related increase in percentage liver/body weight in the rat, especially at doses that did not cause concurrent decreased survival or significant body weight loss. For gerbils, Kjellstrand et al. (1981b) reported a similar value of ~1.25-fold of control percentage liver/body weight as for Sprague-Dawley rats exposed to 150 ppm TCE continuously for 30 days. Woolhiser et al. (2006) also reported inhalation TCE exposure to increase the percentage liver/body weight ratios in female Sprague-Dawley rats, although this strain appeared to be less responsive than others tested for induction of hepatomegaly from TCA exposure and to also be less prone to spontaneous liver cancer.

The size of the liver is under tight control and after cessation of a mitogenic stimulus or one inducing hepatomegaly, the liver will return to its preprogrammed size (see Appendix E). The increase in liver weight from TCE-exposure also appears to be reversible. Kjellstrand et al. (1981b) reported a reduction in liver weight gain increases after cessation of TCE exposure for 5 or 30 days in male and female mice. However, experimental design limitations precluded discernment of the magnitude of decrease. Kjellstrand et al. (1983a) reported that mice exposed to 150 ppm TCE for 30 days and then examined 120 days after the cessation of exposure had liver weights were 1.09-fold of control for TCE-exposed female mice and the same as controls for TCE-exposed male mice. However, the livers were not the same as untreated liver in terms of histopathology. The authors reported that “after exposure to 150 ppm for 30 days, followed by 120 days of rehabilitation, the morphological picture was similar to that of the air-exposure controls except for changes in cellular and nuclear sizes.” Qualitatively, the reduction in liver weight after treatment cessation is consistent with the report of Elcombe et al. (1985) in Alderly Park mice. The authors report that the reversibility of liver effects after the administration of TCE to Alderly Park mice for 10 consecutive days. Effects upon liver weight, DNA concentration, and tritiated thymidine incorporation 24 and 48 hours after the last dose of TCE were reported to still be apparent. However, 6 days following the last dose of TCE, all of these parameters were reported to return to control values with the authors not showing the data to support this assertion. Thus, cessation of TCE exposure would have resulted in a 75% reduction in liver weight by 4 days in mice exposed to the highest TCE concentration. Quantitative comparisons are not possible because Elcombe et al. (1985) did not report data for these results (e.g., how many animals, what treatment doses, and differences in baseline body weights) and such a large decrease in such a short period of time needs to be verified.

#### **4.5.4.2. Cytotoxicity and Histopathology**

Acute exposure to TCE appears to induce low cytotoxicity below subchronically lethal doses. Relatively high doses of TCE appear necessary to induce cytotoxicity after a single exposure with two available studies reported in rats. Okino et al. (1991) reported small increases in the incidence of hepatocellular necrosis in male Wistar rats exposed to 2,000 ppm (8 hours) and 8,000 ppm (2 hours), but not at lower exposures. In addition, “swollen” hepatocytes were noted at the higher exposure when rats were pretreated with ethanol or phenobarbital. Serum transaminases increased only marginally at the 8,000-ppm exposure, with greater increases with pretreatments. Berman et al. (1995) reported hepatocellular necrosis, but not changes in serum markers of necrosis, after single gavage doses of 1,500 and 5,000 mg/kg TCE in female F344 rats. However, they did not report any indications of necrosis after 14 days of treatment at 50–1,500 mg/kg-day nor the extent of necrosis.

At acute and subchronic exposure periods to multiple doses, the induction of cytotoxicity, though usually mild, appears to differ depending on rodent species, strain, dosing vehicle, and

duration of exposure, and the extent of reporting to vary between studies. For instance, Elcombe et al. (1985) and Dees and Travis (1993), which used the B6C3F<sub>1</sub> mouse strain and corn oil vehicle, reported only slight or mild necrosis after 10 days of treatment with TCE at doses up to 1,500 mg/kg-day. Elcombe et al. (1985) also reported cell hypertrophy in the centrilobular region. Dees and Travis (1993) reported some loss of vacuolization in hepatocytes of mice treated at 1,000 mg/kg-day. Laughter et al. (2004) reported that “wild-type” SV129 mice exposed to 1,500 mg/kg TCE exposure for 3 weeks exhibited mild granuloma formation with calcification or mild hepatocyte degeneration, but gave no other details or quantitative information as to the extent of the lesions or what parts of the liver lobule were affected. The authors noted that “wild-type mice administered 1,000 and 1,500 mg/kg exhibited centrilobular hypertrophy” and that “the mice in the other groups did not exhibit any gross pathological changes” after TCE exposure. Channel et al. (1998) reported no necrosis in B6C3F<sub>1</sub> mice treated with 400–1,200 mg/kg-day TCE by corn oil gavage for 2 days to 8 weeks.

However, as stated above, Merrick et al. (1989) reported that corn oil resulted in more hepatocellular necrosis, as described by small focal areas of 3–5 hepatocytes, in male B6C3F<sub>1</sub> mice than use of Emulphor as a vehicle for 4-week TCE gavage exposures. Necrotic hepatocytes were described as surrounded by macrophages and polymorphonuclear cells. The authors reported that visible necrosis was observed in 30–40% of male mice administered TCE in corn oil but not that there did not appear to be a dose-response. For female mice, the extent of necrosis was reported to be 0 for all control and TCE treatment groups using either vehicle. Serum enzyme activities for ALT, AST, and LDH (markers of liver toxicity) showed that there was no difference between vehicle groups at comparable TCE exposure levels for male or female mice. Except for LDH levels in male mice exposed to TCE in corn oil, there was not a correlation with the extent of necrosis and the patterns of increases in ALT and AST enzyme levels.

Ramdhan et al. (2008) assessed TCE-induced hepatotoxicity by measuring plasma ALT and AST activities and histopathology in Sv/129 mice treated by inhalation exposure, which are not confounded by vehicle effects. Despite high variability and only six animals per dose group, all three measures showed statistically significant increases at the high dose of 2,000 ppm (8 hours/day for 7 days), although a nonstatistically significant elevation is evident at the low dose of 1,000 ppm. Even at the highest dose, cytotoxicity was not severe, with ALT and AST measures increased twofold or less and an average histological score <2 (range 0–4).

Using the same paradigm, Ramdhan et al. (2010) also reported increased in AST and ALT liver injury biomarkers to be significantly increased in all exposed mice (Sv/129 wild type, PPAR $\alpha$ -null, and humanized PPAR $\alpha$  mice) relative to controls (41–74 and 36–79% higher, for ALT and AST, respectively). Mean levels within each treatment group were higher, though not statistically significantly different, with exposure to 2,000 vs. 1,000 ppm TCE. Steatosis scores were reported to be significantly higher in the 2,000 vs. 1,000 ppm TCE exposures to

PPAR $\alpha$ -null mice. The authors reported steatosis scored to be significantly correlated with liver triglyceride levels of all mice examined in the study ( $r = 0.75$ ). Macrovesicular steatosis was reported to occur more frequently in hPPAR $\alpha$  than PPAR $\alpha$ -null mice. Necrosis scores were reported to be significantly higher in TCE-exposed mice relative to controls in all three genotype mice and to be significantly higher with 2,000 vs. 1,000 ppm TCE exposure in wild type mice and hPPAR $\alpha$  mice. Inflammation scores were reported to be significantly higher with exposed group than control with 2,000 ppm TCE exposure than controls for each genotype group with a difference between the 2,000 ppm and 1,000 ppm exposure groups in wild type mice.

Kjellstrand et al. ([1983a](#)) exposed male and female NRM1 mice to 150 ppm for 30–120 days. Kjellstrand et al. ([1983a](#)) reported more detailed light microscopic findings from their study and stated that

After 150 ppm exposure for 30 days, the normal trabecular arrangement of the liver cells remained. However, the liver cells were generally larger and often displayed a fine vacuolization of the cytoplasm. The nucleoli varied slightly to moderately in size and shape and had a finer, granular chromatin with a varying basophilic staining intensity. The Kupffer cells of the sinusoid were increased in cellular and nuclear size. The intralobular connective tissue was infiltrated by inflammatory cells. There was not sign of bile stasis. Exposure to TCE in higher or lower concentrations during the 30 days produced a similar morphologic picture. After intermittent exposure for 30 days to a time-weighted-average concentration of 150 ppm or continuous exposure for 120 days, the trabecular cellular arrangement was less well preserved. The cells had increased in size and the variations in size and shape of the cells were much greater. The nuclei also displayed a greater variation in basophilic staining intensity, and often had one or two enlarged nucleoli. Mitosis was also more frequent in the groups exposed for longer intervals. The vacuolization of the cytoplasm was also much more pronounced. Inflammatory cell infiltration in the interlobular connective tissue was more prominent. After exposure to 150 ppm for 30 days, followed by 120 days of rehabilitation, the morphological picture was similar to that of the air-exposure controls except for changes in cellular and nuclear sizes.

Although not reporting comparisons between male and female mice in the results section of the paper for TCE-induced histopathological changes, the authors stated in the discussion section that “However, liver mass increase and the changes in liver cell morphology were similar in TCE-exposed male and female mice.” Kjellstrand et al. ([1983a](#)) did not present any quantitative data on the lesions they described, especially in terms of dose-response. Most of the qualitative description presented was for the 150-ppm exposure level and the authors suggest that lower concentrations of TCE give a similar pathology as those at the 150-ppm level, but do not present data to support that conclusion. Although stating that Kupffer cells were reported to be increased in cellular and nuclear size, no differential staining was applied light microscopy sections to distinguish Kupffer from endothelial cells lining the hepatic sinusoid in this study. Without differential staining, such a determination is difficult at the light microscopic level.

Indeed, Goel et al. (1992) describe proliferation of “sinusoidal endothelial cells” after 1,000 and 2,000 mg/kg-day TCE exposure for 28 days in male Swiss mice. They reported that histologically, “the liver exhibits swelling, vacuolization, widespread degeneration/necrosis of hepatocytes as well as marked proliferation of endothelial cells of hepatic sinusoids at 1,000 and 2,000 mg/kg TCE doses.” Only one figure is given, at the light microscopic level, in which it is impossible to distinguish endothelial cells from Kupffer cells and no quantitative measures or proliferation were examined or reported to support the conclusion that endothelial cells are proliferating in response to TCE treatment. Similarly, no quantitative analysis regarding the extent or location of hepatocellular necrosis was given. The presence or absence of inflammatory cells were not noted by the authors as well. In terms of white blood cell count, the authors note that it was slightly increased at 500 mg/kg-day but decreased at 1,000 and 2,000 mg/kg-day TCE, perhaps indicating macrophage recruitment from blood to liver and kidney, which was also noted to have pathology at these concentrations of TCE.

The inflammatory cell infiltrates described in the Kjellstrand et al. (1983a) study are consistent with invasion of macrophages and well as polymorphonuclear cells into the liver, which could activate resident Kupffer cells. Although not specifically describing the changes as consistent with increased polyploidization of hepatocytes, the changes in cell size and especially the continued change in cell size and nuclear staining characteristics after 120 days of cessation of exposure are consistent with changes in polyploidization induced by TCE. Of note is that in the histological description provided by the authors, although vacuolization is reported and consistent with hepatotoxicity or lipid accumulation, which is lost during routine histological slide preparation, there is no mention of focal necrosis or apoptosis resulting from these exposures to TCE.

Buben and O’Flaherty (1985) reported liver degeneration “as swollen hepatocytes” and to be common with treatment of TCE to Male Swiss-Cox mice after 6 weeks. They reported that “Cells had indistinct borders; their cytoplasm was clumped and a vesicular pattern was apparent. The swelling was not simply due to edema, as wet weight/dry weight ratios did not increase.” Karyorrhexis (the disintegration of the nucleus) was reported to be present in nearly all specimens and suggestive of impending cell death. No Karyorrhexis, necrosis, or polyploidy was reported in controls, but a low score Karyorrhexis was given for 400 mg/kg TCE and a slightly higher one given for 1,600 mg/kg TCE. Central lobular necrosis was reported to be present only at the 1,600 mg/kg TCE exposure level and was assigned a low score. Polyploidy was described as characteristic in the central lobular region but with low scores for both 400 mg/kg and 1,600 mg/kg TCE exposures. The authors reported that “hepatic cells had two or more nuclei or had enlarged nuclei containing increased amounts of chromatin, suggesting that a regenerative process was ongoing” and that there were no fine lipid droplets in TCE exposed animals. The finding of “no polyploidy” in control mouse liver in the study of Buben and O’Flaherty (1985) is unexpected given that binucleate and polyploid hepatocytes are a common

finding in the mature mouse liver. It is possible that the authors were referring to unusually high instances of “polyploidy” in comparison to what would be expected for the mature mouse. The score given by the authors for polyploidy did not indicate a difference between the two TCE exposure treatments and that it was of the lowest level of severity or occurrence. No score was given for centrilobular hypertrophy, although the DNA content and liver weight changes suggested a dose-response. The “Karyrrhexis” described in this study could have been a sign of cell death associated with increased liver cell number or dying of maturing hepatocytes associated with the increased ploidy, and suggests that TCE treatment was inducing polyploidization. Consistent with enzyme analyses, centrilobular necrosis was only seen at the highest dose and with the lowest qualitative score, indicating that even at the highest dose there was little toxicity.

At high doses, Kaneko et al. (2000) reported sporadic necrosis in male Mrl-lpr/lpr mice, which are “genetically liable to autoimmune disease,” exposed to 500–2,000 ppm, 4 hours/day, 6 days/week, for 8 weeks (n = 5). Dose-dependent mild inflammation and associated changes were reported to be found in the liver. The effects on hepatocytes were reported to be minimal by the authors with 500 ppm TCE inducing sporadic necrosis in the hepatic lobule. Slight mobilization and activation of sinusoid lining cells were also noted. These pathological features were reported to increase with dose.

NTP (1990), which used the B6C3F<sub>1</sub> mouse strain, reported centrilobular necrosis in 6/10 male and 1/10 female B6C3F<sub>1</sub> mice treated at a dose of 6,000 mg/kg-day for up to 13 weeks (all of the male mice and 8 of the 10 female mice died in the first week of treatment). At 3,000 mg/kg-day exposure level, although centrilobular necrosis was not observed, 2/10 males had multifocal areas of calcification in their livers, which the authors suggest is indicative of earlier hepatocellular necrosis. However, only 3/10 male mice at this dose survived to the end of the 13-week study.

For the NTP (1990) 2-year study, B6C3F<sub>1</sub> mice were reported to have no treatment-related increase in necrosis in the liver. A slight increase in the incidence of focal necrosis was noted TCE-exposed male mice (8 vs. 2%) with a slight reduction in fatty metamorphosis in treated male mice (zero treated vs. two control animals) and, in female mice, a slight increase in focal inflammation (29 vs. 19% of animals) and no other changes. Therefore, this study did not show concurrent evidence of liver toxicity with TCE-induced neoplasia after 2 years of TCE exposure in mice.

For the more limited database in rats, there appears to be variability in reported TCE-induced cytotoxicity and pathology. Nunes et al. (2001) reported no gross pathological changes in rats gavaged with corn oil or with corn oil plus 2,000 mg/kg TCE for 7 days. Goldsworthy and Popp (1987) gave no descriptions of liver histology in this report for TCE-exposed animals or corn-oil controls. Kjellstrand et al. (1981b) also did not provide histological descriptions for livers of rats in their inhalation study.

Elcombe et al. (1985) provided a description of the histopathology at the light microscopy level in Osborne-Mendel rats and Alderly Park rats exposed to TCE via gavage for 10 days. However, they did not provide a quantitative analysis or specific information regarding the variability of response between animals within group and there was no indication by the authors regarding how many rats were examined by light microscopy. Hematoxylin and eosin sections from Osborne-Mendel rats were reported to show that:

Livers from control rats contained large quantities of glycogen and isolated inflammatory foci, but were otherwise normal. The majority of rats receiving 1,500 mg/kg body weight TCE showed slight changes in centrilobular hepatocytes. The hepatocytes were more eosinophilic and contained little glycogen. At lower doses these effects were less marked and were restricted to fewer animals. No evidence of treatment-related hepatotoxicity (as exemplified by single cell or focal necrosis) was seen in any rat receiving TCE. H&E [hematoxylin and eosin] sections from Alderly Park Rats showed no signs of treatment-related hepatotoxicity after administration of TCE. However, some signs of dose-related increase in centrilobular eosinophilia were noted.

Thus, both mice and rats were reported to exhibit pericentral hypertrophy and eosinophilia as noted from the histopathological examination in Elcombe et al. (1985).

Berman et al. (1995) reported that for female rats exposed to TCE for 14 days hepatocellular necrosis was noted to occur in the 1,500 and 5,000 mg/kg groups in 6/7 and 6/8 female rats, respectively, but not to occur in lower doses. The extent of necrosis was not noted by the authors for the two groups exhibiting a response after 1 day of exposure. Serum enzyme levels, indicative of liver necrosis, were not presented and because only positive results were presented in the paper, presumed to be negative. Therefore, the extent of necrosis was not of a magnitude to affect serum enzyme markers of cellular leakage.

Melnick et al. (1987) reported that the only treatment-related lesion observed microscopically in rats from either dosed-feed or gavage groups was individual cell necrosis of the liver with the frequency and severity of this lesion similar at each dosage levels of TCE microencapsulated in the feed or administered in corn oil. The severity for necrosis was only mild at the 2.2 and 4.8 g/kg feed groups and for the six animals in the 2.8 g/kg group corn oil group. The individual cell necrosis was reported to be randomly distributed throughout the liver lobule with the change to not be accompanied by an inflammatory response. The authors also reported that there was no histologic evidence of cellular hypertrophy or edema in hepatic parenchymal cells. Thus, although there appeared to be TCE-treatment related increases in focal necrosis after 14 days of exposure, the extent was mild even at the highest doses and involved few hepatocytes.

For the 13-week NTP study (1990), only control and high dose F344/N rats were examined histologically. Pathological results were reported to reveal that 6/10 males and 6/10 female rats had pulmonary vasculitis at the highest concentration of TCE. This change was

also reported to have occurred in 1/10 control male and female rats. Most of those animals were also reported to have had mild interstitial pneumonitis. The authors reported that viral titers were positive during this study for Sendai virus.

Kumar et al. (2001b) reported that male Wistar rats exposed to 376 ppm, 4 hours/day, 5 days/week for 8–24 weeks showed evidence of hepatic toxicity. The authors stated that, “after 8 weeks of exposure enlarged hepatocytes, with uniform presence of fat vacuoles were found in all of the hepatocytes affecting the periportal, midzonal, and centrilobular areas, and fat vacuoles pushing the pyknotic nuclei to one side of hepatocytes. Moreover, congestion was not significant. After exposure of 12 and 24 weeks, the fatty changes became more progressive with marked necrosis, uniformly distributed in the entire organ.” No other description of pathology was provided in this report. In regard to the description of fatty change, the authors only did conventional H&E staining of sections with no precautions to preserve or stain lipids in their sections. However, as noted below, the NCI study also reports long-term TCE exposure in rats to result in hepatocellular fatty metamorphosis. The authors provided a table with histological scoring of simply + or – for minimal, mild, or moderate effects and did not define the criteria for that scoring. There is also no quantitative information given as to the extent, nature, or location of hepatocellular necrosis. The authors reported that “no change was observed in glutamic oxoacetate transaminase and glutamic pyruvate transaminase levels of liver in all of the three groups. The GSH level was significantly decreased while “total sulphhydryl” level was significantly increased during 8, 12, and 24 weeks of TCE exposure. The acid and ALPs were significantly increased during 8, 12, and 24 weeks of TCE exposure.” The authors presented a series of figures, which were poor in quality, to demonstrate histopathological TCE-induced changes. No mortality was observed from TCE exposure in any group, despite the presence of liver necrosis.

Thus, in this limited database that spans durations of exposure from days to 24 weeks and uses differing routes of administration, generally high doses for long durations of exposure are required to induce hepatotoxicity from TCE exposure in the rat. The focus of 2-year bioassays in rats has been the detection of a cancer response with little or no reporting of noncancer pathology in most studies. Henschler et al. (1984) and Fukuda et al. (1983) do not report noncancer histopathology, but both reported rare biliary-cell-derived tumors in rats in relatively insensitive assays. For male rats, noncancer pathology in the NCI (1976) study was reported to include increased fatty metamorphosis after TCE exposure and angiectasis or abnormally enlarged blood vessels. Angiectasis can be manifested by hyperproliferation of endothelial cells and dilatation of sinusoidal spaces. For the NTP (1990) study, there was little reporting of non-neoplastic pathology or toxicity and no report of liver weight at termination of the study. In the NTP (1988) study, the 2-year study of TCE exposure reported no evidence of TCE-induced liver toxicity described as non-neoplastic changes in ACI, August, Marshal, and Osborne-Mendel rats. Interestingly, for the control animals of these four strains, there was, in general, a low

background level of focal necrosis in the liver of both genders. Obviously, the negative results in this bioassay for cancer are confounded by the killing of a large portion of the animals accidentally by experimental error, but TCE-induced overt liver toxicity was not reported.

In sum, the cytotoxic effects in the liver of TCE treatment appear to include little or no necrosis in the rodent liver, but rather, a number of histological changes such as mild focal hepatocyte degeneration at high doses, cellular “swelling” or hypertrophy, and enlarged nuclei. Histological changes consistent with increased polyploidization and specific descriptions of TCE-induced polyploidization have been noted in several experiments. Several studies noted proliferation of nonparenchymal cells after TCE exposure as well. These results are more consistently reported in mice, but also have been reported in some studies at high doses in rats, for which fewer studies are available. In addition, the increase in cellular and nuclear sizes appeared to persist after cessation of TCE treatment. In neither rats nor mice is there evidence that TCE treatment results in marked necrosis leading to regenerative hyperplasia.

#### **4.5.4.3. Measures of DNA Synthesis, Cellular Proliferation, and Apoptosis**

The increased liver weight observed in rodents after TCE exposure may result from either increased numbers of cells in the liver, increased size of cells in the liver, or a combination of both. Studies of TCE in rodents have looked at whole-liver DNA content of TCE-treated animals to determine whether the concentration of DNA/g of liver decreases as an indication of hepatocellular hypertrophy ([Dees and Travis, 1993](#); [Buben and O'Flaherty, 1985](#); [Elcombe et al., 1985](#)). While the slight decreases observed in some studies are consistent with hypertrophy, the large variability in controls and lack of dose-response limits the conclusions that can be drawn from these data. In addition, multiple factors beyond hypertrophy affect DNA concentration in whole-liver homogenates, including changes in ploidy and the number of hepatocytes and nonparenchymal cells.

The incorporation of tritiated thymidine or BrdU has also been analyzed in whole-liver DNA and in individual hepatocytes as a measure of DNA synthesis. Such DNA synthesis can occur from either increased numbers of hepatocytes in the liver or increased polyploidization. Section E.1.1 describes polyploidization in human and rodent liver and its impacts on liver function, while Sections E.3.1.4 and E.3.3.1 discuss issues of target cell identification for liver cancer and changes in ploidy as a key event in liver cancer using animals models, respectively. Along with changes in cell size (hypertrophy), cell number (cellular proliferation), and the DNA content per cell (cell ploidy), the rate of apoptosis has also been noted or specifically examined in some studies of TCE and its metabolites. All of these phenomena have been identified in proposed hypotheses as key events possibly related to carcinogenicity. In particular, changes in cell proliferation and apoptosis have been postulated to be part of the mode of action for PPAR $\alpha$ -agonists by Klaunig et al. ([2003](#)) (see Section E.3.4).

In regard to early changes in DNA synthesis, the data for TCE are very limited. Mirsalis et al. (1989) reported measurements of *in vivo-in vitro* hepatocyte DNA repair and S-phase DNA synthesis in primary hepatocytes from male F344 rats and male and female B6C3F<sub>1</sub> mice administered single doses of TCE by gavage in corn oil. They reported negative results 2–12 hours after treatment of 50–1,000 mg/kg TCE in rats and mice (male and female) for UDS and repair using three animals per group. After 24 and 48 hours of 200 or 1,000 mg/kg TCE in male mice (n = 3) and after 48 hours of 200 (n = 3) or 1,000 (n = 4) mg/kg TCE in female mice, similar values of 0.30–0.69% of hepatocytes were reported as undergoing DNA synthesis in primary culture. Only the 1,000 mg/kg TCE dose in male mice at 48 hours was reported to give a result considered to be positive (~2.2% of hepatocytes), but no statistical analyses were performed on these measurements. These results are limited by both the number of animals examined and the relevance of the paradigm.

As noted above, TCE treatment in rodents has been reported to result in hepatocellular hypertrophy and increased centrilobular eosinophilia. Elcombe et al. (1985) reported a small decrease in DNA content with TCE treatment (consistent with hepatocellular hypertrophy) that was not dose-related, increased tritiated thymidine incorporation in whole mouse liver DNA that was that was treatment- but not dose-related (i.e., a two-, two-, and fivefold of control in mice treated with 500, 1,000, and 1,500 mg/kg TCE), and slightly increased numbers of mitotic figures that were treatment but not dose-related and not correlated with DNA synthesis as measured by thymidine incorporation. Elcombe et al. (1985) reported no difference in response between 500 and 1,000 mg/kg TCE treatments for tritiated thymidine incorporation. Dees and Travis (1993) also reported that incorporation of tritiated thymidine in DNA from mouse liver was elevated after TCE treatment with the mean peak level of tritiated thymidine incorporation occurred at 250 mg/kg TCE treatment level and remaining constant for the 500 and 1,000 mg/kg treated groups. Dees and Travis (1993) specifically report that mitotic figures, although very rare, were more frequently observed after TCE treatment, found most often in the intermediate zone, and found in cells resembling mature hepatocytes. They reported that there was little tritiated thymidine incorporation in areas near the bile duct epithelia or close to the portal triad in liver sections from both male and female mice. Channel et al. (1998) reported proliferating cell nuclear antigen (PCNA) positive cells, a measure of cells that have undergone DNA synthesis, was elevated only on day 10 (out of the 21 studied) and only in the 1,200 mg/kg-day TCE exposed group with a mean of ~60 positive nuclei per 1,000 nuclei for six mice (~6%). Given that there was little difference in PCNA positive cells at the other TCE doses or time points studied, the small number of affected cells in the liver could not account for the increase in liver size reported in other experimental paradigms at these doses. The PCNA positive cells as well as “mitotic figures” were reported to be present in centrilobular, midzonal, and periportal regions with no observed predilection for a particular lobular distribution. No data were shown regarding any quantitative estimates of mitotic figures and whether they correlated with PCNA

results. Thus, whether the DNA synthesis phases of the cell cycle indicated by PCNA staining were identifying polyploidization or increased cell number cannot be determined.

For both rats and mice, the data from Elcombe et al. (1985) showed that tritiated thymidine incorporation in total liver DNA observed after TCE exposure did not correlate with mitotic index activity in hepatocytes. Both Elcombe et al. (1985) and Dees and Travis (1993) reported small mitotic indices and evidence of periportal hepatocellular hypertrophy from TCE exposure. Neither mitotic index nor tritiated thymidine incorporation data support a correlation with TCE-induced liver weight increase in the mouse, but rather that the increase is most likely due to hepatocellular hypertrophy. If higher levels of hepatocyte replication had occurred earlier, such levels were not sustained by 10 days of TCE exposure. These data suggest that increased tritiated thymidine levels were targeted to mature hepatocytes and in areas of the liver where greater levels of polyploidization occur (see Section E.1.1). Both Elcombe et al. (1985) and Dees and Travis (1993) show that tritiated thymidine incorporation in the liver was approximately twofold greater than controls between 250 and 1,000 mg/kg TCE, a result consistent with a doubling of DNA. Thus, given the normally quiescent state of the liver, the magnitude of this increase over control levels, even if a result of proliferation rather than polyploidization, would be confined to a very small population of cells in the liver after 10 days of TCE exposure.

Laughter et al. (2004) reported that there was an increase in DNA synthesis after aqueous gavage exposure to 500 and 1,000 mg/kg TCE given as three boluses/day for 3 weeks with BrdU given for the last week of treatment. An examination of DNA synthesis in individual hepatocytes was reported to show that 1 and 4.5% of hepatocytes had undergone DNA synthesis in the last week of treatment for the 500 and 1,000 mg/kg doses, respectively. Again, this level of DNA synthesis is reported for a small percentage of the total hepatocytes in the liver and not reported to be a result of regenerative hyperplasia.

Finally, Dees and Travis (1993) and Channel et al. (1998) reported evaluating changes in apoptosis with TCE treatment. Dees and Travis (1993) enumerated identified by either hematoxylin and eosin or feulgen staining in male and female mice after 10 days of TCE treatment by. Only zero or one apoptosis was observed per 100 high power (400 ×) fields in controls and all dose groups except for those given 1,000 mg/kg-day, in which eight or nine apoptoses per 100 fields were reported. None of the apoptoses were in the intermediate zones where mitotic figures were observed, and all were located near the central veins. This is the same region where one would expect endogenous apoptoses as hepatocytes “stream” from the portal triad toward the central vein (Schwartz-Arad et al., 1989). In addition, this is the same region where Buben and O’Flaherty (1985) noted necrosis and polyploidy. By contrast, Channel et al. (1998) reported no significant differences in apoptosis at any treatment dose (400–1,200 mg/kg-day) examined after any time from 2 days to 4 weeks.

#### 4.5.4.4. Peroxisomal Proliferation and Related Effects

Numerous studies have reported that TCE administered to mice and rats by gavage leads to proliferation of peroxisomes in hepatocytes. Some studies have measured changes in the volume and number of peroxisomes as measures of peroxisome proliferation, while others have measured peroxisomal enzyme activity such as catalase and cyanide-insensitive PCO. Like liver weight, the determination of a baseline level of peroxisomal volume, number, or enzyme activity can be variable and have great effect on the ability to determine the magnitude of a treatment-related effect.

Elcombe et al. (1985) reported increases in the percentage of the cytoplasm occupied by peroxisomes in B6C3F<sub>1</sub> and Alderley Park mice treated for 10 days at 500–1,500 mg/kg-day. Although the increase over controls appeared larger in the B6C3F<sub>1</sub> strain, this is largely due to the twofold smaller control levels in that strain, as the absolute percentage of peroxisomal volume was similar between strains after treatment. All of these results showed high variability, as evidenced from the reported SDs. Channel et al. (1998) found a similar absolute percentage of peroxisomal volume after 10 days treatment in the B6C3F<sub>1</sub> mouse at 1,200 mg/kg-day TCE but with the percentage in vehicle controls similar to the Alderley-Park mice in the Elcombe et al. (1985) study. Interestingly, Channel et al. (1998) found that the increase in peroxisomes peaked at 10 days, with lower values after 6 and 14 days of treatment. Furthermore, the vehicle control levels also varied almost twofold depending on the number of days of treatment. Nakajima et al. (2000) treated male wild-type SV129 mice at 750 mg/kg-day for 14 days, and found even higher baseline values for the percentage of peroxisomal volume, but with an absolute level after treatment similar to that reported by Channel et al. (1998) in B6C3F<sub>1</sub> mice treated at 1,200 mg/kg-day TCE for 14 days. Nakajima et al. (2000) also noted that the treatment-related increases were smaller for female wild-type mice, and that there were no increases in peroxisomal volume in male or female PPAR $\alpha$ -null mice, although vehicle control levels were slightly elevated (not statistically significant). Only Elcombe et al. (1985) examined peroxisomal volume in rats, and reported smaller treatment-related increases in two strains (OM and AP), but higher baseline levels. In particular, at 1,000 mg/kg-day, after 10 days of treatment, the percentage peroxisomal volume was similar in OM and AP rats, with similar control levels as well. While the differences from treatment were not statistically significant, only five animals were used in each group, and variability, as can be seen by the SDs, was high, particularly in the treated animals.

The activities of a number of different hepatic enzymes have also been as markers for peroxisome proliferation and/or activation of PPAR $\alpha$ . The most common of these are catalase and cyanide-insensitive PCO. In various strains of mice (B6C3F<sub>1</sub>, Swiss albino, SV129 wild-type) treated at doses of 500–2,000 mg/kg-day for 10–28 days, increases in catalase activity have tended to be more modest (1.3–1.6-fold of control) as compared to increases in PCO (1.4–7.9-fold of control) (Laughter et al., 2004; Nakajima et al., 2000; Watanabe and Fukui, 2000;

[Goel et al., 1992](#); [Goldsworthy and Popp, 1987](#); [Elcombe et al., 1985](#)). In rats, [Elcombe et al. \(1985\)](#) reported no increases in catalase or PCO activity in Alderley-Park rats treated at 1,000 mg/kg-day TCE for 10 days. In F344 rats, [Goldsworthy and Popp \(1987\)](#) and [Melnick et al. \(1987\)](#) reported increases of up to 2-fold in catalase and 4.1-fold in PCO relative to controls treated at 600–4,800 mg/kg-day for 10–14 days. The changes in catalase were similar to those in mice at similar treatment levels, with 1.1–1.5-fold of control enzyme activities at doses of 1,000–1,300 mg/kg-day ([Melnick et al., 1987](#); [Elcombe et al., 1985](#)). However, the changes in PCO were smaller, with 1.1–1.8-fold of control activity at these doses, as compared to 6.3–7.9-fold of control in mice ([Goldsworthy and Popp, 1987](#); [Melnick et al., 1987](#)).

In SV129 mice, [Nakajima et al. \(2000\)](#) and [Laughter et al. \(2004\)](#) investigated the dependence of these changes on PPAR $\alpha$  by using a null mouse. [Nakajima et al. \(2000\)](#) reported that neither male nor female wild-type or PPAR $\alpha$  null mice had significant increases in catalase after 14 days of treatment at 750 mg/kg-day. However, given the small number of animals (four per group) and the relatively small changes in catalase observed in other (wild-type) strains of mice, this study had limited power to detect such changes. Several other markers of peroxisome proliferation, including acyl-CoA oxidase and CYP4A1 (PCO was not investigated), were induced by TCE in male wild-type mice, but not in male null mice or female mice of either type. Unfortunately, none of these markers have been investigated using TCE in female mice of any other strain, so it is unclear whether the lack of response is characteristic of female mice in general, or just in this strain. Interestingly, as noted above, liver/body weight ratio increases were observed in both sexes of the null mice in this study. [Laughter et al. \(2004\)](#) only quantified activity of the peroxisome proliferation marker, PCO, in their study, and found in null mice a slight decrease (0.8-fold of control) at 500 mg/kg-day TCE and an increase (1.5-fold of control) at 1,500 mg/kg-day TCE after 3 weeks of treatment, with neither statistically significant (4–5 mice per group). However, baseline levels of PCO were almost 2-fold higher in the null mice, and the treated wild-type and null mice differed in PCO activity by only about 1.5-fold.

In sum, oral administration of TCE for up to 28 days causes proliferation of peroxisomes in hepatocytes along with associated increases in peroxisomal enzyme activities in both mice and rats. Male mice tend to be more sensitive in that at comparable doses, rats and female mice tend to exhibit smaller responses. For example, for peroxisomal volume and PCO, the fold-increase in rats appears to be lower by three- to sixfold than that in mice, but, for catalase, the changes were similar between mice in F344 rats. No inhalation or longer-term studies were located, and only one study examined these changes at more than one time-point. Therefore, little is known about the route-dependence, time course, and persistence of these changes. Finally, two studies in PPAR $\alpha$ -null mice ([Laughter et al., 2004](#); [Nakajima et al., 2000](#)) found diminished responses in terms of increased peroxisomal volume and peroxisomal enzyme activities as compared to wild-type mice, although there was some confounding due to baseline differences between null and wild-type control mice in several measures.

#### 4.5.4.5. Oxidative Stress

Several studies have attempted to study the possible effects of “oxidative stress” and DNA damage resulting from TCE exposures. The effects of induction of metabolism by TCE, as well as through co-exposure to ethanol, have been hypothesized to increase levels of “oxidative stress” as a common effect for both exposures (see Sections E.3.4.2.3 and E.4.2.4). Oxidative stress has been hypothesized to be a key event or mode of action for peroxisome proliferators as well, but has been found to be correlated with neither cell proliferation nor carcinogenic potency of peroxisome proliferators (see Section E.3.4.1.1). As a mode of action, it is not defined or specific, as the term “oxidative stress” is implicated as part of the pathophysiologic events in a multitude of disease processes and is part of the normal physiologic function of the cell and cell signaling.

In regard to measures of oxidative stress, Rusyn et al. (2006) noted that although an overwhelming number of studies draw a conclusion between chemical exposure, DNA damage, and cancer based on detection of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a highly mutagenic lesion, in DNA isolated from organs of in vivo treated animals, a concern exists as to whether increases in 8-OHdG represent damage to genomic DNA, a confounding contamination with mitochondrial DNA, or an experimental artifact. As noted in Sections E.2.1.1 and E.2.2.11, studies of TCE which employ the i.p. route of administration can be affected by inflammatory reactions resulting from that routes of administration and subsequent toxicity that can involve oxygen radical formation from inflammatory cells. Finally, as described in Section E.2.2.8, the study by Channel et al. (1998) demonstrated that corn oil as vehicle had significant effects on measures of “oxidative stress” such as thiobarbiturate acid-reactive substances (TBARS).

The TBARS results presented by Channel et al. (1998) indicate suppression of TBARS with increasing time of exposure to corn oil alone with data presented in such a way for 8-OHdG and total free radical changes that the pattern of corn oil administration was obscured. It was not apparent from that study that TCE exposure induced oxidative damage in the liver.

Toraason et al. (1999) measured 8-OHdG and a “free radical-catalyzed isomer of arachidonic acid and marker of oxidative damage to cell membranes, 8-epi-prostaglandin F2 $\alpha$  (8-epiPGF),” excretion in the urine and TBARS (as an assessment of malondialdehyde and marker of lipid peroxidation) in the liver and kidney of male Fischer rats exposed to single i.p. injections in of TCE in Alkamuls vehicle. Using this paradigm, 500-mg/kg TCE was reported to induce Stage II anesthesia and 1,000 mg/kg TCE was reported to induce Level III or IV (absence of reflex response) anesthesia and burgundy-colored urine with 2/6 rats at 24 hours comatose and hypothermic. The animals were sacrificed before they could die and the authors suggested that they would not have survived another 24 hours. Thus, using this paradigm, there was significant toxicity and additional issues related to route of exposure. Urine volume declined significantly during the first 12 hours of treatment and while water consumption was not measured, it was

suggested by the authors to be decreased due to the moribundity of the rats. Given that this study examined urinary markers of “oxidative stress,” the effects on urine volume and water consumption, as well as the profound toxicity induced by this exposure paradigm, limit the interpretation of the study. The issues of bias in selection of the data for this analysis, as well as the issues stated above for this paradigm limit interpretation of these data while the authors suggest that evidence of oxidative damage was equivocal.

#### **4.5.4.6. Bile Production**

Effects of TCE exposure in humans and in experimental animals is presented in Section E.2.6. Serum bile acids (SBA) have been suggested as a sensitive indicator of hepatotoxicity to a variety of halogenated solvents with an advantage of increased sensitivity and specificity over conventional liver enzyme tests that primarily reflect the acute perturbation of hepatocyte membrane integrity and “cell leakage” rather than liver functional capacity (i.e., uptake, metabolism, storage, and excretion functions of the liver) ([Neghab et al., 1997](#); [Bai et al., 1992a](#)) While some studies have reported negative results, a number of studies have reported elevated SBA in organic solvent-exposed workers in the absence of any alterations in normal liver function tests. These variations in results have been suggested to arise from failure of some methods to detect some of the more significantly elevated SBA and the short-lived and reversible nature of the effect ([Neghab et al., 1997](#)). [Neghab et al. \(1997\)](#) reported that occupational exposure to 1,1,2-trichloro-1,2,2-trifluoroethane and TCE has resulted in elevated SBA and that several studies have reported elevated SBA in experimental animals to chlorinated solvents such as carbon tetrachloride, chloroform, hexachlorobutadiene, tetrachloroethylene, 1,1,1-trichloroethane, and TCE at levels that do not induce hepatotoxicity ([Hamdan and Stacey, 1993](#); [Bai et al., 1992b](#); [Bai et al., 1992a](#); [Wang and Stacey, 1990](#)). Toluene, a nonhalogenated solvent, has also been reported to increase SBA in the absence of changes in other hepatobiliary functions ([Neghab and Stacey, 1997](#)). Thus, disturbance in SBA appears to be a generalized effect of exposure to chlorinated solvents and nonchlorinated solvents and not specific to TCE exposure.

[Wang and Stacey \(1990\)](#) administered TCE in corn oil via i.p. injection to male Sprague-Dawley rats with liver enzymes and SBA examined 4 hours after the last TCE treatment. The limitations of i.p injection experiments have already been discussed. While reporting no overt liver toxicity, there was, generally, a reported dose-related increase in cholic acid, chenodeoxycholic acid, deoxycholic acid, taurocholic acid, tauroursodeoxycholic acid with cholic acid, and taurochlolic acid increased at the lowest dose. The authors reported that “examination of liver sections under light microscopy yielded no consistent effects that could be ascribed to trichloroethylene.” In the same study, a rats were also exposed to TCE via inhalation and using this paradigm, cholic acid and taurocholic acid were also significantly elevated but the large variability in responses between rats and the low number of rats tested in this paradigm limit its ability to determine quantitative differences between groups. Nevertheless, without the

complications associated with i.p. exposure, inhalation exposure of TCE at relatively low exposure levels that were not associated with other measures of toxicity *were* associated with increased SBA level.

Hamdan and Stacey (1993) administered TCE in corn oil (1 mmol/kg) in male Sprague-Dawley rats and followed the time-course of SBA elevation, TCE concentration, and TCOH in the blood up to 16 hours. Liver and blood concentration of TCE were reported to peak at 4 hours, while those of TCOH peaked at 8 hours after dosing. TCE levels were not detectable by 16 hours in either blood or liver, while those of TCOH were still elevated. Elevations of SBA were reported to parallel those of TCE with cholic acid, and taurochloate acid was reported to show the highest levels of bile acids. The authors stated that liver injury parameters were checked and were found to be unaffected by TCE exposure, but did not provide the data. Thus, it was TCE concentration and not that of its metabolite that was most closely related to changes in SBA after a single exposure and the effect appeared to be reversible. In an *in vitro* study by Bai and Stacey (1993), TCE was studied in isolated rat hepatocytes with TCE reported to cause a dose-related suppression of initial rates of cholic acid and taurocholic acid, but with no significant effects on enzyme leakage and intracellular calcium contents, further supporting a role for the parent compound in this effect.

#### **4.5.4.7. Summary: TCE-Induced Noncancer Effects in Laboratory Animals**

In laboratory animals, TCE leads to a number of structural changes in the liver, including increased liver weight, small transient increases in DNA synthesis, cytomegaly in the form of “swollen” or enlarged hepatocytes, increased nuclear size probably reflecting polyploidization, and proliferation of peroxisomes. Liver weight increases proportional to TCE dose are consistently reported across numerous studies, and appear to be accompanied by periportal hepatocellular hypertrophy. There is also evidence of increased DNA synthesis in a small portion of hepatocytes at around 10 days *in vivo* exposure. The lack of correlation of hepatocellular mitotic figures with whole-liver DNA synthesis or DNA synthesis observed in individual hepatocytes supports the conclusion that cellular proliferation is not the predominant cause of increased DNA synthesis. The lack of correlation of whole-liver DNA synthesis and those reported for individual hepatocytes suggests that nonparenchymal cells also contribute to such synthesis. Indeed, nonparenchymal cell activation or proliferation has been noted in several studies. Moreover, the histological descriptions of TCE exposed liver are consistent with, and in some cases specifically note, increased polyploidy after TCE exposure. Interestingly, changes in TCE-induced hepatocellular ploidy, as indicated by histological changes in nuclei, have been noted to remain after the cessation of exposure. In regard to apoptosis, TCE has been reported to either not change apoptosis or to cause a slight increase at high doses. Some studies have also noted effects from dosing vehicle alone (such as corn oil in particular) not only on liver pathology, but also on DNA synthesis.

Available data also suggest that TCE does not induce substantial cytotoxicity, necrosis, or regenerative hyperplasia, as only isolated, focal necroses and mild to moderate changes in serum and liver enzyme toxicity markers having been reported. Data on peroxisome proliferation, along with increases in a number of associated biochemical markers, show effects in both mice and rats. These effects are consistently observed across rodent species and strains, although the degree of response at a given mg/kg-day dose appears to be highly variability across strains, with mice on average appearing to be more sensitive.

In addition, like humans, laboratory animals exposed to TCE have been observed to have increased serum bile acids, though the toxicological importance of these effects is unclear.

#### **4.5.5. TCE-Induced Liver Cancer in Laboratory Animals**

For 2-year or lifetime studies of TCE exposure, a consistent hepatocarcinogenic response has been observed using mice of differing strains and genders and from differing routes of exposure. However, some rat studies have been confounded by mortality from gavage error or the toxicity of the dose of TCE administered. In some studies, a relative insensitive strain of rat has been used. However, in general, it appears that the mouse is more sensitive than the rat to TCE-induced liver cancer. Three studies had results that the authors considered to be negative for TCE-induced liver cancer in mice, but have either design and/or reporting limitations, or are in strains and paradigms with apparent low ability for liver cancer induction or detection. Findings from these studies are shown in Tables 4-60 through 4-65, and discussed below.

##### **4.5.5.1. Negative or Inconclusive Studies of Mice and Rats**

Fukuda et al. ([1983](#)) reported a 104-week inhalation bioassay in female Crj:CD-1 (ICR) mice and female Crj:CD (Sprague-Dawley) rats exposed to 0-, 50-, 150-, and 450-ppm TCE (n = 50). There were no reported incidences of mice or rats with liver tumors for controls indicative of relatively insensitive strains and gender used in the study for liver effects. While TCE was reported to induce a number of other tumors in mice and rats in this study, the incidence of liver tumors was <2% after TCE exposure. Of note is the report of cystic cholangioma reported in one group of rats.

**Table 4-60. Summary of liver tumor findings in gavage studies of TCE by NTP (1990)<sup>a</sup>**

Sex	Dose (mg/kg) <sup>b</sup>	Adenoma (overall; terminal <sup>c</sup> )	Adenocarcinoma (overall; terminal <sup>c</sup> )
1/d, 5 d/wk, 103-wk study, F344/N rats			
Male	0	NA <sup>d</sup>	0/49
	500	NA	0/49
	1,000	NA	1/49
Female	0	NA	0/50
	500	NA	1/48
	1,000	NA	1/48
1/d, 5 d/wk, 103-wk study, B6C3F <sub>1</sub> mice			
Male	0	7/48; 6/33	8/48; 6/33
	1,000	14/50; 6/16	31/50; 14/16 <sup>f</sup>
Female	0	4/48; 4/32	2/48; 2/32
	1,000	16/49; 11/23 <sup>e</sup>	13/49; 8/23 <sup>g</sup>

<sup>a</sup>Liver tumors not examined in 13-week study, so data shown only for 103-week study.

<sup>b</sup>Corn oil vehicle.

<sup>c</sup>Terminal values not available for rats.

<sup>d</sup>Data not available.

<sup>e</sup> $p < 0.003$ .

<sup>f</sup> $p < 0.001$ .

<sup>g</sup> $p \leq 0.002$ .

**Table 4-61. Summary of liver tumor findings in gavage studies of TCE by NCI (1976)**

Sex	Dose (mg/kg) <sup>a</sup>	Hepatocarcinoma
1/d, 5 d/wk, 2-yr study, Osborne-Mendel rats		
Males	0	0/20
	549	0/50
	1,097	0/50
Females	0	0/20
	549	1/48
	1,097	0/50
1/d, 5 d/wk, 2-yr study, B6C3F <sub>1</sub> mice		
Males	0	1/20
	1,169	26/50 <sup>b</sup>
	2,339	31/48 <sup>b</sup>
Females	0	0/20
	869	4/50
	1,739	11/47 <sup>b</sup>

<sup>a</sup>Treatment period was 48 week for rats, 66 week for mice. Doses were changed several times during the study based on monitoring of body weight changes and survival. Dose listed here is the TWA dose over the days on which animals received a dose.

<sup>b</sup> $p < 0.01$ .

**Table 4-62. Summary of liver tumor incidence in gavage studies of TCE by NTP (1988)**

Sex	Dose (mg/kg) <sup>a</sup>	Adenoma	Adenocarcinoma
1/d, 5 d/wk, 2-yr study, ACI rats			
Male	0	0/50	1/50
	500	0/49	1/49
	1,000	0/49	1/49
Female	0	0/49	2/49
	500	0/46	0/46
	1,000	0/39	0/39
1/d, 5 d/wk, 2-yr study, August rats			
Male	0	0/50	0/50
	500	0/50	1/50
	1,000	0/48	1/48
Female	0	0/48	2/48
	500	0/48	0/48
	1,000	0/50	0/50
1/d, 5 d/wk, 2-yr study, Marshall rats			
Male	0	1/49	1/49
	500	0/50	0/50
	1,000	0/47	1/47
Female	0	0/49	0/49
	500	0/48	0/48
	1,000	0/46	0/46
1/d, 5 d/wk, 2-yr study, Osborne-Mendel rats			
Male	0	1/50	1/50
	500	1/50	0/50
	1,000	1/49	2/49
Female	0	0/50	0/50
	500	0/48	2/48
	1,000	0/49	2/49

<sup>a</sup>Corn oil vehicle.

**Table 4-63. Summary of liver tumor findings in inhalation studies of TCE by Maltoni et al. (1988; 1986)<sup>a</sup>**

Sex	Concentration (ppm)	Hepatoma
7 hrs/d, 5 d/wk, 8-wk exposure, observed for lifespan, Swiss mice		
Male	0	1/100
	100	3/60
	600	4/72
Female	0	1/100
	100	1/60
	600	0/72
7 hrs/d, 5 d/wk, 78-wk exposure, observed for lifespan, Swiss mice		
Male	0	4/90
	100	2/90
	300	8/90
	600	13/90
Female	0	0/90
	100	0/90
	300	0/90
	600	1/90
7 hrs/d, 5 d/wk, 78-wk exposure, observed for lifespan, B6C3F <sub>1</sub> mice <sup>b</sup>		
Male	0	1/90
	100	1/90
	300	3/90
	600	6/90
Female	0	3/90
	100	4/90
	300	4/90
	600	9/90

<sup>a</sup>Three inhalation experiments in this study found no hepatomas: BT302 (8-week exposure to 0, 100, or 600 ppm in Sprague-Dawley rats); BT303 (8-week exposure to 0, 100, or 600 ppm in Swiss mice); and BT304 (78-week exposure to 0, 100, 300, or 600 ppm in Sprague-Dawley rats).

<sup>b</sup>Female incidences are from experiment BT306, while male incidences are from experiment BT306bis, which was added to the study because of high, early mortality due to aggressiveness and fighting in males in experiment BT306.

**Table 4-64. Summary of liver tumor findings in inhalation studies of TCE by Henschler et al. (1980)<sup>a</sup> and Fukuda et al. (1983)**

Sex	Concentration (ppm)	Adenomas	Adenocarcinomas
6 hrs/d, 5 d/wk, 18-mo exposure, 30-mo observation, Han:NMRI mice (Henschler et al., 1980)			
Males	0	1/30 <sup>b</sup>	1/30
	100	2/29 <sup>b</sup>	0/30
	500	0/29	0/30
Females	0	0/29	0/29
	100	0/30	0/30
	500	0/28	0/28
6 hrs/d, 5 d/wk, 18-mo exposure, 36-mo observation, Han:WIST rats (Henschler et al., 1980)			
Males	0	1/29	0/29
	100	1/30	0/30
	500	0/30	0/30
Females	0	0/28	0/28
	100	1/30	1/30
	500	2/30	0/30
7 hrs/d, 5 d/wk, 2-yr study, Crj:CD (Sprague-Dawley) rats (Fukuda et al., 1983)			
Females	0	0/50	0/50
	50	1/50	0/50
	150	0/47	0/47
	450	0/51	1/50
7 hrs/d, 5 d/wk, 2-yr study, Crj:CD (ICR) mice (Fukuda et al., 1983)			
Females	0	0/49	0/49
	50	0/50	0/50
	150	0/50	0/50
	450	1/46	0/46

<sup>a</sup>Henschler et al. (1980) observed no liver tumors in control or exposed Syrian hamsters.

<sup>b</sup>One additional hepatic tumor of undetermined class not included.

**Table 4-65. Summary of liver tumor findings in gavage studies of TCE by Henschler et al. (1984)<sup>a</sup>**

Sex (TCE concentration)	TCE (Stabilizers if present)	Benign <sup>b</sup>	Malignant <sup>c</sup>
5 d/wk, 18-mo exposure, 24-mo observation, Swiss mice (Henschler et al., 1984)			
Males (2.4g/kg body weight)	Control (none)	5/50	0/50
	TCE (triethanolamine)	7/50	0/50
	TCE (industrial)	9/50	0/50
	TCE (epichlorohydrin [0.8%])	3/50	1/50
	TCE (1,2-epoxybutane [0.8%])	4/50	0/50
	TCE (both epichlorohydrin [0.25%] and 1,2-epoxybutane [0.25%])	5/50	0/50
Females (1.8 g/kg body weight)	Control (none)	1/50	0/50
	TCE (triethanolamine)	7/50	0/50
	TCE (industrial)	9/50	0/50
	TCE (epichlorohydrin [0.8%])	3/50	0/50
	TCE (1,2-epoxybutane [0.8%])	2/50	0/50
	TCE (both epichlorohydrin [0.25%] and 1,2-epoxybutane [0.25%])	4/50	1/50

<sup>a</sup>Henschler et al. (1984) due to poor condition of the animals resulting from the nonspecific toxicity of high doses of TCE and/or the additives, gavage was stopped for all groups during week 35–40, 65 and 69–78, and all doses were reduced by a factor of 2 from the 40<sup>th</sup> week on.

<sup>b</sup>Includes hepatocellular adenomas, hemangioendothelioma, cholangiocellular adenoma.

<sup>c</sup>Includes HCC, malignant hemangiosarcoma, cholangiocellular carcinoma.

Henschler et al. (1980) exposed NMRI mice and WIST random bred rats to 0-, 100-, and 500-ppm TCE for 18 months (n = 30). Control male mice were reported to have one HCC and one hepatocellular adenoma with the incidence rate unknown. In the 100 ppm group, two hepatocellular adenomas and one mesenchymal liver tumor were reported. No liver tumors were reported at any dose of TCE in female mice or controls. For male rats, only one hepatocellular adenomas at 100 ppm was reported. For female rats, no liver tumors were reported in controls, but one adenoma and one cholangiocarcinoma was reported at 100 ppm, and at 500 ppm, two cholangioadenomas, a relatively rare biliary tumor, were reported. The difference in survival in mice, did not affect the power to detect a response, as was the case for rats. However, the low number of animals studied, abbreviated exposure duration, low survival in rats, and absent background response (suggesting low intrinsic sensitivity to this endpoint) suggest a study of limited ability to detect a TCE carcinogenic liver response. Of note is that despite their limitations, both Fukuda et al. (1983) and Henschler et al. (1980) report rare biliary cell derived tumors in TCE-exposed rats.

Van Duuren et al. (1979), exposed mice to 0.5 mg/mouse to TCE via gavage once a week in 0.1 mL trioctanion (n = 30). Inadequate design and reporting of this study limit that ability to use the results as an indicator of TCE carcinogenicity.

The NCI (1976) study of TCE was initiated in 1972 and involved the exposure of Osborne-Mendel rats to varying concentrations of TCE. A low incidence of liver tumors was reported for controls and carbon tetrachloride positive controls in rats from this study. The authors concluded that due to mortality, “the test is inconclusive in rats.” They note the insensitivity of the rat strain used to the positive control of carbon tetrachloride exposure.

The NTP (1990) study of TCE exposure in male and female F344/N rats, and B6C3F<sub>1</sub> mice (500 and 1,000 mg/kg for rats) is limited in the ability to demonstrate a dose-response for hepatocarcinogenicity. For rats, the NTP (1990) study reported no treatment-related, non-neoplastic liver lesions in males and a decrease in basophilic cytological change in female rats. The results for detecting a carcinogenic response in rats were considered to be equivocal because both groups receiving TCE showed significantly reduced survival compared to vehicle controls and because of a high rate (e.g., 20% of the animals in the high-dose group) of death by gavage error.

The NTP (1988) study of TCE exposure in four strains of rats to “diisopropylamine-stabilized TCE” was also considered inadequate for either comparing or assessing TCE-induced liver carcinogenesis in these strains of rats because of chemically induced toxicity, reduced survival, and incomplete documentation of experimental data. TCE gavage exposures of 0, 500, or 1,000 mg/kg-day (5 days/week, for 103 weeks) male and female rats were also marked by a large number of accidental deaths (e.g., for high-dose male Marshal rats, 25 animals were accidentally killed).

Maltoni et al. (1988; 1986) reported the results of several studies of TCE via inhalation and gavage in mice and rats. A large number of animals were used in the treatment groups but the focus of the study was detection of a neoplastic response with only a generalized description of tumor pathology phenotype and limited reporting of non-neoplastic changes in the liver. Accidental death by gavage error was reported not to occur in this study. With regard to effects of TCE exposure on rat survival, “a nonsignificant excess in mortality correlated to TCE treatment was observed only in female rats (treated by ingestion with the compound).”

For rats, Maltoni et al. (1986) reported four liver angiosarcomas (one in a control male rat, one both in a TCE-exposed male and female at 600 ppm TCE for 8 weeks, and one in a female rat exposed to 600-ppm TCE for 104 weeks), but the specific results for incidences of hepatocellular “hepatomas” in treated and control rats were not given. Although the Maltoni et al. (1986) concluded that the small number was not treatment related, the findings were brought forward because of the extreme rarity of this tumor in control Sprague-Dawley rats, untreated or treated with vehicle materials. In rats treated for 104 weeks, there was no report of a TCE treatment-related increase in liver cancer in rats. This study only presented data for positive findings so it did not give the background or treatment-related findings in rats for liver tumors in this study. Thus, the extent of background tumors and sensitivity for this endpoint cannot be determined. Of note is that the Sprague-Dawley strain used in this study was also noted in the

Fukuda et al. (1983) study to be relatively insensitive for spontaneous liver cancer and to also be negative for TCE-induced hepatocellular liver cancer induction in rats. However, like Fukuda et al. (1983) and Henschler et al. (1980), which reported rare biliary tumors in insensitive strains of rat for hepatocellular tumors, Maltoni et al. (1986) reported a relatively rare tumor type, angiosarcoma, after TCE exposure in a relatively insensitive strain for “hepatomas.” As noted above, many of the rat studies were limited by premature mortality due to gavage error or premature mortality (NTP, 1990, 1988; Henschler et al., 1980; NCI, 1976), which was reported not occur in Maltoni et al. (1986).

#### 4.5.5.2. Positive TCE Studies of Mice

In the NCI (1976) study of TCE exposure in B6C3F<sub>1</sub> mice, TCE was reported to increase the incidence of HCCs in both doses and both genders of mice (~1,170 and 2,340 mg/kg for males and 870 and 1,740 mg/kg for female mice). HCC diagnosis was based on histologic appearance and metastasis to the lung. The tumors were described in detail and to be heterogeneous “as described in the literature” and similar in appearance to tumors generated by carbon tetrachloride. The description of liver tumors in this study and tendency to metastasize to the lung are similar to descriptions provided by Maltoni et al. (1986) for TCE-induced liver tumors in mice via inhalation exposure.

The NTP (1990) study of TCE exposure in male and female B6C3F<sub>1</sub> mice (1,000 mg/kg for mice) reported decreased latency of liver tumors, with animals first showing carcinomas at 57 weeks for TCE-exposed animals and 75 weeks for control male mice. The administration of TCE was also associated with increased incidence of HCC (tumors with markedly abnormal cytology and architecture) in male and female mice. Hepatocellular adenomas were described as circumscribed areas of distinctive hepatic parenchymal cells with a perimeter of normal appearing parenchyma in which there were areas that appeared to be undergoing compression from expansion of the tumor. Mitotic figures were sparse or absent but the tumors lacked typical lobular organization. HCCs had markedly abnormal cytology and architecture with abnormalities in cytology cited as including increased cell size, decreased cell size, cytoplasmic eosinophilia, cytoplasmic basophilia, cytoplasmic vacuolization, cytoplasmic hyaline bodies, and variations in nuclear appearance. Furthermore, in many instances several or all of the abnormalities were present in different areas of the tumor and variations in architecture with some of the HCCs having areas of trabecular organization. Mitosis was variable in amount and location. Therefore, the phenotype of tumors reported from TCE exposure was heterogeneous in appearance between and within tumors. However, because it consisted of a single-dose group in addition to controls, this study is of limited utility for analyzing the dose-response for hepatocarcinogenicity. There was also little reporting of non-neoplastic pathology or toxicity and no report of liver weight at termination of the study.

Maltoni et al. (1986) reported the results of several studies of TCE in mice. A large number of animals were used in the treatment groups but the focus of the study was detection of a neoplastic response with only a generalized description of tumor pathology phenotype given and limited reporting of non-neoplastic changes in the liver. There was no accidental death by gavage error reported to occur in mice, but a “nonsignificant” excess in mortality correlated to TCE treatment was observed in male B6C3F<sub>1</sub> mice. TCE-induced effects on body weight were reported to be absent in mice except for one experiment (BT 306 bis) in which a slight nondose-correlated decrease was found in exposed animals. “Hepatoma” was the term used to describe all malignant tumors of hepatic cells, of different subhistotypes, and of various degrees of malignancy and were reported to be unique or multiple, and have different sizes (usually detected grossly at necropsy) from TCE exposure. In regard to phenotype, tumors were described as usual type observed in Swiss and B6C3F<sub>1</sub> mice, as well as in other mouse strains, either untreated or treated with hepatocarcinogens and to frequently have medullary (solid), trabecular, and pleomorphic (usually anaplastic) patterns. Swiss mice from this laboratory were reported to have a low incidence of hepatomas without treatment (1%). The relatively larger number of animals used in this bioassay (n = 90–100), in comparison to NTP standard assays, allows for a greater power to detect a response.

TCE exposure for 8 weeks via inhalation at 100 or 600 ppm may have been associated with a small increase in liver tumors in male mice in comparison to concurrent controls during the life span of the animals. In Swiss mice exposed to TCE via inhalation for 78 weeks, there a reported increase in hepatomas associated with TCE treatment that was dose-related in male but not female Swiss mice. In B6C3F<sub>1</sub> mice exposed via inhalation to TCE for 78 weeks, increases in hepatomas were reported in both males and females. However, the experiment in males was repeated with B6C3F<sub>1</sub> mice from a different source, since in the first experiment, more than half of the mice died prematurely due to excessive fighting. Although the mice in the two experiments in males were of the same strain, the background level of liver cancer was significantly different between mice from the different sources (1/90 vs. 19/90), though the early mortality may have led to some censoring. The finding of differences in response in animals of the same strain but from differing sources has also been reported in other studies for other endpoints. However, for both groups of male B6C3F<sub>1</sub> mice, the background rate of liver tumors over the lifetime of the mice was no greater than about 20%.

There were other reports of TCE carcinogenicity in mice from chronic exposures that were focused primarily on the detection of liver tumors, with limited reporting of tumor phenotype or non-neoplastic pathology. Herren-Freund et al. (1987) reported that male B6C3F<sub>1</sub> mice given 40 mg/L TCE in drinking water had increased tumor response after 61 weeks of exposure. However, concentrations of TCE fell by about half at this dose of TCE during the twice a week change in drinking water solution so the actual dose of TCE the animals received was <40 mg/L. The percentage liver/body weight was reported to be similar for control and

TCE-exposed mice at the end of treatment. However, despite difficulties in establishing accurately the dose received, an increase in adenomas per animal and an increase in the number of animals with HCCs were reported to be associated with TCE exposure after 61 weeks of exposure and without apparent hepatomegaly. Anna et al. (1994) reported tumor incidences for male B6C3F<sub>1</sub> mice receiving 800 mg/kg-day TCE via gavage (5 days/week for 76 weeks). All TCE-treated mice were reported to be alive after 76 weeks of treatment. Although the control group contained a mixture of exposure durations (76–134 weeks) and concurrent controls had a very small number of animals, TCE treatment appeared to increase the number of animals with adenomas and the mean number of adenomas and carcinomas, but with no concurrent TCE-induced cytotoxicity.

#### **4.5.5.3. Summary: TCE-Induced Cancer in Laboratory Animals**

Chronic TCE bioassays have consistently reported increased liver tumor incidences in both sexes of B6C3F<sub>1</sub> mice treated by inhalation and gavage exposure in a number of bioassays. The only inhalation study of TCE in Swiss mice also showed an effect in males. Data in the rat, while not reporting statistically significantly increased risks, are not entirely adequate due to low numbers of animals, inadequate reporting, use of insensitive bioassays, increased systemic toxicity, and/or increased mortality. Notably, several studies in rats noted a few very rare types of liver or biliary tumors (cystic cholangioma, cholangiocarcinoma, or angiosarcomas) in treated animals.

#### **4.5.6. Role of Metabolism in Liver Toxicity and Cancer**

It is generally thought that TCE oxidation by CYPs is necessary for induction of hepatotoxicity and hepatocarcinogenicity (Bull, 2000). Direct evidence for this hypothesis is limited, e.g., the potentiation of hepatotoxicity by pretreatment with CYP inducers such as ethanol and phenobarbital (Okino et al., 1991; Nakajima et al., 1988). Rather, the presumption that CYP-mediated oxidation is necessary for TCE hepatotoxicity and hepatocarcinogenicity is largely based on similar effects (e.g., increases in liver weight, peroxisome proliferation, and hepatocarcinogenicity) having been observed with TCE's oxidative metabolites. The discussion below focuses the similarities and differences between the major effects in the liver of TCE and of the oxidative metabolites CH, TCA, and DCA. In addition, CH is largely converted to TCOH, TCA, and possibly DCA. DCA has been used in human clinical practice for a variety of severe illnesses and no data on liver effects in humans have been reported (U.S. EPA, 2003b). However, as noted in EPA (2003b), data on DCA in humans are scarce and complicated by the fact that available studies have predominantly focused on individuals who have a pre-existing (usually severe) disease.

#### 4.5.6.1. Pharmacokinetics of CH, TCA, and DCA from TCE Exposure

As discussed in Chapter 3, *in vivo* data confirm that CH and TCA, are oxidative metabolites of TCE, with available data on TCA incorporated into the PBPK modeling. In addition, there are indirect data suggesting the formation of DCA. However, direct *in vivo* evidence of the formation of DCA is confounded by its rapid clearance at low concentrations, and analytical artifacts in its detection *in vivo* that have yet to be entirely resolved. PBPK modeling (see Section 3.5) predicts that the proportions of TCE metabolized to CH and TCA varies considerably in mice (ranging from 15 to 97 and 4 to 38%, respectively) and rats (ranging 7–75 and 0.5–22%, respectively). Therefore, a range of smaller concentrations of TCA or CH may be relevant for comparisons with TCE-induced liver effects. For example, for 1,000 mg/kg-day oral doses of TCE, the relevant comparisons would be approximately 0.25–1.5 g/L in drinking water for TCA and CH. For DCA, a corresponding range is harder to determine and has been suggested to be an upper limit of about 12% following oral exposures ([Barton et al., 1999](#)). This is consistent with the range estimated from PBPK modeling attributing all of the “untracked” oxidation (i.e., not producing TCOH or TCA) to DCA (95% CI: 0.2–16%, see Figure 3-22).

Two studies have used analytic methods for DCA that are considered more reliable and less confounded by artifactual formation. Kim et al. ([2009](#)), which was published too late to be incorporated into the PBPK model, used an empirical pharmacokinetic model to analyze data on male B6C3F<sub>1</sub> mice exposed to a single dose of 2,100 mg/kg TCE by gavage. Peak levels of TCA and DCA were found to be 64 and 18 ng/mL, respectively, a difference of more than threefold. The kinetic rate constant they estimated for TCE → DCA were more than five orders of magnitude smaller than the kinetic rate constant estimated for TCE → TCA. These data all suggest that DCA is a minor metabolite of TCE as compared to TCA at high doses of around 2,000 mg/kg. Delinsky et al. ([2005](#)) reported that in male Sprague-Dawley rats, after a single 2,000 mg/kg dose by gavage, peak levels of DCA were 39.5 ng/mL. Delinsky et al. ([2005](#)) did not report TCA levels for comparison. The only data available in rats in this range of gavage doses (coincidentally also in male Sprague-Dawley rats) reported peak levels of TCA of 24 and 60 mg/mL at gavage doses of 600 and 3,000 mg/kg, respectively ([Larson and Bull, 1992b](#)). This suggests a difference between DCA and TCA levels in rats exposed to TCE of about 1,000-fold, albeit with more uncertainty as compared to Kim et al. ([2009](#)), in which both were measured simultaneously in the same animals. However, liver toxicity in both rats and mice is evident at much lower doses, so additional data are needed to inform whether the relative amount of TCA and DCA changes at lower exposures.

#### 4.5.6.2. Comparisons Between TCE and TCA, DCA, and CH Noncancer Effects

##### 4.5.6.2.1. Hepatomegaly—qualitative and quantitative comparisons

As discussed above, TCE causes hepatomegaly in rats, mice, and gerbils under both acute and chronic dosing. Data from a few available studies suggest that oxidative metabolism is important for mediating these effects. Buben and O'Flaherty (1985) collected limited pharmacokinetic data in a sample of the same animals for which liver weight changes were being assessed. While liver weight increases had similarly strong correlations with applied dose and urinary metabolites for doses up to 1,600 mg/kg-day ( $R^2$  of 0.97 for both), above that dose, the linear relationship was maintained with urinary metabolites but not with applied dose. Ramdhan et al. (2008) conducted parallel experiments at TCE 1,000 and 2,000 ppm (8 hours/day, 7 days) in wild-type and CYP2E1-null mice, which did not exhibit increased liver/body weight ratios with TCE treatment and excreted twofold lower amounts of oxidative metabolites TCA and TCOH in urine as compared to wild-type mice. However, among control mice, those with the null genotype had 1.32-fold higher absolute liver weights and 1.18-fold higher liver/body weight ratios than wild-type mice, reducing the sensitivity of the experiment, particularly with only six mice per dose group.

Ramdhan et al. (2010) reported that stated that urinary TCA levels in wild type mice were incorrectly reported by Ramdhan et al. (2008) but were corrected in this study. The authors reported no differences in urinary volume by genotype or exposure but did not show the data. TCA and TCOH were detected in all exposed mice with no significant differences between the 1,000 and 2,000 ppm TCE levels. TCA concentrations were reported to be significantly lower and TCOH levels significantly higher in PPAR $\alpha$ -null mice relative to wild type mice, with no differences in genotype between the sum of total TCA and TCOH concentrations between genotypes. The authors reported that they measured hepatic protein expression of CYP2E1 and ALDH2 enzymes and did not observe a significant difference among controls (data not shown) and that TCE exposure did not alter hepatic CYP2E1 expression but did decrease ALDH2 expression to a comparable extent in all mouse lines (data not shown). Thus, changes in urinary TCA levels in the differing strains were not related to changes in expression of these metabolic enzymes.

As stated above, hepatomegaly was increased by TCE exposure in all three strains. TCE at both 1,000 and 2,000 ppm significantly increased liver weight in the three mouse lines to a similar extent (i.e., 38 and 49% in wild type mice, 20 and 37% in PPAR-null mice, and 28 and 32% in hPPAR $\alpha$  mice). The increases were not statistically significant between doses within each strain. Liver/body weight ratios were also significantly increased with TCE exposure at 1,000 and 2,000 ppm relative to controls (i.e., 38 and 43% in wild type mice, 24 and 36% in PPAR $\alpha$ -null mice, and 27 and 39% in hPPAR $\alpha$  mice, respectively). The difference between 2,000 and 1,000 ppm TCE exposure was statistically significant in PPAR $\alpha$ -null mice. As to the nature of the hepatomegaly induced under these conditions, hepatic triglyceride levels were

reported to be significantly correlated with liver/body weight ratios of all mice used in the study ( $r = 0.54$ ).

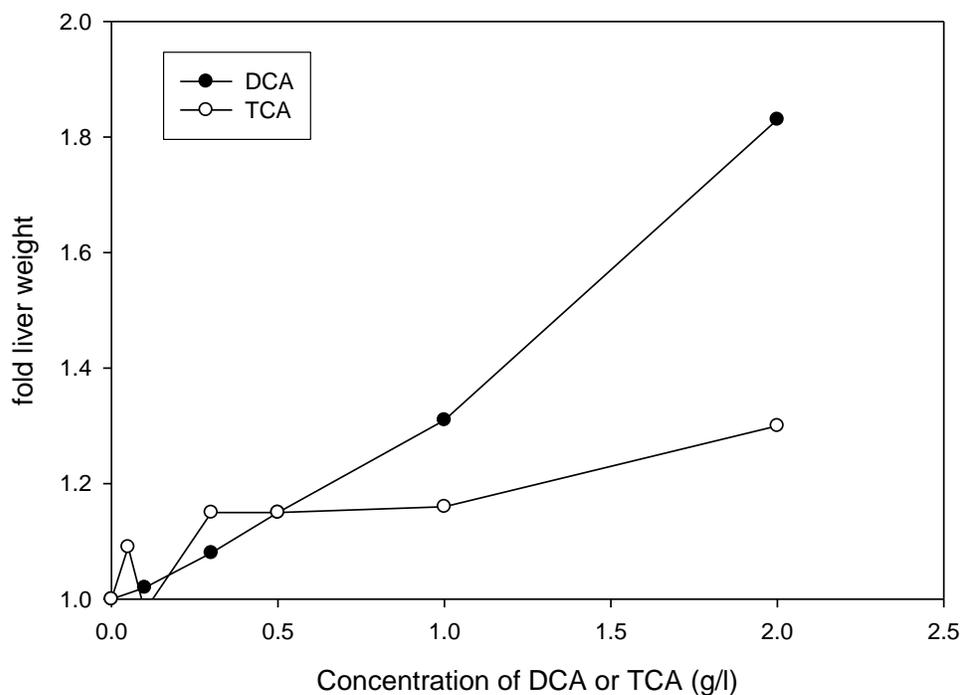
With respect to oxidative metabolites themselves, data from CH studies are not informative—either because data were not shown ([Sanders et al., 1982a](#)) or, because at the time points measured, liver weight increases were substantially confounded by foci and carcinogenic lesions ([Leakey et al., 2003b](#)). TCA and DCA have both been found to cause hepatomegaly in mice and rats, with mice being more sensitive to this effect. DCA also increases liver/body weight ratios in dogs, but TCE and TCA have not been tested in this species ([Cicmanec et al., 1991](#)).

As noted above, TCE-induced changes in liver weight appear to be proportional to the exposure concentration across route of administration, gender and rodent species. As an indication of the potential contribution of TCE metabolites to this effect, a quantitative comparison of the shape of the dose-response curves for liver weight induction for TCE and its metabolites is informative. The analysis below was reported in Evans et al. ([2009](#)).

A number of short-term (<4 weeks) studies of TCA and DCA in drinking water have attempted to measure changes in liver weight induction, with the majority of these studies being performed in male B6C3F<sub>1</sub> mice. Studies conducted from 14 to 30 days show a consistent increase in percentage liver/body weight induction by TCA or DCA. However, as stated in many of the discussions of individual studies (see Appendix E), there is a limited ability to detect a statistically significant change in liver weight change in experiments that use a relatively small number of animals or do not match control and treatment groups for age and weight. The experiments of Buben and O’Flaherty ([1985](#)) used 12–14 mice per group, giving them a greater ability to detect a TCE-induced dose-response. However, many experiments have been conducted with 4–6 mice per dose group. For example, the data from DeAngelo et al. ([2008](#)) for TCA-induced percentage liver/body weight ratio increases in male B6C3F<sub>1</sub> mice were only derived from five animals per treatment group after 4 weeks of exposure. The 0.05 and 0.5 g/L exposure concentrations were reported to give a 1.09- and 1.16-fold of control percentage liver/body weight ratios, which were consistent with the increases noted in the cross-study database above. However, a power calculation shows that the Type II error (which should be >50% and thus, greater than the chances of “flipping a coin”) was only a 6 and 7% and therefore, the designed experiment could accept a false null hypothesis. In addition, some experiments took greater care to age and weight match the control and treatment groups before the start of treatment.

Therefore, given these limitations and the fact that many studies used a limited range of doses, an examination of the combined data from multiple studies ([Kato-Weinstein et al., 2001](#); [Parrish et al., 1996](#); [Carter et al., 1995](#); [Sanchez and Bull, 1990](#); [DeAngelo et al., 1989, 2008](#)) can best inform/discern differences in DCA and TCA dose-response relationships for liver weight induction (described in more detail in Section E.2.4.2). The dose-response curves for

similar concentrations of DCA and TCA are presented in Figure 4-5 for durations of exposure from 14 to 28 days in the male B6C3F<sub>1</sub> mouse, which was the most common sex and strain used. As noted in Appendix E, there appears to be a linear correlation between dose in drinking water and liver weight induction up to 2 g/L of DCA. However, the shape of the dose-response curve for TCA appears to be quite different. Lower concentrations of TCA induce larger increase that does DCA, but the TCE response reaches an apparent plateau while that of DCA continues to increase the response. TCA studies did not show significant duration-dependent difference in liver weight induction in this duration range. Short-duration studies (10–42 days) were selected because: (1) in chronic studies, liver weight increases are confounded by tumor burden; (2) multiple studies are available; and (3) TCA studies do not show significant duration-dependent differences in this duration range.

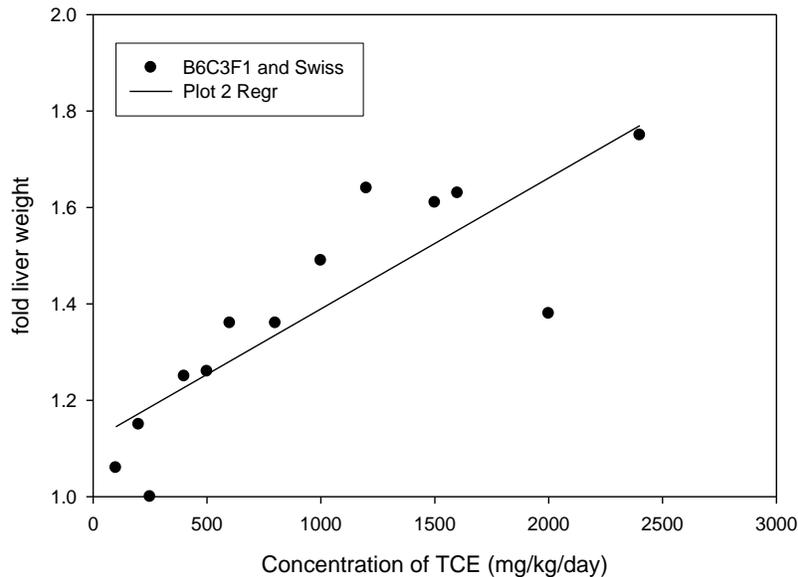
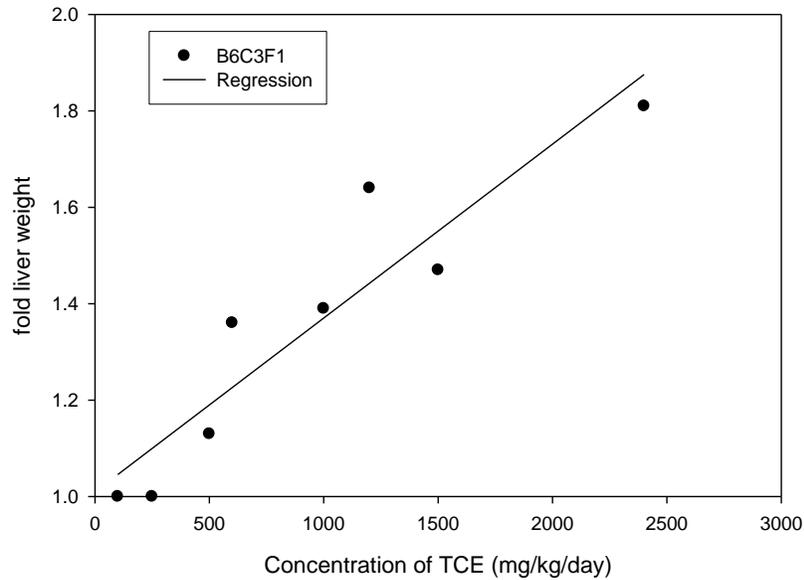


Sources: ([Kato-Weinstein et al., 2001](#); [Parrish et al., 1996](#); [Carter et al., 1995](#); [Sanchez and Bull, 1990](#); [DeAngelo et al., 1989, 2008](#))).

**Figure 4-5. Comparison of average fold-changes in relative liver weight to control and exposure concentrations of 2 g/L or less in drinking water for TCA and DCA in male B6C3F<sub>1</sub> mice for 14–30 days.**

Of interest is the issue of how the dose-response curves for TCA and DCA compare to that of TCE in a similar model and dose range. Since TCA and DCA have strikingly different dose-response curves, which one, if either, best fits that of TCE and thus, can give insight as to which is causative agent for TCE's effects in the liver? The carcinogenicity of chronic TCE

exposure has been predominantly studies in two mouse strains, Swiss and B6C3F<sub>1</sub>, both of which reportedly developed liver tumors. Rather than administered in drinking water, oral TCE studies have been conducted via gavage and generally in corn oil for 5 days of exposure per week. Factors adding to the increased difficulty in establishing the dose-response relationship for TCE across studies and for comparisons to the DCA and TCA database include vehicle effects, the difference between daily and weekly exposures, the dependence of TCE effects in the liver on its metabolism to a variety of agents capable inducing effects in the liver, differences in response between strains, and the inherent increased variability in use of the male mouse model. In particular, these factors would add variability to any effort at a combined analysis, and make a consistent dose-response pattern more difficult to discern. Nonetheless, despite such differences in exposure route, vehicle, etc., a consistent pattern of dose-response emerges from combining the available TCE data. The effects of oral exposure to TCE from 10 to 42 days on liver weight induction is shown below in Figure 4-6 using the data of Elcombe et al. (1985), Dees and Travis (1993), Goel et al. (1992), Merrick et al. (1989), Goldsworthy and Popp (1987), and Buben and O'Flaherty (1985). Oral TCE administration in male B6C3F<sub>1</sub> and Swiss mice appeared to induce a dose-related increase in percentage liver/body weight that was generally proportional to the increase in magnitude of dose, though as expected, with more variability than observed for a similar exercise for DCA or TCA in drinking water. Some of the variability is due to the inclusion of the 10-day studies, since as discussed in Section E.2.4.2, there was a greater increase in TCE-induced liver weight at 28–42 days of exposure Swiss mice than the 10-day data in B6C3F<sub>1</sub> mice, and Kjellstrand et al. (1981b) noted that TCE-induced liver weight increases are still increasing at 10 days inhalation exposure. A strain difference is not evident between the Swiss and B6C3F<sub>1</sub> males, as both the combined TCE data and that for only B6C3F<sub>1</sub> mice show similar correlation with the magnitude of dose and magnitude of percentage liver/body weight increase. The correlation coefficients for the linear regressions presented for the B6C3F<sub>1</sub> data are  $R^2 = 0.861$  and for the combined data sets is  $R^2 = 0.712$ . Comparisons of the slopes of the dose-response curves suggest a greater consistency between TCE and DCA than between TCE and TCA. There did not appear to be evidence of a plateau with higher TCE doses, and the degree of fold-increase rises to higher levels with TCE than with TCA in the same strain of mouse.

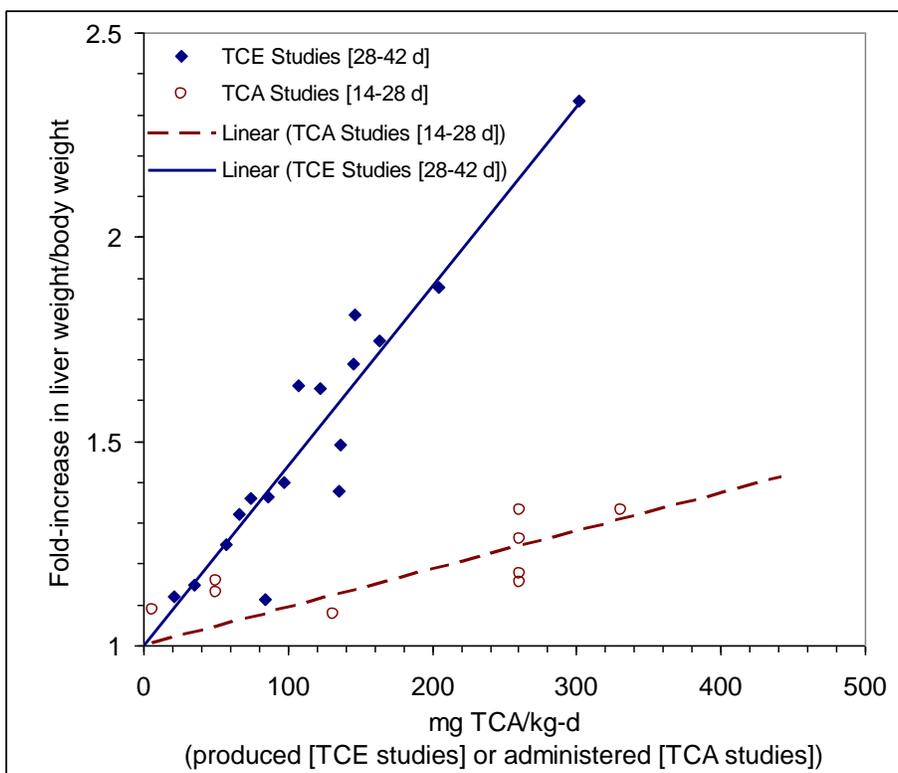


Sources: Dees and Travis ([1993](#)); Merrick et al. ([1989](#)); Goldsworthy and Popp ([1987](#)); Elcombe et al. ([1985](#))

**Figure 4-6. Comparisons of fold-changes in average relative liver weight and gavage dose of (top panel) male B6C3F<sub>1</sub> mice for 10–28 days of exposure and (bottom panel) in male B6C3F<sub>1</sub> and Swiss mice.**

A more direct comparison would be on the basis of dose rather than drinking water concentration. The estimations of internal dose of DCA or TCA from drinking water studies, while varying considerably ([DeAngelo et al., 2008](#); [DeAngelo et al., 1989](#)), nonetheless suggest that the doses of TCE used in the gavage experiments were much higher than those of DCA or TCA. However, only a fraction of ingested TCE is metabolized to DCA or TCA, as, in addition

to oxidative metabolism, TCE is also cleared by GSH conjugation and by exhalation. While DCA dosimetry is highly uncertain (see Sections 3.3 and 3.5), the mouse PBPK model, described in Section 3.5 was calibrated using extensive in vivo data on TCA blood, plasma, liver, and urinary excretion data from inhalation and gavage TCE exposures, and makes robust predictions of the rate of TCA production. If TCA were predominantly responsible for TCE-induced liver weight increases, then replacing administered TCE dose (e.g., mg TCE/kg/day) by the rate of TCA produced from TCE (mg TCA/kg/day) should lead to dose-response curves for increased liver weight consistent with those from directly administered TCA. Figure 4-7 shows this comparison using the PBPK model-based estimates of TCA production for four TCE studies from 28 to 42 days in the male NMRI, Swiss, and B6C3F<sub>1</sub> mice ([Goel et al., 1992](#); [Merrick et al., 1989](#); [Buben and O'Flaherty, 1985](#); [Kjellstrand et al., 1983a](#)) and four oral TCA studies in B6C3F<sub>1</sub> male mice at  $\leq 2$  g/L drinking water exposure ([2008](#); [Kato-Weinstein et al., 2001](#); [Parrish et al., 1996](#); [DeAngelo et al., 1989](#)) from 14 to 28 days of exposure. The selection of the 28–42 day data for TCE was intended to address the decreased opportunity for full expression of response at 10 days. PBPK modeling predictions of daily internal doses of TCA in terms of mg/kg-day produced via TCE metabolism would indeed be lower than the TCE concentrations in terms of mg/kg-day given orally by gavage. The predicted internal dose of TCA from TCE exposure studies are of a comparable range to those predicted from TCA drinking water studies at exposure concentrations in which palpability has not been an issue for estimation of internal dose. Thus, although the TCE data are for higher exposure concentrations, they are predicted to produce comparable levels of TCA internal dose estimated from direct TCA administration in drinking water.



Abscissa for TCE studies consists of the median estimates of the internal dose of TCA predicted from metabolism of TCE using the PBPK model described in Section 3.5 of the TCE risk assessment. Lines show linear regression with intercept fixed at unity. All data were reported fold-change in mean liver weight/body weight ratios, except for Kjellstrand et al. (1983a), which were the fold-change in the ratio of mean liver weight to mean body weight. In addition, in Kjellstrand et al. (1983a), some systemic toxicity as evidence by decreased total body weight was reported in the highest-dose group.

Sources: Kjellstrand et al. (1983a); Goel et al. (1992); Merrick et al. (1989); Maltoni et al., 1988); Buben and O'Flaherty (1985); DeAngelo et al. (1999); DeAngeleo et al. (2008); Kato-Weinstein et al. (2001); Parrish et al. (1996)

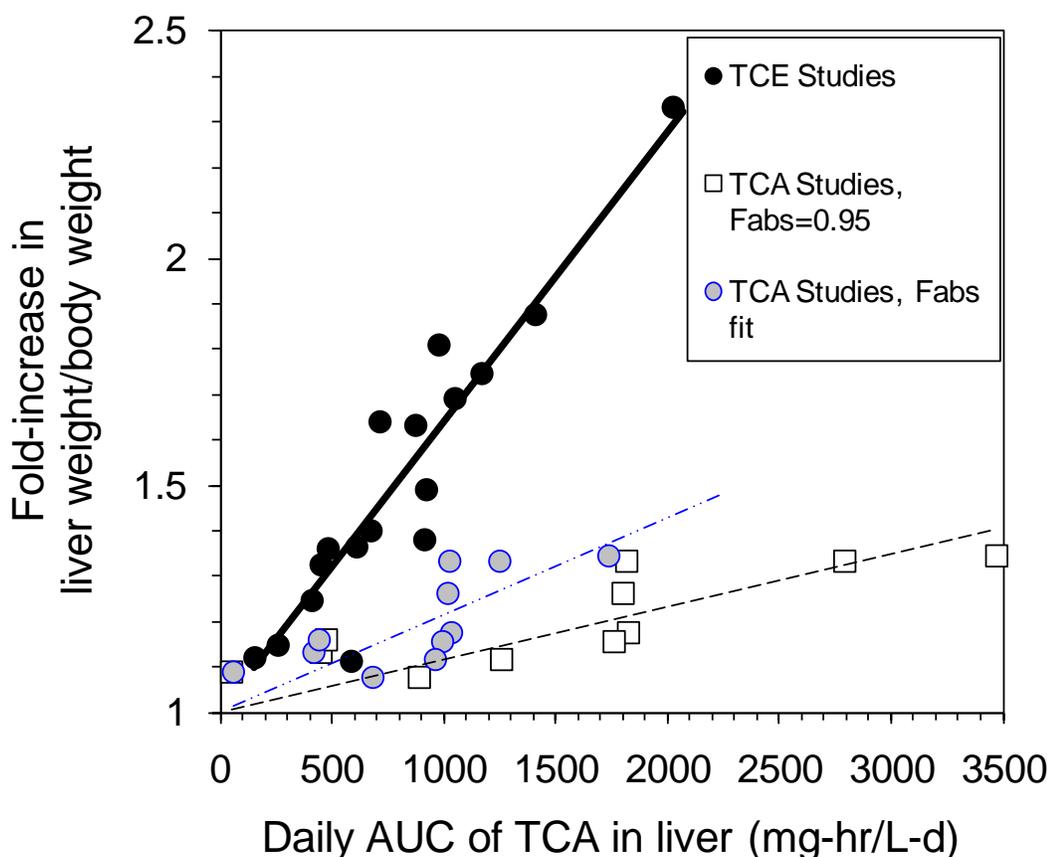
**Figure 4-7. Comparison of fold-changes in relative liver weight for data sets in male B6C3F<sub>1</sub>, Swiss, and NRM1 mice between TCE studies [duration 28–42 days]) and studies of direct oral TCA administration to B6C3F<sub>1</sub> mice [duration 14–28 days]).**

Figure 4-7 clearly shows that for a given amount of TCA produced from TCE, but going through intermediate metabolic pathways, the liver weight increases are substantially greater than, and highly inconsistent with, that expected based on direct TCA administration. In particular, the response from direct TCA administration appears to "saturate" with increasing TCA dose at a level of about 1.4-fold, while the response from TCE administration continues to increase with dose to 1.75-fold at the highest dose administered orally in Buben and O'Flaherty (1985) and over twofold in the inhalation study of Kjellstrand et al. (1983a). Because TCA liver

concentrations are proportional to the dose TCA, and do not depend on whether it is administered in drinking water or internally produced in the liver, the results of the comparison using the TCA liver dose-metric are identical.

Furthermore, while as noted previously, oral studies appear to report a linear relationship between TCE exposure concentration and liver weight induction, the inclusion of inhalation studies on the basis of internal dose led to a highly consistent dose-response curve for among TCE study. Therefore, it is unlikely that differing routes of exposure can explain the inconsistencies in dose-response.

The bioavailability of TCA, which in the above analysis is assumed to be 100%, is another factor that may impact the dose-response. Sweeney et al. (2009), in an analysis of the potential role of TCA in the liver carcinogenesis of tetrachloroethylene, identified a number of previously unpublished TCA kinetic data in mice exposed to TCA via drinking water for 3–14 days. They concluded that fractional absorption of TCA via drinking water exposures is much less than 100%—about 29% at low exposures and decreasing with increasing dose. However, the conclusions of the Sweeney et al. (2009) were based on the Hack et al. (2006) TCE PBPK model, which had a number of deficiencies, as noted in Section 3.5 and Appendix A. Therefore, as discussed in Appendix A, Chiu (2011) reanalyzed those data using the updated TCE PBPK model of Evans et al. (2009) and Chiu et al. (2009) and concluded that while there was evidence of reduced absorption (80–90% at low exposures, and decreasing with increasing dose), it was not as low as that estimated by Sweeney et al. (2009). As discussed in Appendix A, it may be more accurate to characterize the fractional absorption as an empirical parameter reflecting unaccounted-for biological processes as well as experimental variation. Chiu (2011) also reanalyzed the data on TCE- and TCA-induced hepatomegaly using the central estimates of the fractional absorption of TCA inferred from the analysis described above. Figure 4-8 shows the results, comparing a fixed fractional absorption of 95% with the fitted fractional absorption from Chiu (2011), here plotted using AUC of TCA in the liver as the dose-metric. For reference, the dose-response for administered TCA with an assumption of fixed, nearly complete absorption [analogous to the results from Evans et al. (2009), Figure 4-7] is also included. While the reduced fractional absorption inferred from drinking water data reported by Sweeney et al. (2009) accounts for part of the difference in dose-responses between TCE- and TCA-induced hepatomegaly reported by Evans et al. (2009), it does not appear to be able to account for the entire difference. In particular, the fraction of hepatomegaly contributed by TCA is about 0.20 assuming nearly complete absorption, as compared to about 0.33 assuming the best-fitting fractional absorption inferred from the PBPK model-based analysis. The inability of TCA to account for TCE-induced hepatomegaly is confirmed statistically by analysis of variance (ANOVA), with  $p$ -values of  $<10^{-4}$ . Therefore, assuming a reduced TCA bioavailability does not change the conclusion that the available data are inconsistent with the toxicological hypothesis that TCA can fully account for TCE-induced hepatomegaly.



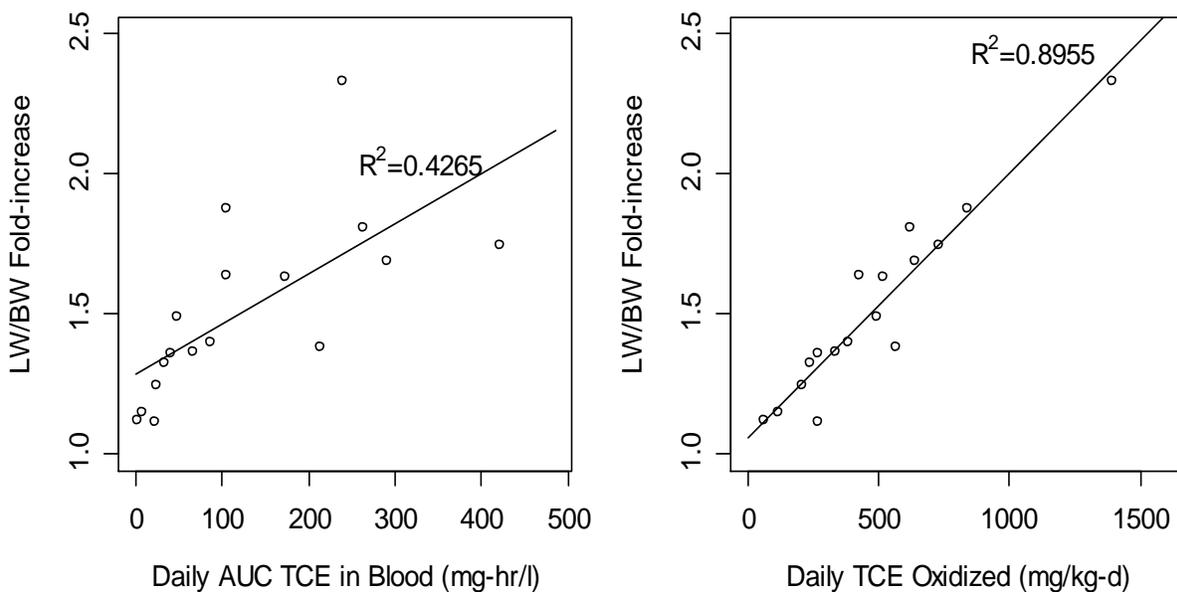
Fold-changes in relative liver weight for data sets in male B6C3F<sub>1</sub>, Swiss, and NRM1 mice between TCE studies (duration 28–42 days) and studies of direct oral TCA administration to B6C3F<sub>1</sub> mice (duration 14–28 days). Linear regressions were compared using ANOVA to assess whether the TCE studies were consistent with the TCA studies, using TCA as the dose-metric. For each analysis of drinking water fraction absorption, ANOVA *p*-values were  $<10^{-4}$  when comparing the assumption that all of the data had a common slope with the assumption that TCE and TCA data had different slopes.

Sources: Kjellstrand et al. (1983a); Goel et al. (1992); Merrick et al. (1989); Buben and O’Flaherty (1985); DeAngelo et al. (2008; 1989); Kato-Weinstein et al. (2001); Parrish et al. (1996); Green (2003).

**Figure 4-8. Comparison of hepatomegaly as a function of AUC of TCA in liver, using values for the TCA drinking water fractional absorption (Fabs).**

Additional analyses do, however, support a role for oxidative metabolism in TCE-induced liver weight increases, and that the parent compound TCE is not the likely active moiety [suggested previously by Buben and O’Flaherty (1985)]. In particular, the same studies are shown in Figure 4-9 using PBPK-model based predictions of the AUC of TCE in blood and total

oxidative metabolism, which produces chloral, TCOH, DCA, and other metabolites in addition to TCA. The dose-response relationship between TCE blood levels and liver weight increase, while still having a significant trend, shows substantial scatter and a low  $R^2$  of 0.43. On the other hand, using total oxidative metabolism as the dose-metric leads to substantially more consistency dose-response across studies, and a much tighter linear trend with an  $R^2$  of 0.90 (see Figure 4-9). A similar consistency is observed using liver-only oxidative metabolism as the dose-metric, with  $R^2$  of 0.86 (not shown). Thus, while the slope is similar between liver weight increase and TCE concentration in the blood and liver weight increase and rate of total oxidative metabolism, the data are a much better fit for total oxidative metabolism.



Lines show linear regression. Use of liver oxidative metabolism as a dose-metric gives results qualitatively similar to (B), with  $R^2 = 0.86$ .

Sources: Kjellstrand et al. (1983a); Goel et al. (1992); Merrick et al. (1989); Buben and O'Flaherty (1985).

**Figure 4-9. Fold-changes in relative liver weight for data sets in male B6C3F<sub>1</sub>, Swiss, and NRM1 mice reported by TCE studies of duration 28–42 days using internal dose-metrics predicted by the PBPK model described in Section 3.5: (A) dose-metric is the median estimate of the daily AUC of TCE in blood, (B) dose-metric is the median estimate of the total daily rate of TCE oxidation.**

Although the qualitative similarity to the linear dose-response relationship between DCA and liver weight increases is suggestive of DCA being the predominant metabolite responsible for TCE liver weight increases, due to the highly uncertain dosimetry of DCA derived from TCE, this hypothesis cannot be tested on the basis of internal dose. Similarly, another TCE metabolite, CH, has also been reported to induce liver tumors in mice; however, there are no adequate comparative data to assess the nature of liver weight increases induced by this TCE metabolite (see Sections E.2.5 and 4.5.6.3.2). Whether its formation in the liver after TCE exposure correlates with TCE-induced liver weight changes cannot be determined.

#### 4.5.6.2.2. Cytotoxicity

As discussed above, TCE has sometimes been reported to cause minimal/mild focal hepatocellular necrosis or other signs of hepatic injury, albeit of low frequency and mostly at doses  $\geq 1,000$  mg/kg-day ([Dees and Travis, 1993](#); [Elcombe et al., 1985](#)) or at exposures  $\geq 1,000$  ppm in air ([Ramdhan et al., 2010](#); [Ramdhan et al., 2008](#)) from 7 to 10 days of exposure. Data from available studies are supportive of a role for oxidative metabolism in TCE-induced cytotoxicity in the liver, though they are not informative as to the actual active moiety(ies). Buben and O'Flaherty ([1985](#)) noted a strong correlation (R-squared between glucose-6-phosphatase inhibition and total urinary oxidative metabolites). Ramdhan et al. ([2008](#)) conducted parallel experiments at TCE 1,000 and 2,000 ppm (8 hours/day, 7 days) in wild-type and CYP2E1-null mice, the latter of which did not exhibit hepatotoxicity (assessed by serum ALT, AST, and histopathology) and excreted twofold lower amounts of oxidative metabolites TCA and TCOH in urine as compared to wild-type mice. In addition, urinary TCA and TCOH excretion was correlated with serum ALT and AST measures, though the R-squared values (square of the reported correlation coefficients) were relatively low (0.54 and 0.67 for TCOH and TCA, respectively). Ramdhan et al. ([2010](#)) reported that TCA and TCOH were detected in the urine of wild type and PPAR $\alpha$ -null and humanized mice after TCE exposure with no significant differences between the 1,000 and 2,000 ppm TCE treatments. TCA concentrations were significantly lower and TCOH concentrations higher in exposed PPAR $\alpha$ -null mice relative to wild type mice. They stated that urinary TCA levels in wild type mice were incorrectly reported by Ramdhan et al. ([2008](#)) but have been corrected in this study. AST and ALT levels were significantly increased in all exposed mice relative to control 41–74 and 36–79% higher for AST and ALT, respectively). Mean levels within each treatment group were higher, though not statistically significantly different, with exposure to 2,000 versus 1,000 ppm TCE. Although increased, such increases were small. Necrosis scores were reported to be significantly higher in TCE-exposed mice relative to controls in all three genotype mice and to be significantly higher with 2,000 vs. 1,000 ppm TCE exposure in wild type mice and hPPAR $\alpha$  mice. Inflammation scores were reported to be significantly higher with exposed group than control with 2,000 ppm TCE exposure than controls for each genotype group with a difference between the 2,000 and

1,000 ppm exposure groups in wild type mice. However, necrosis and inflammation score means at the highest TCE exposure levels in any mouse strain were minimal (only occasional necrotic cells in any lobule) for necrosis and mild for inflammation (<2 foci/field).

With respect to CH (166 mg/kg-day) and DCA (~90 mg/kg-day), Daniel et al. (1992) reported that after drinking water treatment, hepatocellular necrosis and chronic active inflammation were reported to be mildly increased in both prevalence and severity in all treated groups after 104 weeks of exposure. The histological findings, from interim sacrifices (n = 5), were considered by the authors to be unremarkable and were not reported. TCA has not been reported to induce necrosis in the liver under the conditions tested. Relatively high doses of DCA ( $\geq 1$  g/L in drinking water) appear to result in mild focal necrosis with attendant reparative proliferation at lesion sites, but no such effects were reported at lower doses ( $\leq 0.5$  g/L in drinking water) more relevant for comparison with TCE (DeAngelo et al., 1999; Stauber et al., 1998; Sanchez and Bull, 1990). Enlarged nuclei and changes consistent with increased ploidy, are further discussed below in the context of DNA synthesis.

#### **4.5.6.2.3. DNA synthesis and polyploidization**

The effects on DNA synthesis and polyploidization observed with TCE treatment have similarly been observed with TCA and DCA. With respect to CH, George et al. (2000) reported that CH exposure did not alter DNA synthesis in rats and mice at any of the time periods monitored (all well past 2 weeks), with the exception of 0.58 g/L CH at 26 weeks slightly increasing hepatocyte labeling (~two- to threefold of controls) in rats and mice but the percentage labeling still representing  $\leq 3\%$  of hepatocytes.

In terms of whole liver or hepatocyte label incorporation, the most comparable exposure duration between TCE, TCA, and DCA studies is the 10- and 14-day period. Several studies have reported that in this time period, peak label incorporation into individual hepatocytes and whole liver for TCA and DCA have already passed (Pereira, 1996; Carter et al., 1995; Styles et al., 1991; Sanchez and Bull, 1990). A direct time-course comparison is difficult, since data at earlier times for TCE are more limited.

There are conflicting reports of DNA synthesis induction in individual hepatocytes for up to 14 days of DCA or TCA exposure. In particular, Sanchez and Bull (1990) reported tritiated thymidine incorporation in individual hepatocytes up to 2 g/L exposure to DCA or TCA induced little increase in DNA synthesis except in instances and in close proximity to areas of proliferation/necrosis for DCA treatment after 14 days of exposure in male mice. The largest percentage of hepatocytes undergoing DNA synthesis for any treatment group was <1% of hepatocytes. However, they reported treatment- and exposure duration-changes in hepatic DNA incorporation of tritiated thymidine for DCA and TCA. For TCA treatment, the largest increases over control levels for hepatic DNA incorporation (at the highest dose) was a threefold increase after 5 days of treatment and a twofold increase over controls after 14 days of treatment. For

DCA whole-liver tritiated thymidine incorporation was only slightly elevated at necrogenic concentrations and decreased at the 0.3 g/L non-necrogenic level after 14 days of treatment. In contrast to Sanchez and Bull (1990), Stauber and Bull (1997) reported increased tritiated thymidine incorporation for individual hepatocytes after 14 days of treatment with 2 g/L DCA or TCA in male mice. They used a more extended period of tritiated thymidine exposure of 3–5 days and so these results represent aggregate DNA synthesis occurring over a more extended period of time. A “1-day labeling index” was reported as <1% for the highest level of increased incorporation. However, after 14 days, the labeling index was reported to be increased by ~3.5-fold for TCA and ~5.5-fold for DCA over control values. After 28 days, the labeling index was reported to be decreased ~2.3-fold by DCA and increased ~2.5-fold after treatment with TCA. Pereira (1996) reported that for female B6C3F<sub>1</sub> mice, 5-day incorporation of BrdU, as a measure of DNA synthesis, was increased at 0.86 and 2.58 g/L DCA treatment for 5 days (~twofold at the highest dose) but that by Day 12 and 33 levels had fallen to those of controls. For TCA exposures, 0.33, 1.10, and 3.27 g/L TCA all gave a similar ~threefold increase in BrdU incorporation by 5 days, but that by 12 and 33 days were not changed from controls. Nonetheless, what is consistent is that these data report that, similar to TCE-exposed mice at 10 days of exposure, cells undergoing DNA synthesis in DCA- or TCA-exposed mice for up to 14 days of exposure to be confined to a very small population of cells in the liver. Thus, these data are consistent with hypertrophy being primarily responsible for liver weight gains as opposed to increases in cell number in mice.

Interestingly, a lack of correlation between whole liver label incorporation and that in individual hepatocytes has been reported by several studies of DCA (Carter et al., 1995; Sanchez and Bull, 1990). For example, Carter et al. (1995) reported no increase in labeling of hepatocytes in comparison to controls for any DCA treatment group from 5 to 30 days of DCA exposure. Rather than increase hepatocyte labeling, DCA induced no change from Days 5 through 15 but significantly decreased levels between days 20 and 30 for 0.5 g/L that were similar to those observed for the 5 g/L exposures. However, for whole-liver DNA tritiated thymidine incorporation, Carter et al. (1995) reported 0.5g/L DCA treatments to show trends of initial inhibition of DNA tritiated thymidine incorporation followed by enhancement of labeling that was not statistically significant from 5 to 30 days of exposure. Examination of individual hepatocytes does not include the contribution of nonparenchymal cell DNA synthesis that would be detected in whole-liver DNA. As noted above, proliferation of the nonparenchymal cell compartment of the liver has been noted in several studies of TCE in rodents, and thus, this is one possible reason for the reported discrepancy.

Another possible reason for this inconsistency with DCA treatment is polyploidization, as was suggested above for TCE. Although this was not examined for DCA or TCA exposure by Sanchez and Bull (1990), Carter et al. (1995) reported that hepatocytes from both 0.5 and 5 g/L DCA treatment groups had enlarged, presumably polyploidy nuclei, with some hepatocyte nuclei

labeled in the mid-zonal area. There were statistically significant changes in cellularity, nuclear size, and multinucleated cells during 30 days of exposure to DCA. The percentage of mononucleated cells hepatocytes was reported to be similar between control and DCA treatment groups at 5- and 10-day exposure. However, at 15 days and beyond, DCA treatments were reported to induce increases in mononucleated hepatocytes, with later time periods also showing DCA-induced increases in nuclear area, consistent with increased polyploidization without mitosis. The consistent reporting of an increasing number of mononucleated cells between 15 and 30 days could be associated with clearance of mature hepatocytes as suggested by the report of DCA-induced loss of cell nuclei. The reported decrease in the numbers of binucleate cells in favor of mononucleate cells is not typical of any stage of normal liver growth ([Brodsky and Uryvaeva, 1977](#)). The pattern of consistent increase in percentage liver/body weight induced by 0.5 g/L DCA treatment from days 5 through 30 was not consistent with the increased numbers of mononucleate cells and increase nuclear area reported from day 20 onward. Specifically, the large differences in liver weight induction between the 0.5 g/L and 5 g/L treatment groups at all times studied also did not correlate with changes in nuclear size and percentage of mononucleate cells. Thus, increased liver weight was not a function of cellular proliferation, but probably included both aspects of hypertrophy associated with polyploidization and increased glycogen deposition (see below) induced by DCA. Carter et al. ([1995](#)) suggested that although there is evidence of DCA-induced cytotoxicity (e.g., loss of cell membranes and apparent apoptosis), the 0.5 g/L exposure concentration has been shown to increase hepatocellular lesions after 100 weeks of treatment without concurrent peroxisome proliferation or cytotoxicity ([DeAngelo et al., 1999](#)).

In sum, the observation of TCE treatment-related changes in DNA content, label incorporation, and mitotic figures are generally consistent with patterns observed for both TCA and DCA. In all cases, hepatocellular proliferation is confined to a very small fraction of hepatocytes, and hepatomegaly observed with all three treatments probably largely reflects cytomegaly rather than cell proliferation. Moreover, label incorporation likely largely reflects polyploidization rather than hepatocellular proliferation, with a possible contribution from nonparenchymal cell proliferation. As with TCE, histological changes in nuclear sizes and number also suggest a significant degree of treatment-related polyploidization, particularly for DCA.

#### **4.5.6.2.4. Apoptosis**

Both Elcombe et al. ([1985](#)) and Dees and Travis ([1993](#)) reported no changes in apoptosis other than increased apoptosis only at a treatment level of 1,000 mg/kg TCE. Dees and Travis ([1993](#)) reported that increased apoptoses from TCE exposure “did not appear to be in proportion to the applied TCE dose given to male or female mice.” Channel et al. ([1998](#)) reported that there was no significant difference in apoptosis between TCE treatment and control groups with data

not shown. However, the extent of apoptosis in any of the treatment groups, or which groups and timepoints were studied for this effect cannot be determined. While these data are quite limited, it is notable that peroxisome proliferators have been suggested inhibit, rather than increase, apoptosis as part of their carcinogenic mode of action ([Klaunig et al., 2003](#)).

However, for TCE metabolites, DCA has been most studied, though it is clear that age and species affect background rates of apoptosis. Snyder et al. ([1995](#)), in their study of DCA, reported that control mice were reported to exhibit apoptotic frequencies ranging from ~0.04 to 0.085% and that over the 30-day period of their study, the frequency rate of apoptosis declined; it was suggested that this pattern is consistent with reports of the livers of young animals undergoing rapid changes in cell death and proliferation. They reported the rat liver to have a greater than estimated frequency of spontaneous apoptosis (~0.1%) and therefore, greater than that of the mouse. Carter et al. ([1995](#)) reported that after 25 days of 0.5 g/L DCA treatment, apoptotic bodies were reported as well as fewer nuclei in the pericentral zone and larger nuclei in central and midzonal areas. This would indicate an increase in the apoptosis associated with potential increases in polyploidization and cell maturation. However, Snyder et al. ([1995](#)) reported that mice treated with 0.5 g/L DCA over a 30-day period had a similar trend as control mice of decreasing apoptosis with age. The percentage of apoptotic hepatocytes decreased in DCA-treated mice at the earliest time point studied and remained statistically significantly decreased from controls from 5 to 30 days of exposure. Although the rate of apoptosis was very low in controls, treatment with 0.5 g/L DCA reduced it further (~30–40% reduction) during the 30-day study period. The results of this study not only provide a baseline of apoptosis in the mouse liver, which is very low, but also show the importance of taking into account the effects of age on such determinations. The significance of the DCA-induced reduction in apoptosis reported in this study, from a level that is already inherently low in the mouse, for the mode of action for induction of DCA-induced liver cancer is difficult to discern.

#### **4.5.6.2.5. Glycogen accumulation**

As discussed in Sections E.3.2 and E.3.4.2.1, glycogen accumulation has been described to be present in foci in both humans and animals as a result from exposure to a wide variety of carcinogenic agents and predisposing conditions in animals and humans. The data from Elcombe et al. ([1985](#)) included reports of TCE-induced pericentral hypertrophy and eosinophilia for both rats and mice but with “fewer animals affected at lower doses.” In terms of glycogen deposition, Elcombe report “somewhat” less glycogen pericentrally in the livers of rats treated with TCE at 1,500 mg/kg than controls with less marked changes at lower doses restricted to fewer animals. They do not comment on changes in glycogen in mice. Dees and Travis ([1993](#)) reported TCE-induced changes to “include an increase in eosinophilic cytoplasmic staining of hepatocytes located near central veins, accompanied by loss of cytoplasmic vacuolization.” Since glycogen is removed using conventional tissue processing and staining techniques, an

increase in glycogen deposition would be expected to increase vacuolization and thus, the report from Dees and Travis ([1993](#)) is consistent with less, not more, glycogen deposition. Neither study produced a quantitative analysis of glycogen deposition changes from TCE exposure. Although not explicitly discussing liver glycogen content or examining it quantitatively in mice, these studies suggest that TCE-induced liver weight increases did not appear to be due to glycogen deposition after 10 days of exposure and any decreases in glycogen were not necessarily correlated with the magnitude of liver weight gain either.

For TCE and TCA 500 mg/kg treatments in mice for 10 days, changes in glycogen were not reported in the general descriptions of histopathological changes ([Dees and Travis, 1993](#); [Styles et al., 1991](#); [Elcombe et al., 1985](#)) or were specifically described by the authors as being similar to controls ([Nelson et al., 1989](#)). However, for DCA, glycogen deposition was specifically noted to be increased with treatment, although no quantitative analyses was presented that could give information as to the nature of the dose-response ([Nelson et al., 1989](#)).

In regard to cell size, although increased glycogen deposition with DCA exposure was noted by Sanchez and Bull ([1990](#)) to occur to a similar extent in B6C3F<sub>1</sub> and Swiss Webster male mice despite differences in DCA-induced liver weight gain. Lack of quantitative analyses of that accumulation in this study precludes comparison with DCA-induced liver weight gain. Carter et al. ([1995](#)) reported that in control mice, there was a large variation in apparent glycogen content, but did not perform a quantitative analysis of glycogen deposition. The variability of this parameter in untreated animals and the extraction of glycogen during normal tissue processing for light microscopy make quantitative analyses for dose-response difficult unless specific methodologies are employed to quantitatively assess liver glycogen levels as was done by Kato-Weinstein et al. ([2001](#)) and Pereira et al. ([2004a](#)).

Bull et al. ([1990](#)) reported that glycogen deposition was uniformly increased from 2 g/L DCA exposure with photographs of TCA exposure showing slightly less glycogen staining than controls. However, the abstract and statements in the paper suggest that there was increased PAS-positive material from TCA treatment that has caused confusion in the literature in this regard. Kato-Weinstein et al. ([2001](#)) reported that in male B6C3F<sub>1</sub> mice exposed to DCA and TCA, the DCA treatment increased glycogen and TCA decreased glycogen content of the liver by using both chemical measurement of glycogen in liver homogenates and by using ethanol-fixed sections stained with PAS, a procedure designed to minimize glycogen loss.

Kato-Weinstein et al. ([2001](#)) reported that glycogen-rich and -poor cells were scattered without zonal distribution in male B6C3F<sub>1</sub> mice exposed to 2 g/L DCA for 8 weeks. For TCA treatments, they reported centrilobular decreases in glycogen and ~25% decreases in whole liver by 3 g/L TCA. Kato-Weinstein et al. ([2001](#)) reported whole-liver glycogen to be increased ~1.50-fold of control (90 vs. 60 mg glycogen/g liver) by 2 g/L DCA after 8 weeks of exposure to male B6C3F<sub>1</sub> mice, with a maximal level of glycogen accumulation occurring after 4 weeks of DCA exposure. Pereira et al. ([2004a](#)) reported that after 8 weeks of exposure to 3.2 g/L DCA,

liver glycogen content was 2.2-fold of control levels (155.7 vs. 52.4 mg glycogen/g liver) in female B6C3F<sub>1</sub> mice. Thus, the baseline level of glycogen content reported by (~60 mg/g) and the increase in glycogen after DCA exposure was consistent between Kato-Weinstein et al. (2001) and Pereira et al. (2004a). However, the increase in liver weight reported by Kato-Weinstein et al. (2001) of 1.60-fold of control percentage liver/body weight cannot be accounted for by the 1.50-fold of control glycogen content. Glycogen content only accounts for 5% of liver mass so that 50% increase in glycogen cannot account for the 60% increase liver mass induced by 2 g/L DCA exposure for 8 weeks reported by Kato-Weinstein (2001). Thus, DCA-induced increases in liver weight are occurring from other processes as well. Carter et al. (2003) and DeAngelo et al. (1999) reported increased glycogen after DCA treatment at much lower doses after longer periods of exposure (100 weeks). Carter reported increased glycogen at 0.5 g/L DCA and DeAngelo et al. (1999) at 0.03 g/L DCA in mice. However, there is no quantitation of that increase.

#### **4.5.6.2.6. Peroxisome proliferation and related effects**

TCA and DCA have both been reported to induce peroxisome proliferation or increases in related enzyme markers in rodent hepatocytes (Parrish et al., 1996; Mather et al., 1990; DeAngelo et al., 1989, 1997). Between TCA and DCA, both induce peroxisome proliferation in various strains of mice, but it clear that TCA and DCA are weak PPAR $\alpha$  agonists and that DCA is weaker than TCA in this regard (Nelson et al., 1989) using a similar paradigm.

George et al. (2000) reported that CH exposure did not hepatic PCO activity in rats and mice at any of the time periods monitored. It is notable that the only time at which DNA synthesis index was (slightly) increased, at 26 weeks, there remained a lack of induction of PCO. A number of measures that may be related to peroxisome proliferation were investigated in Leakey et al. (2003b). Of the enzymes associated with PPAR $\alpha$  agonism (total CYP, CYP2B isoform, CYP4A, or lauric acid  $\beta$ -hydroxylase activity), only CYP4A and lauric acid  $\beta$ -hydroxylase activity were significantly increased at 15 months of exposure in the dietary-restricted group administered the highest dose (100 mg/kg CH) with no other groups showing a statistically significant increased response (n = 12/group). There is an issue of interpretation of peroxisomal enzyme activities and other enzymes associated with PPAR $\alpha$  receptor activation to be a relevant event in liver cancer induction at a time period in which tumors or foci are already present. Although not statistically significant, the 100 mg/kg CH exposure group of ad-libitum-fed mice also had an increase in CH-induced increases of CYP4A and lauric acid  $\beta$ -hydroxylase activity. Seng et al. (2003) described CH toxicokinetics and peroxisome proliferation-associated enzymes in mice at doses up to 1,000 mg/kg-day for 2 weeks with dietary control or caloric restriction. Lauric acid  $\beta$ -hydroxylase and PCO activities were reported to be induced only at doses >100 mg/kg in all groups, with dietary-restricted mice showing the greatest induction.

Differences in serum levels of TCA, the major metabolite remaining 24 hours after dosing, were reported not to correlate with hepatic lauric acid  $\beta$ -hydroxylase activities across groups.

Direct quantitative inferences regarding the magnitude of response in these studies in comparison to TCE, however, are limited by possible variability and confounding. In particular, many studies used cyanide-insensitive PCO as a surrogate for peroxisome proliferation, but the utility of this marker may be limited for a number of reasons. First, several studies have shown that this activity is not well correlated with the volume or number of peroxisomes that are increased as a result of exposure to TCE or its metabolites ([Nakajima et al., 2000](#); [Nelson et al., 1989](#); [Elcombe et al., 1985](#)). In addition, this activity appears to be highly variable both as a baseline measure and in response to chemical exposures. Laughter et al. ([2004](#)) presented data showing WY-14,643 induced increases in PCO activity that varied up to sixfold between different experiments in wild-type mice. They also showed that, in some instances, PCO activity in untreated PPAR $\alpha$ -null mice was up to sixfold greater than that in wild-type mice. Parrish et al. ([1996](#)) noted that control values between experiments varied as much as a factor of twofold for PCO activity and thus, their data were presented as percentage of concurrent controls. Furthermore, Melnick et al. ([1987](#)) reported that corn oil administration alone can elevate PCO (as well as catalase) activity, and corn oil has also been reported to potentiate the induction of PCO activity of TCA in male mice ([DeAngelo et al., 1989](#)). Thus, quantitative inferences regarding the magnitude of response in these studies are limited by a number of factors. For example, in the studies reported in DeAngelo et al. ([2008](#)), a small number of animals was studied for PCO activity at interim sacrifices (n = 5). PCO activity varied 2.7-fold as baseline controls. Although there was a 10-fold difference in TCA exposure concentration, the increases in PCO activity at 4 weeks were 1.3-, 2.4-, and 5.3-fold of control. More information on the relationship of PCO enzyme activity and its relationship to carcinogenicity is discussed in Section E.3.4 and below.

#### **4.5.6.2.7. Oxidative stress**

Very limited data are available as to oxidative stress and related markers induced by the oxidative metabolites of TCE. As discussed in Appendix E, there are limited data that do not indicate significant oxidative stress and associated DNA damage associated with acute and subacute TCE treatment. In regard to DCA and TCA, Larson and Bull ([1992b](#)) exposed male B6C3F<sub>1</sub> mice or F344 rats to single doses TCA or DCA in distilled water by gavage (n = 4). In the first experiment, TBARS was measured from liver homogenates and assumed to be malondialdehyde. The authors stated that a preliminary experiment had shown that maximal TBARS was increased 6 hours after a dose of DCA and 9 hours after a dose of TCA in mice and that by 24 hours, TBARS concentrations had declined to control values. Time-course information in rats was not presented. A dose of 100 mg/kg DCA (rats or mice) or TCA (mice) did not elevate TBARS concentrations over that of control liver with this concentration of TCA

not examined in rats. For TCA, there was a slight dose-related increase in TBARS over control values starting at 300 mg/kg in mice with the increase in TBARS increasing at a rate that was lower than the magnitude of increase in dose. Of note, is the report that the induction of TBARS in mice is transient and subsided within 24 hours of a single dose of DCA or TCA, that the response in mice appeared to be slightly greater with DCA than TCA at similar doses, and that for DCA, there was similar TBARS induction between rats and mice at similar dose levels.

Austin et al. (1996) is a follow-up publication of the preliminary experiment cited in Larson and Bull (1992b). Male B6C3F<sub>1</sub> mice were treated with single doses of DCA or TCA via gavage with liver examined for 8-OHdG. The authors stated that in order to conserve animals, controls were not employed at each time point. There was a statistically significant increase over controls in 8-OHdG for the 4- and 6-hour time points for DCA (~1.4- and 1.5-fold of control, respectively) but not at 8 hours in mice. For TCA, there was a statistically significant increase in 8-OHdG at 8 and 10 hours for TCA (~1.4- and 1.3-fold of control, respectively).

Consistent results as to low, transient increases in markers of “oxidative stress” were also reported by Parrish et al. (1996), who in addition to examining oxidative stress alone, attempted to examine its possible relationship to PCO and liver weight in male B6C3F<sub>1</sub> mice exposed to TCA or DCA for 3 or 10 weeks (n = 6). The dose-related increase in PCO activity at 21 days for TCA was not increased similarly for DCA. Only the 2.0 g/L dose of DCA was reported to induce a statistically significant increase at 21-days of exposure of PCO activity over control (~1.8-fold of control). After 71 days of treatment, TCA induced dose-related increases in PCO activities that were approximately twice the magnitude as that reported at 21 days. Treatments with DCA at the 0.1 and 0.5 g/L exposure levels produced statistically significant increases in PCO activity of ~1.5- and 2.5-fold of control, respectively. The administration of 1.25 g/L clofibric acid in drinking water, used as a positive control, gave ~six–sevenfold of control PCO activity at 21 and 71 days of exposure. Parrish et al. (1996) reported that laurate hydroxylase activity was elevated significantly only by TCA at 21 days and to approximately the same extent (~1.4–1.6-fold of control) at all doses tested and at 71 days, both the 0.5 and 2.0 g/L TCA exposures resulting in a statistically significant increase in laurate hydroxylase activity (i.e., 1.6- and 2.5-fold of control, respectively). No change was reported after DCA exposure. Laurate hydroxylase activity was within the control values, varying 1.7-fold between 21 and 71 days experiments. Levels of 8-OHdG in isolated liver nuclei were reported to not be altered from 0.1, 0.5, or 2.0 g/L TCA or DCA after 21 days of exposure and this negative result was reported to remain even when treatments were extended to 71 days of treatment. The authors noted that the level of 8-OHdG increased in control mice with age (i.e., ~twofold increase between 71- and 21-day-old control mice). Thus, the increases in PCO activity noted for DCA and TCA were not associated with 8-OHdG levels (which were unchanged) and also not with changes in laurate hydrolase activity observed after either DCA or TCA exposure. Of note, is that the authors report taking steps to minimize artifactual responses for their 8-OHdG

determinations. The authors concluded that their data suggest that peroxisome proliferative properties of TCA were not linked to oxidative stress or carcinogenic response.

#### **4.5.6.3. Comparisons of TCE-Induced Carcinogenic Responses with TCA, DCA, and CH Studies**

##### **4.5.6.3.1. Studies in rats**

As discussed above, data on TCE carcinogenicity in rats, while not reporting statistically significantly increased risks, are not entirely adequate due to low numbers of animals, increased systemic toxicity, and/or increased treatment-related or accidental mortality. Notably, several studies in rats noted a few very rare types of liver or biliary tumors (cystic cholangioma, cholangiocarcinoma, or angiosarcomas) in treated animals. For TCA, DCA, and CH, there are even fewer studies in rats, so there is a very limited ability to assess the consistency or lack thereof in rat carcinogenicity among these compounds.

For TCA, the only available study in rats ([DeAngelo et al., 1997](#)) has been frequently cited in the literature to indicate a lack of response in this species for TCA-induced liver tumors. However, this study does report an apparent dose-related increase in multiplicity of adenomas and an increase in carcinomas over control at the highest dose. The use by DeAngelo et al. ([1997](#)) of a relatively low number of animals per treatment group (n = 20–24) limits this study's ability to determine a statistically significant increase in tumor response. Its ability to determine an absence of treatment-related effects is similarly limited. In particular, a power calculation of the study shows that for most endpoints (incidence and multiplicity of all tumors at all exposure DCA concentrations), the Type II error, which should be >50%, was <8%. The only exception was for the incidence of adenomas and of adenomas and carcinomas for the 0.5 g/L treatment group (58%), at which, notably, there was a reported increase in reported adenomas or adenomas and carcinomas combined over control (15 vs. 4%). Therefore, the likelihood of a false null hypothesis was not negligible. Thus, while suggesting a lower response than for mice for liver tumor induction, this study is inconclusive for determining whether TCA induces a carcinogenic response in the liver of rats.

For DCA, there are two long-term studies in rats ([DeAngelo et al., 1996](#); [Richmond et al., 1995](#)) that appear to have reported the majority of their results from the same data set and that were consequently subject to similar design limitations and DCA-induced neurotoxicity in this species. DeAngelo et al. ([1996](#)) reported increased hepatocellular adenomas and carcinomas in male F344 rats exposed to DCA for 2 years. However, the data from exposure concentrations at the 5 g/L dose had to be discarded and the 2.5 g/L DCA dose had to be continuously lowered during the study due to neurotoxicity. There was a DCA-induced increase in adenomas and carcinomas combined reported for the 0.5 g/L DCA (24.1 vs. 4.4% adenomas and carcinomas combined in treated vs. controls) and an increase at a variable dose started at 2.5 g/L DCA and continuously lowered (28.6 vs. 3.0% adenomas and carcinomas combined in treated vs.

controls). Only combined incidences of adenomas and carcinomas for the 0.5 g/L DCA exposure group was reported to be statistically significant by the authors although the incidence of adenomas was 17.2 vs. 4% in treated vs. control rats. Hepatocellular tumor multiplicity was reported to be increased in the 0.5 g/L DCA group (0.31 adenomas and carcinomas/animal in treated vs. 0.04 in control rats) but was reported by the authors to not be statistically significant. At the starting dose of 2.5 g/L that was continuously lowered due to neurotoxicity, the increased multiplicity of HCCs was reported by the authors to be to be statistically significant (0.25 carcinomas/animals vs. 0.03 in control) as well as the multiplicity of combined adenomas and carcinomas (0.36 adenomas and carcinomas/animals vs. 0.03 in control rats). Issues that affect the ability to determine the nature of the dose-response for this study include: (1) the use of a small number of animals (n = 23, n = 21, and n = 23 at final sacrifice for the 2.0 g/L sodium chloride control, 0.05 g/L and 0.5 g/L treatment groups) that limit the power of the study to both determine statistically significant responses and to determine that there are not treatment-related effects (i.e., power); (2) apparent addition of animals for tumor analysis not present at final sacrifice (i.e., 0.05 and 0.5 g/L treatment groups); and (3) most of all, the lack of a consistent dose for the 2.5 g/L DCA exposed animals.

Similar issues are present for the study of Richmond et al. ([1995](#)), which was conducted by the same authors as DeAngelo et al. ([1996](#)) and appeared to be the same data set. There was a small difference in reports of the results between the two studies for the same data for the 0.5 g/L DCA group in which Richmond et al. ([1995](#)) reported a 21% incidence of adenomas and DeAngelo et al. ([1996](#)) reported a 17.2% incidence. The authors did not report any of the results of DCA-induced increases of adenomas and carcinomas to be statistically significant. The same issues discussed above for DeAngelo et al. ([1996](#)) apply to this study. Similar to the DeAngelo et al. ([1997](#)) study of TCA in rats, the use in these DCA studies ([DeAngelo et al., 1996](#); [Richmond et al., 1995](#)) of relatively small numbers of rats limits the detection of treatment-related effects and the ability to determine whether there were treatment-related effects (Type II error), especially at the low concentrations of DCA exposure.

For CH, George et al. ([2000](#)) exposed male F344/N rats to CH in drinking water for 2 years. Groups of animals were sacrificed at 13, 26, 52, and 78 weeks following the initiation of dosing, with terminal sacrifices at week 104. Only a few animals received a complete pathological examination. The number of animals surviving >78 weeks and the number examined for hepatocellular proliferative appeared to differ (42–44 animals examined, but 32–35 surviving until the end of the experiment). Only the lowest treatment group had increased liver tumors that were marginally significantly increased.

Leuschner and Beuscher ([1998](#)) examined the carcinogenic effects of CH in male and female Sprague-Dawley rats (69–79 g, 25–29 days old at initiation of the experiment) administered 0, 15, 45, and 135 mg/kg CH in unbuffered drinking water 7 days/week (n = 50/group) for 124 weeks in males and 128 weeks in females. Two control groups were

noted in the methods section without explanation as to why they were conducted as two groups. The authors reported no substance-related influence on organ weights and no macroscopic evidence of tumors or lesions in male or female rats treated with CH for 124 or 128 weeks. However, no data were presented on the incidence of tumors in either treatment or control groups. The authors did report a statistically significant increase in the incidence of hepatocellular hypertrophy in male rats at the 135 mg/kg dose (14/50 animals vs. 4/50 and 7/50 in Controls I and II). For female rats, the incidence of hepatocellular hypertrophy was reported to be 10/50 rats (Control I) and 16/50 (Control II) rats with 18/50, 13/50 and 12/50 female rats having hepatocellular hypertrophy after 15, 45, and 135 mg/kg CH, respectively. The lack of reporting in regard to final body weights, histology, and especially background and treatment group data for tumor incidences, limit the interpretation of this study. Whether this paradigm was sensitive for induction of liver cancer cannot be determined.

Therefore, given the limitations in the available studies, a comparison of rat liver carcinogenicity induced by TCE, TCA, DCA, and CH reveals no strong inconsistencies, but nor does it provide much insight into the relative importance of different TCE metabolites in liver tumor induction.

#### **4.5.6.3.2. Studies in mice**

Similar to TCE, the bioassay data in mice for DCA, TCA, and CH are much more extensive and have shown that all three compounds induce liver tumors in mice. Several 2-year bioassays have been reported for CH ([Leakey et al., 2003b](#); [George et al., 2000](#); [Daniel et al., 1992](#)). For many of the DCA and TCA studies, the focus was not carcinogenic dose-response, but rather investigation of the nature of the tumors and potential modes of action in relation to TCE. As a result, studies often employed relatively high concentrations of DCA or TCA and/or were conducted for  $\leq 1$  year. As shown previously in Section 4.5.6.2.1, the dose-response curves for increased liver weight for TCE administration in male mice are more similar to those for DCA administration and TCE oxidative metabolism than for direct TCA administration (inadequate data were available for CH). An analogous comparison for DCA-, TCA-, and CH-induced tumors would be informative, ideally using data from 2-year studies.

##### **4.5.6.3.2.1. TCE carcinogenicity dose-response data**

Unfortunately, the database for TCE, while consistently showing an induction of liver tumors in mice, is very limited for making inferences regarding the shape of the dose-response curve. For many of these experiments, only liver tumor incidence, not multiplicity, was provided. NTP ([1990](#)), Bull et al. ([2002](#)), and Anna et al. ([1994](#)) conducted gavage experiments in which they only tested one dose of  $\sim 1,000$  mg/kg-day TCE. NCI ([1976](#)) tested two doses that were adjusted during exposure to an average of 1,169 and 2,339 mg/kg-day in male mice with only twofold dose spacing in only two doses tested. Maltoni et al. ([1988](#); [1986](#)) conducted

inhalation experiments in two sets of B6C3F<sub>1</sub> mice and one set of Swiss mice at three exposure concentrations that were threefold apart in magnitude between the low and mid-dose and twofold apart in magnitude between the mid- and high dose. However, for one experiment in male B6C3F<sub>1</sub> mice (BT306), the mice fought and suffered premature mortality and for two the experiments in B6C3F<sub>1</sub> mice, although using the same strain, the mice were obtained from differing sources with very different background liver tumor levels. For the Maltoni et al. (1988; 1986) study, a general descriptor of “hepatoma” was used for liver neoplasia rather than describing hepatocellular adenomas and carcinomas so that comparison of that data with those from other experiments is difficult. More importantly, while the number of adenomas and carcinomas may be the same between treatments or durations of exposure, the number of adenomas may decrease as the number of carcinomas increase during the course of tumor progression. Such information is lost by using only a hepatoma descriptor.

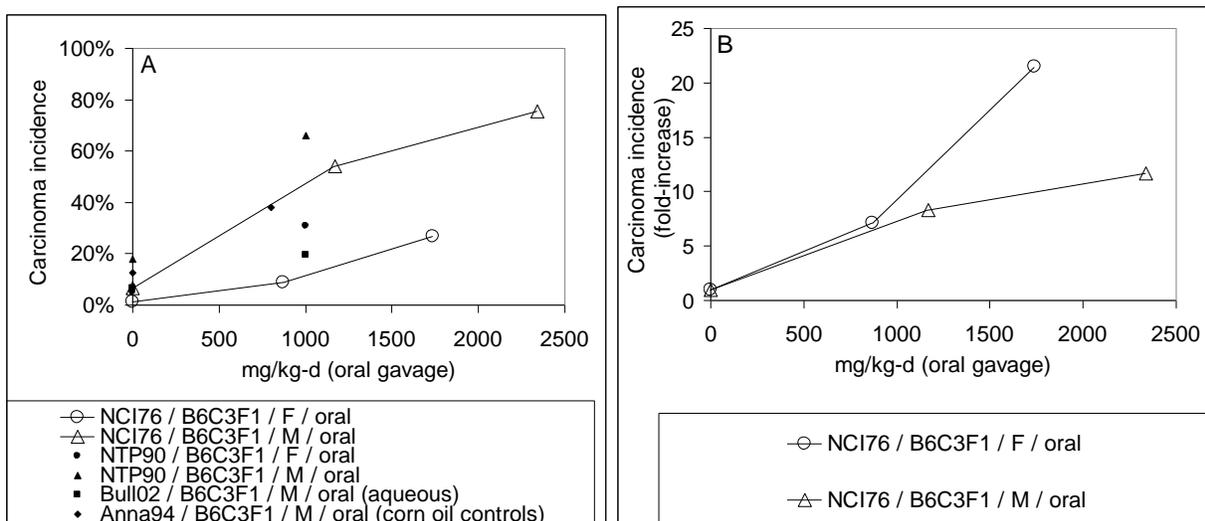
Given the limited database, it would be useful if different studies could be combined to yield a more comprehensive dose-response curve, as was done for liver weight, above. However, this is probably not appropriate for several reasons. First, only the NTP (1990) study was performed with dosing duration and time of sacrifice both being the “standard” 104 weeks. NCI (1976), Maltoni et al. (1988; 1986), Anna et al. (1994), and Bull et al. (2002) all had shorter dosing periods and either longer (Maltoni et al., 1988; Maltoni et al., 1986) or shorter (the other three studies) observation times. Therefore, because of potential dose-rate effects and differences in the degree of expression of TCE-induced tumors, it is difficult to even come up with a comparable administered dose-metric across studies. Moreover, the background tumor incidences are substantially different across experiments, even controlling for mouse strain and sex. For example, across gavage studies in male B6C3F<sub>1</sub> mice, the incidence of HCCs ranged from 1.2 to 16.7% (Anna et al., 1994; NTP, 1990; NCI, 1976) and the incidence of adenomas ranged from 1.2 to 14.6% (Anna et al., 1994; NTP, 1990) in control B6C3F<sub>1</sub> mice. After ~1,000 mg/kg-day TCE treatment, the incidence of carcinomas ranged from 19.4 to 62% (Bull et al., 2002; Anna et al., 1994; NTP, 1990; NCI, 1976), with three of the studies (Anna et al., 1994; NTP, 1990; NCI, 1976) reporting a range of incidences between 42.8 and 62.0%. The incidence of adenomas ranged from 28 to 66.7% (Bull et al., 2002; Anna et al., 1994; NTP, 1990). In the Maltoni et al. (1988; 1986) inhalation study as well, male B6C3F<sub>1</sub> mice from two different sources had very different control incidences of hepatomas (~2 vs. ~20%).

Therefore, only data from the same experiment in which more than a single exposed dose group was used provide reliable data on the dose-response relationship for TCE hepatocarcinogenicity, and incidences from these experiments are shown in Figures 4-10 and 4-11. Except for one of the two Maltoni et al. (1988; 1986) inhalation experiments in male B6C3F<sub>1</sub> mice, all of these data sets show relatively proportional increases with dose, albeit with somewhat different slopes as may be expected across strains and sexes. Direct comparison is difficult, since the “hepatomas” reported by Maltoni et al. (1988; 1986) are much more

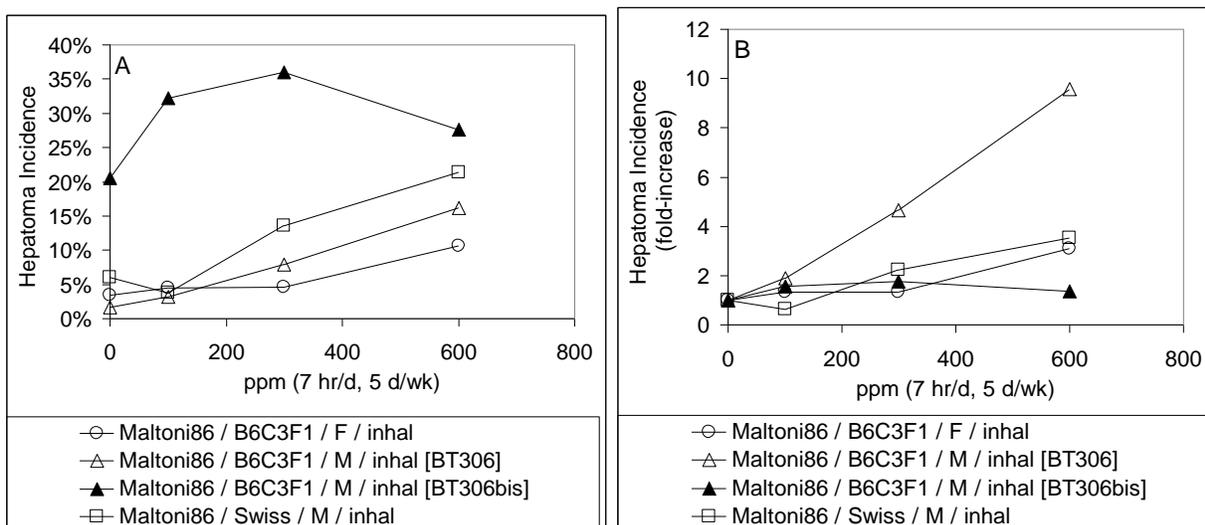
heterogeneous, including neoplastic nodules, adenomas, and carcinomas, than the carcinomas reported by NCI (1976). Nonetheless, although the data limitations preclude a conclusive statement, these data are generally consistent with the linear relationship observed with TCE-induced liver weight changes.

#### 4.5.6.3.2.2. DCA carcinogenicity dose-response data

Pereira (1996) reported that for 82-week exposures to DCA in female B6C3F<sub>1</sub> mice, DCA exposure concentrations of 0, 2, 6.67, and 20 mmol/L (0, 0.26, 0.86, and 2.6 g/L) led to close, proportionally increasing adenoma prevalences of 2.2, 6, 25, and 84.2%, though adenoma multiplicity increased more than linearly between the highest two doses. Unfortunately, too few carcinomas were observed at these doses and duration to meaningfully inform the shape of the dose-response relationship. More useful is DeAngelo et al. (1999), which reported on a study of DCA hepatocarcinogenicity in male B6C3F<sub>1</sub> mice over a lifetime exposure. DeAngelo et al. (1999) used 0.05, 0.5, 1.0, 2.0, and 3.5 g/L exposure concentrations of DCA in their 100-week drinking water study. The number of animals at final sacrifice was generally low in the DCA treatment groups and variable. The multiplicity or number of HCCs/animals was significantly increased over controls in a dose-related manner at all DCA treatments including 0.05 g/L DCA, and a no-observed-effect level (NOEL) was not identified. Between the 0.5 and 3.5 g/L exposure concentrations of DCA, the magnitude of increase in multiplicity was similar to the increases in magnitude in dose. The incidence of HCCs was increased at all doses as well, but was not statistically significant at 0.05 g/L. However, given that the number of mice examined for this response (n = 33), the power of the experiment at this dose was only 16.9% to be able to determine that there was not a treatment-related effect. Indeed, Figure 4-12 replots the data from DeAngelo et al. (1999) with an abscissa drawn to scale (unlike the figure in the original paper, which was not to scale), suggests even a slightly greater-than-linear effect at the lowest dose (0.05 g/L, or 8 mg/kg-day) as compared to the next lowest dose (0.5 g/L, or 84 mg/kg-day), though of course the power of such a determination is limited. The authors did not report the incidence or multiplicity of adenomas for the 0.05 g/L exposure group in the study or the incidence or multiplicity of adenomas and carcinomas in combination. For the animals surviving from 79 to 100 weeks of exposure, the incidence and multiplicity of adenomas peaked at 1 g/L, while HCCs continued to increase at the higher doses. This would be expected where some portion of the adenomas would either regress or progress to carcinomas at the higher doses.

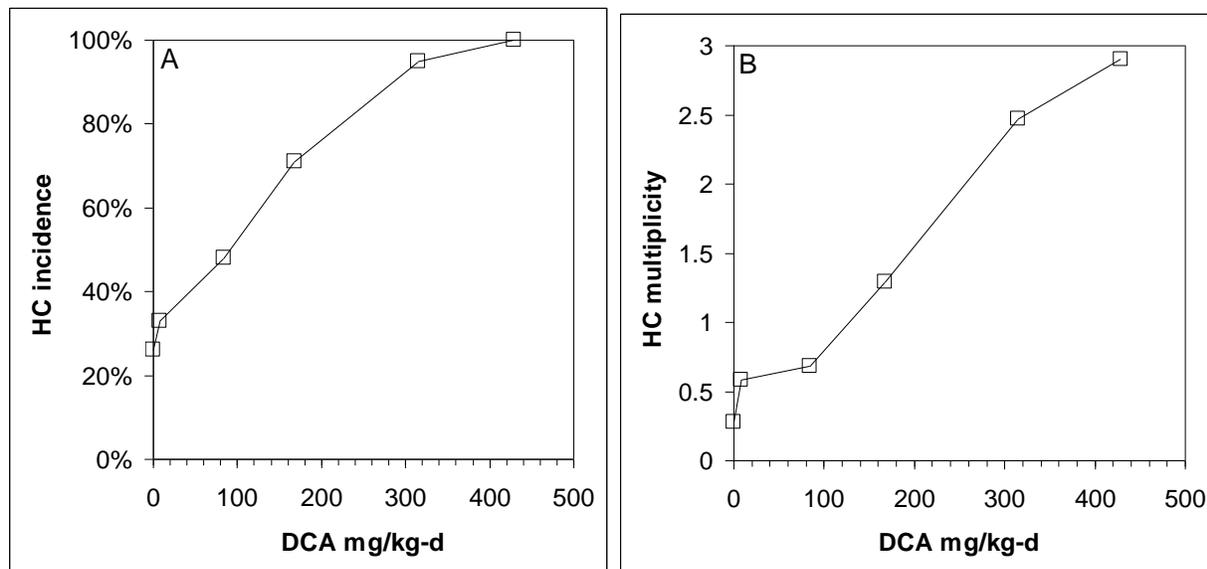


**Figure 4-10. Dose-response relationship, expressed as (A) percentage incidence and (B) fold-increase over controls, for TCE hepatocarcinogenicity in NCI (1976).** For comparison, incidences of carcinomas for NTP (1990), Anna et al. (1994), and Bull et al. (2002) are included, but without connecting lines since they are not appropriate for assessing the shape of the dose-response relationship.



Note that the BT306 experiment reported excessive mortality due to fighting, and so the paradigm was repeated in experiment BT306bis using mice from a different source.

**Figure 4-11. Dose-response relationship, expressed as (A) incidence and (B) fold-increase over controls, for TCE hepatocarcinogenicity in Maltoni et al. (1988; 1986).**



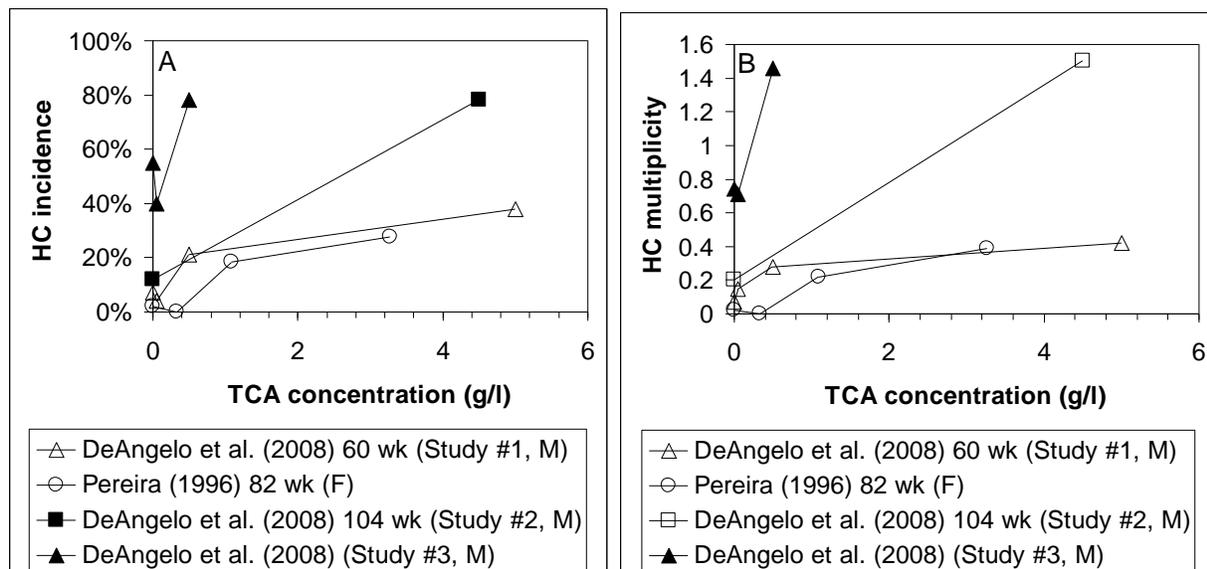
Drinking water concentrations were 0, 0.05, 0.5, 1, 2, and 3.5 g/L, from which daily average doses were calculated using observed water consumption in the study.

**Figure 4-12. Dose-response data for HCCs (A) incidence and (B) multiplicity, induced by DCA from DeAngelo et al. (1999).**

Associations of DCA carcinogenicity with various noncancer, possibly precursor, effects was also investigated. Importantly, the doses that induced tumors in DeAngelo et al. (1999) were reported to not induce widespread cytotoxicity. An attempt was also made to relate differing exposure levels to subchronic changes and peroxisomal enzyme induction. Interestingly, DeAngelo et al. (1999) reported that peroxisome proliferation was significantly increased at 3.5 g/L DCA only at 26 weeks, not correlated with tumor response, and not increased at either 0.05 or 0.5 g/L treatments. The authors concluded that DCA-induced carcinogenesis was not dependent on peroxisome proliferation or chemically sustained proliferation, as measured by DNA synthesis. Slight hepatomegaly was present by 26 weeks in the 0.5 g/L group and decreased with time. By contrast, increases in both percentage liver/body weight and the multiplicity of HCCs increased proportionally with DCA exposure concentration after 79–100 weeks of exposure. DeAngelo et al. (1999) presented a figure comparing the number of HCCs/animal at 100 weeks compared with the percentage liver/body weight at 26 weeks that showed a linear correlation ( $r^2 = 0.9977$ ), while peroxisome proliferation and DNA synthesis did not correlate with tumor induction profiles. The proportional increase in liver weight with DCA exposure was also reported for shorter durations of exposure as noted previously. Therefore, for DCA, both tumor incidence and liver weight appear to increase proportionally with dose.

#### 4.5.6.3.2.3. TCA carcinogenicity dose-response data

Pereira (1996) reported that for 82-week exposures to TCA in female B6C3F<sub>1</sub> mice, TCA exposure concentrations of 0, 2, 6.67, and 20 mmol/L (0, 0.33, 1.1, and 3.3 g/L) led to increasing incidences and multiplicity of adenomas and of carcinomas (see Figure 4-13). DeAngelo et al. (2008) reported the results of three experiments exposing male B6C3F<sub>1</sub> mice to neutralized TCA in drinking water (incidences also in Figure 4-13). Rather than using five exposure levels that were generally twofold apart, as was done in DeAngelo et al. (1999) for DCA, DeAngelo et al. (2008) studied only three doses of TCA that were an order of magnitude apart, which limits the elucidation of the shape of the dose-response curve. In addition, for the 104-week data, DeAngelo et al. (2008) contained two studies, each conducted in a separate laboratories—the two lower doses were studied in one study and the highest dose in another. The first 104-week study was conducted using 2 g/L sodium chloride, or 0.05, 0.5, or 5 g/L TCA in drinking water for 60 weeks (Study #1) while the other two were conducted for a period of 104 weeks (Study #2 with 2.5 g/L neutralized acetic acid or 4.5 g/L TCA exposure groups and Study #3 with deionized water, 0.05 g/L TCA and 0.5 g/L TCA exposure groups). In addition, a relatively small number of animals were used for the determination of a tumor response (n ~ 30 at final necropsy).



Combined HCA + HCC were not reported in Pereira (1996).

Sources: (DeAngelo et al., 2008; Pereira, 1996).

**Figure 4-13. Reported incidences of HCCs and hepatocellular adenomas plus carcinomas (HCA + HCC) in various studies in B6C3F<sub>1</sub> mice.**

In Study #1, the incidence data for adenomas observed at 60 weeks at 0.05, 0.5, and 5.0 g/L TCA were 2.1-, 3.0- and 5.4-fold of control values, with similar fold increases in multiplicity. As shown by Pereira (1996), 60 weeks does not allow for full tumor expression, so whether the dose-response relationship is the same at 104 weeks is not certain. For instance, Pereira (1996) examined the tumor induction in female B6C3F<sub>1</sub> mice and demonstrated that foci, adenoma, and carcinoma development in mice are dependent on duration of exposure (period of observation in controls). In control female mice a 360- vs. 576-day observation period showed that at 360 days, no foci or carcinomas and only 2.5% of animals had adenomas, whereas by 576 days of observation, 11% had foci, 2% adenomas, and 2% had carcinomas. For DCA and TCA treatments, foci, adenomas, and carcinoma incidence and multiplicity did not reach full expression until 82 weeks at the three doses employed. Although the numbers of animals were relatively low and variable at the two highest doses (18–28 mice), there were 50–53 mice studied at the lowest dose level and 90 animals studied in the control group.

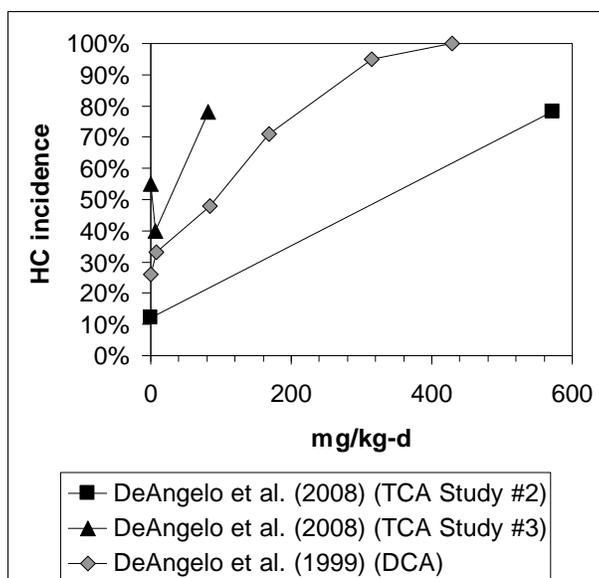
Therefore, the 104-week DeAngelo et al. (2008) data from Studies #2 and #3 would generally be preferred for elucidating the TCA dose-response relationship. However, Study #2 was only conducted at one dose, and although Study #3 used lower doses, it exhibited extraordinarily high control incidences of liver tumors. In particular, while the incidence of adenomas and carcinomas was 12% in Study #2, it was reported to be 64% in Study #3. The mice in Study #3 were of very large size (weighing ~50 g at 45 weeks) as compared to Study #1, Study #2, or most other bioassays in general, and the large background rate of tumors reported is consistent with the body-weight-dependence observed by Leakey et al. (2003a).

To put into context the 64% incidence data for carcinomas and adenomas reported in DeAngelo et al. (2008) for the control group of Study #3, other studies cited in this review for male B6C3F<sub>1</sub> mice show a much lower incidence in liver tumors with: (1) NCI (1976) reporting a colony control level of 6.5% for vehicle and 7.1% incidence of HCCs for untreated male B6C3F<sub>1</sub> mice (n = 70–77) at 78 weeks; (2) Herren-Freund et al. (1987) reporting a 9% incidence of adenomas in control male B6C3F<sub>1</sub> mice with a multiplicity of  $0.09 \pm 0.06$  and no carcinomas (n = 22) at 61 weeks; (3) NTP (1990) reporting an incidence of 14.6% adenomas and 16.6% carcinomas in male B6C3F<sub>1</sub> mice after 103 weeks (n = 48); and (4) Maltoni et al. (1988; 1986) reporting that B6C3F<sub>1</sub> male mice from the “NCI source” had a 1.1% incidence of “hepatoma” (carcinomas and adenomas) and those from “Charles River Co.” had a 18.9% incidence of “hepatoma” during the entire lifetime of the mice (n = 90 per group). The importance of examining an adequate number of control or treated animals before confidence can be placed in those results is illustrated by Anna et al. (1994) in which at 76 weeks, 3/10 control male B6C3F<sub>1</sub> mice that were untreated and 2/10 control animals given corn oil were reported to have adenomas, but from 76 to 134 weeks, 4/32 mice were reported to have adenomas (multiplicity of  $0.13 \pm 0.06$ ) and 4/32 mice were reported to have carcinomas (multiplicity of  $0.12 \pm 0.06$ ). Thus, the reported combined incidence of carcinomas and adenomas of 64% reported by

DeAngelo et al. (2008) for the control mice of Study #3 not only is inconsistent and much higher than those reported in Studies #1 and #2, but also is much higher than reported in a number of other studies of TCE.

Therefore, this large background rate and the increased mortality for these mice limit their use for determining the nature of the dose-response for TCA liver carcinogenicity. At the two lowest doses of 0.05 and 0.5 g/L TCA from Study #3, the differences in the incidences and multiplicities for all tumors were twofold at 104 weeks. However, there was no difference in any of the tumor results (i.e., adenoma, carcinoma, and combinations of adenoma and carcinoma incidence and multiplicity) between the 4.5 g/L dose group in Study #2 and the 0.5 g/L dose group in Study #3 at 104 weeks. By contrast, at 60 weeks of exposure, but within the same study (Study #1), there was a twofold increase in multiplicity for adenomas, and for adenomas and carcinomas combined between the 0.5 and 5.0 g/L TCA exposure groups. These results are consistent with the two highest exposure levels reaching a plateau of response after a long enough duration of exposure for full expression of the tumors (i.e., ~90% of animals having liver tumors at the 0.5 and 5 g/L exposures). However, whether such a plateau would have been observed in mice with a more “normal” body weight, and hence a lower background tumor burden, cannot be determined.

Because of the limitations of different studies, it is difficult to discern whether the liver tumor dose-response curves of TCA and DCA are different in a way analogous to that for liver weight (see Figure 4-14). Certainly, it is clear that at the same concentration in drinking water or estimated applied dose, DCA is more potent than TCA, as DCA induces nearly 100% incidence of carcinomas at a lower dose than TCA. Therefore, like with liver weight gains, DCA has a steeper dose-response function than TCA. However, the evidence for a “plateau” in tumor response at high doses with TCA, as was observed for liver weight, is equivocal, as it is confounded by the highly varying background tumor rates and the limitations of the available study paradigms.



Only carcinomas were reported in DeAngelo et al. (1999), so combined adenomas and carcinomas could not be compared.

Sources: (DeAngelo et al., 2008; DeAngelo et al., 1999).

**Figure 4-14. Reported incidence of HCCs induced by DCA and TCA in 104-week studies.**

DeAngelo et al. (2008) attempted to identify a NOEL for tumorigenicity using tumor multiplicity data and estimated TCA dose. However, it is not an appropriate descriptor for these data, especially given that “statistical significance” of the tumor response is the determinant used by the authors to support the conclusions regarding a dose in which there is no TCA-induced effect. Due to issues related to the appropriateness of use of the concurrent control in Study #3, only the 60-week experiment (i.e., Study #1) is useful for the determination of tumor dose-response. Not only is there not allowance for full expression of a tumor response at the 60-week time point, but a power calculation of the 60-week study shows that the Type II error, which should be >50% and thus, greater than the chances of “flipping a coin,” was 41 and 71% for incidence and 7 and 15% for multiplicity of adenomas for the 0.05 and 0.5 g/L TCA exposure groups. For the combination of adenomas and carcinomas, the power calculation was 8 and 92% for incidence and 6 and 56% for multiplicity at 0.05 and 0.5 g/L TCA exposure. Therefore, the designed experiment could accept a false null hypothesis, especially in terms of tumor multiplicity, at the lower exposure doses and erroneously conclude that there is no response due to TCA treatment.

In terms of correlations with other noncancer, possibly precursor effects, DeAngelo et al. (2008) also reported that PCO activity, which varied 2.7-fold as baseline controls, was 1.3-, 2.4-, and 5.3-fold of control for the 0.05, 0.5, and 5 g/L TCA exposure groups in Study #1 at 4 weeks

and adenomas incidence was 2.1-, 3.0-, and 5.4-fold of control and not similar at the lowest dose level at 60 weeks. However, it is not clear whether the similarity between PCO and carcinogenicity at 60 weeks would persist for tumor incidence at 104 weeks. DeAngelo et al. (2008) provided regression analyses to compare “percent of hepatocellular neoplasia,” as indicated by tumor multiplicity, with TCA dose, represented by estimations of the TCA dose in mg/kg-day, and with PCO activity for the 60- and 104-week data. Whether adenomas and carcinomas combined or individual tumor type were used in these analyses was not reported by the authors. However, it would be preferable to compare “precursor” levels of PCO at earlier time points, rather than at a time when there was already a significant tumor response. In addition, linear regression analyses of these data are difficult to interpret because of the wide dose spacing of these experiments. In such a situation, for a linear regression, control and 5 g/L exposure levels will basically determine the shape of the dose-response curve since the 0.05 and 0.5 g/L exposure levels are so close to the control (zero) value. Thus, dose-response appears to be linear between control and the 5.0 g/L value with the two lowest doses not affectively changing the slope of the line (i.e., “leveraging” the regression). Moreover, at the 5 g/L dose level, there is potential for effects due to palatability, as reported in one study in which drinking water consumption declined at this concentration (DeAngelo et al., 2008). Thus, the value of these analyses is limited by: (1) the use of data from Study #3 in a tumor prone mouse that is not comparable to those used in Studies #1 and #2; (2) the appropriateness of using PCO values from later time points and the variability in PCO control values; (3) the uncertainty of the effects of palatability on the 5 g/L TCA results, which were reported in one study to reduce drinking water consumption; and (4) the dose-spacing of the experiment.

#### **4.5.6.3.2.4. CH carcinogenic dose-response**

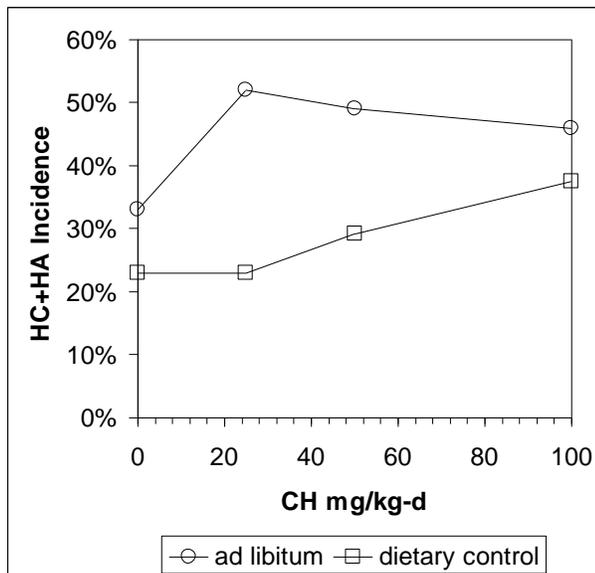
Although a much more limited database in rodents than for TCA or DCA, there is evidence that CH is also a rodent liver hepatocarcinogen [see also Section E.2.5 and Caldwell and Keshava (2006)].

Daniel et al. (1992) exposed adult male B6C3F<sub>1</sub> 28-day-old mice to 1 g/L CH in drinking water for 30 and 60 weeks (n = 5 for interim sacrifice) and for 104 weeks (n = 40). The concentration of CH was 1 g/L and estimated to provide a 166-mg/kg-day dose. It is not clear from the report what control group better matched the CH group, as the mean initial body weights of the groups as well as the number of animals varied considerably in each group (i.e., ~40% difference in mean body weights at the beginning of the study). Liver tumors were increased by CH treatment. The percentage incidence of liver carcinomas and adenomas in the surviving animals was 15% in control and 71% in CH-treated mice and the incidence of HCC was reported to be 46% in the CH-treated group. The number of tumors/animals was also significantly increased with CH treatment. However, because this was a single-dose study, a comparison with the dose-response relationship with TCE, TCA, or DCA is not feasible.

George et al. (2000) exposed male B6C3F<sub>1</sub> mice to CH in drinking water for 2 years. Groups of animals were sacrificed at 26, 52, and 78 weeks following the initiation of dosing, with terminal sacrifices at week 104. Only a few animals received a complete pathological examination. Preneoplastic foci and adenomas were increased in the livers of all CH treatment groups at 104 weeks. The percentage incidences of hepatocellular adenomas were reported to be 21.4, 43.5, 51.3, and 50% in control, 13.5, 65.0 and 146.6 mg/kg-day CH treatment groups, respectively. The percentage incidences of HCCs were reported to be 54.8, 54.3, 59.0 and 84.4% in these same groups. The resulting percentage incidence of hepatocellular adenomas and carcinomas were reported to be 64.3, 78.3, 79.5 and 90.6%. Of concern is the reporting of a 64% incidence of HCCs and adenomas in the control group of mice for this experiment, which is the same as that for another study published by this same laboratory (DeAngelo et al., 2008). DeAngelo et al. (2008) did not identify them as being contemporaneous studies or sharing controls, but a comparison of the control data published by DeAngelo et al. (2008) for TCA and that published by George et al. (2000) for the CH studies shows them to be the same data set. Therefore, as discussed above, this data set was derived from B6C3F<sub>1</sub> mice that were large (~50 g) and resultantly tumor prone, making determinations of the dose-response of CH from this experiment difficult. Therefore, for the purposes of comparison of dose-response relationships, this study has the same limitations as the DeAngelo et al. (2008) study, discussed above.

Leakey et al. (2003b) studied the effects of CH exposure (0, 25, 50, and 100 mg/kg-day, 5 days/week, 104–105 weeks via gavage) in male B6C3F<sub>1</sub> mice with dietary control used to manipulate body growth (n = 48 for 2-year study and n = 12 for the 15-month interim study). Dietary control was reported to decrease background liver tumor rates (decreased by 15–20%) and was reported to be associated with decreased variation in liver-to-body weight ratios, thereby potentially increasing assay sensitivity. In dietary-controlled groups and groups fed ad libitum, liver adenomas and carcinomas (combined) were reported to be increased with CH treatment. With dietary restriction, there was a more discernable CH tumor-response with overall tumor incidence reduced, and time-to-tumor increased by dietary control in comparison to ad-libitum-fed mice. Incidences of hepatocellular adenoma and carcinoma overall rates were reported to be 33, 52, 49, and 46% for control, 25, 50, and 100 mg/kg ad-libitum-fed mice, respectively. For dietary controlled mice the incidence rates were reported to be 22.9, 22.9, 29.2, and 37.5% for controls, 25, 50, and 100 mg/kg CH, respectively. Body weights were matched and carefully controlled in this study. These data are shown in Figure 4-15, relative to control incidences. It is evident from these data that dietary control significantly changes the apparent shape of the dose-response curve, presumably by reducing variability between animals. While the ad libitum dose groups had an apparent “saturation” of response, this was not evident with the dietary controlled group. Of note is that all of the other bioassays for TCE, TCA, DCA, and CH were in ad-libitum-fed mice. Therefore, it is difficult to compare the dose-response curves for CH-treated

mice on dietary restriction to those fed ad libitum. However, the rationale for dietary restriction in the B6C3F<sub>1</sub> mouse is to prevent the types of weight gain and corresponding high background tumor levels observed in DeAngelo et al. (2008) and George et al. (2000). As stated previously, most other studies of TCA, DCA, and TCE had background levels that, while varied, were lower than the ad-libitum-fed mice studied in Leakey et al. (2003b)



Source: Leakey et al. (2003b)

**Figure 4-15. Effects of dietary control on the dose-response curves for changes in liver tumor incidences induced by**

Of note is that incidences of adenomas and carcinomas combined do not show differences in tumor progression as carcinomas may increase and adenomas may regress. Liver weight increases at 15 months did not correlate with 2-year tumor incidences in the ad libitum group, but a consistent dose-response shape between these two measures is evident in the dietary controlled group. However, of note is the reporting of liver weight at 15 months is for a time period in which foci and liver tumors have been reported to have already occurred in other studies, so hepatomegaly in the absence of these changes is hard to detect.

In terms of other noncancer effects that may be associated with tumor induction, it is notable that while dietary restriction reduced the overall level of CH-mediated tumor induction, it led to greater CH-mediated induction of peroxisome proliferation-associated enzymes. Moreover, between control groups, dietary restricted mice appeared to have higher levels of lauric acid  $\omega$ -hydrolase activity than ad-libitum-fed mice. Seng et al. (2003) report that lauric acid  $\beta$ -hydroxylase and PCO were induced only at exposure levels >100 mg/kg CH, again with dietary-restricted groups showing the greatest induction. Such data argue against the role of peroxisome proliferation in CH liver tumor induction in mice.

Leakey et al. (2003b) gave no descriptions of liver pathology, other than incidence of mice with fatty liver changes. Hepatic malondialdehyde concentration in ad-libitum-fed and dietary-controlled mice did not change with CH exposure at 15 months, but the dietary-controlled groups were all approximately half that of the ad-libitum-fed mice. Thus, while overall increased tumors observed in the ad libitum diet correlated with increased malondialdehyde concentration, there was no association between CH dose and malondialdehyde induction for either diet.

Overall, from the CH studies in mice, there is an apparent increase in liver adenomas and carcinomas induced by CH treatment by either drinking water or gavage with all available studies performed in male B6C3F<sub>1</sub> mice. However, the background levels of hepatocellular adenomas and carcinomas in these mice in George et al. (2000) and body-weight data from this study are high, consistent with the association between large body weight and background tumor susceptibility shown with dietary control (Leakey et al., 2003b). With dietary control, Leakey et al. (2003b) report a dose-response relationship between exposure and tumor incidence that is proportional to dose.

#### **4.5.6.3.2.5. Degree of concordance among TCE, TCA, DCA, and CH dose-response relationships**

A quantitative comparison of the carcinogenicity dose-response relationships among TCE, TCA, DCA, and CH—analogue to the quantitative comparison between TCE and TCA hepatomegaly—was considered. This first step in such a comparison would be an examination of the dose-response data for TCE alone to see if they are consistent with a single dose-response relationship. As shown in Figures 4-10 and 4-11, there is substantial variability among the available liver tumor dose-response data that was not observed for hepatomegaly. The strain of mice used in the bioassays had a difference in not only TCE liver tumor response, but also background liver tumor incidence. Differences in exposure paradigms in the bioassays also leads to difference in tumor incidence and reporting. In addition, unlike the case with TCE hepatomegaly data in mice, the TCE dose-response data for liver tumors in mice exposed via inhalation and gavage are not consistent with a common dose-response curve even on an internal dose basis (e.g., Rhomberg, 2000) (Section 5.2). This heterogeneity is also evident for the TCA dose-response data, as shown in Figure 4-13, which may in part be due to the differences in study duration. Furthermore, among all of the available cancer bioassay data for TCE, TCA, DCA, and CH, the control incidences for background liver tumors vary from about 1% to over 50%, and difference of >50-fold that adds substantial uncertainty to any joint analysis. Therefore, differences within and across the databases of these compounds, such as the comparability of study durations, control tumor incidences, and carcinogenic potency, preclude either a quantitative analysis or a definitive conclusion. This question may be better addressed

experimentally where similar animals are exposed to different compounds in the same experimental setting.

#### **4.5.6.3.3. Inferences from liver tumor phenotype and genotype**

A number of studies have investigated tumor phenotypes, such as c-Jun staining, tincture, and dysplasia, or genotypes, such as H-ras mutations, to inform both the identification of the active agents of TCE liver tumor induction as well as what mode(s) of action may be involved.

##### **4.5.6.3.3.1. Tumor phenotype—staining and appearance**

The descriptions of tumors in mice reported by the NCI (1976), NTP (1990), and Maltoni et al. (1988; 1986) studies are also consistent with phenotypic heterogeneity as well as spontaneous tumor morphology (see Section E.3.4.1.5). As noted in Section E.3.1, HCCs observed in humans are also heterogeneous. For mice, Maltoni et al. (1986) described malignant tumors of hepatic cells to be of different subhistotypes, of various degrees of malignancy, and unique or multiple, and have different sizes (usually detected grossly at necropsy) from TCE exposure. In regard to phenotype, tumors were described as usual type observed in Swiss and B6C3F<sub>1</sub> mice, as well as in other mouse strains, either untreated or treated with hepatocarcinogens and to frequently have medullary (solid), trabecular, and pleomorphic (usually anaplastic) patterns. For the NCI (1976) study, the mouse liver tumors were described in detail and to be heterogeneous “as described in the literature” and similar in appearance to tumors generated by carbon tetrachloride. The description of liver tumors in this study and tendency to metastasize to the lung are similar to descriptions provided by Maltoni et al. (1986) for TCE-induced liver tumors in mice via inhalation exposure. The NTP (1990) study reported that TCE exposure is associated with increased incidence of HCC (tumors with markedly abnormal cytology and architecture) in male and female mice. Hepatocellular adenomas were described as circumscribed areas of distinctive hepatic parenchymal cells with a perimeter of normal-appearing parenchyma in which there were areas that appeared to be undergoing compression from expansion of the tumor. Mitotic figures were sparse or absent, but the tumors lacked typical lobular organization. HCCs were reported to have markedly abnormal cytology and architecture with abnormalities in cytology cited as including increased cell size, decreased cell size, cytoplasmic eosinophilia, cytoplasmic basophilia, cytoplasmic vacuolization, cytoplasmic hyaline bodies, and variations in nuclear appearance. Furthermore, in many instances, several or all of the abnormalities were reported to be present in different areas of the tumor and variations in architecture, with some of the HCCs having areas of trabecular organization. Mitosis was variable in amount and location. Therefore, the phenotype of tumors reported from TCE exposure was heterogeneous in appearance between and within tumors from all three of these studies.

Caldwell and Keshava (2006) reported that Bannasch (2001) and Bannasch et al. (2001) describe the early phenotypes of preneoplastic foci induced by many oncogenic agents (DNA-reactive chemicals, radiation, viruses, transgenic oncogenes and local hyperinsulinism) as insulinomimetic. These foci and tumors have been described by tincture (after hematoxylin and eosin staining of structural contents) as primarily eosinophilic (pink, reflecting eosin staining, e.g., staining of intracellular and extracellular protein), basophilic (blue, reflecting hematoxylin staining, e.g., staining of ribosomes and arginine rich basic nucleoprotein such as histones), and to be heterogeneous. Primary eosin staining is associated with a less malignant state of the hepatocyte with increased ribosomal content, decreased glycogen content, and increased basophilia of the cytoplasm by hematoxylin staining to be indicative of a more malignant state or tumor progression (Carter et al., 2003; Bannasch, 2001). Several studies do identify foci and tumors as primarily eosinophilic or basophilic, but do not give specific criteria for how a foci or tumor (which can be and usually is made up of a mixture of phenotypically heterogeneous cells) are assigned to be one category or another. Caldwell and Keshava (2006) noted that the tumors observed after TCE exposure are consistent with the description for the main tumor lines of development described by Bannasch et al. (2001). Thus, the response of liver to DCA (glycogenesis with emergence of glycogen poor tumors) is similar to the progression of preneoplastic foci to tumors induced from a variety of agents and conditions associated with increased cancer risk. Furthermore Caldwell and Keshava (2006) note that Bull et al. (2002) report expression of insulin receptor to be elevated in tumors of control mice or mice treated with TCE, TCA, and DCA but not in nontumor areas, suggesting that this effect is not specific to DCA.

There is a body of literature that has focused on the effects of TCE and its metabolites after rats or mice were exposed to “mutagenic” agents to “initiate” hepatocarcinogenesis and this is discussed in Section E.4.2. TCE and its metabolites were reported to affect tumor incidence, multiplicity, and phenotype when given to mice as a co-exposure with a variety of “initiating” agents and with other carcinogens. Pereira and Phelps (1996) reported that methyl nitrosourea (MNU) alone induced basophilic foci and adenomas. MNU and low concentrations of DCA or TCA in female mice were reported to induce heterogeneous for foci and tumor with a higher concentration of DCA inducing more eosinophilic and a higher concentration of TCA inducing more tumors that were basophilic. Pereira et al. (2001) reported that not only dose, but also gender affected phenotype in mice that had already been exposed to MNU and were then exposed to DCA. As for other phenotypic markers, Lantendresse and Pereira (1997) reported that exposure to MNU and TCA or DCA induced tumors that had some commonalities (i.e., were heterogeneous), but differences were noted for female mice exposed to DCA or TCA as co-exposures with MNU.

With regard to the phenotype of TCA and DCA-induced tumors, Stauber and Bull (1997) reported the for male B6C3F<sub>1</sub> mice, DCA-induced “lesions” contained a number of smaller

lesions that were heterogeneous and more eosinophilic with larger “lesions” tending to be less numerous and more basophilic. For TCA results using this paradigm, the “lesions” were reported to be less numerous, more basophilic, and larger than those induced by DCA. Carter et al. (2003) used tissues from the DeAngelo et al. (1999) study and examined the heterogeneity of the DCA-induced lesions and the type and phenotype of preneoplastic and neoplastic lesions pooled across all time points. Carter et al. (2003) examined the phenotype of liver tumors induced by DCA in male B6C3F<sub>1</sub> mice and the shape of the dose-response curve for insight into its mode of action. They reported a dose-response of histopathologic changes (all classes of premalignant lesions and carcinomas) occurring in the livers of mice at doses of 0.05–3.5 g/L DCA for 26–100 weeks and suggested that foci and adenomas demonstrated neoplastic progression with time at lower doses than observed DCA genotoxicity. Preneoplastic lesions were identified as eosinophilic, basophilic and/or clear cell (grouped with clear cell and mixed cell), and dysplastic. Altered foci were 50% eosinophilic with about 30% basophilic. As foci became larger and evolved into carcinomas, they became increasingly basophilic. The pattern held true throughout the exposure range. There was also a dose and length of exposure related increase in atypical nuclei in “noninvolved” liver. Glycogen deposition was also reported to be dose-dependent with periportal accumulation at the 0.5 g/L exposure level. Carter et al. (2003) suggested that size and evolution into a more malignant state are associated with increasing basophilia, a conclusion consistent with those of Bannasch (1996) and that there is a greater periportal location of lesions suggestive as the location from which they arose. Consistent with the results of DeAngelo et al. (1999), Carter et al. (2003) reported that DCA (0.05–3.5 g/L) increased the number of lesions per animal relative to animals receiving distilled water, that DCA shortened the time to development of all classes of hepatic lesions, and that the phenotype of the lesions were similar to those spontaneously arising in controls. Along with basophilic and eosinophilic lesions or foci, Carter et al. (2003) concluded that DCA-induced tumors also arose from isolated, highly dysplastic hepatocytes in male B6C3F<sub>1</sub> mice chronically exposed to DCA suggesting another direct neoplastic conversion pathway other than through eosinophilic or basophilic foci.

Rather than male B6C3F<sub>1</sub> mice, Pereira (1996) studied the dose-response relationship for the carcinogenic activity of DCA and TCA and characterized their lesions (foci, adenomas, and carcinomas) by tincture in females (the generally less sensitive gender). Like the studies of TCE by Maltoni et al. (1988; 1986), female mice were also reported to have increased liver tumors after TCA and DCA exposures. Pereira (1996) pool lesions were pooled for phenotype analysis so the effect of duration of exposure could not be determined and adenomas could not be separated from carcinomas for “tumors.” However, Pereira (1996) reported that a decrease in the concentration of DCA resulted in a decrease in the number of foci and a shift in the phenotype from primarily eosinophilic foci (i.e., ~95% eosinophilic at 2.58 g/L DCA) to basophilic foci (~57% eosinophilic at 0.26 g/L). For TCA, the number of foci was reported to ~40 basophilic

and ~60 eosinophilic, regardless of dose. Spontaneously occurring foci were more basophilic by a ratio of 7/3. Pereira (1996) described the foci of altered hepatocytes and tumors induced by DCA in female B6C3F<sub>1</sub> mice to be eosinophilic at higher exposure levels, but at lower or intermittent exposures, they were half eosinophilic and half basophilic. Regardless of exposure level, half of the TCA-induced foci were reported to be half eosinophilic and half basophilic with tumors 75% basophilic. In control female mice, the limited numbers of lesions were mostly basophilic, with most of the rest being eosinophilic with the exception of a few mixed tumors. The limitations of descriptions of tincture and especially for inferences regarding peroxisome proliferator from the description of “basophilia” is discussed in Section E.3.4.1.5.

Thus, the results appear to differ between male and female B6C3F<sub>1</sub> mice in regard to tincture for DCA and TCA at differing doses. What is apparent is that the tincture of the lesions is dependent on the stage of tumor progression, agent (DCA or TCA), gender, and dose. Also what is apparent from these studies is that both DCA and TCA are heterogeneous in their tinctural characteristics.

Overall, tumors induced by TCA, DCA, CH, and TCE are all heterogeneous in their physical and tinctural characteristics in a manner this not markedly distinguishable from spontaneous lesions or those induced by a wide variety of chemical carcinogens. For instance, Daniel et al. (1992), which studied DCA and CH carcinogenicity (discussed above), noted that morphologically, there did not appear to be any discernable differences in the visual appearance of the DCA- and CH-induced tumors. Therefore, these data do not provide strong insights into elucidating the active agent(s) for TCE hepatocarcinogenicity or their mode(s) of action.

#### **4.5.6.3.3.2. C-Jun staining**

Stauber and Bull (1997) reported that in male B6C3F<sub>1</sub> mice, the oncoproteins, c-Jun and c-Fos, were expressed in liver tumors induced by DCA but not those induced by TCA. Although Bull et al. (2004) suggested that the negative expression of c-Jun in TCA-induced tumors may be consistent with a characteristic phenotype shown in general by peroxisome proliferators as a class, as pointed out by Caldwell and Keshava (2006), there is no supporting evidence of this. Nonetheless, the observation that TCA and DCA have different levels of oncogene expression led to a number of follow-up studies by this group. No data on oncoprotein immunostaining are available for CH.

Stauber et al. (1998) studied induction of “transformed” hepatocytes by DCA and TCE treatment *in vitro*, including an examination of c-Jun staining. Stauber et al. (1998) isolated primary hepatocytes from 5 to 8-week-old male B6C3F<sub>1</sub> mice (n = 3) and subsequently cultured them in the presence of DCA or TCA. In a separate experiment, 0.5 g/L DCA was given to mice as pretreatment for 2 weeks prior to isolation. The authors assumed that the anchorage-independent growth of these hepatocytes was an indication of an “initiated cell.” After 10 days in culture with DCA or TCA (0, 0.2, 0.5, and 2.0 mM), concentrations of  $\geq 0.5$  mM DCA and

TCA both induced an increase in the number of colonies that was statistically significant, with DCA showing dose-dependence as well as slightly greater overall increases than TCA. In a time-course experiment, the number of colonies from DCA treatment in vitro peaked by 10 days and did not change through days 15–25 at the highest dose and, at lower concentrations of DCA, increased time in culture induced similar peak levels of colony formation by days 20–25 as that reached by 10 days at the higher dose. Therefore, the number of colonies formed was independent of dose if the cells were treated long enough in vitro. However, not only did treatment with DCA or TCA induce anchorage independent growth but untreated hepatocytes also formed larger numbers of colonies with time, although at a lower rate than those treated with DCA. The level reached by untreated cells in tissue culture at 20 days was similar to the level induced by 10 days of exposure to 0.5 mM DCA. The time course of TCA exposure was not tested to see if it had a similar effect with time as did DCA. The colonies observed at 10 days were tested for c-Jun expression with the authors noting that “colonies promoted by DCA were primarily c-Jun positive in contrast to TCA promoted colonies that were predominantly c-Jun negative.” Of the colonies that arose spontaneously from tissue culture conditions, 10/13 (76.9%) were reported to be c-Jun+, those treated with DCA 28/34 (82.3%) were c-Jun+, and those treated with TCA 5/22 (22.7%) were c-Jun+. Thus, these data show heterogeneity in cell in colonies but with more that were c-Jun+ colonies occurring by tissue culture conditions alone than in the presence of DCA, rather than in the presence of TCA.

Bull et al. (2002) administered TCE, TCA, DCA, and combinations of TCA and DCA to male B6C3F<sub>1</sub> mice by daily gavage (TCE) or drinking water (TCA, DCA, and TCA + DCA) for 52–79 weeks, in order to compare a number of tumor characteristics, including c-Jun expression, across these different exposures. Bull et al. (2002) reported lesion reactivity to c-Jun antibody to be dependent on the proportion of the DCA and TCA administered after 52 weeks of exposure. Given alone, DCA was reported to produce lesions in mouse liver for which approximately half displayed a diffuse immunoreactivity to a c-Jun antibody, half did not, and none exhibited a mixture of the two. After TCA exposure alone, no lesions were reported to be stained with this antibody. When given in various combinations, DCA and TCA co-exposure induced a few lesions that were only c-Jun+, many that were only c-Jun–, and a number with a mixed phenotype whose frequency increased with the dose of DCA. For TCE exposure of 79 weeks, TCE-induced lesions were reported to also have a mixture of phenotypes (42% c-Jun+, 34% c-Jun–, and 24% mixed) and to be most consistent with those resulting from DCA and TCA co-exposure but not either metabolite alone.

A number of the limitations of the experiment are discussed in Caldwell et al. (2008b) Specifically, for the DCA- and TCA-exposed animals, the experiment was limited by low statistical power, a relatively short duration of exposure, and uncertainty in reports of lesion prevalence and multiplicity due to inappropriate lesions grouping (i.e., grouping of hyperplastic nodules, adenomas, and carcinomas together as “tumors”), and incomplete histopathology

determinations (i.e., random selection of gross lesions for histopathology examination). For determinations of immunoreactivity to c-Jun, Bull et al. (2002) combined hyperplastic nodules, adenomas, and carcinomas in most of their treatment groups, so differences in c-Jun expression across differing types of lesions were not discernable.

Nonetheless, these data collectively strongly suggest that TCA is not the sole agent of TCE-induced mouse liver tumors. In particular, TCE-induced tumors that were, in order of frequency, c-Jun+, c-Jun-, and of mixed phenotype, while c-Jun+ tumors have never been observed with TCA treatment. Nor do these data support DCA as the sole contributor, since mixed phenotypes were not observed with DCA treatment.

#### **4.5.6.3.3.3. Tumor genotype: H-ras mutation frequency and spectrum**

An approach to determine the potential modes of action of DCA and TCA through examination of the types of tumors each “induced” or “selected” was to examine H-ras activation (Bull et al., 2002; Ferreira-Gonzalez et al., 1995; Anna et al., 1994; Nelson et al., 1989). No data of this type were available for CH. This approach has also been used to try to establish an H-ras activation pattern for “genotoxic” and “nongenotoxic” liver carcinogens compounds and to make inferences concerning peroxisome proliferator-induced liver tumors. However, as noted by Stanley et al. (1994), the genetic background of the mice used and the dose of carcinogen may affect the number of activated H-ras containing tumors which develop. In addition, the stage of progression of “lesions” (i.e., foci vs. adenomas vs. carcinomas) also has been linked the observance of H-ras mutations. Fox et al. (1990) note that tumors induced by phenobarbital (0.05% drinking water [H<sub>2</sub>O], 1 year), chloroform (200 mg/kg corn oil gavage, 2 times weekly for 1 year) or ciprofibrate (0.0125% diet, 2 years) had a much lower frequency of H-ras gene activation than those that arose spontaneously (2-year bioassays of control animals) or induced with the “genotoxic” carcinogen benzidine-2 hydrochloric acid (HCl) (120 ppm, drinking H<sub>2</sub>O, 1 year) in mice. In that study, the term “tumor” was not specifically defined, but a correlation between the incidence of H-ras gene activation and the development of either a hepatocellular adenoma or HCC was reported to be made with no statistically significant difference between the frequency of H-ras gene activation in the hepatocellular adenomas and carcinomas.

Histopathological examination of the spontaneous tumors, tumors induced with benzidine-2 HCl, phenobarbital, and chloroform was not reported to reveal any significant changes in morphology or staining characteristics. Spontaneous tumors were reported to have 64% point mutation in codon 61 (n = 50 tumors examined) with a similar response for benzidine of 59% (n = 22 tumors examined), whereas the mutation rates were 7% (n = 15 tumors examined) for phenobarbital, 21% (n = 24 tumors examined) for chloroform, and 21% (n = 39 tumors examined) for ciprofibrate. The ciprofibrate-induced tumors were reported to be more eosinophilic as were the surrounding normal hepatocytes.

Hegi et al. (1993) tested ciprofibrate-induced tumors in the NIH3T3 cotransfection-nude mouse tumorigenicity assay, which the authors stated was capable of detecting a variety of activated protooncogenes. The tumors examined (ciprofibrate-induced or spontaneously arising) were taken from the Fox et al. (1990) study, screened previously, and found to be negative for H-ras activation. With the limited number of samples examined, Hegi et al. (1993) concluded that ras protooncogene activation or activation of other protooncogenes using the nude mouse assay were not frequent events in ciprofibrate-induced tumors and that spontaneous tumors were not promoted with it. Using the more sensitive methods, the H-ras activation rate was reported to be raised from 21 to 31% for ciprofibrate-induced tumors and from 64 to 66% for spontaneous tumors. Stanley et al. (1994) studied the effect of methylclofenapate (MCP) (25 mg/kg for up to 2 years), a peroxisome proliferator, in B6C3F<sub>1</sub> (relatively sensitive) and C57BL/10J (relatively resistant) mice for H-ras codon 61 point mutations in MCP-induced liver tumors (hepatocellular adenomas and carcinomas). In the B6C3F<sub>1</sub> mice, the number of tumors with codon 61 mutations was 11/46 and for C57BL/10J mice 4/31. Unlike the findings of Fox et al. (1990), Stanley et al. (1994) reported an increase in the frequency of mutation in carcinomas, which was reported to be twice that of adenomas in both strains of mice, indicating that stage of progression was related to the number of mutations in those tumors, although most tumors induced by MCP did not have this mutation.

Anna et al. (1994) reported that the H-ras codon 61 mutation frequency was not statistically different in liver tumors from DCA- and TCE-treated mice from a highly variable number of tumors examined. From their concurrent controls, they reported that H-ras codon 61 mutations in 17% (n = 6) of adenomas and 100% (n = 5) of carcinomas. For historical controls (published and unpublished), they reported mutations in 73% (n = 33) of adenomas and mutations in 70% (n = 30) of carcinomas. For tumors from TCE-treated animals, they reported mutations in 35% (n = 40) of adenomas and 69% (n = 36) of carcinomas, while for DCA-treated animals, they reported mutations in 54% (n = 24) of adenomas and in 68% (n = 40) of carcinomas. Anna et al. (1994) reported more mutations in TCE-induced carcinomas than adenomas. In regard to mutation spectra in H-ras oncogenes in control or spontaneous tumors, the patterns were slightly different but those from TCE treatment were mostly similar to that of DCA-induced tumors (0.5% in drinking water).

The study of Ferreira -Gonzalez (1995) in male B6C3F<sub>1</sub> mice has the advantage of comparison of tumor phenotype at the same stage of progression (HCC), for allowance of the full expression of a tumor response (i.e., 104 weeks), and an adequate number of spontaneous control lesions for comparison with DCA or TCA treatments. However, tumor phenotype at an end stage of tumor progression may not be indicative of earlier stages of the disease process. In spontaneous liver carcinomas, 58% were reported to show mutations in H-61 as compared with 50% of tumor from 3.5 g/L DCA-treated mice and 45% of tumors from 4.5 g/L TCA-treated mice. A number of peroxisome proliferators have been reported to have a much smaller

mutation frequency that spontaneous tumors (e.g., 13–24% H-ras codon 61 mutations after methylclofenopate depending on mouse strain, Stanely et al. [1994]: 21–31% for ciprofibrate-induced tumors and 64–66% for spontaneous tumors, Fox et al. [1990] and Hegi et al. [1993]). Thus, the heterogeneous response for H-ras mutations was similar for spontaneous and DCA-, and TCA-induced HCCs and differed from the reduced H-ras mutation frequencies reported for a number of peroxisome proliferators.

In his review, Bull (2000) suggested that “the report by Anna et al. (1994) indicated that TCE-induced tumors possessed a different mutation spectra in codon 61 of the H-ras oncogene than those observed in spontaneous tumors of control mice.” Bull (2000) stated that “results of this type have been interpreted as suggesting that a chemical is acting by a mutagenic mechanism” but went on to suggest that it is not possible to a priori rule out a role for selection in this process and that differences in mutation frequency and spectra in this gene provide some insight into the relative contribution of different metabolites to TCE-induced liver tumors. Bull (2000) noted that data from Anna et al. (1994), Ferreira-Gonzalez et al. (1995), and Maronpot et al. (1995b) indicated that mutation frequency in DCA-induced tumors did not differ significantly from that observed in spontaneous tumors. Bull (2000) also noted that the mutation spectra found in DCA-induced tumors has a striking similarity to that observed in TCE-induced tumors, and DCA-induced tumors were significantly different than that of TCA-induced liver tumors.

Bull et al. (2002) reported that mutation frequency spectra for the H-ras codon 61 in mouse liver “tumors” induced by TCE (n = 37 tumors examined) were significantly different than that for TCA (n = 41 tumors examined), with DCA-treated mice tumors giving an intermediate result (n = 64 tumors examined). In this experiment, TCA-induced “tumors” were reported to have more mutations in codon 61 (44%) than those from TCE (21%) and DCA (33%). This frequency of mutation in the H-ras codon 61 for TCA is the opposite pattern as that observed for a number of peroxisome proliferators in which the number of mutations at H-ras codon 61 in tumors has been reported to be much lower than spontaneously arising tumors (see above). Bull et al. (2002) noted that the mutation frequency for all TCE, TCA, or DCA tumors was lower in this experiment than for spontaneous tumors reported in other studies (they had too few spontaneous tumors to analyze in this study), but that this study utilized lower doses and was of shorter duration than that of Ferreira-Gonzalez (1995). Furthermore, the disparities from previous studies may also be impacted by lesion grouping, mentioned above, in which lower stages of progression are grouped with more advanced stages.

Overall, in terms of H-ras mutation, TCE-induced tumors appears to be more like DCA-induced tumors (which are consistent with spontaneous tumors), or those resulting from a co-exposure to both DCA and TCA (Bull et al., 2002), than from those induced by TCA. As noted above, Bull et al. (2002) reported the mutation frequency spectra for the H-ras codon 61 in mouse liver tumors induced by TCE to be significantly different than that for TCA, with

DCA-treated mice tumors giving an intermediate result and for TCA-induced tumors to have a H-ras profile that is the opposite than those of a number of other peroxisome proliferators. More importantly, however, these data, along with the measures discussed above, show that mouse liver tumors induced by TCE are heterogeneous in phenotype and genotype in a manner similar to that observed in spontaneous tumors.

#### 4.5.6.3.4. “Stop” experiments

Several stop experiments, in which treatment is terminated early in some dose groups, have attempted to ascertain the whether progression differences exist between TCA and DCA. After 37 weeks of treatment and then a cessation of exposure for 15 weeks, Bull et al. (1990) reported that after a combined 52-week period, liver weight and percentage of liver/body weight were reported to still be statistically significantly elevated after DCA or TCA treatment. The authors partially attribute the remaining increases in liver weight to the continued presence of hyperplastic nodules in the liver. In terms of liver tumor induction, the authors stated that “statistical analysis of tumor incidence employed a general linear model ANOVA with contrasts for linearity and deviations from linearity to determine if results from groups in which treatments were discontinued after 37 weeks were lower than would have been predicted by the total dose consumed.” The multiplicity of tumors (incidence was not used) observed in male mice exposed to DCA or TCA at 37 weeks and then sacrificed at 52 weeks were compared with those exposed for a full 52 weeks. The response in animals that received the shorter duration of DCA exposure was very close to that which would be predicted from the total dose consumed by these animals. By contrast, the response to TCA exposure for the shorter duration was reported by the authors to deviate significantly ( $p = 0.022$ ) from the linear model predicted by the total dose consumed. However, in the prediction of “dose-response,” foci, adenomas, and carcinomas were combined into one measure. Therefore, foci, a certain percentage of which have been commonly shown to spontaneously regress with time, were included in the calculation of total “lesions.” Moreover, only a sample of lesions were selected for histological examination, and as is evident in the sample, some lesions appeared “normal” upon microscopic examination (see below). Therefore, while suggesting that cessation of exposure diminished the number of “lesions,” methodological limitations temper any conclusions regarding the identity and progression of lesion with continuous vs. noncontinuous DCA and TCA treatment.

Additionally, Bull et al. (1990) noted that after stopping treatment, DCA lesions appeared to arrest their progression in contrast to TCA lesions, which appeared to progress. In particular, those in the stop treatment group (at 2 g/L) with 0/19 lesions examined histologically were carcinomas, while in the continuous treatment groups, a significant fraction of lesions examined were carcinomas at the higher exposure (6/23 at 2 g/L). By contrast, at terminal sacrifice, a larger fraction of the lesions examined were carcinomas in the stop treatment groups (3/5 at 2 g/L) than in the continuous treatment group (2/7 and 4/16 at 1 g/L and 2 g/L, respectively).

However, as mentioned above, these inferences are based on examination of only a subset of lesions. Specifically, for TCA treatment, the number of animals examined for determination of which “lesions” were foci, adenomas, and carcinomas was 11/19 mice with “lesions” at 52 weeks, while all 4 mice with lesions after 37 weeks of exposure and 15 weeks of cessation were examined. For DCA treatment, the number of animals examined was only 10/23 mice with “lesions” at 52 weeks, while all 7 mice with lesions after 37 weeks of exposure and 15 weeks of cessation were examined. Most importantly, when lesions were examined microscopically, some did not turn out to be preneoplastic or neoplastic—for example, two lesions appeared “to be histologically normal” and one necrotic.

While limited, the conclusions of Bull et al. (1990) are consistent with later experiments performed by Pereira and Phelps (1996). They noted that in MNU-treated mice that were then treated with DCA, the yield of altered hepatocytes decreases as the tumor yields increase between 31 and 51 weeks of exposure, suggesting progression of foci to adenomas, but that adenomas did not appear to progress to carcinomas. For TCA, Pereira and Phelps (1996) reported that “MNU-initiated” adenomas promoted with TCA continued to progress. However, the use of MNU initiation complicates direct comparisons with treatment with TCA or DCA alone.

No similar data comparing stop and continued treatment of TCE are available to assess the consistency or lack thereof with TCA or DCA. Moreover, the informative of such a comparison would be limited by designs of the available TCA and DCA studies, which have used higher concentrations in conjunction with the much lower durations of exposure. While higher doses allow for responses to be more easily detected, it introduces uncertainty as to the effects of the higher doses alone. In addition, because the overall duration of the experiments is also generally much less than 104 weeks, it is not possible to discern whether the differences in results between those animals in which treatment was suspended in comparison to those in which had not had been conducted would persist with longer durations.

#### **4.5.6.4. Conclusions Regarding the Role of TCA, DCA, and CH in TCE-Induced Effects in the Liver**

In summary, it is likely that oxidative metabolism is necessary for TCE-induced effects in the liver. However, the specific metabolite or metabolites responsible for both noncancer and cancer effects is less clear. TCE, TCA, and DCA exposures have all been associated with induction of peroxisomal enzymes but are all weak PPAR $\alpha$  agonists. The available data strongly support TCA *not* being the sole or predominant active moiety for TCE-induced liver effects. With respect to hepatomegaly, TCE and TCA dose-response relationships are quantitatively inconsistent, for TCE leads to greater increases in liver/body weight ratios than expected from predicted rates of TCA production. In fact, above a certain dose of TCE, liver/body weight ratios are greater than that observed under any conditions studied so far for TCA. Histological

changes and effects on DNA synthesis are generally consistent with contributions from either TCA or DCA, with a degree of polyploidization, rather than cell proliferation, likely to be significant for TCE, TCA, and DCA. With respect to liver tumor induction, TCE leads to a heterogeneous population of tumors, not unlike those that occur spontaneously or that are observed following TCA-, DCA-, or CH-treatment. Moreover, some liver phenotype experiments, particularly those utilizing immunostaining for c-Jun, support a role for both DCA and TCA in TCE-induced tumors, with strong evidence that TCA cannot solely account for the characteristics of TCE-induced tumors. In addition, H-ras mutation frequency and spectrum of TCE-induced tumors more closely resembles that of spontaneous tumors or of those induced by DCA, and were less similar in comparison to that of TCA-induced tumors. The heterogeneity of TCE-induced tumors is similar to that observed to be induced by a broad category of carcinogens, and to that observed in human liver cancer. Overall, then, it is likely that multiple TCE metabolites, and therefore, multiple pathways, contribute to TCE-induced liver tumors.

#### **4.5.7. Mode of Action for TCE Liver Carcinogenicity**

This section will discuss the evidentiary support for several hypothesized modes of action for liver carcinogenicity (including mutagenicity and peroxisome proliferation, as well as several additional proposed hypotheses and key events with limited evidence or inadequate experimental support), following the framework outlined in the *Cancer Guidelines* ([U.S. EPA, 2005e, b](#)).<sup>9</sup>

##### **4.5.7.1. Mutagenicity**

The hypothesis is that TCE acts by a mutagenic mode of action in TCE-induced hepatocarcinogenesis. According to this hypothesis, the key events leading to TCE-induced liver tumor formation constitute the following: TCE oxidative metabolite CH, after being produced in the liver, cause direct alterations to DNA (e.g., mutation, DNA damage, and/or micronuclei induction). Mutagenicity is a well-established cause of carcinogenicity.

##### **4.5.7.1.1. Experimental support for the hypothesized mode of action**

The genotoxicity, as described by the ability of TCE, CH, TCA, and DCA to induce mutations, was discussed previously in Section 4.2. The strongest data for mutagenic potential are for CH, thought to be a relatively short-lived intermediate in the metabolism of TCE that is rapidly converted to TCA and TCOH in the liver (see Section 3.3). CH causes a variety of

---

<sup>9</sup>As recently reviewed ([Guyton et al., 2008](#)), the approach to evaluating mode of action information described in EPA's *Cancer Guidelines* ([U.S. EPA, 2005e, 2005b](#)) considers the issue of human relevance of a hypothesized mode of action in the context of hazard evaluation. This excludes, for example, consideration of toxicokinetic differences across species; specifically, the Cancer Guidelines state, "the toxicokinetic processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the mode of action." In addition, information suggesting quantitative differences in the occurrence of a key event between test species and humans are noted for consideration in the dose-response assessment, but is not considered in human relevance determination.

genotoxic effects in available in vitro and in vivo assays, with particularly strong data as to its ability to induce aneuploidy. It has been argued that CH mutagenicity is unlikely to be the cause of TCE carcinogenicity because the concentrations required to elicit these responses are generally quite high, several orders of magnitude higher than achieved in vivo ([Moore and Harrington-Brock, 2000](#)). For example, peak concentrations of CH in the liver of around 2–3 mg/kg have been reported after TCE administration at doses that are hepatocarcinogenic in chronic bioassays ([Greenberg et al., 1999](#); [Abbas and Fisher, 1997](#)). Assuming a liver density of about 1 kg/L, these concentrations are orders of magnitude less than the minimum concentrations reported to elicit genotoxic responses in the Ames test and various in vitro measures of micronucleus, aneuploidy, and chromosome aberrations, which are in the 100–1,000 mg/L range. However, it is not clear how much of a correspondence is to be expected from concentrations in genotoxicity assays in vitro and concentrations in vivo, as reported in vivo CH concentrations are in whole-liver homogenate while in vitro concentrations are in culture media. In addition, a few in vitro studies have reported positive results at concentrations as low as 1 or 10 mg/L, including Furnus et al. ([1990](#)) for aneuploidy in Chinese hamster CHED cells (10 mg/L), Eichenlaub-Ritter et al. ([1996](#)) for bivalent chromosomes in meiosis I in MF1 mouse oocytes (10 mg/L), and Gibson et al. ([1995](#)) for cell transformation in Syrian hamster embryo cells after a 7-day treatment. Moreover, some in vivo genotoxicity assays of CH reported positive results at doses similar to those eliciting a carcinogenic response in chronic bioassays. For example, Nelson and Bull ([1988](#)) reported increased DNA SSBs at 100 CH mg/kg (oral) in male B6C3F<sub>1</sub> mice, although the result was not replicated by Chang et al. ([1992](#)). In another example, four of six in vivo mouse genotoxicity studies reported that CH induced micronuclei in mouse bone-marrow erythrocytes, with the lowest effective doses in positive studies ranging from 83 to 500 mg/kg [positive: Russo and Levis ([1992a](#)); Russo et al. ([1992](#)); Marrazzini et al. ([1994](#)); Beland et al. ([1999](#)); and negative: Leuschner and Leuschner ([1991](#)); Leopardi et al. ([1993](#))]. However, the use of i.p. administration in these and many other in vivo genotoxicity assays complicates the comparison with carcinogenicity data. Also, it is difficult with the available data to assess the contributions from the genotoxic effects of CH along with those from the genotoxic and nongenotoxic effects of other oxidative metabolites (discussed in Sections 4.5.5.2 and 4.5.5.3).

Furthermore, altered DNA methylation, another heritable mechanism by which gene expression may be altered, is discussed in Section 4.5.7.3.7. As discussed previously, the differential patterns of H-ras mutations observed in liver tumors induced by TCE, TCA, and DCA may be more indicative of tumor selection and tumor progression resulting from exposure to these agents rather than a particular mechanism of tumor induction. The state of the science of cancer and the role of epigenetic changes, in addition to genetic changes, in the initiation and progression of cancer and specifically liver cancer, are discussed in Section E.3.1.

Therefore, while data are insufficient to conclude that a mutagenic mode of action mediated by CH is operant, a mutagenic mode of action, mediated either by CH or by some other oxidative metabolite of TCE, cannot be ruled out.

#### **4.5.7.2. PPAR $\alpha$ Receptor Activation**

The hypothesis is that TCE acts by a PPAR $\alpha$  agonism mode of action in TCE-induced hepatocarcinogenesis. According to this hypothesis, the key events leading to TCE-induced liver tumor formation constitute the following: the TCE oxidative metabolite TCA, after being produced in the liver, activates the PPAR $\alpha$  receptor, which then causes alterations in cell proliferation and apoptosis and clonal expansion of initiated cells. This mode of action is assumed to apply only to the liver.

##### **4.5.7.2.1. Experimental support for the hypothesized mode of action**

Proliferation of peroxisomes and increased activity of a number of related marker enzymes has been observed in rodents treated with TCE, TCA, and DCA. The peroxisome-related effects of TCE are most likely mediated primarily through TCA based on TCE metabolism producing more TCA than DCA and the lower doses of TCA required to elicit a response relative to DCA. However, Bull ([2004a](#)) and Bull et al. ([2004](#)) have recently suggested that peroxisome proliferation occurs at higher exposure levels than those that induce liver tumors for TCE and its metabolites. They report that a direct comparison in the no-effect level or low-effect level for induction of liver tumors in the mouse and several other endpoints shows that, for TCA, liver tumors occur at lower concentrations than peroxisome proliferation *in vivo* but that PPAR $\alpha$  activation occurs at a lower dose than either tumor formation or peroxisome proliferation. A similar comparison for DCA shows that liver tumor formation occurs at a much lower exposure level than peroxisome proliferation or PPAR $\alpha$  activation. *In vitro* transactivation studies have shown that human and murine versions of PPAR $\alpha$  are activated by TCA and DCA, while TCE itself is relatively inactive in the *in vitro* system, at least with mouse PPAR $\alpha$  ([Maloney and Waxman, 1999](#); [Zhou and Waxman, 1998](#)). In addition, Laughter et al. ([2004](#)) reported that the responses of acyl CoA oxidase (ACO), PCO, and CYP4A induction by TCE, TCA, and DCA were substantially diminished in PPAR $\alpha$ -null mice. Therefore, evidence suggests that TCE, through its metabolites TCA and DCA, activate PPAR $\alpha$ , and that at doses relevant to TCE-induced hepatocarcinogenesis, the role of TCA in PPAR $\alpha$  agonism is likely to predominate.

It has been suggested that PPAR $\alpha$  receptor activation is both the mode of action for TCA liver tumor induction as well as the mode of action for TCE liver tumor induction, as a result of the metabolism of TCE to TCA ([Corton, 2008](#); [NRC, 2006](#)). Section E.3.4 addressed the status of the PPAR $\alpha$  mode-of-action hypothesis for liver tumor induction and provides a more detailed discussion. However, as discussed previously and in Section E.2.1.10, TCE-induced increases in

liver weight have been reported in male and female mice that do not have a functional PPAR $\alpha$  receptor ([Nakajima et al., 2000](#)). The dose-response for TCE-induced liver weight increases differs from that of TCA (see Section E.2.4.2). The phenotype of the tumors induced by TCE have been described to differ from those by TCA and to be more like those occurring spontaneously in mice, those induced by DCA, or those resulting from a combination of exposures to both DCA and TCA (see Section E.2.4.4). As to whether TCA induces tumors through activation of the PPAR $\alpha$  receptor, the tumor phenotype of TCA-induced mouse liver tumors has been reported to have a different pattern of H-ras mutation frequency from other peroxisome proliferators (see Section E.2.4.4) ([Bull et al., 2002](#); [Stanley et al., 1994](#); [Hegi et al., 1993](#); [Fox et al., 1990](#)). While TCE, DCA, and TCA are weak peroxisome proliferators, liver weight induction from exposure to these agents has not correlated with increases in peroxisomal enzyme activity (e.g., PCO activity) or changes in peroxisomal number or volume. By contrast, as discussed above, liver weight induction from subchronic exposures appears to be a more accurate predictor of carcinogenic response for DCA, TCA, and TCE in mice (see also Section E.2.4.4). The database for cancer induction in rats is much more limited than that of mice for determination of a carcinogenic response to these chemicals in the liver and the nature of such a response.

While many compounds known to cause rodent liver tumors with long-term treatment also activate the nuclear receptor PPAR $\alpha$ , the mechanisms by which PPAR $\alpha$  activation contributes to tumorigenesis are not completely known ([Yang et al., 2007](#); [NRC, 2006](#); [Klaunig et al., 2003](#)). As reviewed by Keshava and Caldwell ([2006](#)), PPAR $\alpha$  activation leads to a highly pleiotropic response and may play a role in toxicity in multiple organs as well as in multiple chronic conditions besides cancer (obesity, atherosclerosis, diabetes, inflammation). Klaunig et al. ([2003](#)) and NRC ([2006](#)) proposed that the key causal events for PPAR $\alpha$  agonist-induced liver carcinogenesis, after PPAR $\alpha$  activation, are perturbation of cell proliferation and/or apoptosis, mediated by gene expression changes, and selective clonal expansion. It has also been proposed that sufficient evidence for this mode of action consists of evidence of PPAR $\alpha$  agonism (i.e., in a receptor assay) in combination with either light- or electron-microscopic evidence for peroxisome proliferation or both increased liver weight and one more of the in vivo markers of peroxisome proliferation ([Klaunig et al., 2003](#)). However, it should be noted that peroxisome proliferation and in vivo markers such as PCO are not considered causal events ([NRC, 2006](#); [Klaunig et al., 2003](#)), and that their correlation with carcinogenic potency is poor ([Marsman et al., 1988](#)). Therefore, for the purposes of this discussion, peroxisome proliferation and its markers are considered indicators of PPAR $\alpha$  activation, as it is well established that these highly specific effects are mediated through PPAR $\alpha$  ([Klaunig et al., 2003](#); [Peters et al., 1997](#)).

As recently reviewed by Guyton et al. ([2009](#)), recent data suggest that PPAR $\alpha$  activation along with these hypothesized causal events may not be sufficient for carcinogenesis. In particular, Yang et al. ([2007](#)) reported comparisons between mice treated with Wy-14643 and

transgenic mice in which PPAR $\alpha$  was constitutively activated in hepatocytes without the presence of ligand. Yang et al. (2007) reported that, in contrast to Wy-14643-treatment, the transgene did not induce liver tumors at 11 months, despite inducing PPAR $\alpha$ -mediated effects of a similar type and magnitude seen in response to tumorigenic doses of Wy-14643 in wild-type mice (decreased serum fatty acids, induction of PPAR $\alpha$  target genes, altered expression of cell-cycle control genes, and a sustained increase in cellular proliferation). Nonetheless, it is important to discuss the extent to which PPAR $\alpha$  activation mediates the effects proposed by Klaunig et al. (2003) and NRC (2006), even if the hypothesized sequence of key events may not be sufficient for carcinogenesis. Investigation continues into additional events that may also contribute, such as nonparenchymal cell activation and micro-RNA-based regulation of protooncogenes (Shah et al., 2007; Yang et al., 2007). Specifically addressed below are gene expression changes, proliferation, clonal expansion, and mutation frequency or spectrum.

With respect to gene expression changes due to TCE, Laughter et al. (2004) evaluated transcript profiles induced by TCE in wild-type and PPAR $\alpha$ -null mice. As noted in Sections E.3.4.1.3 and E.3.1.2, there are limitations to the interpretation of such studies, some of which are discussed below. Also noted in Appendix E are discussions of how studies of peroxisome proliferators, indicate of the need for phenotypic anchoring, especially since gene expression is highly variable between studies and within studies using the same experimental paradigm. Section E.3.4 also provides detailed discussions of the status of the PPAR $\alpha$  hypothesis. Of note, all null mice at the highest TCE dose (1,500 mg/kg-day) were moribund prior to the end of the planned 3-week experiment (Laughter et al., 2004), and it was proposed that this may reflect a greater sensitivity in PPAR $\alpha$ -null mice to hepatotoxins due to defects in tissue repair abilities. Laughter et al. (2004) also noted that four genes known to be regulated by other peroxisome proliferators also had altered expression with TCE treatment in wild-type, but not null mice. Ramdhan et al. (2010) report that not only do PPAR $\alpha$ -null mice, but also humanized mice (PPAR $\alpha$ -null mice with inserted human PPAR $\alpha$ ) have underlying dysregulation of lipid metabolism and gene expression. However, in a comparative analysis, Bartosiewicz et al. (2001) concluded that TCE induced a different pattern of transcription than two other peroxisome proliferators, di(2-ethylhexyl) phthalate (DEHP) and clofibrate. In addition, Keshava and Caldwell (2006) compared gene expression data from Wy-14643, dibutyl phthalate (DBP), gemfibrozil, and DEHP, and noted a lack of consistent results across PPAR $\alpha$  agonists. Thus, available data are insufficient to conclude that TCE gene expression changes are similar to other PPAR agonists, or even that there are consistent changes (beyond the in vivo markers of peroxisome proliferation, such as ACO, PCO, CYP4A, etc.) among different agonists. It should also be noted that Laughter et al. (2004) did not compare baseline (i.e., control levels of) gene expression between null and wild-type control mice, hindering interpretation of these results (Keshava and Caldwell, 2006). The possible relationship between PPAR $\alpha$  activation and hypomethylation are discussed in Section 4.5.7.3.7.

In terms of proliferation, mitosis itself has not been examined in PPAR $\alpha$ -null mice, but BrdU incorporation, a measure of DNA synthesis that may reflect cell division, polyploidization, or DNA repair, was observed to be diminished in null mice as compared to wild-type mice at 500 and 1,000 mg/kg-day TCE ([Laughter et al., 2004](#)). However, BrdU incorporation in null mice was still about threefold higher than controls, although it was not statistically significantly different due to the small number of animals, high variability, and the two- to threefold higher baseline levels of BrdU incorporation in control null mice as compared to control wild-type mice. Therefore, while PPAR $\alpha$  appears to contribute to the short-term increase in DNA synthesis observed with TCE treatment, these results cannot rule out other contributing mechanisms. However, since it is likely that both cellular proliferation and increased ploidy contribute to the observed TCE-induced increases in DNA synthesis, it is not clear as to whether the observed decrease in BrdU incorporation is due to reduced proliferation, reduced polyploidization, or both.

With respect to clonal expansion, it has been suggested that tumor characteristics such as tincture (i.e., the staining characteristics light microscopy sections of tumor using H&E stains) and oncogene mutation status can be used to associate chemical carcinogens with a particular mode of action such as PPAR $\alpha$  agonism ([NRC, 2006](#); [Klaunig et al., 2003](#)). This approach is problematic primarily because of the lack of specificity of these measures. For example, with respect to tincture, it has been suggested that TCA-induced foci and tumors resemble those of other peroxisome proliferators in basophilia and lack of expression of GGT and GST-pi. However, as discussed in Caldwell and Keshava ([2006](#)), the term “basophilic” in describing foci and tumors can be misleading, because, for example, multiple lineages of foci and tumors exhibit basophilia, including those not associated with peroxisome proliferators ([Carter et al., 2003](#); [Bannasch et al., 2001](#); [Bannasch, 1996](#)). Moreover, a number of studies indicate that foci and tumors induced by other “classic” peroxisome proliferators may have different phenotypic characteristics from that attributed to the class through studies of WY-14643, including DEHP ([Voss et al., 2005](#)) and clofibric acid ([Michel et al., 2007](#)). Furthermore, even the combination of GGT and GST-pi negative, basophilic foci are nonspecific to peroxisome proliferators, as they have been observed in rats treated with AFB1 and AFB1 plus phenobarbital, none of which are peroxisome proliferators ([Grasl-Kraupp et al., 1993](#); [Kraupp-Grasl et al., 1990](#)). Finally, while Bull et al. ([2004](#)) suggested that negative expression of c-Jun in TCA-induced tumors may be consistent with a characteristic phenotype of peroxisome proliferators, no data could be located to support this statement. Therefore, of phenotypic information does not appear to be reliable for associating a chemical with a PPAR $\alpha$  agonism mode of action.

Mutation frequency or spectrum in oncogenes has also been suggested to be an indicator of a PPAR $\alpha$  agonism mode of action being active ([NRC, 2006](#)), with the idea being that specific genotypes are being promoted by PPAR $\alpha$  agonists. Although not a highly specific marker, H-ras codon 61 mutation frequency and spectra data do not support a similarity between mutations in

TCE-, TCA-, or DCA-induced tumors and those due to other peroxisome proliferators. For example, while ciprofibrate and methylclofenopate had lower mutation frequencies than historical controls ([Stanley et al., 1994](#); [Hegi et al., 1993](#)), TCA-induced tumors had mutation frequencies similar to or higher than historical controls ([Bull et al., 2002](#); [Ferreira-Gonzalez et al., 1995](#)). Anna et al. ([1994](#)) and Ferreira-Gonzalez et al. ([1995](#)) also reported TCE and DCA-induced tumors to have mutation frequencies similar to historical controls, although Bull et al. ([2002](#)) reported lower frequencies for these chemicals. However, the data reported by Bull et al. ([2002](#)) consist of mixed lesions at different stages of progression, and such differing stages, in addition to differences in genetic background and dose, can influence the frequency of H-ras mutations ([Stanley et al., 1994](#)). In addition, a greater frequency of mutations was reported in carcinomas than adenomas, and Bull et al. ([2002](#)) stated that this suggested that H-ras mutations were a late event. Moreover, Fox et al. ([1990](#)) noted that tumors induced by phenobarbital, chloroform, and ciprofibrate all had a much lower frequency of H-ras gene activation than those that arose spontaneously, so this marker does not have good specificity. Mutation spectrum is similarly of low utility for supporting a PPAR $\alpha$  agonism mode of action. First, because many peroxisome proliferators been reported to have low frequency of mutations, the comparison of mutation spectrum would be limited to a small fraction tumors. In addition to the low power due to small numbers, the mutation spectrum is relatively nonspecific, as Fox et al. ([1990](#)) reported that of the tumors with mutations, the spectra of the peroxisome proliferator ciprofibrate, historical controls, and the genotoxic carcinogen benzidine-2 HCl were similar.

In summary, TCE clearly activates PPAR $\alpha$ , and some of the effects contributing to tumorigenesis that Klaunig et al. ([2003](#)) and NRC ([2006](#)) propose to be the result of PPAR $\alpha$  agonism are observed with TCE, TCA, or DCA treatment. While this consistency is supportive a role for PPAR $\alpha$ , all of the proposed key causal effects with the exception of PPAR $\alpha$  agonism itself are nonspecific, and may be caused by multiple mechanisms. There is more direct evidence that several of these effects, including alterations in gene expression and changes in DNA synthesis, are mediated by multiple mechanisms in the case of TCE, and a causal linkage to PPAR $\alpha$  specifically is lacking. Therefore, because, as discussed further in the mode of action discussion below, there are multiple lines of evidence supporting the role of multiple pathways of TCE-induced tumorigenesis, the hypothesis that PPAR $\alpha$  agonism and the key causal events proposed by Klaunig et al. ([2003](#)) and NRC ([2006](#)) constitute the sole or predominant mode of action for TCE-induced carcinogenesis is considered unlikely.

Furthermore, as reviewed by Guyton et al. ([2009](#)), recent data strongly suggest that PPAR $\alpha$  and key events hypothesized by Klaunig et al. ([2003](#)) are not sufficient for carcinogenesis induced by the purported prototypical agonist, Wy-14643. Therefore, the proposed PPAR $\alpha$  mode of action is likely “incomplete” in the sense that the sequence of key

events<sup>10</sup> necessary for cancer induction has not been identified. A recent 2-year bioassay of the peroxisome proliferator DEHP showed that it can induce a liver tumor response in mice lacking PPAR $\alpha$  similar to that in wild-type mice ([Ito et al., 2007](#)). Klaunig et al. ([2003](#)) previously concluded that PPAR $\alpha$  agonism was the sole mode of action for DEHP-induced liver tumorigenesis based on the lack of tumors in PPAR $\alpha$ -null mice after 11 months treatment with Wy-14643 ([Peters et al., 1997](#)). They also assumed that due to the lack of markers of PPAR $\alpha$  agonism in PPAR $\alpha$ -null mice after short-term treatment with DEHP ([Ward et al., 1998](#)), a long-term study of DEHP in PPAR $\alpha$ -null mice would yield the same results as for Wy-14643. However, due the finding by Ito et al. ([2007](#)) that PPAR $\alpha$ -null mice exposed to DEHP do develop liver tumors, they concluded that DEHP can induce liver tumors by multiple mechanisms ([Takashima et al., 2008](#); [Ito et al., 2007](#)). Hence, since there is no 2-year bioassay in PPAR $\alpha$ -null mice exposed to TCE or its metabolites, it is not justifiable to use a similar argument based on Peters et al. ([1997](#)) and short-term experiments to suggest that the PPAR $\alpha$  mode of action is operative. Therefore, the conclusion is supported that the hypothesized PPAR $\alpha$  mode of action is inadequately specified because the data do not adequately show the proposed key events individually being required for hepatocarcinogenesis, nor do they show the sequence of key events collectively to be sufficient for hepatocarcinogenesis.

#### 4.5.7.2.2. Quantitative relationships between key events and tumor induction

The issues of whether there is a quantitative relationship between hypothesized key events and tumor induction were recently examined in Guyton et al. ([2009](#)) and are discussed below. Furthermore, IARC has recently concluded that additional mechanistic information has become available, including studies with DEHP in PPAR $\alpha$ -null mice, studies with several transgenic mouse strains, carrying human PPAR $\alpha$  or with hepatocyte-specific constitutively activated PPAR $\alpha$  and a study in humans exposed to DEHP from the environment that has changed its conclusions regarding the relevance of rodent tumor data to human risk ([Grosse et al., 2011](#)). Data from these new studies suggest that many molecular signals and pathways in several cell types in the liver, rather than a single molecular event, contribute to cancer development in rodents with IARC concluding that the human relevance of the molecular events leading to DEHP induced cancer in several target tissues (e.g., liver and testis) in rats or mice could not be ruled out, resulting in the evaluation of DEHP as a Group-2B agent, rather than Group 3.

This following discussion is from Guyton et al. ([2009](#)):

---

<sup>10</sup>As defined by the EPA *Cancer Guidelines* ([2005b](#)) a “key event” is “an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker for such an element,” and the term “mode of action” (MOA) is defined as “a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation.” Therefore, a single key event alone is necessary, but not necessarily sufficient for carcinogenesis; however, the *sequence* of key events constituting a MOA needs to be sufficient for carcinogenesis.

### **Are key or associative events in the PPAR- $\alpha$ activation MOA quantitatively predictive of hepatocarcinogenicity?**

Another question to consider is whether potency for PPAR- $\alpha$  activation or its attendant sequelae is quantitatively associated with carcinogenic activity or potency. If so, differences in sensitivity for carcinogenesis (such as may occur across species) could be predicted using quantitative information about the key events alone. If robust correlations were established, then they could potentially be used either to quantitatively account for pharmacodynamic differences that impact carcinogenic potency or as precursor events in nonlinear dose response assessment. However, there are limitations in the dose-response data available for analyses of quantitative relationships between potencies for precursor events in the proposed PPAR- $\alpha$  activation MOA and for liver tumor induction. Most tumor data, including for the best characterized PPAR- $\alpha$  agonists, are for exposure concentrations inducing well above 50% tumor incidence with less-than-lifetime administration. Precursor events have typically been studied at a single dose, often eliciting a near maximal response, thus precluding benchmark-based comparisons across studies. This is especially true for Wy-14,643, which has been administered most often at only one exposure concentration (1,000 ppm) that elicits a 100% tumor incidence after 1 year or less ([Peters et al., 1997](#)) and that also appears to be necrogenic ([Woods et al., 2007a](#)). On the other hand, hypothesized precursor events such as hepatomegaly, peroxisome proliferation, and increased DNA synthesis appear to have reached their maximal responses at 50 ppm Wy-14,643, with some statistically significant responses as low as 5 ppm ([Marsman et al., 1992](#); [Wada et al., 1992](#)). Potencies across compounds have rarely been compared in a single study using the same experimental paradigm. These deficits in the database notwithstanding, provided below is an assessment of the quantitative predictive power of the potency for four proposed data elements for establishing the hypothesized MOA for hepatocarcinogenesis: PPAR- $\alpha$  activation in mice; and hepatomegaly, DNA synthesis, and increased peroxisome proliferation in rats.

#### **PPAR- $\alpha$ activation in mice**

Table 2 [reproduced as Table 4-66] presents data for four peroxisome proliferators in order of decreasing potency for inducing mouse liver tumors. These compounds were selected because of their importance to environmental human health risk assessments and because data to derive receptor activation potency indicators were available from a single study ([Maloney and Waxman, 1999](#)). The transactivation potencies of MEHP, Wy-14,643, dichloroacetic acid (DCA), and TCA for the mouse PPAR- $\alpha$  were monitored using a luciferase reporter gene containing multiple PPAR response elements derived from the rat hydratase/dehydrogenase promoter in transiently transfected COS-1 monkey kidney cells. The derived potency indicators were compared to the TD<sub>50</sub> (i.e., the daily dose inducing tumors in half of the mice that would otherwise have remained tumor-free) from the Carcinogenic Potency Database (CPDB) of Gold et al. ([2005](#)). Note that for Wy-14,643, the dose listed yielded a maximal response and thus represents an upper limit to the TD<sub>50</sub> (indicated by “<”). Two estimates of PPAR- $\alpha$  transactivation potency are given, the first based on 50% of the maximal response (i.e., EC<sub>50</sub>) and the second based on the effective

concentration required for a 2-fold increase in activity (i.e., EC2-fold) ([Maloney and Waxman, 1999](#)). Orally administered DEHP undergoes presystemic hydrolysis catalyzed by lipase to MEHP in the gut, with mice exhibiting higher lipase activities in the small intestine compared to rats and marmosets ([Ito et al., 2005](#); [Kessler et al., 2004](#); [Pollack et al., 1985](#)). Therefore, because the mouse liver is likely exposed predominantly to MEHP rather than DEHP and unmetabolized -----be explained by pharmacokinetics, i.e., hepatic conversion of DEHP to its mono-ester MEHP, since studies in rats demonstrate that orally administered DEHP undergoes presystemic hydrolysis to MEHP in the gut ([Kessler et al., 2004](#); [Pollack et al., 1985](#)). Possible explanations for these results include one or more of the following: (1) the transactivation assay is not an accurate quantitative indicator of in vivo receptor activation; (2) the rate and nature of effects downstream of PPAR- $\alpha$  activation depends on the ligand; or (3) there are rate-limiting events independent of PPAR- $\alpha$  activation that contribute to mouse hepatocarcinogenesis by the agonists examined.

### **Hepatomegaly, DNA synthesis, and peroxisome proliferation in rats**

Table 1 [reproduced as 4-67] compares potency indicators for various precursor effects at the TD<sub>50</sub> for four PPAR- $\alpha$  agonists and rat hepatocarcinogens. Our analysis of whether there are consistent levels of in vivo precursor effect induction across peroxisome proliferators at the TD<sub>50</sub> does not include all of the data from a similar, prior analysis by Ashby et al. ([1994](#)) for several reasons. First, unlike the CPDB, Ashby et al. ([1994](#)) did not adjust carcinogenicity data for less-than-lifetime dosing, which is relevant for most compounds. Second, for those mouse carcinogens reported in the CPDB, only acute data are available regarding DNA synthesis effects from Ashby et al.. Therefore, our analysis was restricted to rat precursor and potency data for the four compounds Wy-14,643, nafenopin, clofibrate, and DEHP and included both 1-week and 13-week data to separately address transient and sustained changes in DNA synthesis. Even for this small set of compounds, several limitations in the rat database were apparent. Because no single study provided comparative data for the precursor endpoints of interest, four separate reports were used. In the Wada et al. ([1992](#)) and Tanaka et al. ([1992](#)) studies of Wy-14,643 and clofibrate, respectively, administered doses were within 10% of the TD<sub>50</sub>. However, nafenopin data were only available at a single dose of 500 ppm ([Lake et al., 1993](#)), which was linearly interpolated to the TD<sub>50</sub>. The highest administered dose of DEHP was 12,500 ppm ([David et al., 1999](#)), a dose notably below the TD<sub>50</sub>, and thus a lower limit based on the assumption of monotonicity with dose is shown. A further data limitation is that in the CPDB, only the TD<sub>50</sub> for one of the four compounds, DEHP, incorporates data from studies administering more than one dose for two years.

The results shown in Table 1 [reproduced as Table 4-67] indicate that potency for the occurrence of short-term in vivo markers of PPAR- $\alpha$  activation varies widely in magnitude and lacks any apparent correlation with carcinogenic potency. Such differences have been noted previously. Similar to the results presented in Table 1 [reproduced as Table 4-67], Marsman et al. ([1988](#)) noted that although DEHP (12,000 ppm) and Wy-14,643 (1,000 ppm) induced a similar extent of hepatomegaly and peroxisome proliferation (measured either morphologically or biochemically) after 1 year, the frequency of hepatocellular lesions was over 100-fold higher in Wy-14,643- relative to DEHP-exposed rats.

In addition, a higher labeling index was reported for 12,500 ppm DEHP than the maximal level attained after 50–1,000 ppm Wy-14,643 ([David et al., 1999](#); [Tanaka et al., 1992](#); [Wada et al., 1992](#)). We did not examine such differences in maximal responses in our analysis. We also do not present differences in response with dose and time seen among PPAR- $\alpha$  agonists, which are prominent enough to prevent displaying dose-response data on a common scale. For instance, labeling index is increased in a dose-dependent manner at 1 week by clofibrate (1,500, 4,500 and 9,000 ppm) but is decreased compared with controls at 13 weeks at the two higher doses ([Tanaka et al., 1992](#)). Together, these findings underscore the significant chemical-specific quantitative differences in these markers that limit their utility for predicting carcinogenic dose-response relationships.

**Table 4-66. Potency indicators for mouse hepatocarcinogenicity and in vitro transactivation of mouse PPAR $\alpha$  for four PPAR $\alpha$  agonists**

Chemical	Carcinogenic potency indicators (mg/kg-d)	Transactivation potency indicators ( $\mu$ M)	
	TD <sub>50</sub>	EC <sub>50</sub>	EC <sub>twofold</sub>
Hepatocarcinogens			
Wy-14,643	<10.8	0.63	~0.4
DCA	119	~300	~300
TCA	584	~300	~300
DEHP/MEHP	700	~0.7	~0.7

Note: TD<sub>50</sub> = the daily dose inducing tumors in half of the mice that would otherwise have remained tumor-free, estimated from the Carcinogenic Potency Database ([Gold et al., 2005](#)). EC<sub>50</sub> = the effective concentration yielding 50% of the maximal response; EC<sub>twofold</sub> = the effective concentration required for a twofold increase in activity. Transactivation potencies were estimated from Maloney and Waxman ([1999](#)). The “<” symbol denotes an upper limit due to maximal response. A “~” symbol indicates that the transactivation potency was approximated from figures in Maloney and Waxman ([1999](#)).

MEHP = monoethylhexyl phthalate

Source: reproduced from Table 2 of Guyton et al. ([2009](#)).

**Table 4-67. Potency indicators for rat hepatocarcinogenicity and common short-term markers of PPAR $\alpha$  activation for four PPAR $\alpha$  agonists**

Chemical	Tumor TD <sub>50</sub> (ppm in diet)	Fold-increase over control at tumor TD <sub>50</sub>					
		1 wk			13 wks		
		RLW	LI	PCO	RLW	LI	PCO
Wy-14,643	109	1.8	12	13	2.6	6.8	39
Nafenopin	275	1.4	3.6	7.6	1.5	1.12	6.7
Clofibrate	4.225	1.4	4.4	4.2	1.4	0.95	3.7
DEHP	17.900	≥1.4	≥19	≥3.6	≥1.9	≥1.25	≥4.9

Note: For ease of comparison with precursor effect studies, administered doses for the tumor TD<sub>50</sub>s in the Carcinogenic Potency Database were back-converted to equivalent ppm in diet using the formula of Gold et al. (2005), *i.e.*, TD<sub>50</sub> (mg/kg-day) = TD<sub>50</sub>(ppm in diet) × 0.04 (for male rats). Administered doses for precursor data on Wy-14,643 (Wada et al., 1992) and clofibrate (Tanaka et al., 1992) were within 10% of the TD<sub>50</sub>. Because nafenopin precursor data were only available at 0 and 500 ppm (Lake et al., 1993), these doses were linearly interpolated to the TD<sub>50</sub>. Because the highest administered dose of DEHP in precursor effect studies was 12,500 ppm (David et al., 1999), a lower limit is shown, based on the assumption of monotonicity with dose.

RLW = relative liver weight, LI = labeling index, PCO = cyanide insensitive palmitoyl CoA oxidation

Source: reproduced from Table 1 of Guyton et al. (2009).

#### **4.5.7.3. Additional Proposed Hypotheses and Key Events with Limited Evidence or Inadequate Experimental Support**

Several effects that been hypothesized to be associated with liver cancer induction are discussed in more detail below, including increased liver weight, DNA hypomethylation, and pathways involved in glycogen accumulation such as insulin signaling proteins. As discussed above, TCE and its metabolites reportedly increase nuclear size and ploidy in hepatocytes, and these effects likely account for much of the increases in labeling index and DNA synthesis caused by TCE. Importantly, these changes appear to persist with cessation of treatment, with liver weights, but not nuclear sizes, returning to control levels (Kjellstrand et al., 1983a). In addition, glycogen deposition, DNA synthesis, increases in mitosis, or peroxisomal enzyme activity do not appear correlated with TCE-induced liver weight changes.

##### **4.5.7.3.1. Increased liver weight**

Increased liver weight or liver/body weight ratios (hepatomegaly) is associated with increased risk of liver tumors in rodents, but it is relatively nonspecific (Allen et al., 2004). The evidence presented above for TCE and its metabolites suggest a similarity in dose-response between liver weight increases at short-term durations of exposure and liver tumor induction observed from chronic exposure. Liver weight increases may results from several concurrent processes that have been associated with increase cancer risk (e.g., hyperplasia, increased ploidy, and glycogen accumulation) and when observed after chronic exposure may result from the

increased presence of foci and tumors themselves. Therefore, there are inadequate data to adequately define a mode of action hypothesis for hepatocarcinogenesis based on liver weight increases.

#### 4.5.7.3.2. “Negative selection”

As discussed above, TCE, TCA, and DCA all cause transient increases in DNA synthesis. This DNA synthesis has been assumed to result from proliferation of hepatocytes. However, the dose-related TCA- and DCA-induced increases in liver weight not correlate with patterns of DNA synthesis; moreover, there have been reports that DNA synthesis in individual hepatocytes does not correlate with whole liver DNA synthesis measures ([Carter et al., 1995](#); [Sanchez and Bull, 1990](#)). With continued treatment, decreases in DNA synthesis have been reported for DCA ([Carter et al., 1995](#)). More importantly, several studies show that transient DNA synthesis is confined to a very small population of cells in the liver in mice exposed to TCE for 10 days or to DCA or TCA for up to 14 days of exposure. Therefore, generalized mitogenic stimulation is not likely to play a role in TCE-induced liver carcinogenesis.

Bull ([2000](#)) has proposed that the TCE metabolites TCA and DCA may contribute to liver tumor induction through so-called “negative selection” by way of several possible processes. First, it is hypothesized that the mitogenic stimulation by continued TCA and DCA exposure is downregulated in normal hepatocytes, conferring a growth advantage to initiated cells that either do not exhibit the downregulation of response or are resistant to the downregulating signals. This is implausible as both the normal rates of cell division in the liver and the TCE-stimulated increases are very low. Polyploidization has been reported to decrease the normal rates of cell division even further. That the transient and relatively low level of DNA synthesis reported for TCE, DCA, and TCA is reflective of proliferation rather than polyploidization is not supported by data on mitosis. A mechanism for such “downregulation” has not been identified experimentally.

A second proposed contributor to “negative-selection” is direct enhancement by TCA and DCA in the growth of certain populations of initiated cells. While differences in phenotype of end stage tumors have been reported between DCA and TCA, the role of selection and emergence of potentially different foci has not been elucidated. Neither have pathway perturbations been identified that are common to liver cancer in human and rodent for TCE, DCA, and TCA. The selective growth of clones of hepatocytes that may progress fully to cancer is a general feature of cancer and not specific to at TCE, TCA, or DCA mode of action.

A third proposed mechanism by which TCE may enhance liver carcinogenesis within this “negative selection” paradigm is through changing apoptosis. However, as stated above, TCE has been reported to either not change apoptosis or to cause a slight increase at high doses. Rather than increases in apoptosis, peroxisome proliferators have been suggested to inhibit apoptosis as part of their carcinogenic mode of action. However, the age and species studied

appear to greatly affect background rates of apoptosis ([Snyder et al., 1995](#)) with the rat having a greater rate of apoptosis than the mouse. DCA has been reported to induce decreases in apoptosis in the mouse ([Carter et al., 1995](#); [Snyder et al., 1995](#)). However, the significance of the DCA-induced reduction in apoptosis, from a level that is already inherently low in the mouse, for the mode of action for induction of DCA-induced liver cancer is difficult to discern.

Therefore, for a mode of action for hepatocarcinogenesis based on “negative selection,” there are inadequate data to adequately define the mode-of-action hypothesis, or the available data do not support such a mode of action being operative.

#### **4.5.7.3.3. Polyploidization**

Polyploidization may be an important key event in tumor induction. For example, in addition to TCE, partial hepatectomy, nafenopin, methyclofenopate, DEHP, diethylnitrosamine, *N*-nitrosomorpholine, and various other exposures that contribute to liver tumor induction also shift the hepatocyte ploidy distribution to be increasingly diploid or polyploid ([Hasmall and Roberts, 2000](#); [Miller et al., 1996](#); [Vickers and Lucier, 1996](#); [Melchiorri et al., 1993](#); [Styles et al., 1988](#)). As discussed by Gupta (2000), “[w]orking models indicate that extensive polyploidy could lead to organ failure, as well as to oncogenesis with activation of precancerous cell clones.” However, the mechanism(s) by which increased polyploidy enhances carcinogenesis is not currently understood. Due to increased DNA content, polyploid cells will generally have increased gene expression. However, polyploid cells are considered more highly differentiated and generally divide more slowly and are more likely to undergo apoptosis, perhaps thereby indirectly conferring a growth advantage to initiated cells (see Section E.1). Of note is that changes in ploidy have been observed in transgenic mouse models that are also prone to develop liver cancer (see Section E.3.3.1). It is likely that polyploidization occurs with TCE exposure and it is biologically plausible that polyploidization can contribute to liver carcinogenesis, although the mechanism(s) is (are) not known. However, whether polyploidization is necessary for TCE-induced carcinogenesis is not known, as no experiment in which polyploidization specifically is blocked or diminished has been performed and the extent of polyploidization has not been quantified. Therefore, there are inadequate data to adequately define a mode-of-action hypothesis for hepatocarcinogenesis based on polyploidization.

#### **4.5.7.3.4. Glycogen storage**

As discussed above, several studies have reported that DCA causes accumulation of glycogen in mouse hepatocytes. Such glycogen accumulation has been suggested to be pathogenic, as it is resistant to mobilization by fasting ([Kato-Weinstein et al., 1998](#)). In humans, glycogenesis due to glycogen storage disease or poorly controlled diabetes has been associated with increased risk of liver cancer ([Rake et al., 2002](#); [Wideroff et al., 1997](#); [Adami et al., 1996](#);

[La Vecchia et al., 1994](#)). Glycogen accumulation has also been reported to occur in rats exposed to DCA.

For TCE exposure in mice or rats, glycogen content of hepatocytes has been reported to be somewhat less than or the same as controls, or not remarked upon in the studies. TCA exposure has been reported to decrease glycogen content in rodent hepatocytes while DCA has been reported to increase it ([Kato-Weinstein et al., 2001](#)). There is also evidence that DCA-induced increases in glycogen accumulation are not proportional to liver weight increases and only account for a relatively small portion of increases in liver mass. DCA-induced increases in liver weight are not a function of cellular proliferation but probably include hypertrophy associated with polyploidization, increased glycogen deposition, and other factors.

While not accounting for increases in liver weight, excess glycogen can still not only be pathogenic, but also a predisposing condition for hepatocarcinogenesis. Some hypotheses regarding the possible relationship between glycogenesis and carcinogenesis have been posed that lend them biological plausibility. Evert et al. ([2003](#)), using an animal model of hepatocyte exposure to a local hyperinsulinemia from transplanted islets of Langerhans with remaining tissue is hypoinsulinemic, reported that insulin induces alterations resembling preneoplastic foci of altered hepatocytes that develop into hepatocellular tumors in later stages of carcinogenesis. Lingohr et al. ([2001](#)) suggested that normal hepatocytes downregulate insulin-signaling proteins in response to the accumulation of liver glycogen caused by DCA and that the initiated cell population, which does not accumulate glycogen and is promoted by DCA treatment, responds differently from normal hepatocytes to the insulin-like effects of DCA. Bull et al. ([2002](#)) reported increased insulin receptor protein expression in tumor tissues regardless of whether they were induced by TCE, TCA, or DCA. Given the greater activity of DCA relative to TCA on carbohydrate metabolism, it is unclear whether changes in these pathways are causes or simply reflect the effects of tumor progression. Therefore, it is biologically plausible that changes in glycogen status may occur from the opposing actions of TCE metabolites, but changes in glycogen content due to TCE exposure has not been quantitatively studied. The possible contribution of these effects to TCE-induced hepatocarcinogenesis is unclear. Therefore, there are inadequate data to adequately define a mode-of-action hypothesis for TCE-induced hepatocarcinogenesis based on changes in glycogen storage or even data to support increased glycogen storage to result from TCE exposure.

#### **4.5.7.3.5. Inactivation of GST-zeta**

DCA has been shown to inhibit its own metabolism in that pretreatment in rodents prior to a subsequent challenge dose leads to a longer biological half-life ([Schultz et al., 2002](#)). This self-inhibition is hypothesized to occur through inactivation of GST-zeta ([Schultz et al., 2002](#)). In addition, TCE has been shown to cause the same prolongation of DCA half-life in rodents, suggesting that TCE inhibits GST-zeta, probably through the formation of DCA ([Schultz et al.,](#)

[2002](#)). DCA-induced inhibition of GST-zeta has also been reported in humans, with GST-zeta polymorphisms reported to influence the degree of inactivation ([Blackburn et al., 2001](#); [Blackburn et al., 2000](#); [Tzeng et al., 2000](#)). Board et al. ([2001](#)) reported one variant to have significantly higher activity with DCA as a substrate than other GST-zeta isoforms, which could affect DCA susceptibility.

GST-zeta, which is identical to maleylacetoacetate isomerase, is part of the tyrosine catabolism pathway, which is disrupted in Type 1 hereditary tyrosinemia, a disease associated with the development of HCC at a young age ([Tanguay et al., 1996](#)). In particular, GST-zeta metabolizes maleylacetoacetate (MAA) to fumarylacetoacetate (FAA) and maleylacetone (MA) to fumarylacetone ([Cornett et al., 1999](#); [Tanguay et al., 1996](#)). It has been suggested that the increased cancer risk with this disease, as well as through DCA exposure, results from accumulation of MAA and MA, both alkylating agents, or FAA, which displays apoptogenic, mutagenic, aneugenic, and mitogenic activities ([Bergeron et al., 2003](#); [Jorquera and Tanguay, 2001](#); [Kim et al., 2000](#); [Cornett et al., 1999](#); [Tanguay et al., 1996](#)). However, the possible effects of DCA through this pathway will depend on whether MAA, MA, or FAA is the greater risk factor, since inhibition of GST-zeta will lead to greater concentrations of MAA and MA and lower concentrations of FAA. Therefore, if MAA is the more active agent, then DCA may increase carcinogenic risk, while if FAA is the more active, then DCA may decrease carcinogenic risk. Tzeng et al. ([2000](#)) proposed the latter based on the greater genotoxicity of FAA, and in fact suggested that DCA may “merit consideration for trial in the clinical management of hereditary tyrosinemia type 1.”

Therefore, TCE-induced inactivation GST-zeta, probably through formation of DCA, may play a role in TCE-induced hepatocarcinogenesis. However, this mode of action is not sufficiently delineated at this point for further evaluation, as even the question of whether its actions through this pathway may increase or decrease cancer risk has yet to be experimentally tested.

#### **4.5.7.3.6. Oxidative stress**

Several studies have attempted to study the possible effects of “oxidative stress” and DNA damage resulting from TCE exposures. The effects of induction of metabolism by TCE, as well as through co-exposure to ethanol, have been hypothesized to increase levels of “oxidative stress” as a common effect for both exposures (see Section E.4.3.4). In terms of contributing to a carcinogenic mode of action, the term “oxidative stress” is a somewhat nonspecific term, as it is implicated as part of the pathophysiologic events in a multitude of disease processes and is part of the normal physiologic function of the cell and cell signaling. Commonly, it appears to refer to the formation of reactive oxygen species leading to cellular or DNA damage. As discussed above, however, measures of oxidative stress induced by TCE, TCA, and DCA appear to be either not apparent, or at the very most, transient and nonpersistent with continued treatment

([Toraason et al., 1999](#); [Channel et al., 1998](#); [Parrish et al., 1996](#); [Larson and Bull, 1992b](#)).

Therefore, while the available data are limited, there is insufficient evidence to support a role for such effects in TCE-induced liver carcinogenesis.

Oxidative stress has been hypothesized to be part of the mode of action for peroxisome proliferators, but has been found to be correlated with neither cell proliferation nor carcinogenic potency of peroxisome proliferators (see Section E.3.4.1.1). For instance, Parrish et al. ([1996](#)) reported that increases in PCO activity noted for DCA and TCA were not associated with 8-OHdG levels (which were unchanged) and also not with changes laurate hydrolase activity observed after either DCA or TCA exposure. The authors concluded that their data do not support an increase in steady-state oxidative damage to be associated with TCA initiation of cancer and that extension of treatment to time periods sufficient to insure peroxisome proliferation failed to elevate 8-OHdG in hepatic DNA. The authors thus, suggested that peroxisome proliferative properties of TCA were not linked to oxidative stress or carcinogenic response.

#### **4.5.7.3.7. Changes in gene expression (e.g., hypomethylation)**

Studies of gene expression as well as considerations for interpretation of studies of using the emerging technologies of DNA, siRNA, and miRNA microarrays for mode-of-action analyses are included in Sections E.3.1.2 and E.3.4.2.2. Caldwell and Keshava ([2006](#)) and Keshava and Caldwell ([2006](#)) report on both genetic expression studies and studies of changes in methylation status induced by TCE and its metabolites as well as differences and difficulties in the patterns of gene expression between differing PPAR $\alpha$  agonists. In particular are concerns for the interpretation of studies that employ pooling of data as well as interpretation of “snapshots in time of multiple gene changes.” For instance, in the Laughter et al. ([2004](#)) study, it is not clear whether transcription arrays were performed on pooled data as well as the issue of phenotypic anchoring as data on percentage liver/body weight indicates significant variability within TCE treatment groups, especially in PPAR $\alpha$ -null mice. For studies of gene expression using microarrays Bartosiewicz et al. ([2001](#)) used a screening analysis of 148 genes for xenobiotic-metabolizing enzymes, DNA repair enzymes, heat shock proteins, cytokines, and housekeeping gene expression patterns in the liver in response TCE. The TCE-induced gene induction was reported to be highly selective; only Hsp 25 and 86 and CYP were upregulated at the highest dose tested. Collier et al. ([2003](#)) reported differentially expressed mRNA transcripts in embryonic hearts from Sprague-Dawley rats exposed to TCE with sequences downregulated with TCE exposure appearing to be those associated with cellular housekeeping, cell adhesion, and developmental processes. TCE was reported to induce upregulated expression of numerous stress-response and homeostatic genes.

For the Laughter et al. ([2004](#)) study, transcription profiles using macroarrays containing approximately 1,200 genes were reported in response to TCE exposure with 43 genes reported to

be significantly altered in the TCE-treated wild-type mice and 67 genes significantly altered in the TCE-treated PPAR $\alpha$  knockout mice. However, the interpretation of this information is difficult because in general, PPAR $\alpha$  knockout mice have been reported to be more sensitive to a number of hepatotoxins partly because of defects in the ability to effectively repair tissue damage in the liver ([Shankar et al., 2003](#); [Mehendale, 2000](#)) and because a comparison of gene expression profiles between controls (wild-type and PPAR $\alpha$  knockout) were not reported. As reported by Voss et al. ([2006](#)), dose-, time course-, species-, and strain-related differences should be considered in interpreting gene array data. The comparison of differing PPAR $\alpha$  agonists presented in Keshava and Caldwell ([2006](#)) illustrates the pleiotropic and varying liver responses of the PPAR $\alpha$  receptor to various agonists, but did not imply that these responses were responsible for carcinogenesis.

As discussed in Section E.3.3.5, aberrant DNA methylation is a common hallmark of all types of cancers, with hypermethylation of the promoter region of specific tumor suppressor genes and DNA repair genes leading to their silencing (an effect similar to their mutation) and genome-wide hypomethylation ([Pereira et al., 2004b](#); [Ballestar and Esteller, 2002](#); [Berger and Daxenbichler, 2002](#); [Rhee et al., 2002](#); [Herman et al., 1998](#)). Whether DNA methylation is a consequence or cause of cancer is a long-standing issue ([Ballestar and Esteller, 2002](#)). Fraga et al. ([2005](#); [2004](#)) reported global loss of monoacetylation and trimethylation of histone H4 as a common hallmark of human tumor cells; they suggested, however, that genomewide loss of 5-methylcytosine (associated with the acquisition of a transformed phenotype) exists not as a static predefined value throughout the process of carcinogenesis but rather as a dynamic parameter (i.e., decreases are seen early and become more marked in later stages).

DNA methylation is a naturally occurring epigenetic mechanism for modulating gene expression, and disruption of this mechanism is known to be relevant to human carcinogenesis. As reviewed by Calvisi et al. ([2007](#)),

[a]berrant DNA methylation occurs commonly in human cancers in the forms of genome-wide hypomethylation and regional hypermethylation. Global DNA hypomethylation (also known as demethylation) is associated with activation of protooncogenes, such as c-Jun, c-Myc, and c-HA-Ras, and generation of genomic instability. Hypermethylation on CpG islands located in the promoter regions of tumor suppressor genes results in transcriptional silencing and genomic instability.

While clearly associated with cancer, it has not been conclusively established whether these epigenetic changes play a causative role or are merely a consequence of transformation ([Tryndyak et al., 2006](#)). However, as Calvisi et al. ([2007](#)) note, “Current evidence suggests that hypomethylation might promote malignant transformation via multiple mechanisms, including chromosome instability, activation of protooncogenes, reactivation of transposable elements, and loss of imprinting.”

Although little is known about how it occurs, a hypothesis has also been proposed that that the toxicity of TCE and its metabolites may arise from its effects on DNA methylation status. In regard to methylation studies, many are co-exposure studies as they have been conducted in initiated animals with some studies being very limited in their reporting and conduct. Caldwell and Keshava (2006) reviewed the body of work regarding TCE, DCA, and TCA. Methionine status has been noted to affect the emergence of liver tumors (Counts et al., 1996). Tao et al. (2000) and Pereira et al. (2004a) have studied the effects of excess methionine in the diet to see if it has the opposite effects as a deficiency (i.e., a reduction in a carcinogenic response rather than enhancement). However, Tao et al. (2000) reported that the administration of excess methionine in the diet is not without effect and can result in percentage liver/body weight ratios. Pereira et al. (2004a) reported that methionine treatment alone at the 8 g/kg level increased liver weight, decreased lauryl-CoA activity, and increased DNA methylation.

Pereira et al. (2004a) reported that very high levels of methionine supplementation to an AIN-760A diet affected the number of foci and adenomas after 44 weeks of co-exposure to 3.2 g/L DCA. However, while the highest concentration of methionine (8.0 g/kg) was reported to decrease both the number of DCA-induced foci and adenomas, the lower level of methionine co-exposure (4.0 g/kg) increased the incidence of foci. Co-exposure of methionine (4.0 or 8.0 g/kg) with 3.2 g/L DCA was reported to decrease by ~25% DCA-induced glycogen accumulation, increase mortality, but not to have much of an effect on peroxisome enzyme activity (which was not elevated by >33% over control for DCA exposure alone). The authors suggested that their data indicate that methionine treatment slowed the progression of foci to tumors. Given that increasing hypomethylation is associated with tumor progression, decreased hypomethylation from large doses of methionine are consistent with a slowing of progression. Whether these results would be similar for lower concentrations of DCA and lower concentrations of methionine that were administered to mice for longer durations of exposure cannot be ascertained from these data. It is possible that in a longer-term study, the number of tumors would be similar. Finally, a decrease in tumor progression by methionine supplementation is not shown to be a specific event for the mode of action for DCA-induced liver carcinogenicity.

Tao et al. (2000) reported that 7 days of gavage dosing of TCE (1,000 mg/kg in corn oil), TCA (500 mg/kg, neutralized aqueous solution), and DCA (500 mg/kg, neutralized aqueous solution) in 8-week-old female B6C3F<sub>1</sub> mice resulted in not only increased liver weight, but also increased hypomethylation of the promoter regions of c-Jun and c-Myc genes in whole-liver DNA. However, data were shown for 1–2 mice per treatment. Treatment with methionine was reported to abrogate this response only at a 300 mg/kg i.p dose, with 0–100 mg/kg doses of methionine having no effect. Ge et al. (2001a) reported DCA- and TCA-induced DNA hypomethylation and cell proliferation in the liver of female mice at 500 mg/kg and decreased methylation of the c-Myc promoter region in liver, kidney, and urinary bladder. However,

increased cell proliferation preceded hypomethylation. Ge et al. (2002) also reported hypomethylation of the c-Myc gene in the liver after exposure to the peroxisome proliferators 2,4-dichlorophenoxyacetic acid (1,680 ppm), DBP (20,000 ppm), gemfibrozil (8,000 ppm), and Wy-14,643 (50–500 ppm, with no effect at 5 or 10 ppm) after 6 days in the diet. Caldwell and Keshava (2006) concluded that hypomethylation did not appear to be a chemical-specific effect at these concentrations. As noted Section E.3.3.5, chemical exposure to a number of differing carcinogens have been reported to lead to progressive loss of DNA methylation.

After initiation by *N*-methyl-*N*-nitrosourea (25 mg/kg) and exposure to 20 mmol/L DCA or TCA (46 weeks), Tao et al. (2004a) report similar hypomethylation of total mouse liver DNA by DCA and TCA with tumor DNA showing greater hypomethylation. A similar effect was noted for the differentially methylated region-2 of the insulin-like growth factor-II (IGF-II) gene. The authors suggest that hypomethylation of total liver DNA and the IGF-II gene found in nontumorous liver tissue would appear to be the result of a more prolonged activity and not cell proliferation, while hypomethylation of tumors could be an intrinsic property of the tumors. As pointed out by Caldwell and Keshava (2006), overexpression of IGF-II gene in liver tumors and preneoplastic foci has been shown in both animal models of hepatocarcinogenesis and humans, and may enhance tumor growth, acting via the overexpressed IGF-I receptor (Scharf et al., 2001; Werner and Le Roith, 2000).

Diminished hypomethylation was observed in Wy-14643-treated PPAR $\alpha$ -null mice as compared to wild-type mice, suggestive of involvement of PPAR $\alpha$  in mediating hypomethylation (Pogribny et al., 2007), but it is unclear how relevant these results are to TCE and its metabolites. First, the doses of Wy-14643 administered are associated with substantial liver necrosis and mortality with long-term treatment (Woods et al., 2007a), adding confounding factors to the interpretation of their results. Hypomethylation by Wy-14643 progressively increased with time up to 5 months (Pogribny et al., 2007), consistent with the sustained DNA synthesis caused by Wy-14643 and a role for proliferation in causing hypomethylation. Regardless, as discussed above, it is unlikely that PPAR $\alpha$  is the mediator of the observed transient increase in DNA synthesis by DCA, so even if it is important for hypomethylation by TCA, there may be more than one pathway for this effect.

To summarize, aberrant DNA methylation status, including hypomethylation, is clearly associated with both human and rodent carcinogenesis. Hypomethylation itself appears to be sufficient for carcinogenesis, as diets deficient in choline and methionine that induce hypomethylation have been shown to cause liver tumors in both rats and mice (Henning and Swendseid, 1996; Wainfan and Poirier, 1992; Ghoshal and Farber, 1984; Mikol et al., 1983). However, it is not known to what extent hypomethylation is necessary for TCE-induced carcinogenesis. However, as noted by Bull (2004a) and Bull et al. (2004), the doses of TCA and DCA that have been tested for induction of hypomethylation are quite high compared to doses at which tumor induction occurs—at least 500 mg/kg-day. Whether these effects are still manifest

at lower doses relevant to TCE carcinogenicity, particularly with respect to DCA, has not been investigated. Finally, the role of PPAR $\alpha$  in modulating hypomethylation, possibly through increased DNA synthesis as suggested by experiments with Wy-14643, are unknown for TCE and its metabolites.

#### **4.5.7.3.8. Cytotoxicity**

Cytotoxicity and subsequent induction of reparative hyperplasia have been proposed as key events for a number of chlorinated solvents, such as chloroform and carbon tetrachloride. However, as discussed above and discussed by Bull ([2004a](#)) and Bull et al. ([2004](#)), TCE treatment at doses relevant to liver carcinogenicity results in relatively low cytotoxicity. While a number of histological changes with TCE exposure are observed, in most cases necrosis is minimal or mild, associated with vehicle effects, and with relatively low prevalence. This is consistent with the low prevalence of necrosis observed with TCA and DCA treatment at doses relevant to TCE exposure. Therefore, it is unlikely that cytotoxicity and reparative hyperplasia play a significant role in TCE carcinogenicity

#### 4.5.7.4. Mode-of-Action Conclusions

The conclusions regarding the mode of action for TCE-induced liver carcinogenesis described in the preceding sections are summarized in Table 4-68. Overall, although a role for many of the proposed key events discussed above cannot be ruled out, there are inadequate data to support the conclusion that any of the particular mode-of-action hypotheses reviewed above are operant. The available data do suggest that the mode of action of liver tumors induced by TCE is complex, as it is likely that key events from several pathways may operate. Nonetheless, because a collection of key events sufficient to induce liver tumors has not been identified, the answer to the first key question “*1. Is the hypothesized mode of action sufficiently supported in the test animals?*” is “no” at this time. Consequently, the other key questions of “*2. Is the hypothesized mode of action relevant to humans?*” and “*3. Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?*” will not be discussed in a mode-of-action-specific manner. Rather, they are discussed below in more general terms, first qualitatively and then quantitatively, using available relevant data.

##### 4.5.7.4.1. Qualitative human relevance and susceptibility

No data exist that suggest that TCE-induced liver tumorigenesis is caused by processes that are irrelevant in humans. In addition, as discussed above, several of the other effects such as polyploidization, changes in glycogen storage, and inhibition of GST-zeta—are either clearly related to human carcinogenesis or areas of active research as to their potential roles. For example, the effects of DCA on glycogen storage parallel the observation that individuals with conditions that lead to glycogenesis appear to be at an increased risk of liver cancer ([Rake et al., 2002](#); [Wideroff et al., 1997](#); [Adami et al., 1996](#); [La Vecchia et al., 1994](#)). In addition, there may be some relationship between the effects of DCA and the mechanism of increased liver tumor risk in childhood in those with Type 1 hereditary tyrosinemia, though the hypotheses needs to be tested experimentally. Similarly, with respect to PPAR $\alpha$  activation and downstream events hypothesized to be causally related to liver carcinogenesis, it is generally acknowledged that “a point in the rat/mouse key events cascade where the pathway is biologically precluded in humans cannot be identified, in principle” ([NRC, 2006](#); [Klaunig et al., 2003](#)).

In terms of human relevance and susceptibility, it is also useful to briefly review what is known about human HCC. A number of risk factors have been identified for human HCC, including ethanol consumption, hepatitis B and C virus infection, aflatoxin B1 exposure, and, more recently, diabetes and perhaps obesity ([El-Serag and Rudolph, 2007](#)). However, it is also estimated that a substantial minority of HCC patients, perhaps 15–50%, have no established risk factors ([El-Serag and Rudolph, 2007](#)). In addition, cirrhosis is present in a large proportion of HCC patients, but the prevalence of HCC without underlying cirrhosis, while not precisely known, is still significant, with estimates based on relatively small samples ranging from 7 to 54% ([Fattovich et al., 2004](#)).

**Table 4-68. Summary of mode-of-action conclusions for TCE-induced liver carcinogenesis**

Hypothesized MOA postulated key events	Experimental support	Human relevance	Weight-of-evidence conclusion
<b>Mutagenicity (Section 4.5.7.1)</b>			
<p><i>One or more oxidative metabolites produced in situ or delivered systemically to liver.</i></p>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>Multiple in vitro and in vivo studies demonstrate oxidation of TCE, and availability to the liver (see Section 3.3.2).</li> <li>CH is a short-lived intermediate that is rapidly converted to TCA and TCOH.</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>Based on analogy to demonstration that oxidation is necessary for non-cancer effects in the liver. No TCE-specific data.</li> </ul>	<p>Yes: demonstrated in humans in vivo and in human cells in vitro.</p>	<p>Known that both human and rodent liver are exposed to the oxidative metabolites. CH is a short-lived intermediate, whereas TCA and TCOH are more stable.</p>
<p><i>Mutagenicity induced by oxidative metabolites advances acquisition of the multiple critical traits contributing to carcinogenesis.</i></p>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>In rodents, TCE binds to and/or induces damage in DNA and chromosome structure.</li> <li>TCE has a limited ability to induce mutation in bacterial systems, even with metabolic activation that produce oxidative metabolites.</li> <li>Oxidative metabolites, particularly CH, can cause a variety of genotoxic effects (including aneuploidy) in available in vitro and in vivo assays (see Section 4.2.1.5).</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>No TCE-specific data.</li> </ul>	<p>Yes: no basis for discounting in vitro or in vivo genotoxicity results.</p>	<p>Evidence for mutagenicity through CH is the strongest, but difficult to assess genotoxic contributions from nongenotoxic contributions from CH and other oxidative metabolites.</p>
<p><i>Overall Conclusion</i></p>	<p><i>Sufficiency of MOA for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>Mutagenicity is assumed to cause cancer, as a sufficient cause.</li> </ul>	<p>Yes: well established.</p>	<p>Data are inadequate to support a conclusion that a mutagenic MOA mediated by CH is operant; however, a mutagenic MOA, mediated either by CH or other oxidative metabolites of TCE, cannot be ruled out.</p>

**Table 4-68 Summary of mode-of-action conclusions for TCE-induced liver carcinogenesis (continued)**

Hypothesized MOA postulated key events	Experimental support	Human relevance	Weight-of-evidence conclusion
<b>Peroxisome proliferation activated receptor alpha activation (Section 4.5.7.2)</b>			
<ul style="list-style-type: none"> <li>• TCE oxidative metabolites (e.g., TCA), after being produced in the liver, activate PPARα in the liver.</li> <li>• PPARα activation leads to alterations in cell proliferation and apoptosis in the liver.</li> <li>• Alterations in cell proliferation and apoptosis causes clonal expansion of initiated cells.</li> <li>• Increased number of initiated cells causes cancer.</li> </ul>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>• Multiple in vitro and in vivo studies demonstrate oxidation of TCE, and availability of metabolites TCA and DCA to the liver (see Section 3.3.2).</li> <li>• TCE, TCA and DCA activate PPARα, induce peroxisome proliferation and hepatocyte proliferation in mice and rats (e.g., <a href="#">DeAngelo et al., 2008</a>; <a href="#">Laughter et al., 2004</a>; <a href="#">Nakajima et al., 2000</a>; <a href="#">Watanabe and Fukui, 2000</a>; <a href="#">Stauber and Bull, 1997</a>; <a href="#">Pereira, 1996</a>; <a href="#">Dees and Travis, 1994</a>; <a href="#">Goel et al., 1992</a>; <a href="#">Sanchez and Bull, 1990</a>; <a href="#">Goldsworthy and Popp, 1987</a>; <a href="#">Elcombe et al., 1985</a>).</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• No studies of TCE or its metabolites (e.g., cancer bioassays in PPARα-null mice). TCE induces increases in liver weight in male and female mice lacking a functional PPARα receptor (<a href="#">Ramdhan et al., 2010</a>; <a href="#">Nakajima et al., 2000</a>) and in humanized null mice (<a href="#">Ramdhan et al., 2010</a>). Liver tumor response from WY dramatically diminished in PPARα-null mice (<a href="#">Peters et al., 1997</a>); however, liver tumor response from DEHP unchanged in PPARα-null mice (<a href="#">Ito et al., 2007</a>). Thus, inferences regarding TCE are not possible.</li> </ul>	<p>Yes. Humans produce oxidative metabolites of TCE, PPARα is present in the human liver.</p>	<p>Highly likely that PPARα is activated in the liver, but it is unlikely that PPARα agonism and its sequelae constitute the sole or predominant MOA for TCE-induced carcinogenesis.</p>
<p><i>Overall</i></p>	<p><i>Sufficiency of MOA for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• No TCE-specific studies; PPARα activation in a transgenic mouse model caused all the key events in the MOA, but not carcinogenesis, suggesting that the MOA is not sufficient for carcinogenesis (<a href="#">Yang et al., 2007</a>). Consistent with hypothesis that TCE liver carcinogenesis involves multiple mechanisms.</li> </ul>	<p>Yes. No evidence to suggest that key events are implausible in humans.</p>	

**Table 4-68 Summary of mode-of-action conclusions for TCE-induced liver carcinogenesis (continued)**

Hypothesized MOA postulated key events	Experimental support	Human relevance	Weight-of-evidence conclusion
<b>Liver weight increases (Section 4.5.7.3.1)</b>			
<ul style="list-style-type: none"> <li><i>TCE oxidative metabolites, after being produced in the liver, cause liver weight increases.</i></li> <li><i>Further key events not specified.</i></li> </ul>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>Multiple in vitro and in vivo studies demonstrate oxidation of TCE, and availability of metabolites TCA and DCA to the liver (see Section 3.3.2).</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>No studies of TCE or its metabolites.</li> </ul> <p><i>Sufficiency of MOA for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>Hypothesis is inadequately specified for evaluation.</li> </ul>	<p>Yes. Humans produce oxidative metabolites of TCE. No evidence that liver weight changes would not occur in humans.</p>	<p>Data are inadequate to define a MOA hypothesis for hepatocarcinogenesis based on liver weight increases.</p>
<b>Negative selection (Section 4.5.7.3.2)</b>			
<ul style="list-style-type: none"> <li><i>“Negative selection” confers a growth advantage to initiated cells.</i></li> <li><i>Increased number of initiated cells causes cancer.</i></li> </ul>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>Transient DNA synthesis is confined to a very small population of cells in mouse liver (e.g., <a href="#">Laughter et al., 2004</a>; <a href="#">Dees and Travis, 1993</a>; <a href="#">Elcombe et al., 1985</a>), but no data on whether this effect is “selective” of initiated cells.</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>No studies of TCE or its metabolites.</li> </ul> <p><i>Sufficiency of MOA for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>No studies of TCE or its metabolites.</li> </ul>	<p>Yes. Humans produce oxidative metabolites of TCE. No evidence that negative selection would not occur in humans.</p>	<p>Data are inadequate to test the MOA hypothesis for hepatocarcinogenesis based on liver weight increases.</p>
<b>Negative selection (Section 4.5.7.3.3)</b>			
<ul style="list-style-type: none"> <li><i>TCE or its metabolites causes polyploidization of hepatocytes.</i></li> <li><i>Increased ploidy is associated with carcinogenesis.</i></li> </ul>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>Polyploidization likely occurs with TCE exposure, although the evidence is limited (<a href="#">Buben and O’Flaherty, 1985</a>).</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>No studies of TCE or its metabolites.</li> </ul> <p><i>Sufficiency of MOA for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>No studies of TCE or its metabolites.</li> </ul>	<p>Yes. Increased ploidy is associated with cancer in humans as well as rodents.</p>	<p>Although it is biologically plausible that polyploidization can contribute to liver carcinogenesis, inadequate data are available to support this hypothesized MOA for TCE.</p>

**Table 4-68 Summary of mode-of-action conclusions for TCE-induced liver carcinogenesis (continued)**

Hypothesized MOA postulated key events	Experimental support	Human relevance	Weight-of-evidence conclusion
<b>Glycogen storage (Section 4.5.7.3.4)</b>			
<ul style="list-style-type: none"> <li>• <i>Increased glycogen storage.</i></li> <li>• <i>Glycogenesis in humans has been associated with increased risk of liver cancer.</i></li> </ul>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>• DCA increases glycogen deposition (<a href="#">Nelson et al., 1989</a>)</li> <li>• For TCE and TCA, effects on glycogen were either not reported (<a href="#">Dees and Travis, 1993</a>; <a href="#">Styles et al., 1991</a>; <a href="#">Elcombe et al., 1985</a>) or were described as similar to controls (<a href="#">Nelson et al., 1989</a>).</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• No studies of TCE or its metabolites.</li> </ul> <p><i>Sufficiency of MOA for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• No studies of TCE or its metabolites.</li> </ul>	<p>Yes. No evidence of lack of relevance.</p>	<p>Data are inadequate to define a MOA hypothesis for TCE-induced hepatocarcinogenesis based on changes in glycogen storage, or to support changes in glycogen storage as a result of TCE exposure.</p>
<b>Inactivation of GST-zeta (Section 4.5.7.3.5)</b>			
<ul style="list-style-type: none"> <li>• <i>Inactivation of GST-zeta.</i></li> <li>• <i>Hereditary disruption of this pathway in humans has been associated with increased risk of liver cancer, but the active agent has not been identified.</i></li> </ul>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>• TCE prolongs DCA half-life in rodents, suggesting that TCE may inhibit GST-zeta, likely through the formation of DCA (<a href="#">Schultz et al., 2002</a>).</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• No studies of TCE or its metabolites.</li> </ul> <p><i>Sufficiency of MOA for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• No studies of TCE or its metabolites.</li> </ul>	<p>Yes. No evidence of lack of relevance.</p>	<p>Data are inadequate to define a MOA hypothesis for TCE-induced hepatocarcinogenesis based on inactivation of GST-zeta.</p>

**Table 4-68 Summary of mode-of-action conclusions for TCE-induced liver carcinogenesis (continued)**

Hypothesized MOA postulated key events	Experimental support	Human relevance	Weight-of-evidence conclusion
<b>Oxidative stress (Section 4.5.7.3.6)</b>			
<ul style="list-style-type: none"> <li>• <i>Oxidative stress.</i></li> <li>• <i>Further key events not specified.</i></li> </ul>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>• Measures of oxidative stress induced by TCE, TCA, and DCA either do not occur, or are transient and do not persistent with continued treatment (<a href="#">Channel et al., 1998</a>; <a href="#">Parrish et al., 1996</a>; <a href="#">Larson and Bull, 1992b</a>).</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• No studies of TCE or its metabolites.</li> </ul> <p><i>Sufficiency of MOA for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• No studies of TCE or its metabolites.</li> </ul>	<p>Yes. No evidence of lack of relevance.</p>	<p>Available data are limited to support a role for oxidative stress in TCE-induced liver carcinogenesis.</p>
<b>Epigenetic changes (Section 4.5.7.3.7)</b>			
<ul style="list-style-type: none"> <li>• <i>Epigenetic changes, particularly DNA methylation, induced by one or more metabolites (TCA, DCA, and other reactive species) advance acquisition of multiple critical traits contributing to carcinogenesis.</i></li> </ul>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>• TCE, TCA and DCA decrease global DNA methylation and promoter hypomethylation (e.g., of c-myc) in mouse liver (<a href="#">Tao et al., 2004a</a>; <a href="#">Ge et al., 2001b</a>; <a href="#">Tao et al., 1998</a>).</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• No studies of TCE or its metabolites.</li> </ul> <p><i>Sufficiency of MOA for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• No studies of TCE or its metabolites.</li> </ul>	<p>Yes. No evidence of lack of relevance.</p>	<p>Although it is biologically plausible that epigenetic changes contribute to liver carcinogenesis, inadequate data are available to support this hypothesized MOA for TCE.</p>

**Table 4-68 Summary of mode-of-action conclusions for TCE-induced liver carcinogenesis (continued)**

Hypothesized MOA postulated key events	Experimental support	Human relevance	Weight-of-evidence conclusion
<b>Cytotoxicity and reparative hyperplasia(Section 4.5.7.3.8)</b>			
<ul style="list-style-type: none"> <li>• <i>One or more reactive intermediates induces hepatotoxicity through cell death.</i></li> <li>• <i>Cell proliferation increases in the liver to repair damage.</i></li> <li>• <i>Increased cell turnover increases the rate of mutations.</i></li> <li>• <i>Increased proliferation cause clonal expansion of initiated (pre-malignant) cells.</i></li> <li>• <i>Increased number of mutations and/or initiated cells causes cancer.</i></li> </ul>	<p><i>Evidence that TCE or TCE metabolites induces key events:</i></p> <ul style="list-style-type: none"> <li>• TCE treatment at doses relevant to liver carcinogenicity results in relatively low cytotoxicity (<a href="#">Bull, 2004a</a>; <a href="#">Bull et al., 2004</a>).</li> <li>• No evidence that transient increases in DNA synthesis are related to reparative proliferation.</li> </ul> <p><i>Necessity of key events for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• No studies of TCE or its metabolites.</li> </ul> <p><i>Sufficiency of MOA for carcinogenesis:</i></p> <ul style="list-style-type: none"> <li>• No studies of TCE or its metabolites.</li> </ul>	<p>Yes. No evidence of lack of relevance.</p>	<p>It is unlikely that cytotoxicity and reparative hyperplasia play a significant role in TCE carcinogenicity.</p>

However, despite the identification of numerous factors that appear to play a role in the human risk of HCC, the mechanisms are still largely unclear ([Yeh et al., 2007](#)). Interestingly, the observation by Leakey et al. ([2003a](#); [2003b](#)) that body weight significantly and strongly impacts background liver tumor rates in B6C3F<sub>1</sub> mice parallels the observed epidemiologic associations between liver cancer and obesity (review in [El-Serag and Rudolph, 2007](#)). This concordance suggests that similar pathways may be involved in spontaneous liver tumor induction between mice and humans. The extent to which TCE exposure may interact with known risk factors for HCC cannot be determined at this point, but several hypotheses can be posed based on existing data. If TCE affects some of the same pathways involved in human HCC, as suggested in the discussion of several TCE-induced effects above, then TCE exposure may lead a risk that is additive to background.

As discussed above, there are several parallels between the possible key events in TCE-induced liver tumors in mice and what is known about mechanisms of human HCC, though none have been experimentally tested. Altered ploidy distribution and DNA hypomethylation are commonly observed in human HCC ([Calvisi et al., 2007](#); [Lin et al., 2003](#); [Zeppa et al., 1998](#)). Interestingly, El-Serag and Rudolph ([2007](#)) suggested that the risk of HCC increases with cirrhosis in part because the liver parenchymal cells have decreased proliferative capacity, resulting in an altered milieu that promotes tumor cell proliferation. This description suggests a similarity in mode of action, though via different mechanisms, with the “negative selection” hypothesis proposed by Bull ([2000](#)) for TCE and its metabolites although for TCE changes in apoptosis and cell proliferation have not been noted or examined to such an extent to provide evidence of a similar environment. Increased ploidy decreases proliferative capacity, so that may be another mechanism through which the effects of TCE mimic the conditions thought to facilitate the induction of human HCC.

In sum, from the perspective of hazard characterization, the available data support the conclusion that the mode of action for TCE-induced mouse liver tumors is relevant to humans. No data suggest that any of the key events are biologically precluded in humans, and a number of qualitative parallels exist between hypotheses for the mode of action in mice and what is known about the etiology and induction of human HCC. A number of risk factors have been identified that appear to modulate the risk of human HCC, and these may also modulate the susceptibility to the effects from TCE exposure. As noted in Section E.4, TCE exposure in the human population is accompanied not only by external exposures to its metabolites, but brominated analogues of those metabolites that are also rodent carcinogens, a number of chlorinate solvents that are hepatocarcinogenic and alcohol consumption. The types of tumors and the heterogeneity of tumors induced by TCE in rodents parallel those observed in humans (see Section E.3.1.8). The pathways identified for induction of cancer in humans for cancer are similar to those for the induction of liver cancer (see Section E.3.2.1). However, while risk factors have been identified

for human liver cancer that have similarities to TCE-induced effects and those of its metabolites, both the mechanism for human liver cancer induction and that for TCE-induced liver carcinogenesis in rodents are not known.

#### **4.5.7.4.2. Quantitative species differences**

As a precursor to the discussion of quantitative differences between humans and rodents and among humans, it should be noted that an adequate explanation for the difference in response for TCE-liver cancer induction between rats and mice has yet to be established or for that difference to be adequately described given the limitations in the rat database. For TCA, there is only one available long-term study in rats that, while suggestive that TCA is less potent in rats than mice, is insufficient to determine if there was a TCA-induced effect or what its magnitude may be. While some have proposed that the lower rate of TCA formation in rats relative to mice would explain the species difference, PBPK modeling suggests that the differences (three–fivefold) may be inadequate to fully explain the differences in carcinogenic potency. Moreover, inferences from comparing the effects of TCE and TCA on liver weight, using PBPK model-based estimates of TCA internal dose-metrics as a result of TCE or TCA administration, indicate that TCA is not likely to play a predominant role in hepatomegaly. Combined with the qualitative correlation between rodent hepatomegaly and hepatocarcinogenesis observed across many chemicals, this suggests that TCA similarly is not a predominant factor in TCE-induced hepatocarcinogenesis. Indeed, there are multiple lines of evidence that TCA is insufficient to account for TCE-induced tumors, including data on tumor phenotype (e.g., c-Jun immunostaining) and genotype (e.g., H-ras mutation frequency and spectrum). For DCA, only a single experiment in rats is available (reported in two publications), and although it suggests lower hepatocarcinogenic potency in rats relative to mice, its relatively low power limits the inferences that can be made as to species differences.

As TCA induces peroxisome proliferation in the mouse and the rat, some have suggested that difference in peroxisomal enzyme induction is responsible for the difference in susceptibility to TCA liver carcinogenesis. The study of DeAngelo et al. (1989) has been cited in the literature as providing evidence of differences between rats and mice for peroxisomal response to TCA. However, data from the most resistant strain of rat (Sprague-Dawley) have been cited in comparisons of peroxisomal enzyme effects but the Osborne-Mendel and F344 rat were not refractory and showed increased PCO activity so it is not correct to state that the rat is refractory to TCA-induction of peroxisome activity (see Section E.2.3.1.5). In addition, as discussed above, inferences based on PCO activity are limited by its high variability, even in control animals, as well as its not necessarily being predictive of the peroxisome number or cytoplasmic volume.

The same assumption of lower species sensitivity by measuring peroxisome proliferation has been applied to humans, as peroxisome proliferation caused by therapeutic PPAR $\alpha$  agonists

such as fibrates in humans is generally lower (less than twofold induction) than that observed in rodents (20–50-fold induction). However, as mentioned above, it is known that peroxisome proliferation is not a good predictor of potency ([Marsman et al., 1988](#)).

Limited data exist on the relative sensitivity of the occurrence of key events for liver tumor induction between mice and humans and among humans. Pharmacokinetic differences are addressed with PBPK modeling to the extent that data allow, so the discussion here will concentrate on pharmacodynamic differences. Most striking is the difference in “background” rates of liver tumors. Data from NTP indicate that control B6C3F<sub>1</sub> mice in 2-year bioassays have a background incidence of HCCs of 26% in males and 10% in females, with higher incidences for combined hepatocellular adenomas and carcinomas ([Maronpot, 2007](#)). However, as discussed above, Leakey et al. ([2003a](#); [2003b](#)) report that the background incidence rates are very dependent on the weight of the mice. By contrast, the estimated lifetime risk of liver and biliary tract cancer in the United States (about 75% of which are HCCs) is 0.97% for men and 0.43% for women ([Ries et al., 2008](#)). However, regions of the world where additional risk factors (hepatitis infection, aflatoxin exposure) have high prevalence have liver cancer incidences up to more than sixfold greater than the United States ([Ferlay et al., 2004](#)). Therefore, one possible quantitative difference that can be flagged for use in dose-response assessment is the background rate of liver tumors between species. Biologically-based dose-response modeling by Chen ([2000](#)) suggested that the data were consistent with a purely promotional model in which potency would be proportional to background tumor incidence. However, it is notable that male Swiss mice, which have lower background liver tumor rates than the B6C3F<sub>1</sub> strain, were also positive in one long-term bioassay ([Maltoni et al., 1988](#); [Maltoni et al., 1986](#)).

Similarly, in terms of intraspecies susceptibility, to the extent that TCE may independently promote pre-existing initiated cells, it can be hypothesized that those with greater risk for developing HCC due to one more of the known risk factors would have a proportional increase in the any contributions from TCE exposure. In addition, in both humans and mice, males appear to be at increased risk of liver cancer, possibly due to sexually dimorphism in inflammatory responses ([Lawrence et al., 2007](#); [Naugler et al., 2007](#); [Rakoff-Nahoum and Medzhitov, 2007](#)), suggesting that men may also be more susceptible to TCE-induced liver tumorigenesis than women. It has been observed that human HCC is highly heterogeneous histologically, but within patients and between patients, studies are only beginning to distinguish the different pathways that may be responsible for this heterogeneity ([Yeh et al., 2007](#); [Chen et al., 2002b](#); [Feitelson et al., 2002](#)).

Appropriate quantitative data are generally lacking on interspecies differences in the occurrence of most other proposed key events, although many have argued that there are significant quantitative differences between rodents and humans related to PPAR $\alpha$  activation ([NRC, 2006](#); [Klaunig et al., 2003](#)). For instance, it has been suggested that lower levels of PPAR $\alpha$  receptor in human hepatocytes relative to rodent hepatocytes contributes to lower human

sensitivity ([Klaunig et al., 2003](#); [Palmer et al., 1998](#); [Tugwood et al., 1996](#)). However, out of a small sample of human livers ( $n = 6$ ) show similar protein levels to mice ([Walgren et al., 2000b](#)). Another proposed species difference has been ligand affinity, but while transactivation assays showed greater affinity of Wy-14643 and perfluorooctanoic acid for rodent relative to human PPAR $\alpha$ , they showed TCA and DCA had a similar affinities between species ([Maloney and Waxman, 1999](#)). Furthermore, it is not clear that receptor-ligand kinetics (capacity and affinity) are rate-limiting for eliciting hepatocarcinogenic effects, as it is known that maximal receptor occupation is not necessary for a maximal receptor mediated response ([Stephenson, 1956](#)) [see also review by Danhof et al. ([2007](#))].

There is also limited in vivo and in vitro data suggesting that increases in cell proliferation mediated by PPAR $\alpha$  agonists are diminished in humans and other primates relative to rodents ([NRC, 2006](#); [Hoivik et al., 2004](#); [Klaunig et al., 2003](#)). However, Walgren et al. ([2000a](#)) reported that TCA and DCA were not mitogenic in either human or rodent hepatocytes in vitro. Furthermore, TCE, TCA, and DCA all induce only transient increases in cell proliferation, so the relevance to TCE of interspecies differences from PPAR $\alpha$  agonists that to produce sustained proliferation, such as Wy-14643, is not clear. In addition, comparisons between primate and rodent models should take into account the differences in the ability to respond to any mitogenic stimulation (see Section E.3.2). Primate and human liver respond differently (and much more slowly) to a stimulus such as partial hepatectomy.

Recent studies in “humanized” mice (PPAR $\alpha$ -null mice in which a human PPAR $\alpha$  gene was subsequently inserted and expressed in the liver) reported that treatment with a PPAR $\alpha$  agonist lead to greatly lower incidence of liver tumors as compared to wild-type mice ([Morimura et al., 2006](#)). However, these experiments were performed with WY-14643 at a dose causing systemic toxicity (reduced growth and survival), had a duration of <1 year, and involved a limited number of animals. In addition, because liver tumors in mice at <1 year are extremely rare, the finding a one adenoma in WY-14643-treated humanized mice suggests carcinogenic potential that could be further realized with continued treatment ([Keshava and Caldwell, 2006](#)). In addition, Yang et al. ([2007](#)) recently noted that let-7C, a microRNA involved in cell growth and thought to be a regulatory target of PPAR $\alpha$  ([Shah et al., 2007](#)), was inhibited by Wy-14643 in wild-type mice, but not in “humanized mice” in which human PPAR $\alpha$  was expressed throughout the body on a PPAR $\alpha$ -null background. However, these humanized mice had about a 20-fold higher baseline expression of let-7C, as reported in control mice, potentially masking any treatment effects. More generally, it is not known to what extent PPAR $\alpha$ -related events are rate-limiting in TCE-induced liver tumorigenesis, for which multiple pathways appear to be operative. So even if quantitative differences mediated by PPAR $\alpha$  were well estimated, they would not be directly usable for dose-response assessment in the absence of way to integrate the contributions from the different pathways.

In sum, the only quantitative data and inter- and intraspecies susceptibility suitable for consideration in dose-response assessment are differences background liver tumor risk. These may modulate the effects of TCE if RR, rather than additional risk, is the appropriate common inter- and intraspecies metric. However, the extent to which RR would provide a more accurate estimate of human risk is unknown.

## 4.6. IMMUNOTOXICITY AND CANCERS OF THE IMMUNE SYSTEM

Chemical exposures may result in a variety of adverse immune-related effects, including immunosuppression (decreased host resistance), autoimmunity, and allergy-hypersensitivity, and may result in specific diseases such as infections, systemic or organ-specific autoimmune diseases, or asthma. Cell-mediated immune response, such as activation of macrophages, natural killer (NK) cells, and cytokine production, can also influence a broader range of diseases, such as cancer. Measures of immune function (e.g., T-cell counts, immunoglobulin [Ig] E levels, specific autoantibodies, cytokine levels) may provide evidence of an altered immune response that precedes the development of clinically expressed diseases. The first section of this section discusses effects relating to immunotoxicity, including risk of autoimmune diseases, allergy and hypersensitivity, measures of altered immune response, and lymphoid cancers. Studies pertaining to effects in humans are presented first, followed by a section discussing relevant studies in animals. The second section of this section discusses evidence pertaining to TCE in relation to lymphoid tissue cancers, including childhood leukemia.

### 4.6.1. Human Studies

#### 4.6.1.1. Noncancer Immune-Related Effects

##### 4.6.1.1.1. Immunosuppression, asthma, and allergies

In 1982, Lagakos et al. conducted a telephone survey of residents of Woburn, Massachusetts, collecting information on residential history and history of 14 types of medically diagnosed conditions ([Lagakos et al., 1986](#)). The survey included 4,978 children born since 1960 who lived in Woburn before age 19. Completed surveys were obtained from approximately 57% of the town residences with listed phone numbers. Two of the wells providing the town's water supply from 1964 to 1979 had been found to be contaminated with a number of solvents, including tetrachloroethylene (21 ppb) and TCE (267 ppb) ([as cited in Lagakos et al., 1986](#)). Lagakos et al. ([Lagakos et al., 1986](#)) used information from a study by the Massachusetts Department of Environmental Quality and Engineering to estimate the contribution of water from the two contaminated wells to the residence of each participant, based on zones within the town receiving different mixtures of water from various wells, for the period in which the contaminated wells were operating. This exposure information was used to estimate a cumulative exposure based on each child's length of residence in Woburn. A higher cumulative exposure measure was associated with conditions indicative of immunosuppression (e.g., bacterial or viral infections) or hypersensitivity (e.g., asthma). In contrast, a recent study using the National Health and Nutrition Examination Survey data collected from 1999 to 2000 in a representative sample of the U.S. population (n = 550) did not find an association between TCE exposure and self-report of a history of physician-diagnosed asthma (OR: 0.94, 95% CI: 0.77, 1.14) ([Arif and Shah, 2007](#)). TCE exposure, as well as exposure to nine other VOCs, was determined through a passive monitor covering a period of 48–72 hours. No clear trend was

seen with self-reported wheeze episodes (OR: 1.29, 95% CI: 0.98, 1.68 for one to two episodes; OR: 0.21, 95% CI: 0.04, 10.05 for three or more episodes in the past 12 months).

Allergy and hypersensitivity, as assessed with measures of immune system parameters or immune function tests (e.g., atopy) in humans, have not been extensively studied with respect to the effects of TCE (see Table 4-69). Lehmann et al. reported data pertaining to immunoglobulin E (IgE) levels and response to specific antigens in relation to indoor levels of VOCs among children (age 36 months) selected from a birth cohort study in Leipzig, Germany ([Lehmann et al., 2001](#)). Enrollment into the birth cohort occurred between 1995 and 1996. The children in this allergy study represent a higher-risk group for development of allergic disease, with eligibility criteria that were based on low birth weight (between 1,500 and 2,500 g), or cord blood IgE >0.9 kU/L with double positive family history of atopy. These eligibility criteria were met by 429 children; 200 of these children participated in the allergy study described below, but complete data (IgE and VOC measurements) were available for only 121 of the study participants. Lehmann et al. ([2001](#)) measured 26 VOCs via passive indoor sampling in the child's bedroom for a period of 4 weeks around the age of 36 months. The median exposure of TCE was 0.42  $\mu\text{g}/\text{m}^3$  (0.17 and 0.87  $\mu\text{g}/\text{m}^3$  for the 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively). Blood samples were taken at the 36-month study examination and were used to measure the total IgE and specific IgE antibodies directed to egg white, milk, indoor allergens (house dust mites, cats, and molds), and outdoor allergens (timothy-perennial grass and birch trees). There was no association between TCE exposure and any of the allergens tested in this study, although some of the other VOCs (e.g., toluene, 4-ethyltoluene) were associated with elevated total IgE levels and with sensitization to milk or eggs.

**Table 4-69. Studies of immune parameters (IgE antibodies and cytokines) and TCE in humans**

Parameter, source of data	Results	Reference, location, diagnosis period, sample size, age
<b>IgE antibodies</b> blood sample, indoor air sampling of 28 volatile organic chemicals in child's bedroom	TCE exposure not associated with sensitization to indoor or outdoor allergens	Lehmann et al. (2001) Germany. 1997–1999. n = 121 36-mo old children
<b>Cytokine secreting CD3+ T-cell populations</b> cord blood, indoor air sampling of 28 volatile organic chemicals in child's bedroom 4 wks after birth	In CD3+ cord blood cells, some evidence of association between increasing TCE levels and decreased IL-4 >75 <sup>th</sup> percentile OR: 0.6 (95% CI: 0.2, 2.1), <25 <sup>th</sup> percentile OR 4.4 (95% CI: 1.1, 17.8) increased IFN- $\gamma$ >75 <sup>th</sup> percentile OR: 3.6 (95% CI: 0.9, 14.9) <25 <sup>th</sup> percentile OR: 0.7 (95% CI: 0.2, 2.2) Similar trends not seen with tumor necrosis factor- $\alpha$ or IL-2	Lehmann et al. (2002) Germany. 1995–1996. n = 85 newborns
<b>Cytokine secreting CD3+ and CD8+ T-cell populations</b> blood sample, indoor air sampling of 28 volatile organic chemicals in child's bedroom	TCE exposure not associated with percentages of IL-4 CD3+ or IFN- $\gamma$ CD8+ T-cells	Lehmann et al. (2001) Germany. 1995–1999. n = 200 36-mo old children
<b>Cytokine concentration—serum</b> urine sample (TCA concentration), blood sample, questionnaire (smoking history, age, residence), workplace TCE measures (personal samples, four exposed and four nonexposed workers)	Nonexposed workers similar to office controls for all cytokine measures. Compared to nonexposed workers, the TCE exposed workers had decreased IL-4 (mean 3.9 vs. 8.1 pg/mL) increased IL-2 (mean 798 vs. 706 pg/mL) increased IFN- $\gamma$ (mean 37.1 vs. 22.9 pg/mL)	Iavicoli et al. (2005) Italy. n = 35 printers using TCE, 30 nonexposed workers (in same factory, did not use or were not near TCE), 40 office worker controls. All men. Mean age ~33 yrs

IFN = interferon; IL = interleukin

#### 4.6.1.1.2. Generalized hypersensitivity skin diseases, with or without hepatitis

Occupational exposure to TCE has been associated with a severe, generalized skin disorder that is distinct from contact dermatitis in the clinical presentation of the skin disease (which often involves mucosal lesions), and in the accompanying systemic effects that can include lymphadenopathy, hepatitis, and other organ involvement. Kamijima et al. (2007) recently reviewed case reports describing 260 patients with TCE-related generalized skin disorders (Kamijima et al., 2007). Six of the patients were from the United States or Europe, with the remainder occurring in China, Singapore, Philippines, and other Asian countries. One study in Guangdong province, in southeastern China, included >100 of these cases in a single year (Huang et al., 2002). Kamijima et al. (2007) categorized the case descriptions as indicative of hypersensitivity syndrome (n = 124) or a variation of erythema multiforme, Stevens-Johnson syndrome, and toxic epiderma necrolysis (n = 115), with 21 other cases unclassified in either category. The fatality rate, approximately 10%, was similar in the two groups, but the prevalence of fever and lymphadenopathy was higher in the hypersensitivity syndrome patients. Hepatitis was seen in 92–94% of the multiforme, Stevens-Johnson syndrome, and toxic epiderma necrolysis patients, but the estimates within the hypersensitivity syndrome group were more variable (46–94%) (Kamijima et al., 2007).

Some of the case reports reviewed by Kamijima et al. (2007) provided information on the total number of exposed workers, working conditions, and measures of exposure levels. From the available data, generalized skin disease within a worksite occurred in 0.25–13% of workers in the same location, doing the same type of work (Kamijima et al., 2007). The measured concentration of TCE ranged from <50 to >4,000 mg/m<sup>3</sup>, and exposure scenarios included inhalation only and inhalation with dermal exposures. Disease manifestation generally occurred within 2–5 weeks of initial exposure, with some intervals up to 3 months. Most of the reports were published since 1995, and the geographical distribution of cases reflects the newly industrializing areas within Asia.

Kamijima and colleagues recently conducted an analysis of urinary measures of TCE metabolites (TCA and TCOH) in 25 workers hospitalized for hypersensitivity skin disease in 2002 (Kamijima et al., 2008). Samples taken within 15 days of the last exposure to TCE exposure were available for 19 of the 25 patients, with a mean time of 8.4 days. Samples from the other patients were not used in the analysis because the half-life of U-TCA is 50–100 hours. In addition, 3–6 healthy workers doing the same type of work in the factories of the affected worker, and 2 control workers in other factories not exposed to TCE were recruited in 2002–2003 for a study of breathing zone concentration of volatile organochlorines and urinary measures of TCE metabolites. Worksite measures of TCE concentration were also obtained. Adjusting for time between exposure and sample collection, mean urinary concentration at the time of last exposure among the 19 patients was 206 mg/mL for TCA. Estimates for TCOH were not presented because of the shorter half-life for this compound. U-TCA levels in the

healthy exposed workers varied among the 4 factories, with means ( $\pm$  SDs) of 41.6 ( $\pm$  18.0), 131 ( $\pm$ 90.2), 180 ( $\pm$ 92), and 395 ( $\pm$ 684). The lower values were found in a factory in which the degreasing machine had been partitioned from the workers after the illnesses had occurred. TCE concentrations (personal TWAs) at the factories of the affected workers ranged from 164 to 2,330 mg/m<sup>3</sup> (30–431 ppm). At the two factories with no affected workers in the past 3 years, the mean personal TWA TCE concentrations were 44.9 mg/m<sup>3</sup> (14 ppm) and 1,803 mg/m<sup>3</sup> (334 ppm). There was no commonality of additives or impurities detected among the affected factories that could explain the occurrence of the hypersensitivity disorder.

To examine genetic influences on disease risk, Dai et al. (2004) conducted a case-control study of 111 patients with TCE-related severe generalized dermatitis and 152 TCE-exposed workers who did not develop this disease. Patients were recruited from May 1999 to November 2003 in Guangdong Province, and were employed in approximately 80 electronic and metal-plating manufacturing plants. Initial symptoms occurred within 3 months of exposure. The comparison group was drawn from the same plants as the cases, and had worked for >3 months without development of skin or other symptoms. Mean age in both groups was approximately 23 years. A blood sample was obtained from study participants for genotyping of TNF- $\alpha$ , TNF- $\beta$ , and interleukin (IL)-4 genotypes. The genes were selected based on the role of TNF and of IL-4 in hypersensitivity and inflammatory responses. The specific analyses included two polymorphisms in the promoter region of TNF- $\alpha$  (G  $\rightarrow$  A substitution at position -308); and a G  $\rightarrow$  A substitution at position -238), a polymorphism at the first intron on TNF- $\beta$ , and a polymorphism in the promoter region of IL-4 (C  $\rightarrow$  T substitution at -590). There was no difference in the frequency of the TNF- $\alpha$ <sup>-238</sup>, TNF- $\beta$ , or IL-4 polymorphisms between cases and controls, but the wild-type TNF- $\alpha$ <sup>-308</sup> genotype was somewhat more common among cases (94% in cases and 86% in controls).

Kamijima et al. (2007) note the similarities, particular with respect to specific skin manifestations, of the case presentations of TCE-related generalized skin diseases to conditions that have been linked to specific medications (e.g., carbamazepine, allopurinol, antibacterial sulfonamides), possibly in conjunction with reactivation of specific latent herpes viruses. A previous review by these investigators discussed insights with respect to drug metabolism that may be useful in developing hypotheses regarding susceptibility to TCE-related generalized skin disorders (Nakajima et al., 2003). Based on consideration of metabolic pathways and intermediaries, variability in CYP2E1, UDP-glucuronyltransferase, GST, and N-acetyl transferase (NAT) activities could be hypothesized to affect the toxicity of TCE. NAT2 is most highly expressed in liver, and the “slow” acetylation phenotype (which arises from a specific mutation) has been associated with adverse effects of medications, including drug-induced lupus (Lemke and McQueen, 1995) and hypersensitivity reactions (Spielberg, 1996). There are limited data pertaining to genetic or other sources of variability in these enzymes on risk of TCE-related generalized skin diseases, however. In a study in Guangdong province, CYP1A1, GSTM1,

GSTP1, GSTT1, and NAT2 genotypes in 43 cases of TCE-related generalized skin disease were compared to 43 healthy TCE-exposed workers ([Huang et al., 2002](#)). The authors reported that the NAT2 slow acetylation genotype was associated with disease, but the data pertaining to this finding were not presented.

#### **4.6.1.1.3. Cytokine profiles and lymphocyte subsets**

Cytokines are produced by many of the immune regulatory cells (e.g., macrophages, dendritic cells), and have many different effects on the immune system. The T-helper Type 1 (Th1) cytokines, are characterized as “pro-inflammatory” cytokines, and include TNF- $\alpha$  and interferon (IFN)- $\gamma$ . Although this is a necessary and important part of the innate immune response to foreign antigens, an aberrant pro-inflammatory response may result in a chronic inflammatory condition and contribute to development of scarring or fibrotic tissue, as well as to autoimmune diseases. Th2 cytokines are important regulators of humoral (antibody-related) immunity. IL-4 stimulates production of IgE and thus influences IgE-mediated effects such as allergy, atopy, and asthma. Th2 cytokines can also act as “brakes” on the inflammatory response, so the balance between different types of cytokine production is also important with respect to risk of conditions resulting from chronic inflammation. Several studies have examined cytokine profiles in relation to occupational or environmental TCE exposure (see Table 4-69).

The Lehmann et al. ([2001](#)) study of 36-month-old children (described above) also included a blood sample taken at the 3-year study visit, which was used to determine the percentages of specific cytokine producing T-cells in relation to the indoor VOCs exposures measured at birth. There was no association between TCE exposure and either IL-4 CD3+ or IFN- $\gamma$  CD8+ T-cells ([Lehmann et al., 2001](#)).

Another study by Lehmann et al. ([2002](#)) examined the relationship between indoor exposures to VOCs and T-cell subpopulations measured in cord blood of newborns. The study authors randomly selected 85 newborns (43 boys and 42 girls) from a larger cohort study of 997 healthy, full-term babies, recruited between 1997 and 1999 in Germany. Exclusion criteria included a history in the mother of an autoimmune disease or infectious disease during the pregnancy. Twenty-eight VOCs were measured via passive indoor sampling in the child’s bedroom for a period of 4 weeks after birth (a period that is likely to reflect the exposures during the prenatal period close to the time of delivery). The levels were generally similar or slightly higher than the levels seen in the previous study using samples from the bedrooms of the 36-month-old children. The highest levels of exposure were seen for limonene (median 24.3  $\mu\text{g}/\text{m}^3$ ),  $\alpha$ -pinene (median 19.3  $\mu\text{g}/\text{m}^3$ ), and toluene (median 18.3  $\mu\text{g}/\text{m}^3$ ), and the median exposure of TCE was 0.6  $\mu\text{g}/\text{m}^3$  (0.2 and 1.0  $\mu\text{g}/\text{m}^3$  for the 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively). Flow cytometry was used to measure the presence of CD3 T-cells obtained from the cord blood labeled with antibodies against IFN- $\gamma$ , tumor necrosis factor- $\alpha$ , IL-2, and IL-4. There was some evidence of a decreased level of IL-2 with higher TCE exposure in the

univariate analysis, with median percentage of IL-2 cells of 46.1 and 33.0% in the groups that were below the 75<sup>th</sup> percentile and above the 75<sup>th</sup> percentile of TCE exposure, respectively. In analyses adjusting for family history of atopy, gender, and smoking history of the mother during pregnancy, there was little evidence of an association with either IL-2 or IFN- $\gamma$ , but there was a trend of increasing TCE levels associated with decreased IL-4 and increased IFN- $\gamma$ .

Iavicoli et al. (2005) examined cytokine levels in 35 TCE-exposed workers (Group A) from a printing area of a factory in Italy. Their work involved use of TCE in degreasing. Two comparison groups were included. Group B consisted of 30 other factory workers who were not involved in degreasing activities and did not work near this location, and Group C consisted of 40 office workers at the factory. All study participants were male and had worked at their present position for at least 3 years, and all were considered healthy. Personal breathing zone air samples from four workers in Group A and four workers in Group B were obtained in three consecutive shifts (24 total samples) to determine air concentration of TCE. A urine sample was obtained from each Group A and Group B worker (end of shift at end of work week) for determination of TCA concentrations (corrected for creatinine), and blood samples were collected for assessment of IL-2, IL-4, and IFN- $\gamma$  concentrations in serum using enzyme-linked immunosorbent assays. Among exposed workers, the mean TCE concentration was approximately 35 mg/m<sup>3</sup> (30.75  $\pm$  SD 9.9, 37.75  $\pm$  23.0, and 36.5  $\pm$  8.2 mg/m<sup>3</sup> in the morning, evening, and night shifts, respectively). The U-TCA concentrations were much higher in exposed workers compared with nonexposed workers (mean  $\pm$  SD, Group A 13.3  $\pm$  5.9 mg/g creatinine; Group B 0.02  $\pm$  0.02 mg/g creatinine). There was no difference in cytokine levels between the two control groups, but the exposed workers differed significantly (all *p*-values < 0.01 using Dunnett's test for multiple comparisons) from each of the two comparison groups. The observed differences were a decrease in IL-4 levels (mean 3.9, 8.1, and 8.1 pg/mL for Groups A, B, and C, respectively), and an increase in IL-2 levels (mean 798, 706, and 730 pg/mL for Groups A, B, and C, respectively) and in IFN- $\gamma$  levels (mean 37.1, 22.9, and 22.8 pg/mL for Groups A, B, and C, respectively).

The available data from these studies ([Iavicoli et al., 2005](#); [Lehmann et al., 2002](#); [Lehmann et al., 2001](#)) provide some evidence of an association between increased TCE exposure and modulation of immune response involving an increase in pro-inflammatory cytokines (IL-2, IFN- $\gamma$ ) and a decrease in Th2 (allergy-related) cytokines (e.g., IL-4). These observations add support to the influence of TCE in immune-related conditions affected by chronic inflammation.

Lan et al. (2010) examined lymphocyte subsets among 80 TCE-exposed workers and 96 controls in Guangdong, China. Six factories using TCE for cleaning metals, optical lenses, or circuit boards were included in this study. These factories did not use other solvents (benzene, styrene, ethylene oxide, formaldehyde, or epichlorohydrin), based on an exposure screening using Dräger tubes and 3M Badges. Eighty workers from these factories and 96 unexposed controls (frequency matched by sex and 5-year age groups to controls) from clothes

manufacturers, a food production factory, and a hospital, were included in the study. The study was conducted in 2006. Study participants provided a blood sample, buccal cells, postshift and overnight urine samples, and completed a questionnaire with demographic, alcohol and smoking history, and occupational history data. A blood sample was used for a complete blood count and differential lymphocyte subset analysis. At the time of the blood draw, a clinical examination, including measurement of height and weight, and symptoms of recent respiratory infection (which could affect the differential blood cell counts) was conducted. TCE monitoring was conducted using full-shift personal air exposure measurements. The median level of exposure, based on the mean of two measurements taken for each participant in the month before the blood draw, among the 80 TCE-exposed workers was 12 ppm. The analysis used this level to categorize workers into high ( $\geq 12$  ppm; mean 38 ppm) and low ( $< 12$  ppm; mean 5 ppm) exposures. Among the controls, the mean TCE exposure was  $< 0.03$  ppm. The total number of lymphocytes, T cells, CD4+ T cells, CD8+ T cells, B cells and NK cells was significantly lower among TCE-exposed workers compared with controls, with the largest decrease seen in the higher exposure group. For example, the age- and sex-adjusted lymphocyte count was 2,154, 2,012, and 1,671 cells/ $\mu\text{L}$  blood in the controls,  $< 12$  and  $\geq 12$  ppm groups, respectively (trend  $p = < 0.0001$ ). Plasma concentrations of soluble CD27 and CD30, two costimulators involved in the regulation of T cells, were also decreased in both exposure groups compared with controls. Similar patterns were seen when limited to the 77 workers with exposure levels  $< 100$  ppm, and when limited to the 60 workers with exposure levels  $< 25$  ppm. Granulocytes, monocytes and platelet counts did not differ by exposure. The authors noted that the immunosuppression and decreased lymphocyte activation seen in this study provide support the biological plausibility of a role of TCE exposure in NHL.

#### **4.6.1.1.4. Autoimmune disease**

##### **4.6.1.1.4.1. Disease clusters and geographic-based studies**

Reported clusters of diseases have stimulated interest in environmental influences on systemic autoimmune diseases. These descriptions include investigations into reported clusters of systemic lupus erythematosus ([Dahlgren et al., 2007](#); [Balluz et al., 2001](#)) and Wegener granulomatosis ([Albert et al., 2005](#)). Wegener granulomatosis, an autoimmune disease involving small vessel vasculitis, usually with lung or kidney involvement, is a very rare condition, with an incidence rate of 3–14 per million per year ([Mahr et al., 2006](#)). TCE was one of several groundwater contaminants identified in a recent study investigating a cluster of seven cases of Wegener granulomatosis around Dublin, Pennsylvania. Because of the multiple contaminants, it is difficult to attribute the apparent disease cluster to any one exposure.

In addition to the study of asthma and infectious disease history among residents of Woburn, Massachusetts ([Lagakos et al., 1986](#)) (see Section 4.6.1.1.1), Byers et al. ([1988](#)) provided data pertaining to immune function from 23 family members of leukemia patients in

Woburn, Massachusetts. Serum samples were collected in May and June of 1984 and in November of 1985 (several years after 1979, when the contaminated wells had been closed). Total lymphocyte counts and lymphocyte subpopulations (CD3, CD4, and CD8) and the CD4/CD8 ratio were determined in these samples, and in samples from a combined control group of 30 laboratory workers and 40 residents of Boston selected through a randomized probability area sampling process. The study authors also assessed the presence of antinuclear antibodies (ANA) or other autoantibodies (antismooth muscle, antiovarian, antithyroglobulin, and antimicrosomal antibodies) in the family member samples and compared the results with laboratory reference values. The age distribution of the control group, and stratified analyses by age, are not provided. The lymphocyte subpopulations (CD3, CD4, and CD8) were higher and the CD4/CD8 ratio was lower in the Woburn family members compared to the controls in both of the samples taken in 1984. In the 1985 samples, however, the lymphocyte subpopulation levels had decreased and the CD4/CD8 ratio had increased; the values were no longer statistically different from the controls. None of the family member serum samples had antithyroglobulin or antimicrosomal antibodies, but 10 family-member serum samples (43%) had ANA (compared to <5% expected based on the reference value). Because the initial blood sample was taken in 1984, it is not possible to determine the patterns at a time nearer to the time of the exposure. The co-exposures that occurred also make it difficult to infer the exact role of TCE in any alterations of the immunologic parameters.

Kilburn and Warshaw ([1992a](#)) reported data from a study of contamination by metal-cleaning solvents (primarily TCE) and heavy metals (e.g., chromium) of the aquifer of the Santa Cruz River in Tucson, Arizona ([1992a](#)). Exposure concentrations >5 ppb (6–500 ppb) had been documented in some of the wells in this area. A study of neurological effects was undertaken between 1986 and 1989 ([Kilburn and Warshaw, 1993b](#)), and two of the groups within this larger study were also included in a study of symptoms relating to systemic lupus erythematosus. Residents of Tucson (n = 362) were compared to residents of southwest Arizona (n = 158) recruited through a Catholic parish. The Tucson residents were selected from the neighborhoods with documented water contamination (>5 ppb TCE for at least 1 year between 1957 and 1981). Details of the recruitment strategy are not clearly described, but the process included recruitment of patients with lupus or other rheumatic diseases ([Kilburn and Warshaw, 1993b, 1992a](#)). The prevalence of some self-reported symptoms (malar rash, arthritis/arthralgias, Raynaud syndrome, skin lesions, and seizure or convulsion) was significantly higher in Tucson, but there was little difference between the groups in the prevalence of oral ulcers, anemia, low white blood count or low platelet count, pleurisy, alopecia, or proteinuria. The total number of symptoms reported was higher in Tucson than in the other southwest Arizona residents (14.3 vs. 6.4% reported four or more symptoms, respectively). Low-titer (1:80) ANA were seen in 10.6 and 4.7% of the Tucson and other Arizona residents, respectively ( $p = 0.013$ ). However, since part of the Tucson

study group was specifically recruited based on the presence of rheumatic diseases, it is difficult to interpret these results.

#### 4.6.1.1.4.2. Case-control studies

Interest in the role of organic solvents, including TCE, in autoimmune diseases was spurred by the observation of a scleroderma-like disease characterized by skin thickening, Raynaud's phenomenon, and acroosteolysis and pulmonary involvement in workers exposed to vinyl chloride ([Gama and Meira, 1978](#)). A case report in 1987 described the occurrence of a severe and rapidly progressive case of systemic sclerosis in a 47-year-old woman who had cleaned X-ray tubes in a tank of TCE for approximately 2.5 hours ([Lockey et al., 1987](#)).

One of the major impediments to autoimmune disease research is the lack of disease registries, which makes it difficult to identify incident cases of specific diseases. There are no cohort studies of the incidence of autoimmune diseases in workers exposed to TCE. Most of the epidemiologic studies of solvents and autoimmune disease rely on general measures of occupational exposures to solvents, organic solvents, or chlorinated solvents exposures. A two- to threefold increased risk of systemic sclerosis (scleroderma) ([Maitre et al., 2004](#); [Garabrant et al., 2003](#); [Aryal et al., 2001](#)), rheumatoid arthritis ([Sverdrup et al., 2005](#); [Lundberg et al., 1994](#)), undifferentiated connective tissue disease ([Lacey et al., 1999](#)), and antineutrophil-cytoplasmic antibody (ANCA)-related vasculitis ([Beaudreuil et al., 2005](#); [Lane et al., 2003](#)) has generally been seen in these studies, but there was little evidence of an association between solvent exposure and systemic lupus erythematosus in two recent case-control studies ([Finckh et al., 2006](#); [Cooper et al., 2004](#)).

Two case-control studies of scleroderma ([Bovenzi et al., 2004](#); [Maitre et al., 2004](#)) and two of rheumatoid arthritis ([Olsson et al., 2004](#); [Olsson et al., 2000](#)) provide data concerning solvent exposure that occurred among metal workers or in jobs that involved cleaning metal (i.e., types of jobs that were likely to use TCE as a solvent). There was a twofold increased risk among male workers in the two studies of rheumatoid arthritis from Sweden ([Olsson et al., 2004](#); [Olsson et al., 2000](#)). The results from the smaller studies of scleroderma were more variable, with no exposed cases seen in one study with 93 cases and 206 controls ([Maitre et al., 2004](#)), and an OR of 5.2 (95% CI: 0.7, 37) seen in a study with 56 cases and 171 controls ([Bovenzi et al., 2004](#)).

Five other case-control studies provide data specifically about TCE exposure, based on industrial hygienist review of job history data (see Table 4-70). Three of these studies are of scleroderma ([Garabrant et al., 2003](#); [Diot et al., 2002](#); [Nietert et al., 1998](#)), one is of undifferentiated connective tissue disease ([Lacey et al., 1999](#)), and one is of small vessel vasculitides involving ANCA's ([Beaudreuil et al., 2005](#)).

These studies included some kind of expert review of job histories, but only two studies included a quantification of exposure (e.g., a cumulative exposure metric, or a "high" exposure

group) ([Diot et al., 2002](#); [Nietert et al., 1998](#)). Most of the studies present data stratified by sex, and as expected, the prevalence of exposure (either based on type of job or on industrial hygienist assessment) is considerably lower in women compared with men. In men, the studies generally reported ORs between 2.0 and 8.0, and in women, the ORs were between 1.0 and 2.0. The incidence rate of scleroderma in the general population is approximately 5–10 times higher in women compared with men, which may make it easier to detect large RRs in men.

The EPA conducted a meta-analysis of the three scleroderma studies with specific measures of TCE ([Garabrant et al., 2003](#); [Diot et al., 2002](#); [Nietert et al., 1998](#)), examining separate estimates for males and for females. The resulting combined estimate for “any” exposure, using a random effects model to include the possibility of nonrandom error between studies ([DerSimonian and Laird, 1986](#)), was OR: 2.5 (95% CI: 1.1, 5.4) for men and OR: 1.2 (95% CI: 0.58, 2.6) in women. (Because the “any” exposure variable was not included in the published report, Dr. Paul Nietert provided the EPA with a new analysis with these results, e-mail communication from Paul Nietert to Glinda Cooper, November 28, 2007.)

Specific genes may influence the risk of developing autoimmune diseases, and genes involving immune response (e.g., cytokines, major histocompatibility complex, B- and T-cell activation) have been the focus of research pertaining to the etiology of specific diseases. The metabolism of specific chemical exposures may also be involved ([Cooper et al., 1999](#)). Povey et al. ([2001](#)) examined polymorphisms of two CYP genes, CYP2E1 and CYP2C19, in relation to solvent exposure and risk of developing scleroderma. These specific genes were examined because of their hypothesized role in metabolism of many solvents, including TCE. Seven scleroderma patients who reported a history of solvent exposure were compared to 71 scleroderma patients with no history of solvent exposure and to 106 population-based controls. The CYP2E1\*3 allele and the CYP2E1\*4 allele were more common in the seven solvent-exposed patients (each seen in two of the seven patients; 29%) than in either of the comparison groups (approximately 5% for CYP2E1\*3 and 14% for CYP2E1\*4). The authors present these results as observations that require a larger study for corroboration and further elucidation of specific interactions.

**Table 4-70. Case-control studies of autoimmune diseases with measures of TCE exposure**

Disease, source of data	Results: exposure prevalence, OR, 95% CI	Reference, location, sample size, age
Scleroderma		
Structured interview (specific jobs and materials; jobs held $\geq 1$ yrs). Exposure classified by self-report and by expert review (JEM).	<p>Men            Maximum intensity 30% cases, 10% controls; OR: 3.3 (95% CI: 1.0, 10.3)            Cumulative intensity 32% cases, 21% controls; OR: 2.0 (95% CI: 0.7, 5.3)            Maximum probability 16% cases, 3% controls; OR: 5.1 (95% CI: not calculated)</p> <p>Women            Maximum intensity 6% cases, 7% controls; OR: 0.9 (95% CI: 0.3, 2.3)            Cumulative intensity 10% cases, 9% controls; OR: 1.2 (95% CI: 0.5, 2.6)            Maximum probability 4% cases, 5% controls; OR: 0.7 (95% CI: 0.2, 2.2)</p>	Nietert et al. (1998) South Carolina. Prevalent cases, 178 cases (141 women, 37 men), 200 hospital-based controls. Mean age at onset 45.2 yrs
Structured interview (specific jobs and materials; jobs held $\geq 6$ mo). Exposure classified by expert review.	<p>Men and women            Any exposure: cases 16%, controls 8%; OR: 2.4 (95% CI: 1.0, 5.4)            High exposure:<sup>a</sup> cases 9%, controls 1%; OR: 7.6 (95% CI: 1.5, 37.4)</p> <p>Men            Any exposure: cases 64%, controls 27%; OR: 4.7 (95% CI: 0.99, 22.0)</p> <p>Women            Any exposure: cases 9%, controls 4%; OR: 2.1 (95% CI: 0.65, 6.8)</p>	Diot et al. (2002) France. Prevalent cases, 80 cases (69 women, 11 men), 160 hospital controls. Mean age at diagnosis 48 yrs
Structured interview (specific jobs and materials; jobs held $\geq 3$ mo). Exposure classified by self-report and by expert review.	<p>Women            Self report: cases 1.3%, controls 0.7%; OR: 2.0 (95% CI: 0.8, 4.8)            Expert review: cases 0.7%, controls 0.4%; OR: 1.9 (95% CI: 0.6, 6.6)</p>	Garabrant et al. (2003) Michigan and Ohio. Prevalent cases, 660 cases (all women), 2,227 population controls. <sup>b</sup> Ages 18 and older
Undifferentiated connective tissue disease		
Structured interview (specific jobs and materials; jobs held $\geq 3$ mo). Exposure classified by self-report and by expert review.	<p>Women            Self report: cases 0.5%, controls 0.7%; OR: 0.88 (95% CI: 0.11, 6.95)            Expert review: cases 0.5%, controls 0.4%; OR: 1.67 (95% CI: 0.19, 14.9)</p>	Lacey et al. (1999), Michigan and Ohio. Prevalent cases, 205 cases (all women), 2,095 population controls. Ages 18 and older
ANCA-related diseases <sup>c</sup>		
Structured interview (specific jobs and materials; jobs held $\geq 6$ mo). Exposure classified by expert review.	<p>Men and women (data not presented separately by sex)            cases 18.3%, controls 17.5%; OR: 1.1 (95% CI: 0.5, 2.4)</p>	Beaudreuil et al. (2005) France. Incident cases, 60 cases (~50% women), 120 hospital controls. Mean age 61 yrs

<sup>a</sup>Cumulative exposure defined as product of probability  $\times$  intensity  $\times$  frequency  $\times$  duration scores, summed across all jobs; scores of  $>1$  classified as “high.”

<sup>b</sup>Total n; n with TCE data: self-report 606 cases, 2,138 control; expert review 606 cases, 2,137 controls.

<sup>c</sup>Diseases included Wegener glomerulonephritis (n = 20), microscopic polyangiitis (n = 8), pauci-immune glomerulonephritis (n = 10), uveitis (n = 6), Churg-Strauss syndrome (n = 4), stroke (n = 4), and other diseases (no more than 2 each).

#### 4.6.1.2. Cancers of the Immune System, Including Childhood Leukemia

##### 4.6.1.2.1. Description of studies

Human studies have reported cancers of the immune system resulting from TCE exposure. Lymphoid tissue neoplasms arise in the immune system and result from events that occur within immature lymphoid cells in the bone marrow or peripheral blood (leukemias), or more mature cells in the peripheral organs (NHL). As such, the distinction between lymphoid leukemia and NHL is largely distributional with overlapping entities, such that a particular lymphoid neoplasm may manifest both lymphomatous and leukemic features during the course of the disease ([Weisenburger, 1992](#)). The broad category of lymphomas can be divided into specific types of cancers, including NHL, Hodgkin lymphoma, multiple myeloma, and various types of leukemia (e.g., acute and chronic forms of lymphoblastic and myeloid leukemia). The classification criteria for these cancers has changed over the past 30 years, reflecting improved understanding of the underlying stem cell origins of these specific subtypes. Lymphomas are grouped according to the World Health Organization (WHO) classification as B-cell neoplasms, T-cell/NK-cell neoplasms, and Hodgkin lymphoma, formerly known as Hodgkin disease ([Harris et al., 2000](#)). For example, hairy cell leukemia, CLL, NHL, and multiple myeloma may arise from mature B cells and are types of NHLs according to the WHO's lymphoma classification system (Morton et al., 2007, 2006). Most of the studies of TCE exposure evaluate NHL defined as lymphosarcoma, reticulum-cell sarcoma, and other lymphoid tissue neoplasms with recently published studies reporting on total B-cell or specific B-cell neoplasms.

Numerous studies are found in the published literature on NHL and either broad exposure categories or occupational title. The NHL studies generally report positive associations with organic solvents or job title as aircraft mechanic, metal cleaner or machine tool operator, and printers, although associations are not observed consistently across all studies, specific solvents are not identified, and different lymphoma classifications are adopted ([Cocco et al., 2010](#); [Orsi et al., 2010](#); [Schenk et al., 2009](#); [Wang et al., 2009](#); ['t Mannetje et al., 2008](#); [Karunanayake et al., 2008](#); [Richardson et al., 2008](#); [Alexander et al., 2007b](#); [Boffetta and de Vocht, 2007](#); [Seidler et al., 2007](#); [Vineis et al., 2007](#); [Dryver et al., 2004](#); [Chiu and Weisenburger, 2003](#); [Lynge et al., 1997](#); [Tatham et al., 1997](#); [Figgs et al., 1995](#); [Blair et al., 1993](#)). A major use of TCE is the degreasing, as vapor or cold state solvent, of metal and other products with potential exposure in jobs in the metal industry, printing industry, and aircraft maintenance or manufacturing industry ([Bakke et al., 2007](#)). The recent NHL case-control study of Purdue et al. ([2009](#)) examined degreasing tasks, specifically, and reported an increasing positive trend between NHL risk in males and three degreasing exposure surrogates: average frequency (hours/year) ( $p = 0.02$ ), maximal frequency (hours/year), ( $p = 0.06$ ), or cumulative number of hours ( $p = 0.04$ ).

As described in Appendix B, the EPA conducted a thorough and systematic search of published epidemiological studies of cancer risk and TCE exposure using the PubMed,

TOXNET<sup>®</sup>, and EMBASE<sup>®</sup> bibliographic database. The EPA also requested unpublished data pertaining to TCE from studies that may have collected these data but did not include it in their published reports. ATSDR and state health department peer-reviewed studies were also reviewed. Information from each of these studies relating to specified design and analysis criteria was abstracted. These criteria included aspects of study design, representativeness of study subjects, participation rate/loss to follow-up, latency considerations, potential for biases related to exposure misclassification, disease misclassification, and surrogate information, consideration of possible confounding, and approach to statistical analysis. All studies were considered for hazard identification, but those studies more fully meeting the objective criteria provided the greater weight for identifying a cancer hazard.

The body of evidence on NHL and TCE is comprised of occupational cohort studies, population-based case-control studies, and geographic studies. Four case-control studies and four geographic studies also examined childhood leukemia and TCE. Most studies reported observed risk estimates and associated CIs for NHL and overall TCE exposure. The studies included a broad but sometimes slightly different group of lymphosarcoma, reticulum-cell sarcoma, and other lymphoid tissue neoplasms, with the exception of the Nordstrom et al. (1998) case-control study, which examined hairy cell leukemia, now considered a NHL, the Zhao et al. (2005) cohort study, which reported only results for *all* lymphohematopoietic cancers, including nonlymphoid types and excluding CLL, and the Greenland et al. (1994) nested case-control study which reported results for NHL and Hodgkin lymphoma combined. Persson and Fredrikson (1999) do not identify the classification system for defining NHL, and Hardell et al. (1994) define NHL using the Rappaport classification system. Miligi et al. (2006) used an NCI classification system and considered CLLs and NHL, classified as lymphosarcoma, reticulosarcoma, and other lymphoid tissue neoplasms, together, while Cocco et al. (2010), used the WHO classification system, which reclassifies lymphocytic leukemias and NHLs as lymphomas of B-cell or T-cell origin. EPA staff, additionally, was able to obtain results generally consistent with the traditional NHL definition from Dr. Cocco, although lymphomas not otherwise specified were excluded (Cocco, 2010). The cohort studies [except for Zhao et al. (2005)] and the nested case-control study of Greenland et al. (1994) have some consistency in coding NHL, with NHL defined as lymphosarcoma and reticulum-cell sarcoma (ICD code 200) and other lymphoid tissue neoplasms (ICD code 202) using the ICD Revisions 7, 8, or 9. Revisions 7 and 8 are essentially the same with respect to NHL; under Revision 9, the definition of NHL was broadened to include some neoplasms previously classified as Hodgkin lymphomas (Banks, 1992). Wang et al. (2009) refer to their cases as “NHL” cases and according to the ICD-O classification system that they used, their cases are more specifically NHL subtypes such as diffuse, lymphosarcoma, or follicular lymphoma (9590–9642, 9690–9701) or mast cell tumors (9740–9750) which is consistent with the traditional definition of NHL (i.e., ICD-7, -8, -9 codes 200 + 202) (Morton et al., 2003). NHL cases in Purdue et al. (2011) were also classified

according to ICD-O (2<sup>nd</sup> Edition converted to ICO-O 3<sup>rd</sup> Edition codes), included diffuse, follicular T-cell and all other NHL subtypes, which is generally consistent with the traditional definition of NHL, although this grouping does not include the malignant lymphomas of unspecified type coded as M-9590-9599. Fewer studies in published papers presented this information for cell-specific lymphomas, leukemia, leukemia cell type, or multiple myeloma ([Gold et al., 2011](#); [Cocco et al., 2010](#); [Costantini et al., 2008](#); [Radican et al., 2008](#); [Boice et al., 2006b](#); [Hansen et al., 2001](#); [Raaschou-Nielsen et al., 2001](#); [Boice et al., 1999](#); [Morgan et al., 1998](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#)).

The seven cohort studies with data on the incidence of lymphopoietic and hematopoietic cancer in relation to TCE exposure range in size from 803 ([Hansen et al., 2001](#)) to 86,868 ([Chang et al., 2005](#)), and were conducted in Denmark, Sweden, Finland, Taiwan, and the United States (see Table 4-71; for additional study descriptions, see Appendix B). Some subjects in the Hansen et al. ([2001](#)) study are also included in a study reported by Raaschou-Nielsen et al. ([2003](#)); however, any contribution from the former to the latter are minimal given the large differences in cohort sizes of these studies ([Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#)). The exposure assessment techniques used in all studies except Chang et al. ([2005](#)) and Sung et al. ([2007](#)) included a detailed JEM ([Zhao et al., 2005](#); [Blair et al., 1998](#)), biomonitoring data ([Hansen et al., 2001](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#)), or reference to industrial hygiene records on TCE exposure patterns and factors that affected exposure, indicating a high probability of TCE exposure potential ([Raaschou-Nielsen et al., 2003](#)) with high probability of TCE exposure to individual subjects. Subjects in Chang et al. ([2005](#)) and Sung et al. ([2007](#)), two studies with overlapping subjects employed at an electronics plant in Taiwan, have potential exposure to several solvents including TCE; all subjects are presumed as “exposed” because of employment in the plant although individual subjects would be expected to have differing exposure potentials. The lack of attribution of exposure intensity to individual subjects yields a greater likelihood for exposure misclassification compared to the six other studies with exposure assessment approaches supported by information on job titles, tasks, and industrial hygiene monitoring data. Incidence ascertainment in two cohorts began 21 ([Blair et al., 1998](#)) and 38 years ([Zhao et al., 2005](#)) after the inception of the cohort. Specifically, Zhao et al. ([2005](#)) noted that their results may not accurately reflect the effects of carcinogenic exposure that resulted in nonfatal cancers before 1988. Because of the issues concerning case ascertainment raised by this incomplete coverage, observations must be interpreted in light of possible bias reflecting incomplete ascertainment of incident cases.

**Table 4-71. Incidence cohort studies of TCE exposure and lymphopoietic and hematopoietic cancer risk**

Population exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description <sup>b</sup>
	RR (95% CI) <sup>a</sup>	n <sup>a</sup>	RR (95% CI) <sup>a</sup>	n <sup>a</sup>	RR (95% CI) <sup>a</sup>	n <sup>a</sup>	RR (95% CI) <sup>a</sup>	n <sup>a</sup>	
Aerospace workers (Rocketdyne), California									Zhao et al. (2005)
	Any TCE exposure	Not reported		Not reported					n = 5,049 (2,689 with high cumulative TCE exposure), began work before 1980, worked at least 2 yrs, alive with no cancer diagnosis in 1988, follow-up from 1988 to 2000, JEM (intensity), internal referents (workers with no TCE exposure). Leukemia and multiple myeloma observations included in NHL category.
	Low cumulative TCE score			1.0 (referent)	28				
	Medium cumulative TCE score			0.88 (0.47, 1.65)	16				
	High cumulative TCE score			0.20 (0.03, 1.46)	1				
	(p for trend)			(0.097)					
Electronic workers, Taiwan									Chang et al. (2005); Sung et al. (2007)
	All employees	0.67 (0.42, 1.01)	22						n = 88,868 (n = 70,735 female), follow-up 1979–1997, does not identify TCE exposure to individual subjects (Chang et al., 2005).
	Males	0.73 (0.27, 1.60)	6	Not reported		Not reported		Not reported	
	Females	0.65 (0.37, 1.05)	16	Not reported		Not reported		Not reported	
	Females					0.78 (0.49, 1.17)	23	Not reported	n = 63,982 females, follow-up 1979–2001, does not identify TCE exposure to individual subjects (Sung et al., 2007).

**Table 4-71. Incidence cohort studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)**

Population exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description <sup>b</sup>	
	RR (95% CI) <sup>a</sup>	n <sup>a</sup>	RR (95% CI) <sup>a</sup>	n <sup>a</sup>	RR (95% CI) <sup>a</sup>	n <sup>a</sup>	RR (95% CI) <sup>a</sup>	n <sup>a</sup>		
Blue-collar workers, Denmark										
	Any exposure	1.1 (1.0, 1.6)	229	1.2 (1.0, 1.5)	96	1.2 (0.9, 1.4)	82	1.03 (0.70, 1.47)	31	n = 40,049 (14,360 with presumed higher level exposure to TCE), worked for at least 3 mo, follow-up from 1968 to 1997, documented TCE use <sup>c</sup> . EPA based the lymphopoietic cancer category on summation of ICD codes 200–204.
	Subcohort w/higher exposure <sup>d</sup>	Not reported		1.5 (1.2, 2.0)	65	Not reported		Not reported		
	Employment duration									
	1–4.9 yrs			1.5 (1.1, 2.1)	35					
	≥5 yrs			1.6 (1.1, 2.2)	30					
Biologically-monitored workers, Denmark										
	Any TCE exposure	2.0 (1.1, 3.3)	15	3.1 (1.3, 6.1)	8	2.0 (0.7, 4.4)	6	0.71 (0.02, 3.98)	1	n = 803, U-TCA or air TCE samples, follow-up 1968–1996 [subset of Raaschlou-Nielsen et al. (2003) cohort]. EPA based the lymphopoietic cancer category on summation of ICD codes 200–204.
	Cumulative exposure (Ikeda), males	Not reported				Not reported		Not reported		
	<17 ppm-yr			3.9 (0.8, 11)	3					
	≥17 ppm-yr			3.1 (0.6, 9.1)	3					
	Mean concentration (Ikeda), males	Not reported				Not reported		Not reported		
	<4 ppm			3.9 (1.1, 10)	4					
	4+ ppm			3.2 (1.1, 10)	4					
	Employment duration, males	Not reported				Not reported		Not reported		
	<6.25 yr			2.5 (0.3, 9.2)	2					
	≥6.25 yr			4.2 (1.1, 11)	4					

**Table 4-71. Incidence cohort studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)**

Population exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description <sup>b</sup>
	RR (95% CI) <sup>a</sup>	n <sup>a</sup>							
Aircraft maintenance workers, Hill Air Force Base, Utah									Blair et al. ( <a href="#">1998</a> )
TCE Subcohort	Not reported		Not reported		Not reported		Not reported		n = 10,461 men and 3,605 women (total n = 14,066, n = 7,204 with TCE exposure), employed at least 1 yr from 1952 to 1956, follow-up 1973–1990, JEM (intensity), internal referent (workers with no chemical exposures).
Males, cumulative exposure									
0	1.0 (referent)		1.0 (referent)		1.0 (referent)		1.0 (referent)	9	
<5 ppm-yr	0.8 (0.4, 1.7)	12	0.9 (0.3, 2.6)	8	0.4 (0.1, 2.0)	2	0.8 (0.1, 12.7)	1	
5–25 ppm-yr	0.7 (0.3, 1.8)	7	0.7 (0.2, 2.6)	4		0	3.8 (0.4, 37.4)	3	
>25 ppm-yr	1.4 (0.6, 2.9)	17	1.0 (0.4, 2.9)	7	0.9 (0.2, 3.7)	4	5.1 (0.6, 43.7)	5	
Females, cumulative exposure									
0	1.0 (referent)		1.0 (referent)		1.0 (referent)		1.0 (referent)		
<5 ppm-yr	1.2 (0.3, 4.4)	3	0.6 (0.1, 5.0)	1		0	Not reported	2	
5–25 ppm-yr	1.9 (0.4, 8.8)	2		0	2.4 (0.3, 21.8)	1	Not reported	1	
>25 ppm-yr	0.9 (9.2, 3.3)	3	0.9 (0.2, 4.5)	2		0	Not reported	1	
Biologically-monitored workers, Finland									Anttila et al. ( <a href="#">1995</a> )
Any TCE exposure	1.51 (0.92, 2.33)	20	1.81 (0.78, 3.56)	8	1.08 (0.35, 2.53)	5	1.62 (0.44, 4.16)	4	n = 3,089 men and women, U-TCA samples, follow-up 1967–1992.
Mean air-TCE (Ikeda extrapolation)									
<6 ppm	1.36 (0.65, 2.49)	10	2.01 (0.65, 4.69)	5	0.39 (0.01, 2.19)	1	1.48 (0.18, 5.35)	2	
6+ ppm	2.08 (0.95, 3.95)	9	1.40 (0.17, 5.04)	2	2.65 (0.72, 6.78)	4	2.41 (0.29, 6.78)	2	

**Table 4-71. Incidence cohort studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)**

Population exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description <sup>b</sup>
	RR (95% CI) <sup>a</sup>	n <sup>a</sup>							
Biologically-monitored workers, Sweden									Axelsson et al. ( <a href="#">1994</a> )
Males	1.17 (0.47, 2.40)	7	1.56 (0.51, 3.64)	5	Not reported		0.57 (0.01, 3.17)	1	n = 1,421 men and 249 women (total 1,670), U-TCA samples, follow-up 1958–1987. EPA based the lymphopoietic cancer category includes ICD-7 200–203.
0–17 ppm (Ikeda extrapolation)	Not reported		1.44 (0.30, 4.20)	3	Not reported				
18–35 ppm (Ikeda extrapolation)			(0, 8.58)	0					
≥36 ppm (Ikeda extrapolation)			6.25 (0.16, 34.8)	1					
Females	Not reported		Not reported		Not reported				

<sup>a</sup>n = number of observed cases.

<sup>b</sup>SIRs using an external population referent group unless otherwise noted.

<sup>c</sup>Exposure assessment based on industrial hygiene data on TCE exposure patterns and factors that affect such exposure ([Raaschou-Nielsen et al., 2002](#)), with high probability of TCE exposure potential to individual subjects. Companies included iron and metal (48%), electronics (11%), painting (11%), printing (8%), chemical (5%), dry cleaning (5%), and other industries.

<sup>d</sup>Defined as at least 1 year duration and first employed before 1980.

Eighteen cohort or PMR studies describing mortality risks from lymphopoietic and hematopoietic cancer are summarized in Table 4-72 (for additional study descriptions, see Appendix B). Two studies examined cancer incidence, Radican et al. (2008), who updated mortality in Blair et al. (1998) cohort, and Zhao et al. (2005), and are identified above. In 10 of the 18 studies presenting mortality risks (Clapp and Hoffman, 2008; Sung et al., 2007; ATSDR, 2004a; Chang et al., 2003; Henschler et al., 1995; Sinks et al., 1992; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988; Wilcosky et al., 1984), a relatively limited exposure assessment methodology was used, study participants may not represent the underlying population, or there was a low exposure prevalence of TCE exposure. For reasons identified in the systematic review, these studies are given less weight in the overall evaluation of the literature than the eight other cohort studies that better met the ideals of evaluation criteria (Radican et al., 2008; Boice et al., 2006b; Zhao et al., 2005; Boice et al., 1999; Ritz, 1999a; Blair et al., 1998 and extended follow-up by; Morgan et al., 1998; Greenland et al., 1994).

Case-control studies of NHL from United States (Connecticut), Germany, Italy, Sweden, and Canada were identified, and are summarized in Table 4-73 (for additional study descriptions, see Appendix B). These studies identified cases from hospital records (Cocco et al., 2010; Costantini et al., 2008; Seidler et al., 2007; Mester et al., 2006; Miligi et al., 2006; Persson and Fredrikson, 1999; Hardell et al., 1994; Siemiatycki, 1991); the SEER Cancer Registry—Connecticut residents (Wang et al., 2009), Iowa, Los Angeles County, and Seattle and Detroit metropolitan area residents (Purdue et al., 2011), or Seattle and Detroit metropolitan area residents (Gold et al., 2011); or the Swedish Cancer Registry (Nordström et al., 1998), and hospital or population controls. These studies assign potential occupational TCE exposure to cases and controls using self-reported information obtained from a mailed questionnaire (Persson and Fredrikson, 1999; Nordström et al., 1998; Hardell et al., 1994) or from direct interview with study subjects, with industrial hygienist ratings of exposure potential and a JEM (Purdue et al., 2011; Cocco et al., 2010; Wang et al., 2009; Costantini et al., 2008; Seidler et al., 2007; Miligi et al., 2006; Siemiatycki, 1991). Additionally, large multiple center lymphoma case-control studies examine specific types of NHL (Purdue et al., 2011; Cocco et al., 2010; Wang et al., 2009; Miligi et al., 2006), leukemia (Costantini et al., 2008), or multiple myeloma (Purdue et al., 2011; Cocco et al., 2010; Costantini et al., 2008).

**Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk**

Population, exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description <sup>b</sup>
	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	
Computer manufacturing workers (IBM), New York									Clapp and Hoffman ( <a href="#">2008</a> )
Males	2.24 (1.01, 4.19)	9	Not reported		Not reported		Not reported	3	n = 115 cancer deaths from 1969 to 2001, proportional cancer mortality ratio, does not identify TCE exposure to individual subjects. EPA based the lymphopoietic cancer category on “all lymphatic cancers.”
Females	Not reported	0	Not reported		Not reported		Not reported	0	
Aerospace workers (Rocketdyne), California									Boice et al. ( <a href="#">2006b</a> )
Any TCE (utility/eng flush)	0.74 (0.34, 1.40)	9	0.21 (0.01, 1.18)	1	1.08 (0.35, 2.53)	5	0.50 (0.01, 2.77)	1	n = 41,351 (1,111 Santa Susana workers with any TCE exposure), employed on or after 1948–1999, worked ≥6 mo, follow-up to 1999, JEM without quantitative estimate of TCE intensity.

**Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)**

Population, exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description <sup>b</sup>
	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	
Aerospace workers (Rocketdyne), California (continued)									Zhao et al. (2005)
Any TCE exposure	Not reported		Not reported	60	Not reported		Not reported		n = 6,044 (n = 2,689 with high cumulative level exposure to TCE), began work and worked at least 2 yrs in 1950 or later–1993, follow-up to 2001, JEM (intensity), internal referents (workers with no TCE exposure). Leukemia and multiple myeloma observations included in NHL category.
Low cumulative TCE score			1.0 (referent)	27					
Medium cumulative TCE score			1.49 (0.86, 2.57)	27					
High TCE score			1.30 (0.52, 3.23)	6					
(p for trend)			(0.370)						
View-Master employees, Oregon									ATSDR (2004a)
Males	0.58 (0.11, 1.69)	3	0.69 (0.08, 2.49)	2	0.50 (0.01, 2.79)	1			n = 616 deaths from 1989 to 2001, PMR, does not identify TCE exposure to individual subjects. EPA based the NHL cancer category on “other lymphopoietic tissue” which included NHL and multiple myeloma.
Females	0.64 (0.28, 1.26)	8	0.52 (0.14, 1.33)	4	0.67 (0.14, 1.96)	3			

**Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)**

Population, exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description <sup>b</sup>
	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	
Electronic workers, Taiwan									Chang et al. (2003)
All employees									n = 88,868 (n = 70,735 female), began work 1978–1997, follow-up 1985–1997, does not identify TCE exposure to individual subjects.
Males	Not reported		1.27 (0.41, 2.97)	5	0.44 (0.05, 1.59)	2	Not reported		
Females	Not reported		1.14 (0.55, 2.10)	10	0.54 (0.23, 1.07)	8	Not reported		
Aerospace workers (Lockheed), California									Boice et al. (1999)
Routine TCE									n = 77,965 (n = 2,267 with routine TCE exposure and n = 3,016 with intermittent-routine TCE exposure), began work ≥1960, worked at least 1 yr, follow-up from 1960 to 1996, JEM without quantitative estimate of TCE intensity.
Any TCE exposure	1.5 (0.81, 1.60)	36	1.19 (0.65, 1.99)	14	1.05 (0.54, 1.84)	12	0.91 (0.34, 1.99)	6	
Routine-intermittent									
Any TCE exposure	Not reported		Not reported		Not reported				
Exposure duration	Not reported				Not reported				
0 yr			1.0 (referent)	32			1.0 (referent)	24	
<1 yr			0.74 (0.32, 1.72)	7			0.45 (0.13, 1.54)	3	
1–4 yrs			1.33 (0.64, 2.78)	10			1.48 (0.64, 3.41)	8	
≥5 yrs			1.62 (0.82, 3.22)	14			0.51 (0.15, 1.76)	3	
p for trend			0.20				>0.20		

**Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)**

Population, exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description <sup>b</sup>
	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	
Uranium-processing workers (Fernald), Ohio									Ritz ( <a href="#">1999a</a> )
Any TCE exposure	Not reported		Not reported		Not reported		Not reported		n = 3,814 (n = 2,971 with TCE), began work 1951–1972, worked ≥3 mo, follow-up to 1989, internal referents (workers with no TCE exposure).
No TCE exposure	1.0 (referent)		Not reported		Not reported		Not reported		
Light TCE exposure, >2 yrs	1.45 (0.68, 3.06) <sup>c</sup>	18	Not reported		Not reported		Not reported		
Moderate TCE exposure, >2 yrs	1.17 (0.15, 9.00) <sup>c</sup>	1	Not reported		Not reported		Not reported		
Aerospace workers (Hughes), California									Morgan et al. ( <a href="#">1998</a> )
TCE subcohort	0.99 (0.64, 1.47)	25	0.96 (0.20, 2.81) <sup>d</sup>	3	1.05 (0.50, 1.93)	10	1.08 (0.35, 2.53) <sup>e</sup>	5	n = 20,508 (4,733 with TCE exposure), worked ≥6 mo 1950–1985, follow-up to 1993, external and internal (all non-TCE exposed workers) workers referent, JEM (intensity).
TCE subcohort			1.01 (0.46, 1.92) <sup>e</sup>	9					
Low intensity (<50 ppm)	1.07 (0.51, 1.96)	10	1.79 (0.22, 6.46) <sup>d</sup>	2	0.85 (0.17, 2.47)	3			
High intensity (>50 ppm)	0.95 (0.53, 1.57)	15	0.50 (0.01, 2.79) <sup>d</sup>	1	1.17 (0.47, 2.41)	7			
TCE subcohort (Cox Analysis)									
Never exposed	1.0 (referent)	82	1.0 (referent)	8	1.0 (referent)	32			
Ever exposed	1.05 (0.67, 1.65) <sup>f</sup>	25	1.36 (0.35, 5.22) <sup>d,f</sup>	3	0.99 (0.48, 2.03) <sup>f</sup>	10			
Peak									
No/Low	1.0 (referent)	90	1.0 (referent)	9	1.0 (referent)	35			
Medium/High	1.08 (0.64, 1.82)	17	1.31 (0.28, 6.08) <sup>d</sup>	2	1.10 (0.49, 2.49)	7			

**Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)**

Population, exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description <sup>b</sup>	
	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>		
Aerospace workers (Hughes), California (continued)										
Cumulative										
Referent	1.0 (referent)	82	1.0 (referent)	8	1.0 (referent)	32				
Low	1.09 (0.56, 2.14)	10	2.25 (0.46, 11.1) <sup>d</sup>	2	0.69 (0.21, 2.32)	3				
High	1.03 (0.59, 1.79)	15	0.81 (0.10, 6.49) <sup>d</sup>	1	1.14 (0.5, 2.60)	7				
Aircraft maintenance workers, Hill Air Force Base, Utah										
TCE subcohort	1.1 (0.7, 1.8) <sup>g</sup>	66	2.0 (0.9, 4.6) <sup>g</sup>	28	0.6 (0.3, 1.2) <sup>g</sup>	16	1.3 (0.5, 3.4)	14	n = 14,066 (n = 7,204 ever exposed to TCE), employed at least 1 yr from 1952 to 1956, follow-up to 1990 (Blair et al., 1998) or to 2000 (Radican et al., 2008), JEM, internal referent (workers with no chemical exposures).	
Males, cumulative exposure										
0	1.0 (referent)		1.0 (referent)		1.0 (referent)		1.0 (referent)			
<5 ppm-yr	1.1 (0.6, 2.1)	21	1.8 (0.6, 5.4)	10	1.0 (0.3, 3.2)	7	1.0 (0.2, 4.2)	4		
5–25 ppm-yr	1.0 (0.4, 2.1)	11	1.9 (0.6, 6.3)	6		0	0.8 (0.1, 4.4)	2		
>25 ppm-yr	1.3 (0.7, 2.5)	21	1.1 (0.3, 3.8)	5	1.2 (0.4, 3.6)	7	1.2 (0.3, 4.7)	4		
Females, cumulative exposure										
0	1.0 (referent)				1.0 (referent)		1.0 (referent)			
<5 ppm-yr	1.5 (0.6, 4.0)	6	3.8 (0.8, 18.9)	3	0.4 (0.1, 3.2)	1	3.2 (0.5, 19.8)	2		
5–25 ppm-yr	0.7 (0.1, 4.9)	1		0		0	4.3 (0.4, 23.4)	1		
>25 ppm-yr	1.1 (0.4, 3.0)	6	3.6 (0.8, 16.2)	4	0.3 (0.1, 2.4)	1	1.3 (0.1, 13.2)	1		
TCE subcohort	1.06 (0.75, 1.51) <sup>h</sup>	106	1.36 (0.77, 2.39) <sup>h</sup>	46	0.64 (0.35, 1.18) <sup>h</sup>	27	1.35 (0.62, 2.93)	25		

**Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)**

Population, exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description <sup>b</sup>
	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	
Aircraft maintenance workers, Hill Air Force Base, Utah (continued)									
Males, cumulative exposure	1.12 (0.72, 1.73)	88	1.56 (0.79, 4.21)	37	0.77 (0.37, 1.62)	24	1.08 (0.43, 2.71)	19	
0	1.0 (referent)		1.0 (referent)		1.0 (referent)		1.0 (referent)		
<5 ppm-yr	1.04 (0.63, 1.74)	34	1.83 (0.79, 4.21)	18	0.86 (0.36, 2.02)	11	0.69 (0.21, 2.27)	5	
5–25 ppm-yr	1.06 (0.49, 1.88)	21	1.17 (0.42, 3.24)	7	0.51 (0.16, 1.63)	4	1.58 (0.53, 4.71)	7	
>25 ppm-yr	1.25 (0.75, 2.09)	33	1.50 (0.61, 3.69)	12	0.87 (0.35, 2.14)	9	1.19 (0.40, 3.54)	7	
Females, cumulative exposure	1.00 (0.55, 1.83)	18	1.18 (0.49, 2.85)	9	0.36 (0.10, 1.32)	3	2.37 (0.67, 8.44)	6	
0	1.0 (referent)		1.0 (referent)		1.0 (referent)		1.0 (referent)		
<5 ppm-yr	1.10 (0.48, 2.54)	7	1.48 (0.47, 4.66)	4	0.35 (0.05, 2.72)	1	2.20 (0.40, 12.02)	2	
5–25 ppm-yr	0.38 (0.05, 2.79)	1		0		0	2.79 (0.31, 25.05)	1	
>25 ppm-yr	1.11 (0.53, 2.31)	10	1.30 (0.45, 3.77)	5	0.48 (0.10, 2.19)	2	2.38 (0.53, 10.67)	3	
Cardboard manufacturing workers, Arnsburg, Germany									Henschler et al. (1995)
TCE-exposed subjects	1.10 (0.03, 6.12)	1							n = 169 TCE exposed and n = 190 unexposed men, employed ≥1 yr from 1956 to 1975, follow-up to 1992, local population referent, qualitative exposure assessment.
Unexposed subjects from same factory	1.11 (0.03, 6.19)	1							

**Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)**

Population, exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description <sup>b</sup>
	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	
GE plant, Pittsfield, Massachusetts			0.76 (0.24, 2.42) <sup>ij</sup>	15	1.1 (0.46, 2.66) <sup>i</sup>	22			Greenland et al. ( <a href="#">1994</a> )
									Nested case-control study, n = 512 cancer (cases) and 1,202 noncancer (controls) male deaths reported to pension fund between 1969 and 1984 among workers employed <1984 and with job history record, JEM-ever held job with TCE exposure. Hodgkin lymphoma in NHL grouping.
Cardboard manufacturing workers, Atlanta, Georgia									Sinks et al. ( <a href="#">1992</a> )
	0.3 (0.0, 1.6)	1	Not reported		Not reported		Not reported		n = 2,050, employed on or before 1957–1988, follow-up to 1988, Material Data Safety Sheets used to identify chemicals used in work areas.

**Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)**

Population, exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description <sup>b</sup>
	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	
U.S. Coast Guard employees									Blair et al. (1989)
Marine inspectors	1.57 (0.91, 2.51)	17	1.75 (0.48, 4.49)	4	1.55 (0.62, 3.19)	7	Not reported		n = 3,781 males (1,767 marine inspectors), employed 1942–1970, follow-up to 1980. TCE and nine other chemicals identified as potential exposures; no exposure assessment to individual subjects.
Noninspectors	0.60 (0.24, 1.26)	7	0.41 (0.01, 2.30)	1	0.66 (0.14, 1.94)	3	Not reported		
Aircraft manufacturing employees, Italy									Costa et al. (1989)
All male subjects	0.80 (0.41, 1.40)	12	Not reported		Not reported		Not reported		n = 7,676 males, employed on or before 1954–1981, followed to 1981, job titles of white- and blue-collar workers, technical staff, and administrative clerks, does not identify TCE exposure to individual subjects.
Aircraft manufacturing, San Diego, California									Garabrant et al. (1988)
All employees	0.82 (0.56, 1.15)	32	0.82 (0.44, 1.41) <sup>d</sup>	13	0.82 (0.47, 1.32)	10	Not reported		n = 14,067, employed at least 4 yrs with company and ≥1 d at San Diego plant from 1958 to 1982, followed to 1982, does not identify TCE exposure to individual subjects.
			0.65 (0.21, 1.52) <sup>k</sup>	5					

**Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)**

Population, exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description <sup>b</sup>
	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	RR (95% CI)	n <sup>a</sup>	
Solvent-exposed rubber workers									Wilcosky et al. ( <a href="#">1984</a> )
	2.4 <sup>i</sup>	3	0.81	3					Nested case-control study, n = 9 lymphosarcoma and 10 leukemia (cases) and 20% random sample of all other deaths (controls) between 1964 and 1973 in cohort of n = 6,678, exposure assessment by company record for use in work area.

<sup>a</sup>n = number of observed cases.

<sup>b</sup>Unless otherwise noted, all studies reported standardized mortality ratios using an external population referent group.

<sup>c</sup>Logistic regression analysis with 15 lag for TCE exposure ([Ritz, 1999a](#)).

<sup>d</sup>In Morgan et al. ([1998](#)) and Garabrant et al. ([1988](#)), this category was based on lymphosarcoma and reticulosarcoma.

<sup>e</sup>As presented in Mandel et al. ([2006](#)) for NHL, this category defined as ICD-7, ICDA-8, and ICD-9 codes of 200 and 202. As presented in Alexander et al. ([2006](#)) for multiple myeloma.

<sup>f</sup>Risk ratio from Cox Proportional Hazard Analysis, stratified by age and sex, from Environmental Health Strategies ([1997](#)) Final Report to Hughes Corporation (Communication from Paul A. Cammer, President, Trichloroethylene Issues Group to Cheryl Siegel Scott, U.S. EPA, December 22, 1997).

<sup>g</sup>Estimated RRs from Blair et al. ([1998](#)) from Poisson regression models adjusted for date of hire, calendar year of death and sex.

<sup>h</sup>Estimated RRs from Radican et al. ([2008](#)) from Cox proportional hazard models adjusted for age and sex.

<sup>i</sup>OR from nested case-control analysis.

<sup>j</sup>Lymphomas, lymphosarcomas, reticulosarcomas, and Hodgkin lymphoma (ICDA-8 200-202) in Greenland et al. ([1994](#)).

<sup>k</sup>Other lymphatic and hematopoietic tissue neoplasms ([Garabrant et al., 1988](#)).

**Table 4-73. Case-control studies of TCE exposure and lymphopietic cancer, leukemia or multiple myeloma**

Population	Cancer type and exposure group	OR (95% CI)	n exposed cases	Reference(s)
Men and women aged 20–74 in Iowa, Los Angeles County (California), Seattle and Detroit metropolitan areas	<b>NHL</b>			Gold et al. ( <a href="#">2011</a> ); Purdue et al. ( <a href="#">2011</a> )
	Any TCE exposure			
	Possible	1.1 (0.9, 1.3)	545	
	Probable	1.4 (0.8, 2.4)	45	
	Average weekly exposure <sup>a</sup>			
	0 ppm-hr/wk	1.0	341	
	1–60 ppm-hr/wk	1.6 (0.7, 3.8)	15	
	61–150 ppm-hr/wk	0.5 (0.2, 1.4)	7	
	>150 ppm-hr/wk	2.5 (1.1, 6.1)	23	
	( <i>p</i> for linear trend)	0.02		
	Cumulative exposure <sup>a</sup>			
	0	1.0	341	
	1–46,800 ppm-hr	1.4 (0.6, 3.3)	14	
	46,801–112,320 ppm-hr	0.6 (0.2, 1.7)	7	
	>112,320 ppm-hr	2.3 (1.0, 5.0)	24	
	( <i>p</i> for linear trend)	0.08		
	<b>NHL types</b>			
	Probable TCE exposure			
	Diffuse	0.9 (0.5, 2.0)	155	
	Follicular	2.1 (1.0, 4.2)	13	
CLL	2.7 (1.2, 5.8)	11		

**Table 4-73. Case-control studies of TCE exposure and lymphopoietic cancer, leukemia or multiple myeloma (continued)**

Population	Cancer type and exposure group	OR (95% CI)	n exposed cases	Reference(s)
Men and women aged 20–74 (continued)	<b>Multiple myeloma</b>			Gold et al. (2011); Purdue et al. (2011) (continued)
	Any TCE exposure	1.4 (0.9, 2.1)	66	
	High confidence exposure <sup>b</sup>	1.7 (1.0, 2.7)	43	
	Cumulative exposure <sup>b</sup>			
	0	1.0	139	
	1–471 ppm-hr	1.1 (0.4, 2.9)	6	
	472–3,000 ppm-hr	1.6 (0.7, 3.5)	11	
	3,001–7,644 ppm-hr	1.5 (0.6, 3.9)	7	
	7,645–570,000 ppm-hr	2.3 (1.1, 5.0)	17	
	<i>(p</i> for linear trend)	0.03		
Men and women aged ≥17 yrs in Czech Republic, Finland, France, Germany, Ireland, Italy, and Spain (EpiLymph study)	<b>All Centers:</b>			Cocco et al. (2010)
	<b>B-cell NHL<sup>b</sup></b>			
	Any TCE exposure	0.8 (0.6, 1.1)	71	
	Cumulative Exposure			
	Low	0.9 (0.6, 1.6)	26	
	Medium	0.5 (0.3, 0.9),	16	
	High	1.0 (0.6, 1.6)	29	
		<i>(p</i> for linear trend)	0.16	

**Table 4-73. Case-control studies of TCE exposure and lymphopietic cancer, leukemia or multiple myeloma (continued)**

Population	Cancer type and exposure group	OR (95% CI)	n exposed cases	Reference(s)
Men and women aged ≥17 yrs in Czech Republic, Finland, France, Germany, Ireland, Italy, and Spain (Epilymph study) (continued)	<b>NHL types<sup>c</sup></b>			Cocco et al. (2010) (continued)
	Diffuse large B-cell	0.7 (0.4, 1.1)	17	
	Follicular	1.2 (0.6, 2.3)	11	
	CLL	0.9 (0.5, 1.5)	18	
	Multiple myeloma	0.6 (0.3, 1.2)	9	
	T-cell lymphoma	0.9 (0.4, 2.2)	6	
	<b>German centers:</b>			Seidler et al. (2007); Mester et al. (2006)
	<b>NHL</b>			
	Any TCE exposure	Not reported		
	Cumulative TCE			
	0 ppm-yr	1.0	610	
	>0– ≤4 ppm-yr	0.7 (0.4, 1.1)	40	
	4.4– <35 ppm-yr	0.7 (0.5, 1.2)	32	
	High exposure, >35 ppm-yr	2.1 (1.0, 4.8)	21	
( <i>p</i> for linear trend)	0.14			
>35 ppm-yr, 10-yr lag	2.2 (1.0, 4.9)			
Women aged 21–84 in Connecticut, United States	<b>NHL</b>			Wang et al. (2009)
	Any TCE exposure	1.2 (0.9, 1.8)	77	
	Low intensity TCE exposure	1.1 (0.8, 1.6)	64	
	Medium-high intensity TCE exposure	2.2 (0.9, 5.4)	13	
	( <i>p</i> for linear trend)	0.06		
	Low probability TCE exposure	1.1 (0.7, 1.8)	43	
	Medium-high probability TCE exposure	1.4 (0.9, 2.4)	34	
	( <i>p</i> for linear trend)	0.37		
	Low intensity TCE exposure/low probability	0.9 (0.6, 1.5)	30	Wang et al. (2009) (continued)
	Low intensity/medium-high probability	1.4 (0.9, 2.4)	34	
Medium-high intensity/low probability	2.2 (0.9, 5.4)	13		
Medium-high intensity/medium-high probability		0		

**Table 4-73. Case-control studies of TCE exposure and lymphopoietic cancer, leukemia or multiple myeloma (continued)**

Population	Cancer type and exposure group	OR (95% CI)	n exposed cases	Reference(s)
Population in eight Italian regions	<b>NHL</b>			Miligi et al. (2006); Costantini et al. (2008)
	Any TCE exposure	Not reported		
	TCE exposure intensity			
	Very low/low	0.8 (0.5, 1.3)	35	
	Medium/high	1.2 (0.7, 2.0)	35	
	( <i>p</i> for linear trend)	0.8		
	Duration exposure, medium/high TCE intensity			
	≤15 yrs	1.1 (0.6, 2.1)	22	
	>15 yrs	1.0 (0.5, 2.6)	12	
	( <i>p</i> for linear trend)	0.72		
	<b>Other NHL</b>			
	TCE exposure intensity, medium/high			
	Small lymphocytic NHL	0.9 (0.4, 2.1)	7	
	Follicular NHL	Not presented	3	
	Diffuse NHL	1.9 (0.9, 3.7)	13	
	Other NHL	1.2 (0.6, 2.4)	11	
Multiple myeloma	0.9 (0.3, 2.4)	27		

**Table 4-73. Case-control studies of TCE exposure and lymphopoietic cancer, leukemia or multiple myeloma (continued)**

Population	Cancer type and exposure group	OR (95% CI)	n exposed cases	Reference(s)
Population in eight Italian regions (continued)	<b>Leukemia</b>			Miligi et al. (2006); Costantini et al. (2008) (continued)
	Any TCE exposure	Not reported		
	TCE exposure intensity			
	Very low/low	1.0 (0.5, 1.8)	17	
	Medium/high	0.7 (0.4, 1.5)	11	
	<b>CLL</b>			
	Any TCE exposure	Not reported		
	TCE exposure intensity			
Population of Örebro and Linköping, Sweden	<b>B-cell NHL</b>			Persson and Fredrikson (1999)
	Any TCE exposure	1.2 (0.5, 2.4)	16	
Population of Sweden	<b>Hairy cell lymphoma</b>			Nordstrom et al. (1998)
	Any TCE exposure	1.5 (0.7, 3.3)	9	
Population of Umea, Sweden	<b>NHL</b>			Hardell et al. (1994)
	Any exposure to TCE	7.2 (1.3, 42)	4	
Population of Montreal, Canada	<b>NHL</b>			Siemiatycki et al. (1991)
	Any TCE exposure	1.1 (0.6, 2.3) <sup>d</sup>	6	
	Substantial TCE exposure	0.8 (0.2, 2.5) <sup>d</sup>	2	

<sup>a</sup>For Purdue et al. (2011), OR for subjects interviewed using computer-assisted personal interview with job modules and includes subjects assessed as unexposed or with probably exposure, defined as holding one or more jobs with an assigned probability of TCE exposure of  $\geq 50\%$ .

<sup>b</sup>For Gold et al. (2011) subjects with jobs assessed with low confidence considered as unexposed.

<sup>c</sup>For Cocco et al. (2010), OR for subjects with high confidence assessment of TCE exposure.

<sup>d</sup>90% CI.

Four geographic-based studies on NHL in adults are summarized in Table 4-74 (for additional study descriptions, see Appendix B) and subjects in three studies are identified based upon their residence in a community where TCE was detected in water serving the community (ATSDR, 2006a; [Cohn et al., 1994b](#); [Vartiainen et al., 1993](#)). Both Cohn et al. (1994b) and ATSDR (2006a) also present estimates for childhood leukemia and these observations are discussed below with other studies reporting on childhood leukemia. A subject is assumed to have a probability of exposure due to residence likely receiving water containing TCE. Most studies do not include statistical models of water distribution networks, which may influence TCE concentrations delivered to a home, nor a subject's ingestion rate to estimate TCE exposure to individual study subjects. ATSDR (2006a) adopts exposure modeling of soil vapor contamination to define study area boundaries and to identify census tracts with a higher probability of exposure to volatile organic solvents without identifying exposure concentrations to TCE and other solvents. In these studies, one level of exposure to all subjects in a geographic area is assigned, although there is some inherent measurement error and misclassification bias because not all subjects are exposed uniformly.

NHL risk is statistically significantly elevated in three studies in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review [3.1, 95% CI: 1.3, 6.1 ([Hansen et al., 2001](#)); 1.5, 95% CI: 1.2, 2.0, subcohort with higher exposure ([Raaschou-Nielsen et al., 2003](#)), 2.3, 95% CI: 1.0, 5.0, >112,320-ppm hours cumulative TCE exposure, 2.5, 95% CI: 1.1, 6.1, >150-ppm hours average weekly TCE exposure ([Purdue et al., 2011](#))]. Two of these incidence studies report statistically significant associations for NHL for subjects with longer employment duration as a surrogate of TCE exposure [ $\geq 6.25$  year, 4.2, 95% CI: 1.1, 11 ([Hansen et al., 2001](#));  $\geq 5$  year, 1.6, 95% CI: 1.1, 2.2 ([Raaschou-Nielsen et al., 2003](#))] and Purdue et al. (2011) report a positive trend with NHL and cumulative TCE exposure ( $p = 0.08$ ) or average weekly TCE exposure ( $p = 0.02$ ). Hansen et al. (2001) also examined two other exposure surrogates, cumulative exposure and exposure intensity, with estimated risk larger in low exposure groups than for high exposure groups. A fourth study from Sweden reports a large and imprecise risk with TCE [7.2, 95% CI: 1.3, 42 ([Hardell et al., 1994](#))] based on four exposed cases. Cohort mortality studies and other case-control studies, except Cocco et al. (2010), observed a 10–50% increased risk between NHL and any TCE exposure [1.2, 95% CI: 0.65, 1.99 ([Boice et al., 1999](#)); 1.36, 95% CI: 0.35, 5.22 ([Morgan et al., 1998](#)); 1.5, 95% CI: 0.7, 3.3 ([Nordström et al., 1998](#)); 1.2, 95% CI: 0.5, 2.4 ([Persson and Fredrikson, 1999](#)); 1.36, 95% CI: 0.77, 2.39 ([Radican et al., 2008](#)); 1.1, 95% CI: 0.6, 2.3 ([Siemiatycki, 1991](#)); 1.2, 95% CI: 0.9, 1.8 ([Wang et al., 2009](#))].

**Table 4-74. Geographic-based studies of TCE and NHL or leukemia in adults**

Population	Exposure group	NHL		Leukemia		Reference <sup>a</sup>
		RR (95% CI)	n exposed cases	RR (95% CI)	n exposed cases	
Two study areas in Endicott, New York		0.54 (0.22, 1.12)	7	0.79 (0.34, 1.55)	8	ATSDR ( <a href="#">2006a</a> )
Residents of 13 census tracts in Redlands, California		1.09 (0.84, 1.38)	111	1.02 (0.74, 1.35)	77	Morgan and Cassady ( <a href="#">2002</a> )
Population in New Jersey	Males, maximum estimated TCE concentration (ppb) in municipal drinking water					Cohn et al. ( <a href="#">1994b</a> )
	<0.1	1.00	493	1.00	438	
	0.1–0.5	1.28 (1.10, 1.48)	272	0.85 (0.71, 1.02)	162	
	≥5.0	1.20 (0.94, 1.52)	78	1.10 (0.84, 1.90)	63	
	Females, maximum estimated TCE concentration (ppb) in municipal drinking water					
	<0.1	1.00	504	1.00; 315		
	0.1–0.5	1.02 (0.87, 1.2)	26	1.13 (0.93, 1.37)	156	
>5.0	1.36 (1.08, 1.70)	87	1.43 (1.43, 1.90)	56		
Population in Finland	Residents of Hausjarvi	0.6 (0.3, 1.1)	14	1.2 (0.8, 1.7)	33	Vartiainen et al. ( <a href="#">1993</a> )
	Residents of Huttula	1.4 (1.0, 2.0)	13	0.7 (0.4, 1.1)	19	

<sup>a</sup>No geographic-based study reported an RR estimate for multiple myeloma except Vartiainen et al. ([1993](#)) who observed SIRs of 0.7 (95% CI: 0.3, 1.3) and 0.6 (95% CI; 0.2, 1.3) for residents of Hausjarvi and Huttula, respectively..

ORs are higher for diffuse or follicular NHL, primarily B-cell lymphomas, than for all NHLs in both studies that examine forms of lymphoma, although based on few exposed cases and inconsistently reported ([Purdue et al., 2011](#); [Cocco et al., 2010](#); [Miligi et al., 2006](#)) (see Table 4-74). Observations in the two other studies of B-cell lymphomas ([Wang et al., 2009](#); [Persson and Fredrikson, 1999](#)) appear consistent with Miligi et al. (2006) and Purdue et al. (2011). Together, these observations suggest that the associations between TCE and specific NHL types are stronger than the associations seen with other forms of NHL, and that disease misclassification may be introduced in studies examining TCE and NHL as a broader category. Mortality observations in other occupational cohorts ([Sung et al., 2007](#); [Boice et al., 2006b](#); [ATSDR, 2004a](#); [Chang et al., 2003](#); [Ritz, 1999a](#); [Henschler et al., 1995](#); [Greenland et al., 1994](#); [Costa et al., 1989](#); [Garabrant et al., 1988](#); [Wilcosky et al., 1984](#)) included a risk estimate of 1.0 in 95% CIs; these studies neither add to nor detract from the overall weight of evidence given their lower likelihood for TCE exposure due to inferior exposure assessment approaches, lower prevalence of exposure, lower statistical power, and fewer exposed deaths.

Seven studies presented estimated risks for leukemia and overall TCE exposure: Antilla et al. (1995); Blair et al. (1998) and its update by Radican et al. (2008); Morgan et al. (1998); Boice et al. (1999); Boice et al. (2006b); Hansen et al. (2001); and Raaschou-Nielsen et al. (2003). Only three studies also presented estimated risks for a high exposure category ([Blair et al., 1998](#); [Morgan et al., 1998](#); [Anttila et al., 1995](#)). Three case-control studies presented estimated risk for leukemia categories and overall TCE exposure or low or high TCE exposure category ([Purdue et al., 2011](#); [Cocco et al., 2010](#); [Costantini et al., 2008](#)). Risk estimates in these cohort studies ranged from 0.64 (95% CI: 0.35, 1.18) ([Radican et al., 2008](#)) to 2.0 (95% CI: 0.7, 4.44) ([Hansen et al., 2001](#)). The largest study, with 82 observed incident leukemia cases, reported an RR estimate of 1.2 (95% CI: 0.9, 1.4) ([Raaschou-Nielsen et al., 2003](#)). Case-control studies which examined all leukemias ([Costantini et al., 2008](#)) or CLL ([Purdue et al., 2011](#); [Cocco et al., 2010](#); [Costantini et al., 2008](#)), and TCE exposure are quite limited in statistical power. Risk estimates in the four case-control studies ranged from 0.7 (95% CI: 0.4, 1.5) for all leukemias and medium to high exposure intensity [[Costantini et al., 2008](#)] to 2.7 (95% CI: 1.2, 5.8) for CLL] and probable TCE exposure ([Purdue et al., 2011](#)).

Eight cohort studies presented estimated risks for multiple myeloma and overall TCE exposure Antilla et al. (1995); Axelson et al. (1994); Blair et al. (1998) and its update by Radican et al. (2008); Morgan et al. (1998); Boice et al. (1999); Boice et al. (2006b); Hansen et al. (2001); and Raaschou-Nielsen et al. (2003). Only three studies also presented estimated risks for a high exposure category ([Radican et al., 2008](#); [Boice et al., 1999](#); [Anttila et al., 1995](#)). Three case-control studies presented estimated risk for multiple myeloma and overall TCE exposure or low or high TCE exposure category ([Gold et al., 2011](#); [Cocco et al., 2010](#); [Costantini et al., 2008](#)). Risk estimates in these cohort studies ranged from 0.57 (95% CI: 0.01, 3.17) ([Axelson et al., 1994](#)) to 1.62 (95% CI: 0.44, 4.16) ([Anttila et al., 1995](#)). The largest cohort study, with

31 observed incident multiple myeloma cases, reported an RR estimate of 1.03 (95% CI: 0.70, 1.47) ([Raaschou-Nielsen et al., 2003](#)). The largest case-control study of 43 exposed multiple myeloma cases with high confidence TCE exposure reported an OR of 1.7 (95% CI: 1.0, 2.7) and a positive trend with increasing cumulative TCE exposure ( $p = 0.03$ ) ([Gold et al., 2011](#)).

The number of studies of childhood lymphoma including acute lymphatic leukemia and TCE is much smaller than the number of studies of TCE and adult lymphomas, and consists of four case-control studies ([Costas et al., 2002](#); [Shu et al., 1999](#); [McKinney et al., 1991](#); [Lowengart et al., 1987](#)) and four geographic-based studies ([ATSDR, 2008b](#), 2006a; [ADHS, 1995](#); [Cohn et al., 1994b](#); [Aickin et al., 1992](#); [ADHS, 1990](#)) (see Table 4-75). An additional publication, focusing on ras mutations, based on one of the case-control studies is also available ([Shu et al., 2004](#)). All four case-control studies evaluate maternal exposure, and three studies also examine paternal occupational exposure ([Shu et al., 2004](#); [Shu et al., 1999](#); [McKinney et al., 1991](#); [Lowengart et al., 1987](#)). There are relatively few cases with maternal exposure (range 0–16) in these case-control studies, and only Shu et al. (2004; 1999) used a large number ( $n = 136$ ) of cases with paternal exposure. The small numbers of exposed case parents limit examination of possible susceptibility time windows. Overall, evidence for association between parental TCE exposure and childhood leukemia is not robust or conclusive.

The results from the studies of Costas et al. (2002) and Shu et al. (2004; 1999) suggest a fetal susceptibility to maternal exposure during pregnancy, with RRs observed for this time period equal or higher than the RRs observed for periods before conception or after birth (see Table 4-75). The studies by Lowengart et al. (1987) and McKinney et al. (1991) do not provide informative data pertaining to this issue due to the small number ( $n = <3$ ) of exposed case mothers. A recent update of a cohort study of electronics workers at a plant in Taiwan (2005; [Chang et al., 2003](#)) reported a fourfold increased risk (3.83; 95% CI: 1.17, 12.55) ([Sung et al., 2008](#)) for childhood leukemia risk among the offspring of female workers employed during the 3 months before to 3 months after conception. Exposures at this factory included TCE, perchloroethylene, and other organic solvents ([Sung et al., 2008](#)). The lack of TCE assignment to individual subjects in this study decrease its weight in the overall analysis.

**Table 4-75. Selected results from epidemiologic studies of TCE exposure and childhood leukemia**

	<b>RR (95% CI)</b>	<b>n observed events</b>	<b>Reference(s)</b>
<b>Cohort studies (solvents)</b>			
Childhood leukemia among offspring of electronic workers			Sung et al. ( <a href="#">2008</a> )
Nonexposed	1.0 <sup>a</sup>	9	
Exposed pregnancy to organic solvents	3.83 (1.17, 12.55)	6	
<b>Case-control studies</b>			
Children's Cancer Group Study (children ≤15 yrs old)			
<b>Acute lymphocytic leukemia</b>			
Maternal occupational exposure to TCE			Shu et al. ( <a href="#">1999</a> )
Anytime	1.8 (0.8, 4.1)	15	
Preconception	1.8 (0.8, 5.2)	9	
During pregnancy	1.8 (0.5, 6.4)	6	
Postnatal	1.4 (0.5, 4.1)	9	
Paternal occupational exposure to TCE			
Anytime	1.1 (0.8, 1.5)	136	
Preconception	1.1 (0.8, 1.5)	100	
During pregnancy	0.9 (0.6, 1.4)	56	
Postnatal	1.0 (0.7, 1.3)	77	
<b>K-ras + acute lymphocytic leukemia</b>			Shu et al., ( <a href="#">2004</a> )
Maternal occupational exposure to TCE			
Anytime	1.8 (0.6, 4.8)	5	
Preconception	2.0 (0.7, 6.3)	4	
During pregnancy	3.1 (1.0, 9.7)	4	
Postnatal		0	
Paternal occupational exposure to TCE			
Anytime	0.6 (0.3, 1.4)	9	
Preconception	0.6 (0.3, 1.5)	8	
During pregnancy	0.3 (0.1, 1.2)	2	
Postnatal	0.4 (0.1, 1.4)	3	

**Table 4-75. Selected results from epidemiologic studies of TCE exposure and childhood leukemia (continued)**

	<b>RR (95% CI)</b>	<b>n observed events</b>	<b>Reference(s)</b>
Residents of ages ≤19 in Woburn, Massachusetts			Costas et al. ( <a href="#">2002</a> )
<b>Maternal exposure 2 yrs before conception to diagnosis</b>			
Never	1.00	3	
Least	5.00 (0.75, 33.5)	9	
Most	3.56 (0.51, 24.8)	7	
( <i>p</i> for linear trend)	≥0.05		
<b>Maternal exposure 2 yrs before conception</b>			
Never	1.00	11	
Least	2.48 (0.42, 15.2)	4	
Most	2.82 (0.30, 26.4)	4	
( <i>p</i> for linear trend)	≥0.05		
<b>Birth to diagnosis</b>			
Never	1.00	7	
Least	1.82 (0.31, 10.8)	7	
Most	0.90 (0.18, 4.56)	5	
( <i>p</i> for linear trend)	≥0.05		
<b>Maternal exposure during pregnancy</b>			
Never	1.00	9	
Least	3.53 (0.22, 58.1)	3	
Most	14.3 (0.92, 224)	7	
( <i>p</i> for linear trend)	<0.05		
Population ≤14 yrs of age in 3 areas north England, United Kingdom			McKinney et al. ( <a href="#">1991</a> )
<b>Acute lymphocytic leukemia and NHL</b>			
<b>Maternal occupation exposure to TCE</b>			
Preconception	1.16 (0.13, 7.91)	2	
<b>Paternal occupational exposure to TCE</b>			
Preconception	2.27 (0.84, 6.16)	9	
Periconception and gestation	4.49 (1.15, 21)	7	
Postnatal	2.66 (0.82, 9.19)	7	

**Table 4-75. Selected results from epidemiologic studies of TCE exposure and childhood leukemia (continued)**

	<b>RR (95% CI)</b>	<b>n observed events</b>	<b>Reference(s)</b>
Los Angeles Cancer Surveillance Program			Lowengart et al. ( <a href="#">1987</a> )
<b>Acute lymphocytic and nonlymphocytic leukemia, ≤10 yrs old</b>			
Maternal occupational exposure to TCE		0	
Paternal occupational exposure to TCE			
One yr before pregnancy	2.0 ( <i>p</i> = 0.16)	6/3 <sup>b</sup>	
During pregnancy	2.0 ( <i>p</i> = 0.16)	6/3 <sup>b</sup>	
After delivery	2.7 (0.64, 15.6)	8/3 <sup>b</sup>	
<b>Geographic-based studies</b>			
Two study areas in Endicott, New York			ATSDR ( <a href="#">2006a</a> )
<b>Leukemia, ≤19 yrs old</b>	Not reported	<6	
Population in New Jersey			
<b>Acute lymphocytic leukemia</b>			
Maximum estimated TCE concentration (ppb) in municipal drinking water			Cohn et al. ( <a href="#">1994b</a> )
Males			
<0.1	1.00	45	
0.1–0.5	0.91 (0.53, 1.57)	16	
≥5.0	0.54 (0.17, 17.7)	3	
Females			
<0.1	1.00	25	
0.1–0.5	1.85 (1.03, 3.70)	22	
≥5.0	2.36 (1.03, 5.45)	7	
Resident of Tucson Airport Area, Arizona			ADHS ( <a href="#">1995</a> , <a href="#">1990</a> )
<b>Leukemia, ≤19 yrs old</b>			
1970–1986	1.48 (0.74, 2.65)	11	
1987–1991	0.80 (0.31, 2.05)	3	
Resident of West Central Phoenix, Arizona			Aickin et al. ( <a href="#">1992</a> )
<b>Leukemia, ≤19 yrs old</b>	1.95 (1.43, 2.63)	38	

<sup>a</sup>Internal referents, live born children among female workers not exposed to organic solvents.

<sup>b</sup>Discordant pairs.

The evidence for an association between childhood leukemia and paternal exposure to solvents is quite strong ([Colt and Blair, 1998](#)); however, for studies of TCE exposure, the small numbers of exposed case fathers in two studies ([McKinney et al., 1991](#); [Lowengart et al., 1987](#)) and, for all three studies, likelihood of misclassification resulting from a high percentage of paternal occupation information obtained from proxy interviews, limits observation interpretations. Both Lowengart et al. ([1987](#)) and McKinney et al. ([1991](#)) provide some evidence for a two- to fourfold increase of childhood leukemia risk and paternal occupational exposure although the population study of Shu et al. ([2004](#); [1999](#)), with 13% of case father's occupation reported by proxy respondents, does not appear to support the earlier and smaller studies.

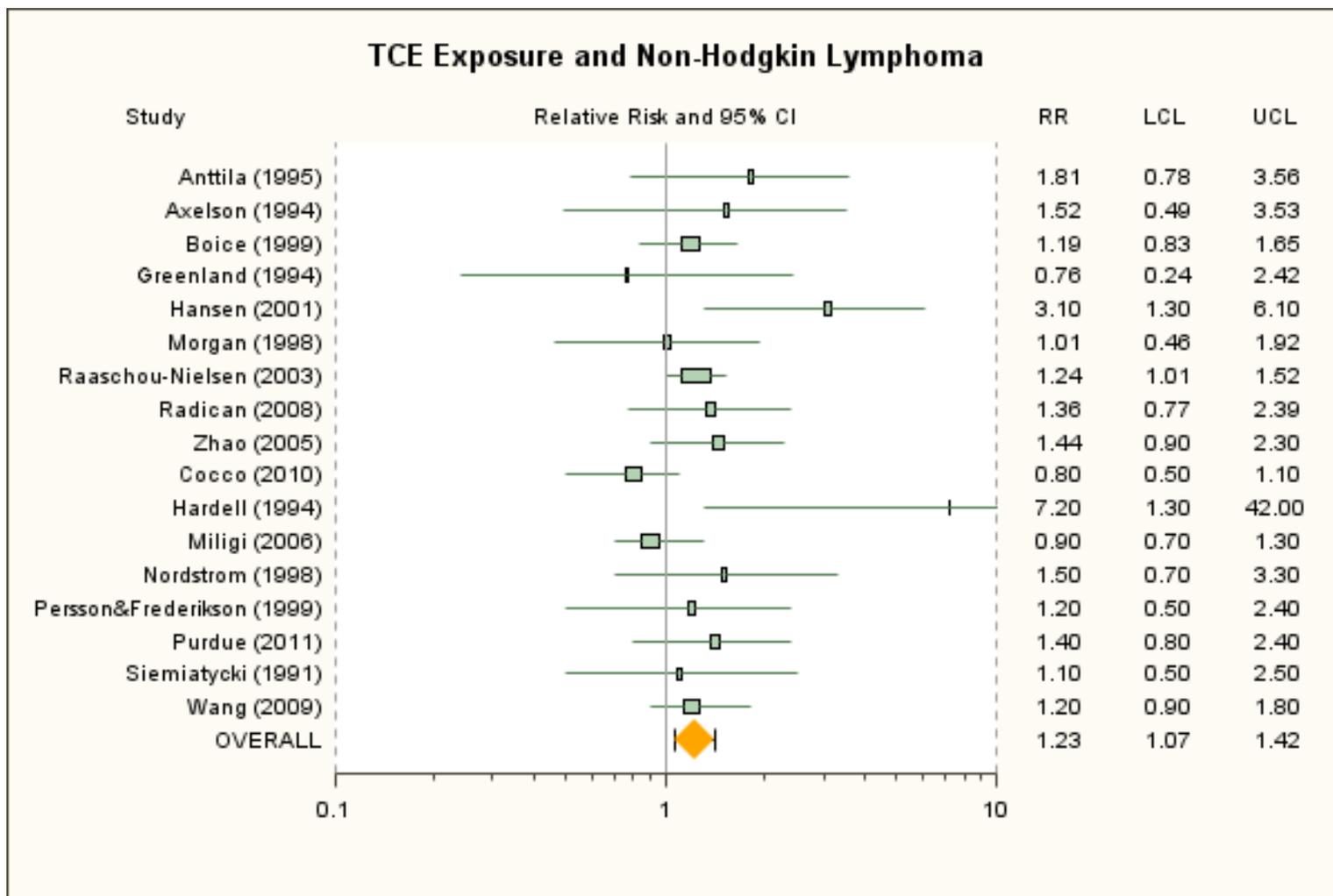
The geographic-based studies for adult lymphopoietic (see Table 4-74) or childhood leukemias (see Table 4-75) do not greatly contribute to the overall weight of evidence. While some studies observed statistically significantly elevated risks for NHL or childhood cancer, these studies generally fulfilled only the minimal of evaluation criteria with questions raised about subject selection ([Morgan and Cassady, 2002](#)), their use of less sophisticated exposure assessment approaches and associated assumption of an average exposure to all subjects (all studies), and few cases with high level parental exposure (all studies).

#### 4.6.1.2.2. Meta-analysis of NHL risk

Meta-analysis is adopted as a tool for examining the body of epidemiologic evidence on NHL and TCE exposure and to identify possible sources of heterogeneity. The meta-analysis of NHL examines 17 cohort and case-control studies identified through a systematic review and evaluation of the epidemiologic literature on TCE exposure ([Purdue et al., 2011](#); [Cocco et al., 2010](#); [Wang et al., 2009](#); [Radican et al., 2008](#); [Miligi et al., 2006](#); [Zhao et al., 2005](#); [Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#); [Boice et al., 1999](#); [Persson and Fredrikson, 1999](#); [Morgan et al., 1998](#); [Nordström et al., 1998](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#); [Greenland et al., 1994](#); [Hardell et al., 1994](#); [Siemiatycki, 1991](#)) and two studies as alternatives ([Boice et al., 2006b](#); [Blair et al., 1998](#)). These 19 studies of NHL and TCE had high likelihood of exposure, were judged to have met, to a sufficient degree, the criteria of epidemiologic design and analysis, and reported estimated risks for overall TCE exposure; 13 of these studies, also, presented estimated NHL risk with high level TCE exposure ([Purdue et al., 2011](#); [Cocco et al., 2010](#); [Wang et al., 2009](#); [Radican et al., 2008](#); [Miligi et al., 2006](#); [Zhao et al., 2005](#); [Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#); [Boice et al., 1999](#); [Morgan et al., 1998](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#); [Siemiatycki, 1991](#)). Full details of the systematic review, criteria to identify studies for including in the meta-analysis, and meta-analysis methodology and findings are discussed in Appendices B and C.

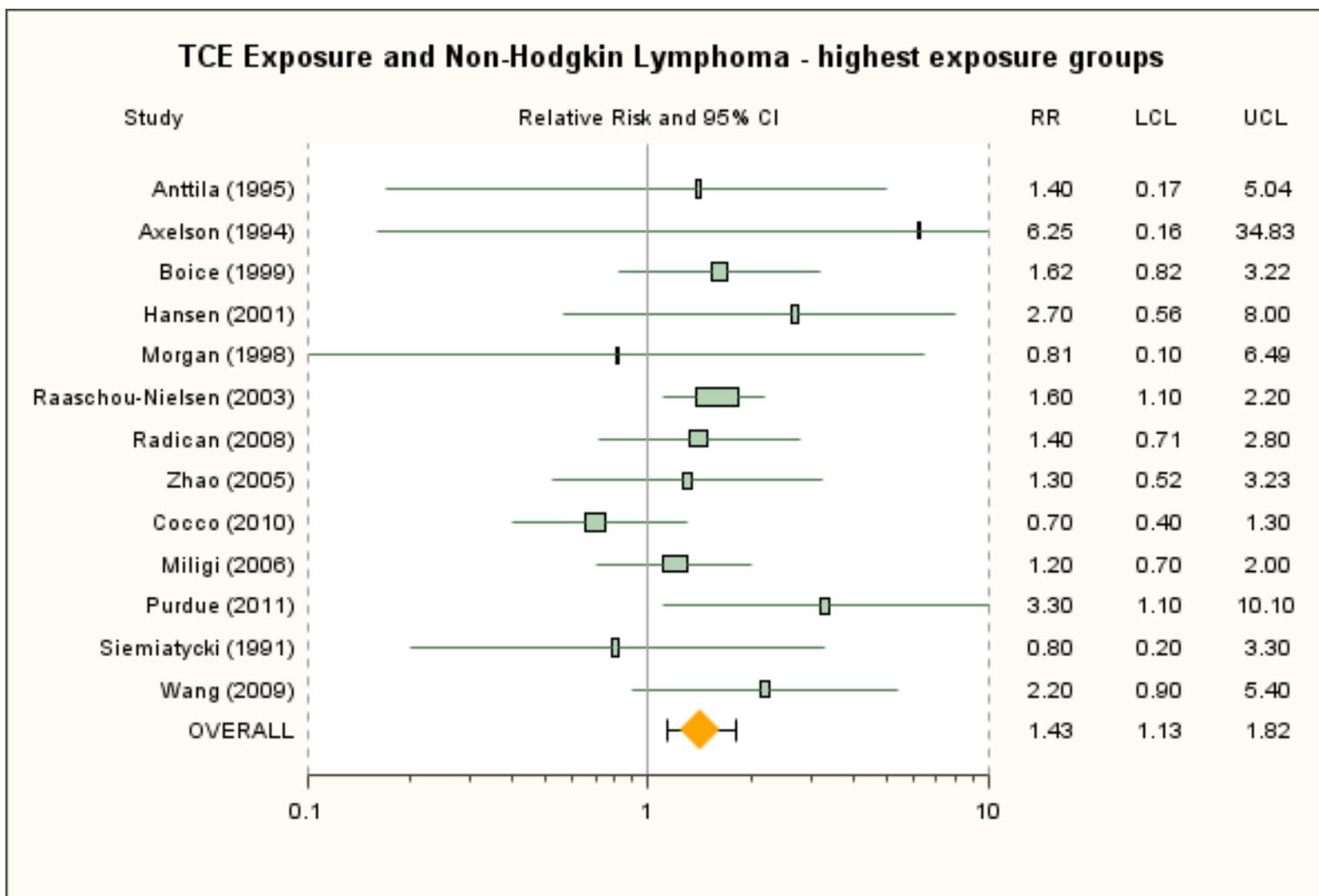
The meta-analyses of the overall effect of TCE exposure on NHL suggest a small, robust, and statistically significant increase in NHL risk. The summary estimate from the primary random effect meta-analysis (RR<sub>m</sub>) was 1.23 (95% CI: 1.07, 1.42) (see Figure 4-16). This result and its statistical significance were not influenced by individual studies. Removal of individual studies resulted in RR<sub>m</sub> estimates between 1.18 ([with the removal of Hansen et al., 2001](#)) and 1.27 (with the removal of Miligi et al. ([2006](#)) or Cocco et al. ([2010](#))), and lower 95% CIs excluded 1.0 (all *p*-values were *p* < 0.02). The result is similarly not sensitive to individual risk ratio estimate selections. Use of six alternative selections, individually, resulted in RR<sub>m</sub> estimate that ranged from 1.20 (95% CI: 1.03, 1.39) (with estimated overall RR for incidence in [Zhao et al., 2005](#)) to 1.28 (95% CI: 1.09, 1.49) (with Raaschou-Nielsen et al. ([2003](#)) subgroup). Nor was the RR<sub>m</sub> estimate highly sensitive to restriction of the meta-analysis to only those studies for which RR estimates for the traditional definition of NHL were available. An alternate

analysis that omitted Miligi (which included CLLs), Nordstrom (which was a study of hairy cell leukemias), Persson and Frederikson (for which the classification system not specified), and Greenland (which included Hodgkin lymphomas) and which included Boice ([2006b](#)) instead of Zhao (which included all lymphohematopoietic cancers) yielded an RRM estimate of 1.27 (95% CI: 1.05, 1.55). Meta-analysis of the highest exposure groups, either duration, intensity, or their product, cumulative exposure, results in an RRM of 1.43 (95% CI: 1.13, 1.82), which is greater than the RRM from the overall exposure analysis, and provides additional support for an association between NHL and TCE (see Figure 4-17). No single study was overly influential; removal of individual studies resulted in RRM estimates that were all statistically significant (all with  $p \leq 0.025$ ) and that ranged from 1.38 [with the removal of Purdue et al. ([2011](#))] to 1.57 [with the removal of Cocco et al. ([2010](#))]. In addition, the RRM estimate was not highly sensitive to alternate RR estimate selections. Use of the nine alternate selections, individually, resulted in RRM estimates that were all statistically significant (all with  $p < 0.025$ ) and all in the narrow range from 1.40 (95% CI: 09, 1.80) [with Blair et al. ([1998](#)) incidence RR instead of Radican et al. ([2008](#)) mortality hazard ratio] to 1.49 (95% CI: 1.14, 1.93) [with Hansen et al. ([2001](#)) duration]. The highest exposure category groups have a reduced likelihood for exposure misclassification because they are believed to represent a greater differential TCE exposure compared to people identified with overall TCE exposure. Observation of greater risk associated with higher exposure category compared to overall (typically any vs. none) exposure comparison additionally suggests an exposure-response gradient between NHL and TCE, although estimation of a level of exposure associated with the RRM is not possible.



The summary estimate is in the bottom row. Symbol sizes reflect relative weights of the studies. The horizontal midpoint of the bottom diamond represents the RRm estimate.

**Figure 4-16. Meta-analysis of NHL and overall TCE exposure.**



The summary estimate is in the bottom row. Symbol sizes reflect relative weights of the studies. The horizontal midpoint of the bottom diamond represents the RR<sub>m</sub> estimate.

**Figure 4-17. Meta-analysis of NHL and TCE exposure—highest exposure groups.**

Low-to-moderate heterogeneity in RR<sub>m</sub> is observed across the results of the 17 studies in the meta-analysis of the overall effect of TCE and the 13 studies with highest exposure groups, but it was not statistically significant ( $p = 0.16$  and  $p = 0.30$ , respectively). The  $I^2$ -values were 26% for overall exposure and 14% for highest exposure groups, suggesting low-to-moderate and low heterogeneity, respectively. To investigate the heterogeneity, subgroup analyses were done examining the cohort and case-control studies separately. Difference between cohort and case-control studies could explain much of the observed heterogeneity. In the subgroup analysis of overall exposure and of highest exposure groups, increased risk of NHL was strengthened in analysis limited to cohort studies and reduced in the case-control study analysis. Examination of heterogeneity in cohort and case-control studies of overall exposure separately was not statistically significant in either case ( $I^2$ -values for the cohort studies were 12%, suggesting low heterogeneity and 27% for the case-control studies, suggesting low-to-moderate heterogeneity), although some may be present given that statistical tests of heterogeneity are generally insensitive in cases of minor heterogeneity. Subgroup analyses examining the cohort and case-control studies highest exposure groups, separately, showed no residual heterogeneity in the cohort subgroup ( $I^2 = 0\%$ ) and moderate heterogeneity in the case-control subgroup ( $I^2$ -value was 53%) that was not statistically significant ( $p = 0.08$ ). Although no further attempt was made to quantitatively investigate potential sources of heterogeneity, the removal of the Cocco et al. (2010) study, an influential study, eliminates all of the heterogeneity, suggesting that the RR estimate for the highest exposure group from that study is a relative outlier.

In general, sources of heterogeneity are uncertain and may reflect several features known to influence epidemiologic studies. Study design itself is unlikely to be an underlying cause of heterogeneity and, to the extent that it may explain some of the differences across studies, is more probably a surrogate for some other difference(s) across studies that may be associated with study design. Furthermore, other potential sources of heterogeneity may be masked by the broad study design subgroupings. The true source(s) of heterogeneity across these studies is an uncertainty.

One reason may be differences in exposure assessment and in overall TCE exposure concentration between cohort and case-control studies. Several cohort and case-control studies included TCE assignment from information on job and task exposures, e.g., a JEM (Purdue et al., 2011; Cocco et al., 2010; Wang et al., 2009; Radican et al., 2008; Boice et al., 2006b; Miligi et al., 2006; Zhao et al., 2005; Boice et al., 1999; Morgan et al., 1998; Siemiatycki, 1991), or from an exposure biomarker in either breath or urine (Hansen et al., 2001; Anttila et al., 1995; Axelson et al., 1994). Three case-control studies (Persson and Fredrikson, 1999; Nordström et al., 1998; Hardell et al., 1994) relied on self-reported TCE exposure. No information is available to judge the degree of possible misclassification bias associated with a particular exposure assessment approach; it is quite possible that in some cohort studies, in which past exposure is inferred from various data sources, exposure misclassification may be as great as in population- or hospital-

based case-control studies. In addition, a low overall TCE exposure prevalence is anticipated in population case-control studies, which would typically assess a large number of workplaces and operations, where exposures are less well defined, and where case and control subjects identified as exposed to TCE probably have minimal contact ([NRC, 2006](#)). Observed higher risk ratios with higher exposure categories in NHL case-control studies support exposure differences as a source of heterogeneity.

Diagnostic inaccuracies are likely another source of heterogeneity in the meta-analysis through study differences in NHL groupings and in lymphoma classification schemes, although restricting the meta-analysis to only those studies for which RR estimates based on the traditional NHL definition were available did not eliminate all heterogeneity. All studies include a broad but slightly different group of lymphosarcoma, reticulum-cell sarcoma, and other lymphoid tissue neoplasms (Codes 200 and 202), except Nordstrom et al. ([1998](#)), Zhao et al. ([2005](#)), and Greenland et al. ([1994](#)). Cohort studies have some consistency in coding NHL, with NHL defined as lymphosarcoma and reticulum-cell sarcoma (200) and other lymphoid tissue neoplasms (202) using the ICD, Revision 7, 200 and 202—four studies ([Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#)), ICD-Adapted, Revision 8 ([Blair et al., 1998](#)), and ICD-7, -8, -9, and -10, per the version in use at the time of death (as presented in Morgan et al. ([1998](#)) [as presented in Mandel et al. ([2006](#)), Boice et al. ([1999](#)), Radican et al. ([2008](#))], as does the case-control study of Siemiatycki ([1991](#)) whose coding scheme for NHL is consistent with ICD 9, 200 and 202. Case-control studies, on the other hand, have adopted other classification systems for defining NHL including the NCI Working Formulation ([Miligi et al., 2006](#)), Rappaport ([Hardell et al., 1994](#)), or else do not identify the classification system for defining NHL ([Persson and Fredrikson, 1999](#)). Cocco et al. ([2010](#)) used the WHO/Revised European-American Lymphoma (REAL) classification system, which reclassifies lymphocytic leukemias and NHLs as lymphomas of B-cell or T-cell origin and considers CLLs and multiple myelomas as (non-Hodgkin) lymphomas; however, U.S. EPA was able to obtain results generally consistent with the traditional NHL definition from Dr. Cocco, although lymphomas not otherwise specified were excluded. Wang et al. ([2009](#)) defined NHL using ICD-O-2 codes (M-9590-9595, 9670-9688, 9690-9698, 9700-9723), which is consistent with the traditional definition of NHL (i.e., ICD-7, -8, -9 codes 200 + 202). Purdue et al. ([2011](#)) used ICD-O-3 codes 967-972, which is generally consistent with the traditional definition of NHL, although this grouping does not include the malignant lymphomas of unspecified type coded as M-9590-9599.

There is some evidence of potential publication bias in this data set; however, it is uncertain that this is actually publication bias rather than an association between SE and effect size resulting for some other reason, e.g., a difference in study populations or protocols in the smaller studies. Furthermore, if there is publication bias in this data set, it does not appear to account completely for the finding of an increased NHL risk.

NRC (2006) deliberations on TCE commented on two prominent evaluations of the then-current epidemiologic literature using meta-analysis techniques. These studies were by Wartenberg et al. (2000), and by Kelsh et al. (2005), submitted by Exponent-Health Sciences to NRC during their deliberations and subsequently published in a paper on NHL (Mandel et al., 2006) and a paper on multiple myeloma and leukemia (Alexander et al., 2006). The NRC found weaknesses in the techniques used in each of these studies, and suggested that EPA conduct a new meta-analysis of the epidemiologic data on TCE using objective and transparent criteria so as to improve on the past analyses. EPA staff conducted their analysis according to NRC (2006) suggestions for transparency, systematic review criteria, and examination of both cohort and case-control studies. The EPA analysis of NHL analysis considered a larger number of studies than in the previous analyses (Mandel et al., 2006; Wartenberg et al., 2000), includes recently published studies (Purdue et al., 2011; Cocco et al., 2010; Wang et al., 2009; Radican et al., 2008; Boice et al., 2006b; Miligi et al., 2006; Zhao et al., 2005), and combines both cohort and case-control studies.

#### **4.6.2. Animal Studies**

The immunosuppressive and immunomodulating potential of TCE has not been fully evaluated in animal models across various exposure routes, over various relevant durations of exposure, across representative lifestages, and/or across a wide variety of endpoints. Nevertheless, the studies that have been conducted indicate a potential for TCE-induced immunotoxicity, both following exposures in adult animals and during immune system development (i.e., in utero and preweaning exposures).

##### **4.6.2.1. Immunosuppression**

A number of animal studies have indicated that moderate to high concentrations of TCE over long periods have the potential to result in immunosuppression in animal models, dependant on species and gender. These studies are described in detail below and summarized in Table 4-76.

###### **4.6.2.1.1. Inhalation exposures**

Mature cross-bred dogs (5/group) were exposed to 0-, 200-, 500-, 700-, 1,000-, 1,500-, or 2,000 ppm TCE for 1 hour or to 700 ppm TCE for 4 hours, by tracheal intubation under i.v. sodium pentobarbital anesthesia. An additional group of dogs was exposed by venous injection of 50 mg/kg TCE administered at a rate of 1 mL/minute (Hobara et al., 1984). Blood was sampled pre- and postexposure for erythrocyte and leukocyte counts. Marked, transient decreases in leukocyte counts were observed at all exposure levels 30 minutes after initiation of exposure. At the end of the exposure period, all types of leukocytes were decreased (by 85%); neutrophils were decreased 33%, and lymphocytes were increased 40%. There were no treatment-related changes in erythrocyte counts, hematocrit values, or thrombocyte counts.

**Table 4-76. Summary of TCE immunosuppression studies**

Exposure route/vehicle, duration, dose	NOAEL; LOAEL <sup>a</sup>	Results	Reference, species/strain sex/number
<b>Inhalation exposure studies<sup>b</sup></b>			
Single 1-hr exposure to all dose groups; plus single 4-hr exposure at 700 ppm <sup>c</sup> 0, 200, 500, 700, 1,000, 1,500, or 2,000 ppm	LOAEL: 200 ppm	Marked transient ↓ leukocyte counts at all exposure levels 30 min after initiating exposure. At end of exposure, 85% ↓ leukocyte counts (33% ↓ neutrophils, 40% ↓ lymphocytes).	Hobara et al. (1984) Dog, cross-bred, both sexes, 5/group
Single 3-hr exposure. Also, 3 hrs/d on 5 d at lowest dose 0, 2.6, 5.2, 10.6, 25.6, or 48 ppm	NOAEL: 2.6 ppm LOAEL: 5.2 ppm	Challenged with <i>Streptococcus zooepidemicus</i> to assess susceptibility to infection and <i>Klebsiella pneumoniae</i> to assess bacterial clearance. For single exposure: dose-related statistically significant ↑ mortality at ≥5.2 ppm over 14 d. Statistically significant ↓ in bactericidal activity at 10.6 ppm.	Aranyi et al. (1986) Mouse, CD-1 females, 4–5 wks old, approximately 30 mice/group, 5–10 replications; for pulmonary bactericidal activity assay, 17–24 mice/group
Single 3-hr exposure. 0, 5, 10, 25, 50, 100, 200 ppm	NOAEL: 25 ppm LOAEL: 50 ppm	Challenged with <i>Streptococcus zooepidemicus</i> to assess susceptibility to infection and bacterial clearance. For single exposure: dose-related statistically significant ↑ mortality at ≥50 ppm over 20 d. Dose dependent responses also observed in the clearance of bacteria from the lung at ≥50 ppm, the number of mice with delayed bacterial clearance at various postinfection time points at ≥50 ppm, and the phagocytic function of alveolar macrophages at 200 ppm.	Selgrade and Gilmour (2010) Mouse, CD-1 females, 5–6 wks old, at least 38 mice/group
Single 3-hr exposure, 50–200 ppm <sup>d</sup>		Challenged with <i>Streptococcus zooepidemicus</i> . Dose-related ↑ mortality, bacterial antiphagocytic capsule formation, and bacterial survival. Dose-related impairment of alveolar macrophages; increased neutrophils in bronchoalveolar fluid at 3 d postinfection.	Park et al. (1993) (abstract) Mouse, CD-1, (sex and number/group not specified)
<b>4-wk, 6 hrs/d, 5 d/wk 0, 100, 300, or 1,000 ppm</b>	<b>NOAEL: 300 ppm LOAEL: 1,000 ppm</b>	<b>At 1,000 ppm, 64% ↓ plaque-forming cell assay response.</b>	<b>Woolhiser et al. (2006) Rat, Sprague-Dawley, female, 16/group</b>

**Table 4-76. Summary of TCE immunosuppression studies (continued)**

Exposure route/vehicle, duration, dose	NOAEL; LOAEL <sup>a</sup>	Results	Reference, species/strain sex/number
<b>Oral exposure studies</b>			
Gavage in 10% Emulphor, 14 d, daily, 0, 24, or 240 mg/kg-d	LOAEL: 24 mg/kg-d	Statistically significant ↓ cell-mediated immune response to SRBC at both dose levels.	Sanders et al. (1982b) Mouse, CD-1, male, 9–12/group
<b>Drinking water with 1% Emulphor, 4–6 mo</b> <b>0, 0.1, 1.0, 2.5, or 5.0 mg/mL</b>	<b>LOAEL: 0.1 mg/kg-d</b>	<b>In females, humoral immunity ↓ at 2.5 and 5 mg/mL TCE, whereas cell-mediated immunity ↓ and bone marrow stem cell colonization ↓ at all four concentrations. The males were relatively unaffected after both 4 and 6 mo.</b>	<b>Sanders et al. (1982b)</b> <b>Mouse, CD-1, male and female, 7–25/group</b>
Gavage, 14 d, 0, 14.4, or 144 mg/kg-d CH	NOAEL: 144 mg/kg-d	No treatment-related effects.	Kauffmann et al. (1982) Mouse, CD-1, male, 12/group
Drinking water, 90 d, 0, 0.07, or 0.7 mg/mL CH. (M: 0, 16, or 160 mg/kg-d; F: 0, 18, or 173 mg/kg-d)	NOAEL: 0.07 mg/mL LOAEL: 0.7 mg/mL	Statistically significant ↓ cell-mediated immune response (plasma hemagglutination titers and spleen antibody-producing cells of mice sensitized to SRBC) in females at 0.7 mg/mL.	Kauffmann et al. (1982) Mouse, CD-1, male and female, 15–20/group
Drinking water, From mating to PND 21 or 56, (Emulphor concentration not provided) 0 (Emulphor), 1, or 10 ppm	LOAEL: 1 ppm	At 10 ppm, ↓ body weight and length at PND 21. IgM antibody response to SRBC challenge suppressed in both ♂ and ♀ pups at 10 ppm, and ♂ pups at 1 ppm, ↓ in splenic CD4+CD8-T-cells. At 56 PND, striking ↑ in NK cell activity seen at both doses.	Adams et al. (2003) (abstract) Mouse, B6C3F <sub>1</sub> , both sexes, numbers of pups not stated
Drinking water, from GDs 0 to 3 or 8 wks of age, 0, 1,400, or 14,000 ppb	LOAEL: 1,400 ppb	Suppressed PFC responses in both sexes and ages at 14,000 ppb, in males at both ages at 1,400 ppb, and in females at 8 wks at 1,400 ppb. Numbers of spleen B220+ cells ↓ at 3 wks at 14,000 ppb. Pronounced ↑ thymus T-cell populations at 8 wks.	Peden-Adams et al. (2006) Mouse, B6C3F <sub>1</sub> , dams and both sexes offspring, 5 litters/group; 5–7 pups/group at 3 wks; 4–5 pups/sex/group at 8 wks
Drinking water, from GD 0 to 7–8 wks of age; 0, 0.5, or 2.5 mg/mL	LOAEL: 0.5 mg/mL	At 0.5 mg/mL: statistically significant ↓ postweaning weight; statistically significant ↑ IFN $\gamma$ produced by splenic CD4+ cells at 5–6 wks; statistically significant ↓ splenic CD8+ and B220+ lymphocytes; statistically significant ↑ IgG2a and histone; statistically significant altered CD4–/CD8– and CD4+/CD8+ thymocyte profile At 2.5 mg/mL: statistically significant ↓ postweaning weight; statistically significant ↑ IFN $\gamma$ produced by splenic CD4+ and CD8+ cells at 4–5 and 5–6 wks; statistically significant ↓ splenic CD4+, CD8+, and B220+ lymphocytes; statistically significant altered CD4+/CD8+ thymocyte profile.	Blossom and Doss (2007) Mouse, MRL +/+, dams and both sexes offspring, 3 litters/group; 8–12 pups/group

**Table 4-76. Summary of TCE immunosuppression studies (continued)**

Exposure route/vehicle, duration, dose	NOAEL; LOAEL <sup>a</sup>	Results	Reference, species/strain sex/number
Drinking water, from GD 0 to PND 42; 0 or 0.1 mg/mL; maternal dose = 25.7 mg/kg-d; offspring PNDs 24–42 dose = 31.0 mg/kg-d	LOAEL: 0.1 mg/mL	At 0.1 mg/mL: at PND 20, statistically significant ↑ thymocyte cellularity and distribution, associated with statistically significant ↑ in thymocyte subset distribution; statistically significant ↑ reactive oxygen species generation in total thymocytes; statistically significant ↑ in splenic CD4+ T-cell production of IFN-γ and IL-2 in females and TNF-α in males at PND 42.	Blossom et al. (2008) Mouse, MRL +/+, dams and both sexes offspring, 8 litters/group; 3–8 pups/group
Drinking water, from GD 0 to 12 mo of age; 0 (1% Emulphor), 1,400, or 14,000 ppb	LOAEL: 1,400 ppb	At 1,400 ppb: splenic CD4–/CD8– cells statistically significant ↑ in females; thymic CD4+/CD8+ cells statistically significant ↓ in males; 18% ↑ in male kidney weight. At 14,000 ppb: thymic T-cell subpopulations (CD8+, CD4/CD8–, CD4+) statistically significant ↓ in males.	Peden-Adams et al. (2008) Mouse, MRL +/+, dams and both sexes offspring, unknown number litters/group, 6–10 offspring/sex/group
<b>i.p. injection exposure studies</b>			
3 d, single daily injection, 0, 0.05, 0.5, or 5 mmol/kg-d	NOAEL: 0.05 mmol/kg-d LOAEL: 0.5 mmol/kg-d	↓ NK cell activity at 0.5 and 5 mmol/kg-d. ↓ splenocyte counts at 5 mmol/kg-d.	Wright et al. (1991) Rat, Sprague-Dawley
3 d, single daily injection, 0 or 10 mmol/kg-d	LOAEL: 10 mmol/kg-d	↓ NK cell activity and ↓ spleen weights at 10 mmol/kg-d.	Wright et al. (1991) Mouse, B6C3F <sub>1</sub>

<sup>a</sup>NOAEL and LOAEL are based upon reported study findings.

<sup>b</sup>**Bolded studies** carried forward for consideration in dose-response assessment (see Chapter 5).

<sup>c</sup>Inhalation, tracheal intubation under anesthesia.

<sup>d</sup>Exact dose levels not specified.

↓, ↑ = decreased, increased; PFC = plaque-forming cell; SRBC = sheep red blood cells

In a study that examined the effects of a series of inhaled organic chemical air contaminants on murine lung host defenses, Aranyi et al. ([Aranyi et al., 1986](#)) exposed female CD-1 mice to single 3-hour exposures of TCE at time-weighted concentrations of 0, 2.6, 5.2, 10.6, 25.6, or 48 ppm. Additionally, at the dose at which no adverse treatment-related effect occurred with a single exposure (i.e., 2.6 ppm), a multiple exposure test (5 days, 3 hours/day) was conducted. Susceptibility to *Streptococcus zooepidemicus* aerosol infection and pulmonary bactericidal activity to inhaled *Klebsiella pneumoniae* were evaluated. There was a significant ( $p < 0.0001$ ) treatment by concentration interaction for mortality, with the magnitude of the effect increasing with concentration. A significant ( $p < 0.0001$ ) treatment by concentration interaction was also found for bactericidal activity. Single 3-hour exposures at 10.6, 25.6, and 48 ppm resulted in significant increases in mortality, although increases observed after single exposures at 5.2 or 2.6 ppm or five exposures at 2.6 ppm were not significant. Pulmonary bactericidal activity was significantly decreased after a single exposure at 10.6 ppm, but single exposures to 2.6 or 5.2 ppm resulted in significant increases.

Suppression of pulmonary host defenses and enhanced susceptibility to respiratory bacterial infection was studied in female CD-1 mice by Selgrade and Gilmour ([2010](#)). The mice (5–6 weeks of age; at least 38 per exposure group) were exposed via inhalation for 3 hours to concentrations of 0, 5, 10, 25, 50, 100, or 200 ppm TCE. The mice were then challenged by aerosol doses of *S. zooepidemicus*. Bacterial clearance (based upon organisms present in lung lavage fluid) and a phagocytic index (percentage of phagocytic cells in lung lavage fluid and the number of bacteria ingested per phagocytic cell) were assessed. Mortality due to infection was significantly increased with TCE exposure concentration at exposures of 50 ppm and higher (NOAEL = 25 ppm). Dose-dependent responses were also observed for the clearance of bacteria from the lung at  $\geq 25$  ppm, the number of mice with delayed bacterial clearance at various postinfection time points at  $\geq 25$  ppm, and the phagocytic function of alveolar macrophages at 200 ppm. The higher NOAEL for mortality observed in this study compared to Aranyi et al. ([1986](#)) (i.e., 25 vs. 5 ppm) was attributed to the use of unencapsulated bacteria in this study; the study authors suggested that this may be more representative of the human condition.

In a host-resistance assay, CD-1 mice (sex and number/group not specified) exposed to TCE by inhalation for 3 hours at 50–200 ppm were found to be more susceptible to increased infection following challenge with *S. zooepidemicus* administered via aerosol ([Park et al., 1993](#)). Dose-related increases in mortality, bacterial antiphagocytic capsule formation, and bacterial survival were observed. Alveolar macrophage phagocytosis was impaired in a dose-responsive manner, and an increase in neutrophils in bronchoalveolar lavage fluid was observed in exposed mice 3 days post infection.

A guideline (OPPTS 870.3800) 4-week inhalation immunotoxicity study was conducted in female Sprague-Dawley rats ([Woolhiser et al., 2006](#)). The animals (16/group) were exposed to TCE at nominal levels of 0, 100, 300, or 1,000 ppm for 6 hours/day, 5 days/week. Effects on

the immune system were assessed using an antigen response assay, relevant organs weights, histopathology of immune organs, and hematology parameters. Four days prior to study termination, the rats were immunized with sheep red blood cells (SRBC), and within 24 hours following the last exposure to TCE, a plaque-forming cell (PFC) assay was conducted to determine effects on splenic anti-SRBC IgM response. Minor, transient effects on body weight and food consumption were noted in treated rats for the first 2 weeks of exposure. Mean relative liver and kidney weights were significantly ( $p = 0.05$ ) increased at 1,000 ppm as compared to control, while lung, spleen, and thymus weights were similar to control. No treatment-related effects were observed for hematology, white blood cell differential counts, or histopathological evaluations (including spleen, thymus, and lung-associated lymph nodes). At 1,000 ppm, rats demonstrated a 64% decrease in PFC assay response. LDH, total protein levels, and cellular differentiation counts evaluated from bronchoalveolar lavage (BAL) samples were similar between control and treated groups. A phagocytic assay using BAL cells showed no alteration in phagocytosis, although these data were not considered fully reliable since: (1) the number of retrieved macrophage cells was lower than expected and pooling of samples was conducted and (2) samples appear to have been collected at 24 hours after the last exposure (rather than within approximately 2 hours of the last exposure), thereby allowing for possible macrophage recovery. The NOAEL for this study was considered by the study authors to be 300 ppm, and the LOAEL was 1,000 ppm; however, the effect level may have actually been lower. It is noted that the outcome of this study does not agree with the studies by Aranyi et al. (1986) and Park et al. (1993), both of which identified impairment of macrophage phagocytic activity in BAL following inhalation TCE exposures.

#### 4.6.2.1.2. Oral exposures

In a study by Sanders et al. (1982b), TCE was administered to male and female CD-1 mice for 4 or 6 months in drinking water at concentrations of 0, 0.1, 1, 2.5, or 5 mg/mL (Sanders et al., 1982b). In females, humoral immunity was suppressed at 2.5 and 5 mg/mL, while cell-mediated immunity and bone marrow stem cell activity were inhibited at all dose levels. Male mice were relatively unaffected either at 4 or 6 months, even though a preliminary study in male CD-1 mice (exposed to TCE for 14 days by gavage at 0, 24, or 240 mg/kg-day) had demonstrated a decrease in cell-mediated immune response to SRBC in male mice at both treatment levels.

A significant decrease in humoral immunity (as measured by plasma hemagglutination titers and the number of spleen antibody producing cells of mice sensitized to sheep erythrocytes) was observed by Kaufmann et al. (1982) in female CD-1 mice (15–20/group) following a 90-day drinking water exposure to 0, 0.07, or 0.7 mg/mL (equivalent to 0, 18, or 173 mg/kg) CH, a metabolite of TCE. Similar responses were not observed in male CD-1 mice

exposed for 90 days in drinking water (at doses of 0, 16, or 160 mg/kg-day), or when administered CH by gavage to 12/group for 14 days at 14.4 or 144 mg/kg-day.

The potential for developmental immunotoxicity was assessed in B6C3F<sub>1</sub> mice administered TCE in drinking water at dose levels of 0, 1,400 or 14,000 ppb from GD 0 to either 3 or 8 weeks of age ([Peden-Adams et al., 2006](#); [Adams et al., 2003 \[preliminary data\]](#)). At 3 and 8 weeks of age, offspring lymphocyte proliferation, NK cell activity, SRBC-specific IgM production (PFC response), splenic B220+ cells, and thymus and spleen T-cell immunophenotypes were assessed. Delayed-typed hypersensitivity and autoantibodies to double-stranded DNA (dsDNA) were evaluated in offspring at 8 weeks of age. Observed positive responses consisted of suppressed PFC responses in males at both ages and both TCE treatment levels, and in females at both ages at 14,000 ppb and at 8 weeks of age at 1,400 ppb. Spleen numbers of B220+ cells were decreased in 3-week-old pups at 14,000 ppb. Pronounced increases in all thymus T-cell subpopulations (CD4+, CD8+, CD4+/CD8+, and CD4-/CD8-) were observed at 8 weeks of age. Delayed hypersensitivity response was increased in 8-week-old females at both treatment levels and in males at 14,000 ppb only. No treatment-related increase in serum anti-dsDNA antibody levels was found in the offspring at 8 weeks of age.

In a study designed to examine potential susceptibility of the young ([Blossom and Doss, 2007](#)), TCE was administered to groups of pregnant MRL +/+ mice in drinking water at occupationally-relevant levels of 0, 0.5, or 2.5 mg/mL. A total of 3 litters per treatment group were maintained following delivery (i.e., a total of 11 pups at 0 mg/mL TCE, 8 pups at 0.5 mg/mL TCE, and 12 pups at 2.5 mg/mL TCE), and TCE was continuously administered to the offspring until young adulthood (i.e., 7–8 weeks of age). Although there were no effects on reproduction, offspring postweaning body weights were significantly decreased in both treated groups. Additionally, TCE exposure was found to modulate the immune system following developmental and early life exposures. Decreased spleen cellularity and reduced numbers of CD4+, CD8+, and B220+ lymphocyte subpopulations were observed in the postweaning offspring. Thymocyte development was altered by TCE exposures, as evidenced by significant alterations in the proportions of double-negative subpopulations and inhibition of in vitro apoptosis in immature thymocytes. TCE was also shown to induce a dose-dependent increase in CD4+ and CD8+ T-lymphocyte IFN $\gamma$  in peripheral blood by 4–5 weeks of age, although these effects were no longer observed at 7–8 weeks of age. Serum antihistone autoantibodies and total IgG<sub>2a</sub> were significantly increased in treated offspring; however, no histopathological signs of autoimmunity were observed in the liver and kidneys at sacrifice.

This increase in T-cell hyperactivity was further explored in a study by Blossom et al. ([2008](#)). In this study, MRL +/+ mice were treated with 0 or 0.1 mg/mL TCE in the drinking water. Based on drinking water consumption data, average maternal doses of TCE were 25.7 mg/kg-day, and average offspring (PNDs 24–42) doses of TCE were 31.0 mg/kg-day. Treatment was initiated at the time of mating, and continued in the females (8/group) throughout

gestation and lactation. Pups were weaned at PND 24, and the offspring were continued on drinking water treatment in a group-housed environment until study termination (PND 42). Subsets of offspring were sacrificed at PNDs 10 and 20, at which time developmental and functional endpoints in the thymus were evaluated (i.e., total cellularity, CD4<sup>+</sup>/CD8<sup>+</sup> ratios, CD24 differentiation markers, and double-negative subpopulation counts). Indicators of oxidative stress were measured in the thymus at PNDs 10 and 20, and in the brain at PND 42. Mitogen-induced intracellular cytokine production by splenic CD4<sup>+</sup> and CD8<sup>+</sup> T-cells was evaluated in juvenile mice and brain tissue was examined at PND 42 for evidence of inflammation. Behavioral testing was also conducted; these methods and results are described in Section 4.3. TCE treatment did not affect reproductive capacity, parturition, or ability of dams to maintain litters. The mean body weight of offspring was not different between the control and treated groups. Evaluation of the thymus identified a significant treatment-related increase in cellularity, accompanied by alterations in thymocyte subset distribution, at PND 20 (sexes combined). TCE treatment also appeared to promote T-cell differentiation and maturation at PND 42, and ex vivo evaluation of cultured thymocytes indicated increased reactive oxygen species generation. Evaluation of peripheral blood indicated that splenic CD4<sup>+</sup> T-cells from TCE-exposed PND 42 mice produced significantly greater levels of IFN- $\gamma$  and IL-2 in males and TNF- $\alpha$  in both sexes. There was no effect on cytokine production on PND 10 or 20. The dose of TCE that resulted in adverse offspring outcomes in this study (i.e., 0.1 mg/mL, equivalent to 25.7–31.0 mg/kg-day) is comparable to that which has been previously demonstrated to result in immune system alterations and autoimmunity in adult MRL  $+/+$  mice (i.e., 0.1 mg/mL, equivalent to 21 mg/kg-day ([Griffin et al., 2000b](#))).

Another study that examined the effects of developmental exposure to TCE on the MRL $+/+$  mouse was conducted by Peden-Adams et al. ([2008](#)). In this study, MRL/MpJ (i.e., MRL  $+/+$ ) mice (unspecified number of dams/group) were exposed to TCE (solubilized with 1% Emulphor) in drinking water at levels of 0, 1,400, or 14,000 ppb from GD 0 and continuing until the offspring were 12 months of age. TCE concentrations in the drinking water were reported to be analytically confirmed. Endpoints evaluated in offspring at 12 months of age included final body weight; spleen, thymus, and kidney weights; spleen and thymus lymphocyte immunophenotyping (CD4 or CD8); splenic B-cell counts; mitogen-induced splenic lymphocyte proliferation; serum levels of autoantibodies to dsDNA and glomerular antigen, periodically measured from 4 to 12 months of age; and urinary protein measures. Reported sample sizes for the offspring measurements varied from 6 to 10 per sex per group; the number of source litters represented within each sample was not specified. The only organ weight alteration was an 18% increase in kidney weight in the 1,400 ppb males. Splenic CD4<sup>-</sup>/CD8<sup>-</sup> cells were altered in female mice (but not males) at 1,400 ppm only. Splenic T-cell populations, numbers of B220<sup>+</sup> cells, and lymphocyte proliferation were not affected by treatment. Populations of thymic T-cell subpopulations (CD8<sup>+</sup>, CD4<sup>-</sup>/CD8<sup>-</sup>, and CD4<sup>+</sup>) were significantly decreased in male but not

female mice following exposure to 14,000-ppb TCE, and CD4<sup>+</sup>/CD8<sup>+</sup> cells were significantly reduced in males by treatment with both TCE concentrations. Autoantibody levels (anti-dsDNA and anti-glomerular antigen) were not increased in the offspring over the course of the study, indicating that TCE did not contribute to the development of autoimmune disease markers following developmental exposures that continued into adult life.

Overall, the studies by Peden-Adams et al. (2008; 2006), Blossom and Doss (2007), and Blossom et al. (2008), which examined various immunotoxicity endpoints following exposures that spanned the critical periods of immune system development in the rodent, were generally not designed to assess issues such as posttreatment recovery, latent outcomes, or differences in severity of response that might be attributed to the early life exposures.

#### **4.6.2.1.3. i.p. administration**

Wright et al. reported that following 3 days of single i.p. injections of TCE in Sprague-Dawley rats at 0, 0.05, 0.5, or 5 mmol/kg-day and B6C3F<sub>1</sub> mice at 0 or 10 mmol/kg-day, NK cell activity was depressed in the rats at the mid- and high-dose levels, and in the mice at the high-dose level (Wright et al., 1991). Also at the highest dose levels tested, decreased splenocyte counts and relative spleen weight were observed in the rats and mice, respectively. In vitro assays demonstrated treatment-related decreases in splenocyte viability, inhibition of lipopolysaccharide-stimulated lymphocyte mitogenesis, and inhibited NK cell activity suggesting the possibility that compromised immune function may play a role in carcinogenic responses of experimental animals treated with TCE.

#### **4.6.2.2. Hypersensitivity**

Evidence of a treatment-related increase in delayed hypersensitivity response has been observed in guinea pigs following dermal exposures with TCE and in mice following exposures that occurred both during development and postnatally (see Table 4-77).

In a modified guinea pig maximization test, Tang et al. (2002) evaluated the contact allergenicity potential of TCE and three metabolites (TCA, TCOH, and CH) in four animals (FMMU strain, sex not specified) per group (Tang et al., 2002). Edema and erythema indicative of skin sensitization (and confirmed by histopathology) were observed. Sensitization rates were reported to be 71.4% for TCE and 58.3% for TCA, as compared to a reference positive control response rate (i.e., 100% for 2,4-dinitrochlorobenzene). In this study, the mean response scores for TCE, TCA, and 2,4-dinitrochlorobenzene were 2.3, 1.1, and 6.0, respectively. TCE was judged to be a strong allergen and TCA was a moderate allergen, according to the criteria of Magnusson and Kligman (1969). TCOH and CH were not found to elicit a dermal hypersensitivity response.

**Table 4-77. Summary of TCE hypersensitivity studies<sup>a</sup>**

Exposure route/vehicle, duration, dose	NOAEL; LOAEL <sup>b</sup>	Results	Reference, species/strain sex/number
Induction by single intradermal injection, then challenge by dermal application at 21 d  0 or 0.1 mL induction; 0 or 0.2 mL challenge  TCE, TCA, TCOH, and CH		Edema and erythema (confirmed by histopathology) indicative of skin sensitization for TCE (strong sensitizer) and TCA (moderate sensitizer)	Tang et al. (2002) Guinea pig, FMMU strain, sex not specified, 4/group
Intradermal injection, 0, 167, 500, 1,500, or 4,500 mg/kg  Dermal patch, 0 or 900 mg/kg  Hypersensitivity: total dose from induction through challenge <340 mg/kg	Intradermal NOAEL: 500 mg/kg Intradermal LOAEL: 1,500 mg/kg  Dermal patch NOAEL: 900 mg/kg	Intradermal injection: At 1,500 mg/kg: statistically significant ↑ AST; at 4,500 mg/kg, statistically significant ↑ ALT and AST, statistically significant ↓ total protein and globulin; fatty degeneration of liver  Dermal patch: no effects of treatment  Hypersensitivity: sensitization rate of 66% (strong sensitizer), with edema and erythema; statistically significant ↑ ALT, AST, and LDH; statistically significant ↑ relative liver weight; statistically significant ↓ albumin, IgA, and GGT; hepatic lesions (ballooning changes)	Tang et al. (2008) Guinea pig, FMMU strain, female, 5–6/group for intradermal/dermal patch study, 10/group for hypersensitivity study, female
<b>Drinking water, from GD 0 to 8 wks of age</b> <b>0, 1,400, or 14,000 ppb</b>	<b>LOAEL: 1,400 ppb</b>	<b>Statistically significant ↑ swelling of foot pad in females at 1,400 and in both sexes at 14,000 ppb</b>	<b>Peden-Adams et al. (2006)</b> <b>Mouse, B6C3F<sub>1</sub>, both sexes, 5 litters/group; 4–5 pups/sex/group at 8 wks<sup>c</sup></b>

<sup>a</sup>**Bolded study** carried forward for consideration in dose-response assessment (see Chapter 5).

<sup>b</sup>NOAEL and LOAEL are based upon reported study findings.

<sup>c</sup>Subset of immunosuppression study.

Immune-mediated hepatitis associated with dermal hypersensitivity reactions in the guinea pig following TCE exposures was characterized by Tang et al. (2008). In this study, FMMU strain female guinea pigs (5–6/group) were treated with intradermal injection of 0, 167, 500, 1,500, or 4,500 mg/kg TCE or with a dermal patch containing 0 or 900 mg/kg TCE and sacrificed at 48 hours posttreatment. At the intradermal dose of 1,500 mg/kg, a significant increase ( $p < 0.05$ ) in serum AST level was observed. At 4,500 mg/kg, significantly ( $p < 0.01$ ) increased ALT and AST levels were reported, and total protein and globulin decreased significantly ( $p < 0.05$ ). Histopathological examination of the liver revealed fatty degeneration, hepatic sinusoid dilation, and inflammatory cell infiltration. No changes were observed at the intradermal doses of  $\leq 500$  mg/kg, or the dermal patch dose of 900 mg/kg. A Guinea Pig Maximization Test was also conducted according to the procedures of Magnusson and Kligman on 10 FMMU females/group, in which the total TCE dosage from induction through challenge phases was below 340 mg/kg. TCE treatment resulted in dermal erythema and edema, and the sensitization rate was 66% (i.e., classified as a strong sensitizer). Significant increases ( $p < 0.05$ ) in ALT, AST, LDH, and relative liver weight, and significant decreases ( $p < 0.05$ ) in albumin, IgA, and GGT were observed. Additionally, hepatic lesions (diffuse ballooning changes without lymphocyte infiltration and necrotic hepatocytes) were noted. It was concluded that TCE exposure to guinea pigs resulted in delayed type hypersensitivity reactions with hepatic injury that was similar to occupational medicamentosa-like dermatitis disorders observed in human occupational studies.

Also, as indicated in Section 4.6.2.1.2, in a developmental immunotoxicity-type study in B6C3F<sub>1</sub> mice, administration of TCE in drinking water at dose levels of 0, 1,400, or 14,000 ppb from GD 0 through to 8 weeks of age resulted in an increased delayed hypersensitivity response in 8-week-old female offspring at both treatment levels and in males at the high dose of 14,000 ppb (Peden-Adams et al., 2006).

In an in vitro study that evaluated a number of chlorinated organic solvents, nonpurified rat peritoneal mast cells (NPMC) and rat basophilic leukemia (RBL-2H3) cells were sensitized with anti-DNP (dinitrophenol) monoclonal IgE antibody and then stimulated with DNP-conjugated bovine serum albumin plus TCE (Seo et al., 2008). TCE enhanced antigen-induced histamine release from NPMC and RBL-2H3 cells in a dose-related manner, and increased IL-4 and TNF- $\alpha$  production from the RBL-2H3 cells. In an in vivo study, i.p.-injected TCE was found to markedly enhance passive cutaneous anaphylaxis reaction in antigen-challenged rats. These results suggest that TCE increases histamine release and inflammatory mediator production from antigen-stimulated mast cells via the modulation of immune responses; TCE exposure may lead to the enhancement of allergic disease through this response.

#### 4.6.2.3. Autoimmunity

A number of studies have been conducted to examine the effects of TCE exposure in mouse strains (i.e., MRL +/+, MRL -lpr, or NZB × NZW) which are all known to be genetically susceptible to autoimmune disease. The studies have demonstrated the potential for TCE to induce autoimmune disease (as demonstrated in Table 4-78, which summarizes those studies which assessed serology, ex vivo assays of cultured splenocytes, and/or clinical or histopathology). These and other studies conducted in susceptible mouse strains have proven to be useful tools in exploring various aspects of the mode of action for this response.

Khan et al. ([1995](#)) used the MRL +/+ mouse model to evaluate the potential for TCE and one of its metabolites, DCAC to elicit an autoimmune response. Female mice (4–5/group) were dosed by i.p. injection with 10 mmol/kg TCE or 0.2 mmol/kg DCAC every 4<sup>th</sup> day for 6 weeks and then sacrificed. Spleen weights and IgG were increased. ANA and anti-ssDNA (single-stranded DNA) antibodies were detected in the serum of TCE- and DCAC-treated mice; anticardiolipin antibodies were detected in the serum of DCAC-treated mice. A greater magnitude of response observed with DCAC treatment suggested that the metabolite may be important to the mechanism of TCE-induced autoimmunity.

Other studies in female MRL +/+ mice (8/group) examined exposure via drinking water. In one of these studies, mice were treated with 2.5 or 5.0 mg/mL (455 or 734 mg/kg-day) TCE in drinking water for up to 22 weeks ([Griffin et al., 2000a](#); [Gilbert et al., 1999](#)). Serial sacrifices were conducted at weeks 4, 8, and 22. Significant increases in ANA and total serum immunoglobulin were found at 4 weeks of TCE treatment (indicating an autoimmune response), but not at 22 weeks. Increased expression of the activation marker C44 on splenic CD4+ cells was observed at 4 weeks, with the highest expression seen in the highest exposure group. In addition, at 4 weeks, splenic T-cells from treated mice secreted more IFN- $\gamma$  and less IL-4 than control T-cells (significant at 0.5 and 2.5 mg/mL), consistent with a Th1 immune or inflammatory response. By 22 weeks of TCE treatment, a specific immune serum antibody response directed against dichloroacetylated proteins was activated in hepatic tissues, indicating the presence of protein adducts.

In a subsequent study that assessed occupationally relevant concentrations, TCE was administered to female MRL +/+ mice (8/group) in drinking water at treatment levels of 0.1, 0.5, or 2.5 mg/mL (21, 100, or 400 mg/kg-day) for 4 and 32 weeks ([Griffin et al., 2000b](#)). At 4 weeks, significant increases in serum antinuclear antibody levels were observed at 0.1 and 0.5 mg/kg-day. A dose-related increase in the percentage of activated CD4+ T-cells in lymph nodes of treated mice was observed at 32 weeks, and a dose-related increase in secretion of IFN- $\gamma$  by the CD4+ T-cells was also observed at 4 and 32 weeks. There was a slight but statistically significant increase in serum ALT levels at 32 weeks at 0.5 mg/mL. Histopathological evaluation at 32 weeks revealed extensive hepatic lymphocytic cell infiltration at 0.5 and 2.5 mg/mL; all treated groups contained significantly more hepatocyte reactive changes (i.e.,

presence of multinucleated hepatocytes, variations in hepatocyte morphology, and hepatocytes in mitosis) than controls.

A similar response was observed by Cai et al. following chronic (48 weeks) exposure of TCE to female MRL +/+ mice (5/group) in drinking water at 0 or 0.5 mg/mL (approximately 60 µg/g/day) (Cai et al., 2008). After 11 weeks of treatment, a statistically significant decrease in body-weight gain was observed. After 24 weeks of exposure, serum ANA was consistently elevated in treated mice as compared to controls, although statistical significance was not achieved. Apparent treatment-related effects on serum cytokines included decreased IL-6 after 36 and 48 weeks, decreased TNF- $\alpha$  after 48 weeks, and increased granulocyte colony stimulating factor (G-CSF) after 36 weeks of treatment. After 36 weeks of treatment, ex vivo cultured splenocytes secreted higher levels of IFN- $\gamma$  than control splenocytes. Although there were no observed effects on serum aminotransferase liver enzymes at termination, statistically significant incidences of hepatocytic necrosis and leukocyte infiltration (including CD3+ T lymphocytes) into liver lobules were observed in treated mice after 48 weeks of exposure. Hepatocyte proliferation was also increased. TCE treatment for 48 weeks also induced necrosis and extensive infiltration of leukocytes in the pancreas, infiltration of leukocytes into the perivascular and peribronchial regions of the lungs, and thickening of the alveolar septa in the lungs. At 36 and 48 weeks of exposure, massive perivascular infiltration of leukocytes (including CD3+ T lymphocytes) was observed in the kidneys, and immunoglobulin deposits were found in the glomeruli.

**Table 4-78. Summary of autoimmune-related studies of TCE and metabolites in mice and rats (by sex, strain, and route of exposure)<sup>a</sup>**

Number/group, vehicle, dose, duration	NOAEL; LOAEL <sup>b</sup>	Results			Reference
		Serology	Ex vivo assays of cultured splenocytes	Clinical and histopathology	
<b>Autoimmune-prone: female MRL +/- mice, drinking water</b>					
8 per group, 0, 2.5, or 5 mg/mL TCE (average 0, 455, or 734 mg/kg-d), 4, 8, or 22 wks	LOAEL: 2.5 mg/mL	Increased ANA at 4 and 8 wks, no difference between groups at 22 wks	Increased activated CD4+ T-cells and IFN- $\gamma$ secretion across doses at 4 wks, these effects were reversed at 22 wks; decreased IL-4 secretion (4 and 22 wks)	No evidence of liver or renal damage, based on serum ALT, SDH, and BUN.	Griffin et al. (2000a)
<b>8 per group, 0, 0.1, 0.5, or 2.5 mg/mL TCE (0, 21, 100, or 400 mg/kg-d), 4 or 32 wks</b>	<b>LOAEL: 0.1 mg/mL</b>	<b>Increased ANA in all treated groups at 4 wks, but not at 32 wks</b>	<b>Increased activated CD4+ T-cells (32 wks), IFN-<math>\gamma</math> secretion (4 and 32 wks), no effect on IL-4 secretion</b>	<b>Extensive hepatic mononuclear cellular infiltrate in 0.5 and 2.5 mg/mL groups, and hepatocyte reactive changes in all treated groups at 32 wks.</b>	<b>Griffin et al. (2000b)</b>
6–8 per group, 0, 0.1, or 0.9 mg/mL trichloroacetaldehyde hydrate (0, 24, or 220 mg/kg-d) or TCA (0, 27, or 205 mg/kg-d), 4 wks	LOAEL: 0.1 mg/mL	Increased ANA and antihistone antibodies at 0.9 mg/mL trichloroacetaldehyde hydrate <sup>c</sup>	Increased activated CD4+ T-cells at 0.1 and 0.9 g/mL doses of both metabolites. At 0.9 mg/mL, increased IFN- $\gamma$ secretion, no effect on IL-4 secretion	No evidence of liver or kidney damage, based on serum ALT, liver and kidney histology.	Blossom et al. (2004)
8 per group, 0, 0.1, 0.3, or 0.9 mg/mL trichloroacetaldehyde hydrate (0, 13, 46, or 143 mg/kg-d), 40 wks	LOAEL: 0.9 mg/mL	Slightly suppressed anti-ssDNA, anti-dsDNA, and antihistone antibody expression; differences not statistically significant	Increased activated CD4+ T-cells and increased INF- $\gamma$ secretion, no effect on IL-4 secretion	Diffuse alopecia, skin inflammation and ulceration, mononuclear cell infiltration, mast cell hyperplasia, dermal fibrosis. Statistically significant increase at 0.9 mg/mL dose group, but also increased at lower doses. No liver or kidney histopathology effects seen.	Blossom et al. (2007)

**Table 4-78. Summary of autoimmune-related studies of TCE and metabolites (by sex, strain, and route of exposure) (continued)**

Number/group, vehicle, dose, duration	NOAEL; LOAEL <sup>b</sup>	Results			Reference
		Serology	Ex vivo assays of cultured splenocytes	Clinical and histopathology	
5 per group, 0 or 0.5 mg/mL TCE (mean 60 µg/g-d), 48 wks	LOAEL: 0.5 mg/mL	Increased ANA after 24 wks but not statistically significant	Increased INF-γ secretion after 36 wks but not statistically significant	Hepatic necrosis; hepatocyte proliferation; leukocyte infiltrate in the liver, lungs, and kidneys; no difference in serum aminotransferase liver enzymes.	Cai et al. (2008)
<b>Autoimmune-prone: male and female offspring MRL +/- mice, drinking water</b>					
3 litters/group, 8–12 offspring/group; 0, 0.5, or 2.5 mg/mL, GD 0 to 7–8 wks of age	LOAEL: 0.5 mg/mL	Increased antihistone antibodies and total IgG <sub>2a</sub> in treated groups	Dose-dependent increase in IFN-γ secretion at 4–5 wks of age but not 7–8 wks of age	No histopathological effects in liver or kidneys.	Blossom and Doss (2007)
8 litters/group, 8–12 offspring/group; 0 or 0.1 mg/mL; maternal dose = 25.7 mg/kg-d; offspring PNDs 24–42 dose = 31.0 mg/kg-d; GD 0 to PND 42	LOAEL: 0.1 mg/mL	Not evaluated	Increased IFN-γ and IL-2 in females, increased TNF-α in both sexes	Not evaluated	Blossom et al. (2008)
Unknown number of litters/group, 6–10 offspring/sex/group; 0 (1% Emulphor), 1,400, or 14,000 ppb; GD 0 to 12 mo of age	NOAEL: 1,400 ppb	No increase in autoantibody levels	Not evaluated	Not evaluated	Peden-Adams et al. (2008)

**Table 4-78. Summary of autoimmune-related studies of TCE and metabolites (by sex, strain, and route of exposure) (continued)**

Number/group, vehicle, dose, duration	NOAEL; LOAEL <sup>b</sup>	Results			Reference
		Serology	Ex vivo assays of cultured splenocytes	Clinical and histopathology	
<b>Autoimmune-prone: female MRL +/+ mice, i.p. injection</b>					
4–5 per group, 0 (corn oil), 10 mmol/kg TCE, or 0.2 mmol/kg DCAC, every 4 <sup>th</sup> d for 6 wks	LOAEL: 10 mmol/kg TCE, 0.2 mmol/kg DCAC	In both groups, increased ANA and anti-ssDNA antibodies. In DCAC group, anticardiolipin antibodies. No difference in antihistone, -Sm, or -DNA antibodies	Not evaluated	Not evaluated	Khan et al. (1995)
6 per group, 0 (corn oil), 0.2 mmol/kg DCAC, or 0.2 mmol/kg dichloroacetic anhydride, 2 times per wk for 6 wks	LOAEL: 0.2 mmol/kg TCE, 0.2 mmol/kg dichloroacetic anhydride	In both treated groups, increased ANA	In both treated groups, increased IL-1 $\alpha$ , IL-1 $\beta$ , IL-3, IL-6, IFN- $\gamma$ , G-CSF and KC secretion; decreased IL-5. In DCAC group, increased IL-17 and INF- $\alpha^d$	In both treated groups, increased lymphocytes in spleen, thickening of alveolar septa with lymphocytic interstitial infiltration.	Cai et al. (2006)
<b>Autoimmune-prone: female NZB <math>\times</math> NZW mice, drinking water</b>					
6 per group, 0, 1,400, or 14,000 ppb TCE <sup>e,f</sup> , 27 wks exposure	LOAEL: 1,400 ppb	Increased anti-dsDNA antibodies at 19 wks and at 32–32 wks in the 1,400 ppb group	Not evaluated	At 14,000 ppb, proteinuria increased beginning at 20 wks; renal pathology scores increased, no evidence of liver disease.	Gilkeson et al. (2004)
10 per group, 0, 1,400, or 14,000 ppb TCE <sup>f</sup> , 27 wks exposure	LOAEL: 1,400 ppb	Increased anti-dsDNA antibodies at 19 wks and at 32–32 wks in the 1,400 ppb group	No effect on splenocyte NK activity	No effect on renal pathology score; liver disease not examined.	Keil et al. (2009)

**Table 4-78. Summary of autoimmune-related studies of TCE and metabolites (by sex, strain, and route of exposure) (continued)**

Number/group, vehicle, dose, duration	NOAEL; LOAEL <sup>b</sup>	Results			Reference
		Serology	Ex vivo assays of cultured splenocytes	Clinical and histopathology	
<b>Autoimmune-prone: male MRL—<i>lpr/lpr</i> mice, inhalation</b>					
5 per group, 0, 500, 1,000, or 2,000 ppm TCE, 4 hrs/d, 6 d/wk, 8 wks	LOAEL: 500 ppm			At ≥500 ppm, dose-related liver inflammation, splenomegaly and hyperplasia of lymphatic follicles; at 1,000 ppm, immunoblastic cell formation in lymphatic follicles, no changes in thymus.	Kaneko et al. (2000)
<b>Autoimmune-inducible: female brown Norway Rat, gavage</b>					
6–8 per group, 0, 100, 200, 400 mg/kg, 5 d/wk, 6 wks followed by 1 mg/kg HgCl <sub>2</sub> challenge	NOAEL 500 mg/kg	Not reported <sup>g</sup>	Not evaluated	Not evaluated	White et al. (2000)
<b>Nonautoimmune-prone: female B6C3F<sub>1</sub> mice, drinking water</b>					
6 per group, 0, 1,400, or 14,000 ppb TCE, <sup>e,f</sup> 30 wks exposure	LOAEL: 1,400 ppb	Anti-dsDNA increased in 1,400 ppb group beginning at age 32 wks and in the 14,000 ppb group beginning at age 26 wks	No effect on splenocyte NK activity	No renal disease observed.	Gilkeson et al. (2004)

**Table 4-78. Summary of autoimmune-related studies of TCE and metabolites (by sex, strain, and route of exposure) (continued)**

Number/group, vehicle, dose, duration	NOAEL; LOAEL <sup>b</sup>	Results			Reference
		Serology	Ex vivo assays of cultured splenocytes	Clinical and histopathology	
10 per group, 0, 1,400, or 14,000 ppb TCE, <sup>f</sup> 30 wks exposure	LOAEL: 1,400 ppb	Anti-dsDNA increased beginning at 26 wks in the 14,000 ppb group and at 32 wks of age in the 1,400 ppb group; increases in anti-ssDNA antibodies seen in both groups at 32 wks. Anti-glomerular antigen were not affected	No effect on splenocyte NK activity	Increased renal pathology scores in 1,400 ppb group; Significant decrease in thymus weight in both groups	Keil et al. (2009)

<sup>a</sup>**Bolded studies** carried forward for consideration in dose-response assessment (see Chapter 5); selected endpoints, based on those reported across the majority of studies. Lupus-prone mouse strains develop lupus-like condition spontaneously, with virtually complete penetrance. The autoimmune-inducible (Brown Norway) rat has been used as a model of mercuric chloride induced glomerulonephritis and experimental autoimmune myasthenia gravis.

<sup>b</sup>NOAEL and LOAEL are based upon reported study findings.

<sup>c</sup>No difference reported in anti-dsDNA, -ssDNA, -ribonucleosome, -SSA, -SSB, -Sm, -Jo-1, or -Scl-70 antibodies.

<sup>d</sup>No difference reported in secretion of other cytokines measured: IL-2, IL-4, IL-10, IL-12, TNF- $\alpha$ , granulocyte monocyte colony stimulating factor, macrophage inflammatory protein-1 $\alpha$ , and RANTES (CCL-5).

<sup>e</sup>Dose levels cited in the report (Gilkeson et al., 2004) were incorrect; corrections provided by personal communication from Margie Peden-Adams (Medical University of South Carolina) to Glinda Cooper (U.S. EPA) on 13 August 2008; dose levels in this table are correctly reported.

<sup>f</sup>Dose in mg/kg-day not given.

<sup>g</sup>Anti-dsDNA tests were described in the methods section; no effect of TCE on serum IgE levels was seen, and it is not clear if the additional serological tests were conducted in the TCE portion of this study or if they were conducted but not reported because no effect was seen.

G-CSF = granulocyte colony stimulating factor; KC = keratinocyte-derived chemokine; SDH = sorbitol dehydrogenase

To examine the role of metabolic activation in the autoimmune response, Griffin et al. (2000c) treated MRL +/+ mice with 2.5 mg/mL (300 mg/kg-day) TCE in drinking water for 4 weeks (Griffin et al., 2000c). Immune responses were examined in the presence or absence of subcutaneous doses of 200 mg/kg-day diallyl sulfide, a specific inhibitor of CYP2E1, which is known to be a primary CYP that is active in TCE metabolism. With diallyl sulfide cotreatment that resulted in a decreased level of CYP2E1 apoprotein in liver microsomes, the enhanced mitogen-induced proliferative capacity of T-cells was inhibited and the reduction in IL-4 levels secreted by CD4+ T-cells was reversed for TCE-treated MRL +/+ mice. This study suggests that metabolism of TCE by CYP2E1 is responsible, at least in part, for the treatment-related CD4+ T-cell alterations.

The TCE metabolite, trichloroacetaldehyde (TCAA) or trichloroacetaldehyde hydrate (TCAH), was also evaluated in MRL +/+ mice (Blossom et al., 2007; Blossom and Gilbert, 2006; Gilbert et al., 2004) in order to determine if outcomes similar to the immunoregulatory effects of TCE would be observed, and to attempt to further characterize the role of metabolism in the mode of action for TCE. At concentrations ranging from 0.04 to 1 mM, TCAA stimulated proliferation of murine Th1 cells treated with anti-CD3 antibody or antigen in vitro. At similar concentrations, TCAA induced phenotypic alterations consistent with upregulation of CD28 and downregulation of CD62L in cloned memory Th1 cells and DC4+ T-cells from untreated MRL +/+ mice. Phosphorylation of activating transcription factor 2 (ATF-2) and c-Jun (two components of the activator protein-1 transcription factor) was also observed with TCAA-induced Th1 cell activation. Higher concentrations of TCAA formed a Schiff base on T-cells, which suppressed the ability of TCAA to phosphorylate ATF-2. These findings suggested that TCAA may promote T-cell activation by stimulating the mitogen-activated protein kinase pathway in association with Schiff base formation on T-cell surface proteins (Gilbert et al., 2004).

In order to determine whether metabolites of TCE could mediate the immunoregulatory effects previously observed with TCE treatment (i.e., the generation of lupus and autoimmune hepatitis, associated with activation of IFN- $\gamma$ -producing CD4+ T-cells), Blossom et al. (2004) administered TCE metabolites, TCAH and TCA, to MRL +/+ mice (6–8/group) in drinking water for 4 weeks. Drinking water concentrations were 0, 0.1, or 0.9 mg/mL; average daily doses were calculated as 0, 24, or 220 mg/kg-day for TCAH and 0, 27, or 205 mg/kg-day for TCA. These treatment levels were considered to be physiologically relevant and to reflect occupational exposure. A phenotypic analysis of splenic and lymph node cells, cytokine profile analysis, evaluation of apoptosis in CD4+ T-cells, and examination of serum markers of autoimmunity (anti-ssDNA, antihistone, or ANA) were conducted. Exposure to TCAH or TCA at both treatment levels was found to promote CD4+ T-cell activation, as shown by significant ( $p < 0.05$ ) increases in the percentage of CD62L<sup>lo</sup> CD4+ T-cells in the spleens and lymph nodes of the MRL +/+ mice. Increased levels of IFN- $\gamma$  were secreted by CD4+ T-cells from mice

treated by TCAH and TCA. No significant changes in body weight were observed; spleen weights were similar between control and treated mice with the exception of a significant decrease in spleen weight from mice treated with 0.9 mg/mL TCA. Liver and kidney histology were not affected, and serum ALT levels were similar for control and treated mice. A generalized trend towards an increase in serum autoantibodies (anti-ssDNA) was observed in TCAH-treated mice, and slight but significant increases in antihistone and antinuclear antibody production were observed in mice treated with 0.9 mg/mL-day TCAH.

The autoimmune response of female MRL *+/+* mice to DCAC, a metabolite of TCE, and to dichloroacetic anhydride (DCAA) a similar acylating agent, was evaluated by Cai et al. (2006). Six mice/group were injected intraperitoneally, twice weekly for 6 weeks, with 0.2 mmol/kg DCAC or DCAA in corn oil. Body weight gain was significantly decreased after 5 or 6 weeks treatment with DCAC and DCAA. DCAC treatment resulted in significant increases in total serum IgG (77% increase over control) and IgG1 (172% increase over control), as well as the induction of DCAC-specific IgG and IgG1. Serum IgM levels were significantly decreased by 25 and 18% in DCAC and DCAA-treated mice, respectively. IgE levels were increased 100% over controls in DCAC-treated mice. Of eight Th1/Th2 cytokines measured, only IL-5 was decreased in DCAC- and DCAA-treated mice. Serum ANA were detected in both DCAC- and DCAA-treated mice. Treatment-related increases in cytokine and chemokine secretion in cultured splenocytes were observed for DCAC and DCAA (IL-1, G-CSF, keratinocyte-derived chemokine, IL-3, and IL-6). DCAC-treated splenocytes also secreted more IL-17 and IFN- $\alpha$  than controls. Histopathological changes were observed in the spleens of DCAC and DCAA-treated mice (lymphocyte population increases in the red pulp). With both DCAC and DCAA treatment, the alveolar septa were thickened in the lungs, moderate levels of lymphocytic interstitial infiltrates were present in tissues, and alveolar capillaries were clogged with erythrocytes. These findings were attributed both to the predisposition of the MRL *+/+* mice towards autoimmune disease and to the treatment-related induction of autoimmune responses.

Fas-dependant activation-induced cell death leading to autoimmune disease has been shown to be related to impaired Fas or FasL ligand expression in humans and mice, and defects in the Fas-signaling pathways have been described in autoimmune disease models. The study by Blossom and Gilbert (2006) examined the effects of TCAH on Fas-dependent autoimmune cell death). In this study, TCAH: (1) inhibited apoptosis of antigen-activated cells; (2) did not protect CD4<sup>+</sup> T-cells from Fas-independent apoptosis; (3) did not inhibit autoimmune cell death induced by direct engagement of the Fas receptor; (4) inhibited the expression of FasL but not Fas on the surface of activated CD4<sup>+</sup> T-cell; (5) increased release of FasL from CD4<sup>+</sup> cells in a metalloprotein-dependent manner; and (6) increased metalloprotein MMP-7 expression.

Gilbert et al. (2006) studied the effect of treatment on apoptosis in CD4<sup>+</sup> T-lymphocytes isolated from MRL *+/+* female mice that had been exposed to TCE (0, 0.1, 0.5, or 2.5 mg/mL) in

the drinking water for 4 or 32 weeks or to TCAH (0.1, 0.3, or 0.9 mg/mL) in drinking water for 4 or 40 weeks. After only 4 weeks, decreased activation-induced apoptosis was associated with decreased FasL expression in the CD4<sup>+</sup> T-cells, suggesting that TCE- and TCAH-induced autoimmune disease was promoted through suppression of the process that would otherwise delete activated self-reactive T-lymphocytes. By 32 weeks of treatment, TCE had induced autoimmune hepatitis, which was associated with the promotion of oxidative stress, the formation of liver protein adducts, and the stimulated production of antibodies to those adducts. TCAH-treated mice did not exhibit autoimmune hepatitis by 40 weeks, but developed a dose-dependent alopecia and skin inflammation ([Blossom et al., 2007](#)). TCAH appeared to modulate the CD4<sup>+</sup> T-cell subset by promoting the expression of an activated/effector phenotype with an increased capacity to secrete the proinflammatory cytokine IFN- $\gamma$ . A 4-week exposure to TCAH attenuated activation-induced cell death and the expression of the death receptor Fas in CD4<sup>+</sup> cells; these effects were not seen after a 40-week exposure period. Differences in response were tentatively attributed to higher levels of metalloproteinases (specifically MMP-7) at 4 weeks of treatment, suggesting a possible mechanism for the promotion of skin pathology by TCAH.

The role of protein adduct formation in autoimmune response has been pursued by various researchers. Halmes et al. ([1997](#)) administered a single i.p. dose of TCE in corn oil to male Sprague-Dawley rats (2/group) at 0 or 1,000 mg/kg. Using antiserum that recognizes TCE covalently bound to protein, a single 50 kDa microsomal adduct was detected by Western blot in livers of treated rats. Using affinity chromatography, a 50 kDa dichloroacetyl protein was also isolated from rat plasma. The protein was reactive immunochemically with anti-CYP2E1 antibodies. The data suggest that the protein adduct may be CYP2E1 that has been released from TCE-damaged hepatocytes.

Cai et al. ([2007](#)) examined the role of protein haptization in the induction of immune responses. In this study, MRL +/+ mice were immunized with albumin adducts of various TCE reactive intermediates of oxidative metabolism. Serum immunoglobulins and cytokine levels were measured to evaluate immune responses against the haptized albumin. Antigen-specific IgG responses (subtypes: IgG1, IgG2a, and IgG2b) were found. Serum levels of G-CSF were increased in immunized mice, suggesting macrophage activation. Following immunization with formyl-albumin, lymphocyte infiltration in the hepatic lobule and portal area was increased. This study suggests that proteins that are haptized by metabolites of TCE may act as antigens to induce humoral immune responses and T-cell-mediated hepatitis.

A possible role for oxidative stress in inflammatory autoimmune disease was proposed by Khan et al. ([2001](#)). A study was performed in which female MRL +/+ mice were treated with 10 mmol/kg TCE or 0.2 mmol/kg DCAC via i.p. injection every 4<sup>th</sup> day for 2, 4, 6, or 8 weeks. Antimalondialdehyde serum antibodies, a marker of lipid peroxidation and oxidative stress, were measured and were found to increase by 4 weeks of treatment, marginally for TCE and

significantly for DCAC. It was reported that antimalondialdehyde antibodies has also been found to be present in the serum of systemic lupus erythematosus-prone MRL-lpr/lpr mice.

In another study that addressed the association of oxidative and nitrosative stress, and the role of lipid peroxidation and protein nitration, in TCE-mediated autoimmune response, Wang et al. (2007b) treated female MRL +/+ mice with 0.5 mg/mL TCE in drinking water for 48 weeks. The formation of antibodies in the serum to lipid peroxidation-derived aldehyde protein adducts was evaluated. With TCE treatment, the serum levels of antimalondialdehyde and anti-4-hydroxynonenal protein adduct antibodies, inducible nitric oxide synthase, and nitrotyrosine were increased. These were associated with increases in antinuclear-, anti-ssDNA-, and anti-dsDNA antibodies. The involvement of lipid peroxidation-derived aldehyde protein adducts in TCE autoimmunity was further explored, using female MRL +/+ mice that were administered by i.p. injections of TCE at 10 mmol/kg, either every 4<sup>th</sup> day for 6 or 12 weeks (Wang et al., 2007a) or once per week for 4 weeks (Wang et al., 2008). Significant increases in malondialdehyde and 4-hydroxynonenal protein adducts, as well as significant induction of specific antibodies directed against these antigens were observed in both studies. Wang et al. (2008) also demonstrated a significant proliferation of CD4+ T-cells in TCE-treated mice, and splenic lymphocytes from TCE-treated mice released more IL-2 and IFN- $\gamma$  when stimulated with MDA- or 4-hydroxynonenal-adducted mouse serum albumin. Overall, the result of these studies suggest a role for lipid peroxidation aldehydes in the induction and/or exacerbation of autoimmune response in the MRL +/+ animal model, and the involvement of Th1 cell activation.

In studies conducted in other rodent strains, less consistent outcomes have been observed. Inhalation exposure of an autoimmune-prone strain of male mice (MRL-lpr/lpr) to 0, 500, 1,000, or 2,000 ppm TCE for 4 hours/day, 6 days/week, for 8 weeks resulted in depressed serum IgG levels and increased numbers of lymphoblastoid cells (Kaneko et al., 2000). Also at 2,000 ppm, changes in T-cell helper to suppressor cell ratios were observed. At histopathological evaluation, dose-dependent inflammation and associated changes were noted in the liver at  $\geq 500$  ppm, hyperplasia of the lymphatic follicles of the spleen and splenomegaly were observed at  $\geq 500$  ppm, and the spleen exhibited the development of an immunoblastic-cell-like structure at 1,000 ppm.

A 26-week drinking water study of TCE in NZB  $\times$  NZW (NZBWF1) autoimmune-prone mice demonstrated an increase in anti-dsDNA antibodies at 19 weeks and at 32 and 34 weeks in the 1,400 ppb group, and increased kidney disease at 14,000 ppb (i.e., increased proteinuria at 20 weeks; increased renal pathology scores were noted at termination, based upon glomerular proliferation, inflammation, and necrosis) (Gilkeson et al., 2004).<sup>11</sup> Also in that study, a small increase in anti-dsDNA antibody production, without kidney disease, was observed in B6C3F<sub>1</sub>

---

<sup>11</sup>The study was reported in symposium proceedings. Dose levels cited in the proceedings were incorrect; however, corrections were provided by personal communication from Margie Peden-Adams (Medical University of South Carolina) to Glinda Cooper (U.S. EPA) on 13 August 2008, and dose levels are correctly reported here.

mice, with statistically significant ( $p < 0.05$ ) or borderline ( $p = 0.07$ ) effects seen in the 1,400 ppb group at observations between 32 and 39 weeks of age, and in the 14,000 ppb group at observations between 26 and 39 weeks of age.

Keil et al. (2009) also assessed the effects of TCE exposure on NZBWF1 mice, comparing the responses to those of TCE-exposed B6C3F<sub>1</sub> mice, which are not autoimmune prone (Keil et al., 2009). In this study, groups of NZBWF1 and B6C3F<sub>1</sub> female mice (10/dose level) were administered 0, 1,400, or 14,000 ppb TCE in the drinking water. Treatment was initiated at 9 weeks of age and continued until 36 weeks of age for the NZBWF1 mice and until 39 weeks of age for the B6C3F<sub>1</sub> mice. Body weight; spleen, thymus, liver, and kidney weight; spleen and thymus cellularity; and renal pathology were assessed. Splenic lymphocyte proliferation, autoantibody production (anti-dsDNA, anti-ssDNA, and antiglomerular), total serum IgG, NK cell activity, and mitogen-induced lymphocyte proliferation were conducted. Administration of TCE did not result in alterations in NK cell activity or T- or B-cell proliferation in either strain of mice. In the NZBWF1 mice, there was little evidence of an increase or of an acceleration in ssDNA antibody production with TCE exposure, but as was seen in the earlier study by these investigators (Gilkeson et al., 2004), dsDNA antibodies were increased at 19 weeks and at 32–34 weeks in the 1,400 ppb group. However, antiglomerular antibody levels were increased in NZBWF1 mice early in the study, returning to control levels by 23 weeks of age. In the B6C3F<sub>1</sub> mice, the number of activated T-cells (CD4<sup>++</sup>/CD44<sup>+</sup>) was increased (significantly at 14,000 ppb;  $p \leq 0.05$ ) and thymus weights were significantly decreased ( $p \leq 0.05$ ) in a dose-responsive manner. Renal pathology (as indicated by renal score based on assessment of glomerular inflammation, proliferation, crescent formation, and necrosis) was significantly increased ( $p \leq 0.05$ ) at 1,400 ppb. Also in the B6C3F<sub>1</sub> mice, autoantibodies to dsDNA were increased relative to controls beginning at 26 weeks in the 14,000 ppb group and at 32 weeks of age in the 1,400 ppb group; increases in anti-ssDNA antibodies were seen in both groups at 32 weeks. Antiglomerular antibodies were not affected in B6C3F<sub>1</sub> mice. In summary, the authors concluded that this study showed that 27–30 weeks of TCE drinking water administration to NZBWF1 (autoimmune-prone) mice did not contribute to the progression of autoimmune disease, while similar administration to B6C3F<sub>1</sub> (nonautoimmune-prone) mice increased the expression of a number of markers that are associated with autoimmune disease. This study is important in that it demonstrates that autoimmune responses to TCE exposure in animal models are not solely dependent upon a genetic predisposition to autoimmune disease.

White et al. (2000) conducted a study in female Brown Norway rats, which have been shown to be susceptible to development of chemically-induced IgE mediated glomerulonephritis that is similar to the nephritic damage seen in systemic lupus erythematosus. TCE administered by gavage 5 days/week at 100, 200, or 400 mg/kg did not increase in IgE levels after 6 weeks exposure, or after an additional challenge with 1 mg/kg HgCl<sub>2</sub>.

Several studies have examined the potential for autoimmune response following oral exposures during pre- and postnatal immune system development, as described in Section 4.6.2.1.2. Peden-Adams et al. (2008; 2006) conducted two such studies. In the first study, B6C3F<sub>1</sub> mice were treated with either 1,400 or 14,000 ppb TCE in drinking water from GD 0 to postnatal week 8 (Peden-Adams et al., 2006). No treatment-related increases in serum anti-dsDNA antibody levels were observed in the 8-week-old offspring, although it is noted that the mouse strain used in the experiment is not an autoimmune-prone animal model. A more recent study (Peden-Adams et al., 2008) exposed pregnant MRL +/+ mice to TCE in drinking water at levels of 0, 1,400, or 14,000 ppb from GD 0 and continued the exposures until the offspring were 12 months of age. Consistent with the findings of the 2006 publication, autoantibody levels (anti-dsDNA and antiglomerular) were not increased in the offspring over the course of the study. Contrasting with these negative studies, the lupus-prone MRL +/+ mouse model was utilized in two additional drinking water studies with developmental exposures in which there was some indication of a positive association between developmental exposures to TCE and the initiation of autoimmune disease. Blossom and Doss (2007) administered TCE to pregnant MRL +/+ mice in drinking water at levels of 0, 0.5, or 2.5 mg/mL and continued administration to the offspring until approximately 7–8 weeks of age. TCE exposure induced a dose-dependent increase in T-lymphocyte IFN- $\gamma$  in peripheral blood at 4–5 weeks of age, but this effect was not observed in splenic T-lymphocytes at 7–8 weeks of age. Serum antihistone autoantibodies and total IgG<sub>2a</sub> were significantly increased in the TCE-treated offspring; however, histopathological evaluation of the liver and kidneys did not reveal any treatment-related signs of autoimmunity. In a study by Blossom et al. (2008), pregnant MRL +/+ mice were administered TCE in the drinking water at levels of 0 or 0.1 mg/mL from GD 0 through lactation, and continuing postweaning in the offspring until GD 42. Significant treatment-related increases in pro-inflammatory cytokines (IFN- $\gamma$  and Il-2 in males and TNF- $\alpha$  in both sexes) produced by splenic CD4+ T-cells were observed in PND 42 offspring.

In summary, TCE treatment induces and exacerbates autoimmune disease in genetically susceptible strains of mice, and has also been shown to induce signs of autoimmune disease in a nongenetically predisposed strain. Although the mechanism for this response is not fully understood, a number of studies have been conducted to examine this issue. The primary conclusion to date is that metabolism of the TCE to its chloral or DCA metabolites is at least partially responsible for activating T-cells or altering T-cell regulation and survival associated with polyclonal disease in susceptible mice strains.

#### **4.6.2.4. Cancers of the Immune System**

Cancers of the immune system that have been observed in animal studies and are associated with TCE exposure are summarized in Tables 4-79 and 4-80. The specific cancer

types observed are malignant lymphomas, lymphosarcomas, and reticulum cell sarcomas in mice and leukemias in rats.

**Table 4-79. Malignant lymphomas incidence in mice exposed to TCE in gavage and inhalation exposure studies**

Cancer type, species, and sex	Prevalence in exposure groups: n affected/n total (% affected)						Reference
	Vehicle control		1,000 mg/kg-d				
<b>Gavage exposure</b>							
Malignant lymphomas	Vehicle control		1,000 mg/kg-d				NTP (1990)
B6C3F <sub>1</sub> mice, male	11/50 (22%)		13/50 (26%)				
B6C3F <sub>1</sub> mice, female	7/48 (15%)		13/49 (27%)				
Lymphosarcomas and reticulum cell sarcomas	Vehicle control		Low dose		High dose		NCI (1976) <sup>b</sup>
B6C3F <sub>1</sub> mice, male	1/20 (5%)		4/50 (8%)		2/48 (4%)		
B6C3F <sub>1</sub> mice, female	1/20 (5%)		5/50 (10%)		5/47 (11%)		
Malignant lymphomas	Control	TCE-pure	TCE-indust	TCE-EPC	TCE-BO	TCE-EPC-BO	Henschler et al. (1984) <sup>c</sup>
Swiss (ICR/HA) mice, male	19/50 (38%)	16/50 (32%)	17/49 (35%)	11/49 (22%)	11/49 (22%)	12/49 (24%)	
Swiss (ICR/HA) mice, female	28/50 (56%)	21/50 (42%)	19/50 (38%)	20/50 (40%)	23/48 (48%)	18/50 (36%)	
<b>Inhalation exposure</b>							
Malignant lymphomas	Control		96		480		Henschler et al. (1980) <sup>d</sup>
Han:NMRI mice, male	7/30 (23%)		7/29 (24%)		6/30 (20%)		
Han:NMRI mice, female <sup>e</sup>	9/29 (31%)		17/30 (57%)		18/28 (64%)		

<sup>a</sup>After 103 weeks of gavage exposure, beginning at 8 weeks of age.

<sup>b</sup>After 90 weeks of gavage exposure, beginning at 5 weeks of age. Low dose is 1,200 mg/kg-d for male mice, 900 mg/kg-d for female mice (5 days/week). High dose is 2,400 mg/kg-d for male mice, 1,800 mg/kg-d for female mice (5 days/week).

<sup>c</sup>After 72 weeks of gavage exposure (corn oil), beginning at 5 weeks of age. Male mice received 2,400 mg/kg-d, female mice received 1,800 mg/kg-d. Stabilizers were added in the percentage w/w: TCE-EPC, 0.8%, TCE-BO, 0.8%, TCE-EPC-BO, 0.25 and 0.25%.

<sup>d</sup>After 78 weeks of inhalation exposure. Administered daily concentration: low dose is 96 (mg/m<sup>3</sup>) and high dose is 480 (mg/m<sup>3</sup>), equivalent to 100 and 500 ppm (100 ppm = 540 mg/m<sup>3</sup>), adjusted for 6 hours/day, 5 days/week exposure.

<sup>e</sup>Statistically significant by Cochran-Armitage trend test ( $p < 0.05$ ).

Sources: NTP (1990) Tables 8 and 9; NCI (1976) Table "XXXa"; Henschler et al. (1980) Table 3a.

**Table 4-80. Leukemia incidence in rats exposed to TCE in gavage and inhalation exposure studies**

Species and sex	Prevalence in exposure groups: n affected/n total (% affected)				Reference
	Control	50 mg/kg	250 mg/kg	600 ppm	
<b>Gavage exposure</b>					Maltoni et al. (1988; 1986) <sup>a</sup>
Sprague-Dawley rats, male	0/30 (0%)	2/30 (6.7%)	3/30 (10.0%)		
Sprague-Dawley rats, female	1/30 (3.3%)	0/30 (0%)	0/30 (0%)		
	Control	500 mg/kg	1,000 mg/kg		
					NTP (1988) <sup>b</sup>
August rats, female	0/50 (0%)	1/50 (2%)	5/50 (10%)		
<b>Inhalation exposure</b>	Control	100 ppm	300 ppm	600 ppm	Maltoni et al. (1988; 1986) <sup>c</sup>
Sprague-Dawley rats, male	9/135 (6.7)	13/130 (10.0)	14/130 (10.8)	15/130 (11.5)	
Sprague-Dawley rats, female	7/145 (4.8)	9/130 (6.9)	2/130 (1.5)	11/130 (8.5)	

<sup>a</sup>After 52 weeks of gavage exposure, beginning at 13 weeks of age, olive oil vehicle. Percentage affected and starting n given in reported; EPA calculated n affected.

<sup>b</sup>After 104 weeks of gavage exposure, beginning at 6.5–8 weeks of age, corn oil vehicle.

<sup>c</sup>After 104 weeks of inhalation exposure, BT304 and BT304bis. Percentage affected and starting n given in reported; EPA calculated n affected.

In the NCI (1976) study, the results for Osborne-Mendel rats were considered inconclusive due to significant early mortality, but exposure to B6C3F<sub>1</sub> mice were also analyzed. Limited increases in lymphomas over controls were observed in both sexes of mice exposed (see Table 4-79). The NCI study (1976) used technical-grade TCE, which contained two known carcinogenic compounds as stabilizers (epichlorohydrin and 1,2-epoxybutane). A later study (Henschler et al., 1984) in which mice were given TCE that was pure, industrial, and stabilized with one or both of these stabilizers did not find significant increases in lymphomas over historical controls. A gavage study by NTP (1988), which used TCE stabilized with diisopropylamine, did not see an increase in lymphomas in all four strains of rats (ACI, August, Marshall, and Osborne-Mendel). The final NTP study (1990) in male and female F344 rats and B6C3F<sub>1</sub> mice, using epichlorohydrin-free TCE, again reported early mortality in male rats. This study did not observe a significant increase in lymphomas over that of controls. Henschler et al. (1980) tested NMRI mice, WIST rats, and Syrian hamsters of both sexes, and observed a variety of tumors in both sexes, consistent with the spontaneous tumor incidence in these strains (Deerberg et al., 1974; Deerberg and Müller-Peddinghaus, 1970). Henschler et al. (1980) did not show an increase in lymphomas in rats or hamsters of either sex. Background levels of lymphomas in this mouse strain are high, making it difficult to determine if the increased lymphomas in female mice is a treatment effect. In a follow-up study, Henschler et al. (1984) examined the role of stabilizers of TCE in the lymphomas demonstrated in female mice in the

1980 paper. Each exposure group had ~50 SPF-bred ICR/HA-Swiss mice and exposure was for 18 months. Background incidence of tumors was high in all groups. Focusing just on malignant lymphomas (see Table 4-79), the high background incidence in unexposed animals again makes it difficult to determine if there is TCE and/or stabilizer-related incidence of lymphomas. There are no data at any other timepoint than 18 months. A high mortality rate in all animals as well as the increased incidence of ‘background’ lymphomas in that report was also a problem and may have been related to the shorter time frame.

Maltoni et al. (1988; 1986) reported a nonsignificant increase in leukemias in male rats exposed via inhalation. Maltoni et al. (1988; 1986) demonstrates a borderline higher frequency of leukemias in male Sprague-Dawley rats following exposure by ingestion for 52 weeks, believed by the authors to be related to an increase in lymphoblastic lymphosarcomas (see Table 4-80). The gavage study by NTP (1988), which used TCE stabilized with diisopropylamine, observed leukemia in female August rats with a positive trend, but was not significantly greater than the vehicle controls.

In summary, overall there is limited available data in animals on the role of TCE in lymphomas and leukemias. There are few studies that analyze for lymphomas and/or leukemias. Lymphomas were described in four studies (NTP, 1990; Henschler et al., 1984; Henschler et al., 1980; NCI, 1976), but study limitations (high background rate) in most studies make it difficult to determine if these are TCE-induced. Three studies found positive trends in leukemia in specific strains and/or gender (Maltoni et al., 1988; NTP, 1988; Maltoni et al., 1986). Due to study limitations, these trends cannot be determined to be TCE-induced.

### **4.6.3. Summary**

#### **4.6.3.1. Noncancer Effects**

The human and animal studies of TCE and immune-related effects provide strong evidence for a role of TCE in autoimmune disease and in a specific type of generalized hypersensitivity syndrome. The data pertaining to immunosuppressive effects is weaker. It should also be noted that immune-related and inflammatory effects, particularly cell-mediated immunity involving cytokine production and activation of macrophages and NK cells, may influence a variety of other conditions of considerable public health importance, including cancer (tumor surveillance) and atherosclerosis. Thus, the relevance of immune-related effects of TCE are not limited to diseases affecting organs and tissues within the immune system. The relation between systemic autoimmune diseases, such as scleroderma, and occupational exposure to TCE has been reported in several recent studies. A meta-analysis of scleroderma studies (Garabrant et al., 2003; Diot et al., 2002; Nietert et al., 1998) conducted by the EPA resulted in a statistically significant combined OR for any exposure in men (OR: 2.5, 95% CI: 1.1, 5.4), with a lower RR seen in women in women (OR: 1.2, 95% CI: 0.58, 2.6). The incidence of systemic sclerosis among men is very low (approximately 1 per 100,000 per year), and is approximately 10 times

lower than the rate seen in women ([Cooper and Stroehla, 2003](#)). Thus, the human data, at this time, do not allow for the determination of whether the difference in effect estimates between men and women reflects the relatively low background risk of scleroderma in men, gender-related differences in exposure prevalence or in the reliability of exposure assessment ([Messing et al., 2003](#)), a gender-related difference in susceptibility to the effects of TCE, or chance. Changes in levels of inflammatory cytokines were reported in an occupational study of degreasers exposed to TCE ([Iavicoli et al., 2005](#)) and a study of infants exposed to TCE via indoor air ([Lehmann et al., 2002](#); [Lehmann et al., 2001](#)). Experimental studies support the biological plausibility of these effects. Numerous studies have demonstrated accelerated autoimmune responses in autoimmune-prone mice ([Cai et al., 2008](#); [Blossom et al., 2007](#); [Blossom et al., 2004](#); [Griffin et al., 2000a](#); [Griffin et al., 2000b](#)). With shorter exposure periods, effects include changes in cytokine levels similar to those reported in human studies. More severe effects, including autoimmune hepatitis, inflammatory skin lesions, and alopecia, were manifest at longer exposure periods, and interestingly, these effects differ somewhat from the “normal” expression in these mice. Immunotoxic effects, including increases in anti-dsDNA antibodies in adult animals and decreased PFC response with prenatal and neonatal exposure, have been also reported in B6C3F<sub>1</sub> mice, which do not have a known particular susceptibility to autoimmune disease ([Peden-Adams et al., 2006](#); [Gilkeson et al., 2004](#)). Recent mechanistic studies have focused on the roles of various measures of oxidative stress in the induction of these effects by TCE ([Wang et al., 2008](#); [Wang et al., 2007b](#)).

There have been a large number of case reports of a severe hypersensitivity skin disorder, distinct from contact dermatitis and often accompanied by hepatitis, associated with occupational exposure to TCE, with prevalences as high as 13% of workers in the same location ([Kamijima et al., 2008](#); [Kamijima et al., 2007](#)). Evidence of a treatment-related increase in delayed hypersensitivity response accompanied by hepatic damage has been observed in guinea pigs following intradermal injection ([Tang et al., 2008](#); [Tang et al., 2002](#)), and hypersensitivity response was also seen in mice exposed via drinking water pre- and postnatally (GD 0 through to 8 weeks of age) ([Peden-Adams et al., 2006](#)).

Human data pertaining to TCE-related immunosuppression resulting in an increased risk of infectious diseases is limited to the report of an association between reported history of bacteria or viral infections in Woburn, Massachusetts ([Lagakos et al., 1986](#)). Evidence of localized immunosuppression, as measured by pulmonary response to bacterial challenge (i.e., risk of Streptococcal pneumonia-related mortality and clearance of *Klebsiella* bacteria) was seen in an acute exposure study in CD-1 mice ([Aranyi et al., 1986](#)). A 4-week inhalation exposure in Sprague-Dawley rats reported a decrease in PFC response at exposures of 1,000 ppm ([Woolhiser et al., 2006](#)).

#### 4.6.3.2. Cancer

Associations observed in epidemiologic studies of lymphoma and TCE exposure suggest a causal relation between TCE exposure and NHL. Issues of study heterogeneity, potential publication bias, and weaker exposure-response results contribute uncertainty to the evaluation of the available data.

In a review of the NHL studies, studies in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs, biomarker monitoring, or industrial hygiene data on TCE exposure patterns and factors that affect such exposure) and which met, to a sufficient degree, the standards of epidemiologic design and analysis were identified. These studies generally reported excess RR estimates for NHL between 0.8 and 3.1 for overall TCE exposure. Statistically significant elevated RR estimates with NHL and overall TCE exposure were observed in two cohort ([Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#)) and one case-control ([Hardell et al., 1994](#)) study. Both cohort studies reported statistically significant associations with NHL for subjects with longer employment duration as a surrogate of TCE exposure as does a second case-control study with high-quality exposure-assessment methodology reported statistically significant associations with highest cumulative TCE exposure or highest average-weekly TCE exposure ([Purdue et al., 2011](#)). [Hardell et al. \(1994\)](#) reported a strong but imprecise association, in part reflecting possible bias from subject-reported exposure history and few exposed cases. Other identified studies reported a 10–50% elevated RR estimate with overall TCE exposures that were not statistically significant, except for two population case-control studies of NHL, one of which did not report RR estimates with overall TCE exposure but did for medium-high intensity or cumulative TCE exposure ([Purdue et al., 2011](#); [Cocco et al., 2010](#); [Wang et al., 2009](#); [Radican et al., 2008](#); [Miligi et al., 2006](#); [Zhao et al., 2005](#); [Boice et al., 1999](#); [Persson and Fredrikson, 1999](#); [Morgan et al., 1998](#); [Nordström et al., 1998](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#); [Greenland et al., 1994](#); [Siemiatycki, 1991](#)). Fifteen additional studies were given less weight because of their lesser likelihood of TCE exposure and other design limitations that would decrease study power and sensitivity ([Clapp and Hoffman, 2008](#); [ATSDR, 2006a](#); [Chang et al., 2005](#); [ATSDR, 2004a](#); [Chang et al., 2003](#); [Morgan and Cassady, 2002](#); [Ritz, 1999a](#); [Henschler et al., 1995](#); [Cohn et al., 1994b](#); [Vartiainen et al., 1993](#); [Sinks et al., 1992](#); [Blair et al., 1989](#); [Costa et al., 1989](#); [Garabrant et al., 1988](#); [Wilcosky et al., 1984](#)) The observed lack of association with NHL in these studies likely reflects study design and exposure assessment limitations and is not considered inconsistent with the overall evidence on TCE and NHL.

Consistency of the association between TCE exposure and NHL is further supported by the results of meta-analyses of 17 studies reporting risk estimates for overall TCE exposure that met the meta-analysis inclusion criteria. These meta-analyses found a statistically significant increased RR<sub>m</sub> estimate for NHL of 1.23 (95% CI: 1.07, 1.42) for overall TCE exposure. The analysis of NHL was robust to the removal of individual studies and the use of alternate RR

estimates from individual studies, and in only one cases was the resulting RRm no longer statistically significant (lower 95% confidence bounds of 1.00). Some evidence heterogeneity was observed, particularly between cohort and case-control studies, but it was not statistically significant ( $p = 0.10$ ); and, in addition, there was some evidence of potential publication bias. Analyzing the cohort and case-control studies separately resolved most of the heterogeneity, but the result for the summary case-control studies was only a 7% increased RR estimate and was not statistically significant. The sources of heterogeneity are uncertain but may be the result of some bias associated with exposure assessment and/or disease classification, or from differences between cohort and case-control studies in average TCE exposure.

Exposure-response relationships are examined in the TCE epidemiologic studies only to a limited extent. Many studies examined only overall “exposed” vs. “unexposed” groups and did not provide exposure information by level of exposure. Others do not have adequate exposure assessments to confidently distinguish between levels of exposure. The NHL case-control study of Purdue et al. (2011) reported a statistically significant trend with TCE exposure ( $p = 0.02$  for average-weekly TCE exposure), and NHL risk in Boice et al. (1999) appeared to increase with increasing exposure duration ( $p = 0.20$  for routine-intermittent exposed subjects). The borderline statistically significant trend with TCE intensity in the case-control study of Wang et al. (2009 [ $p = 0.06$ ]) and with cumulative TCE exposure in the case-control study of Purdue et al. (2011 [ $p = 0.08$ ]) is consistent with that observed with average weekly TCE exposure in Purdue et al. (2011). Further support was provided by meta-analyses using only the highest exposure groups, which yielded a higher RRm estimate (1.43 [95% CI: 1.13, 1.82]) than for overall TCE exposure (1.23 [95% CI: 1.07, 1.42]).

Few risk factors are recognized for NHL, with the exception of viruses, immunosuppression, or smoking, which are associated with specific NHL subtypes (Besson et al., 2006). Associations between NHL and TCE exposure are based on groupings of several subtypes. Two of the seven NHL case-control studies adjusted for age, sex, and smoking in statistical analyses (Wang et al., 2009; Miligi et al., 2006), two others adjusted for age and sex (Purdue et al., 2011; Cocco et al., 2010), and the other three case-control studies presented only unadjusted OR estimates (Persson and Fredrikson, 1999; Nordström et al., 1998; Hardell et al., 1994).

Animal studies describing rates of lymphomas and/or leukemias in relation to TCE exposure (NTP, 1990; Maltoni et al., 1988; NTP, 1988; Maltoni et al., 1986; Henschler et al., 1984; Henschler et al., 1980; NCI, 1976) are available. Henschler et al. (1980) reported statistically significant increases in lymphomas in female Han:NMRI mice treated via inhalation. While Henschler et al. (1980) suggested that these lymphomas were of viral origin specific to this strain, subsequent studies reported increased lymphomas in female B6C3F<sub>1</sub> mice treated via corn oil gavage (NTP, 1990) and leukemias in male Sprague-Dawley and female August rats (Maltoni et al., 1988; NTP, 1988; Maltoni et al., 1986). However, these tumors had relatively

modest increases in incidence with treatment, and were not reported to be increased in other studies.

## **4.7. RESPIRATORY TRACT TOXICITY AND CANCER**

### **4.7.1. Epidemiologic Evidence**

#### **4.7.1.1. Chronic Effects: Inhalation**

Two reports of a study of 1,091 gun-manufacturing workers are found on noncancer pulmonary toxicity ([Saygun et al., 2007](#); [Cakmak et al., 2004](#)). A subset of these workers (n = 411) had potential exposure to multiple organic solvents including toluene, acetone, butanol, xylene, benzene, and TCE used to clean gun parts; however, both papers lacked information on exposure concentration. Mean exposure duration in [Cakmak et al. \(2004\)](#) was 17 years (SD = 7.9) for nonsmokers and 16 years (SD = 7.1) for smokers. [Cakmak et al. \(2004\)](#) indicated effects of smoking and exposure to solvents, with smoking having the most important effect on asthma-related symptoms (smoking, OR = 2.8, 95% CI: 2.0, 3.8; solvent exposure, OR = 1.4, 95% CI: 1.1, 1.9). Similarly, smoking, but not solvent exposure, was shown as a statistically significant predictor of lung function decrements. [Saygun et al. \(2007\)](#) reported on a 5-year follow-up of 393 of the original 1,091 subjects, 214 of who were exposed to solvents. Of the 393 original subjects, the prevalence of definitive asthma symptoms, a more rigorous definition than used by [Cakmak et al. \(2004\)](#), was 3.3% among exposed and 1.1% among nonexposed subjects,  $p > 0.05$ . [Saygun et al. \(2007\)](#) presents observations on lung function tests for 697 current workers, a group which includes the 393 original study subjects. Smoking, but not solvent exposure, was a predictor of mean annual forced expiratory volume (FEV<sub>1</sub>) decrease.

#### **4.7.1.2. Cancer**

Cancers of the respiratory tract including the lung, bronchus, and trachea were examined in 25 cohort, community studies and case-control studies of TCE. Twelve of the 25 studies approached standards of epidemiologic design and analysis identified in the review of the epidemiologic body of literature on TCE and cancer ([Radican et al., 2008](#); [Boice et al., 2006b](#); [Zhao et al., 2005](#); [Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#); [Boice et al., 1999](#); [Blair et al., 1998](#); [Morgan et al., 1998](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#); [Greenland et al., 1994](#); [Siemiatycki, 1991](#)). Cancers at other sites besides lung, bronchus, and trachea in the respiratory system are more limitedly reported in these studies. Some information is available on laryngeal cancer; however, only 9 of the 16 occupational cohort studies providing information on lung cancer also reported findings for this site. Case-control studies of lung or laryngeal cancers and occupational title or organic solvent exposure were found in the literature. Two case-control studies of lung cancer, one population-based and the other nested within a cohort, were of TCE exposure specifically. Lung and laryngeal cancer risk ratios reported in cohort, community and case-control studies are found in Table 4-81.

**Table 4-81. Selected results from epidemiologic studies of TCE exposure and lung cancer**

Exposure group	RR (95% CI)	Number of observable events	Reference
<b>Cohort studies—incidence</b>			
Aerospace workers (Rocketdyne)			Zhao et al. ( <a href="#">2005</a> )
Any exposure to TCE	Not reported		
Low cumulative TCE score	1.00 <sup>a</sup>	43	
Medium cumulative TCE score	1.36 (0.86, 2.14)	35	
High TCE score	1.11 (0.60, 2.06)	14	
<i>p</i> for trend	0.60		
All employees at electronics factory (Taiwan)	1.07 (0.72, 1.52)	30	Chang et al. ( <a href="#">2005</a> )
Danish blue-collar worker with TCE exposure			Raaschou-Nielsen et al. ( <a href="#">2003</a> )
Any exposure, all subjects	1.4 (1.32, 1.55)	632	
Any exposure, males	1.4 (1.28, 1.51)	559	
Any exposure, females	1.9 (1.48, 2.35)	73	
Employment duration			
<1 yr	1.7 (1.46, 1.93)	209	
1–4.9 yrs	1.3 (1.16, 1.52)	218	
≥5 yrs	1.4 (1.23, 1.63)	205	
Biologically-monitored Danish workers			Hansen et al. ( <a href="#">2001</a> )
Any TCE exposure, males	0.8 (0.5, 1.3)	16	
Any TCE exposure, females	0.7 (0.01, 3.8)	1	
Cumulative exposure (Ikeda)			
<17 ppm-yr			
≥17 ppm-yr			
Mean concentration (Ikeda)			
<4 ppm			
4+ ppm			
Employment duration			
<6.25 yr			
≥6.25 yr			
Aircraft maintenance workers (Hill Air Force Base, Utah)			Blair et al. ( <a href="#">1998</a> )
TCE subcohort	Not reported		
Males, cumulative exposure			
0	1.0 <sup>a</sup>		
<5 ppm-yr	1.0 (0.6, 2.0)	24	
5–25 ppm-yr	0.8 (0.4, 1.6)	11	
>25 ppm-yr	0.8 (0.4, 1.7)	15	

**Table 4-81. Selected results from epidemiologic studies of TCE exposure and lung cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
Females, cumulative exposure				Blair et al. (1998) (continued)
0		1.0 <sup>a</sup>		
<5 ppm-yr			1	
5–25 ppm-yr			1	
>25 ppm-yr			1	
Biologically-monitored Finnish workers				Anttila et al. (1995)
All subjects		0.92 (0.59, 1.35)	25	
Mean air-TCE (Ikeda extrapolation)				
<6 ppm		1.02 (0.58, 1.66)	16	
6+ ppm		0.83 (0.33, 1.71)	7	
Biologically-monitored Swedish workers				Axelson et al. (1994)
Any TCE exposure, males		0.69 (0.31, 1.30)	9	
Any TCE exposure, females		Not reported		
<b>Cohort and PMR-mortality</b>				
Computer manufacturing workers (IBM), New York				Clapp and Hoffman (2008)
Males		1.03 (0.71, 1.42)	35	
Females		0.95 (0.20, 2.77)	3	
Aerospace workers (Rocketdyne)				Boice et al. (2006b)
Any TCE (utility or engine flush workers)		1.24 (0.92, 1.63)	51	
Engine flush—duration of exposure				
Referent		1.0 <sup>a</sup>	472	
0 yr (utility workers with TCE exposure)		0.5 (0.22, 1.00)	7	
<4 yrs		0.8 (0.50, 1.26)	27	
≥4 yrs		0.8 (0.46, 1.41)	24	
Any exposure to TCE		Not reported		
Low cumulative TCE score		1.00 <sup>a</sup>	99	
Medium cumulative TCE score		1.05 (0.76, 1.44)	62	
High TCE score		1.02 (0.68, 1.53)	33	
<i>p</i> for trend		0.91		
View-Master employees				ATSDR (2004a)
Males		0.81 (0.42, 1.42) <sup>b</sup>	12	
Females		0.99 (0.71, 1.35) <sup>b</sup>	41	

**Table 4-81. Selected results from epidemiologic studies of TCE exposure and lung cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
United States uranium-processing workers (Fernald)				Ritz ( <a href="#">1999a</a> )
Any TCE exposure		Not reported		
Light TCE exposure, >2-yr duration <sup>c</sup>		Not reported		
Moderate TCE exposure, >2-yr duration <sup>c</sup>		Not reported		
Aerospace workers (Lockheed)				Boice et al. ( <a href="#">1999</a> )
Routine exposure		0.76 (0.60, 0.95)	78	
Routine-intermittent exposure <sup>a</sup>		Not reported	173	
Duration of exposure				
0 yr		1.0	288	
<1 yr		0.85 (0.65, 1.13)	66	
1–4 yrs		0.98 (0.74, 1.30)	63	
≥5 yrs		0.64 (0.46, 0.89)	44	
Trend test		$p < 0.05$		
Aerospace workers (Hughes)				Morgan et al. ( <a href="#">1998</a> )
TCE subcohort		1.10 (0.89, 1.34)	97	
Low intensity (<50 ppm)		1.49 (1.09, 1.99)	45	
High intensity (>50 ppm)		0.90 (0.67, 1.20)	52	
TCE subcohort (Cox Analysis) <sup>b</sup>				
Never exposed		1.00 <sup>a</sup>	291	
Ever exposed		1.14 (0.90, 1.44)	97	
Peak				
No/Low		1.00 <sup>a</sup>	324	
Medium/High		1.07 (0.82, 1.40)	64	
Cumulative				
Referent		1.00 <sup>a</sup>	291	
Low		1.47 (1.07, 2.03)	45	
High		0.96 (0.72, 1.29)	52	
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. ( <a href="#">1998</a> )
TCE subcohort				
Any TCE exposure		0.9 (0.6, 1.3) <sup>a</sup>	109	

**Table 4-81. Selected results from epidemiologic studies of TCE exposure and lung cancer (continued)**

Exposure group	RR (95% CI)	Number of observable events	Reference
Males, cumulative exposure			Blair et al. (1998) (continued)
0	1.0 <sup>a</sup>	51	
<5 ppm-yr	1.0 (0.7, 1.6)	43	
5–25 ppm-yr	0.9 (0.5, 1.6)	23	
>25 ppm-yr	1.1 (0.7, 1.8)	38	
Females, Cumulative exp			
0	1.0 <sup>a</sup>	2	
<5 ppm-yr	0.6 (0.1, 2.4)	2	
5–25 ppm-yr	0.6 (0.1, 4.7)	11	
>25 ppm-yr	0.4 (0.1, 1.8)	2	
TCE subcohort			Radican et al. (2008)
Any TCE exposure	0.83 (0.63, 1.08)	166	
Males, cumulative exposure	0.91 (0.67, 1.24)	155	
0	1.0 <sup>a</sup>	66	
<5 ppm-yr	0.96 (0.67, 1.37)		
5–25 ppm-yr	0.71 (0.46, 1.11)	31	
>25 ppm-yr	1.00 (0.69, 1.45)	58	
Females, cumulative exposure	0.53 (0.27, 1.07)	11	
0	1.0 <sup>a</sup>		
<5 ppm-yr	0.69 (0.27, 1.77)	5	
5–25 ppm-yr	0.65 (0.16, 2.73)	2	
>25 ppm-yr	0.39 (0.14, 1.11)	4	
Cardboard manufacturing workers in Arnsburg, Germany			Henschler et al. (1995)
TCE-exposed workers	1.38 (0.55, 2.86)	7	
Unexposed workers	1.06 (0.34, 2.47)	5	
Deaths reported to GE pension fund (Pittsfield, Massachusetts)	1.01 (0.69, 1.47) <sup>d</sup>	139	Greenland et al. (1994)
U.S. Coast Guard employees			Blair et al. (1998)
Marine inspectors	0.52 (0.31, 0.82)	18	
Noninspectors	0.81 (0.55, 1.16)	30	
Aircraft manufacturing employees (Italy)			Costa et al. (1989)
All employees	0.99 (0.73, 1.32)	99	
Aircraft manufacturing plant employees (San Diego, California)			Garabrant et al. (1988)
All subjects	0.80 (0.68, 0.95)	138	

**Table 4-81. Selected results from epidemiologic studies of TCE exposure and lung cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
Lamp manufacturing workers (GE)		0.58 (0.27, 1.27)	6	Shannon et al. (1988)
Rubber industry workers (Ohio)		0.64 ( $p > 0.05$ ) <sup>c</sup>	11	Wilcosky et al. (1984)
<b>Case-control studies</b>				
Population of Montreal, Canada				Siemiatycki et al. (1991)
	Any TCE exposure	0.9 (0.6, 1.5) <sup>e</sup>	21	
	Substantial TCE exposure	0.6 (0.3, 1.2) <sup>e</sup>	9	
<b>Geographic-based studies</b>				
Two study areas in Endicott, New York		1.28 (0.99, 1.62)	68	ATSDR (2006a)
Residents of 13 census tracts				Morgan and Cassidy (2002)
	In Redlands, California	0.71 (0.61, 0.81) <sup>f</sup>	356	
Iowa residents with TCE in water supply				Isacson et al. (1985)
	Males			
	<0.15 µg/L	343.1 <sup>g</sup>	1,181	
	≥0.15 µg/L	345.7 <sup>g</sup>	299	
	Females			
	<0.15 µg/L	58.7 <sup>g</sup>	289	
	≥0.15 µg/L	47.8 <sup>g</sup>	59	

<sup>a</sup>Internal referents, workers not exposed to TCE.

<sup>b</sup>Risk ratio from Cox Proportional Hazard Analysis, stratified by age, sex, and decade (EHS, 1997).

<sup>c</sup>OR from nested case-control study.

<sup>d</sup>OR from nested case-control analysis.

<sup>e</sup>90% CI.

<sup>f</sup>99% CI.

<sup>g</sup>Average annual age-adjusted incidence (per 100,000).

Lung cancer RRs were reported in 11 of 12 cohort studies of aircraft manufacturing, aircraft maintenance, aerospace, and metal workers, with potential exposure to TCE as a degreasing agent, and in occupational cohort studies employing biological markers of TCE exposures. All 11 studies had a high likelihood of TCE exposure in individual study subjects and were judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis (Radican et al., 2008; Boice et al., 2006b; Zhao et al., 2005; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994). Lung cancer risks were not reported for Fernald uranium processing workers with potential TCE exposure (Ritz, 1999a), a study of less weight than the other 11 studies. The incidence study of Raaschou-Nielsen et al. (2003) was the largest cohort, with 40,049 subjects identified as potentially exposed to TCE in several industries (primarily, in the iron/metal and electronic industries), including 14,360 who had presumably higher level exposures to TCE. The study included 632 lung cancer cases and reported a 40%

elevated incidence in TCE exposed males and females combined (95% CI: 1.32, 1.55), with no exposure duration gradient. The 95% CIs in other studies of lung cancer incidence included a risk ratio of 1.0 ([Zhao et al., 2005](#); [Hansen et al., 2001](#); [Blair et al., 1998](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#)). Lung cancer mortality risks in studies of TCE exposure to aircraft manufacturing, aircraft maintenance, and aerospace workers included a RR of 1.0 in their 95% CIs ([Radican et al., 2008](#); [Boice et al., 2006b](#); [Zhao et al., 2005](#); [Blair et al., 1998](#); [Morgan et al., 1998](#)). Boice et al. (1999) observed a 24% decrement (95% CI: 0.60, 0.95) for subjects with routine TCE exposure. Exposure-response analyses using internal controls (unexposed subjects at the same company) showed a statistically significant decreasing trend between lung cancer risk and routine or intermittent TCE exposure duration. The routine or intermittent category is broader and includes more subjects with potential TCE exposure. Five other studies with internal controls do not provide evidence of either an increasing or decreasing pattern between TCE and lung cancer incidence or mortality ([Radican et al., 2008](#); [Boice et al., 2006b](#); [Zhao et al., 2005](#); [Blair et al., 1998](#); [Morgan et al., 1998](#)).

The population studied by Garabrant et al. (1988), ATSDR (2004a) and Chang et al. (2005) are all employees (white- and blue-collar) at a manufacturing facility or plant with potential TCE exposures. Garabrant et al. (1988) observed a 20% deficit in lung cancer mortality (95% CI: 0.68, 0.95) in their study of all employees working for  $\geq 4$  years at an aircraft manufacturing company. Blair et al. (1998), a study of Coast Guard marine inspectors with potential for TCE exposure but lacking assessment to individual subjects, observed a 48% deficit in lung cancer mortality (95% CI: 0.31, 0.82). Confidence intervals (95% CI) in Costa et al. (1989), Chang et al. (2005), and ATSDR (2004a) included a risk of 1.0. TCE exposure was not known for individual subjects in these studies. A wide potential for TCE exposure is likely ranging from subjects with little to no TCE exposure potential to those with some TCE exposure potential. Exposure misclassification bias, typically considered as a negative bias, is likely greater in these studies compared to studies adopting more sophisticated exposure assessment approaches, which are able to assign quantitative exposure metrics to individual study subjects. All three studies were of lower likelihood for TCE exposure, in addition to limited statistical power and other design limitations, and these aspects, in addition to potential exposure misclassification bias, were alternative explanations of observed findings.

One population case-control study examined the relationship between lung cancer and TCE exposure ([Siemiatycki, 1991](#)) with risk ratios of 0.9 (95% CI: 0.6, 1.5) for any TCE exposure and 0.6 (95% CI: 0.3, 1.2) for substantial TCE exposure after adjustment for cigarette smoking. TCE exposure prevalence in cases in this study was 2.5% for any exposure. Only 1% had “substantial” (author’s term) exposure, limiting the sensitivity of this study. RRs  $>2.0$  could only be detected with sufficient (80%) statistical power. The finding of no association of lung cancer with TCE exposure, therefore, is not surprising. One nested case-control study of rubber

workers observed a smoking unadjusted risk of 0.64 (95% CI: not presented in paper) in those who had >1 year cumulative exposure to TCE ([Wilcosky et al., 1984](#)).

Three geographic-based studies reported lung cancer incidence or mortality risks for drinking water contamination with TCE (ATSDR, 2006a; [Morgan and Cassidy, 2002](#); [Isacson et al., 1985](#)). Morgan and Cassidy (2002) observed a RR of 0.71 (99% CI: 0.61, 0.81) for lung cancer among residents of Redlands (San Bernardino County), California, whose drinking water was contaminated with TCE and perchlorate. However, ATSDR (2006a) reported a 28% increase (95% CI: 0.99, 1.62) in lung cancer incidence among residents living in a area in Endicott, New York, whose drinking water was contaminated with TCE and other solvents. No information on smoking patterns is available for individual lung cancer cases as identified by the New York State Department of Health (NYS DOH) for other cancer cases in this study ([ATSDR, 2008b](#)). Isacson et al. (1985) presented lung cancer age-adjusted incidence rates for Iowa residents by TCE level in drinking water supplies and did not observe an exposure-response gradient. Exposure information is inadequate in all three of these studies, with monitoring data, if available, based on few samples and for current periods only, and no information on water distribution, consumption patterns, or temporal changes. Thus, TCE exposure potential to individual subjects was not known with any precision, introducing misclassification bias, and greatly limiting their ability to inform evaluation of TCE and lung cancer.

Laryngeal cancer risks are presented in a limited number of cohort studies involving TCE exposure. No case-control or geographic-based studies of TCE exposure were found in the published literature. All but one of the cohort studies providing information on laryngeal cancer observed less than five incident cases or deaths. Accordingly, these studies are limited for examining the relationship between TCE exposure and laryngeal cancer. Risk ratios for laryngeal cancer are found in Table 4-82.

**Table 4-82. Selected results from epidemiologic studies of TCE exposure and laryngeal cancer**

Exposure group		RR (95% CI)	Number of observable events	Reference
<b>Cohort studies—incidence</b>				
Aerospace workers with TCE exposure		Not reported		Zhao et al. ( <a href="#">2005</a> )
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al. ( <a href="#">2003</a> )
	Any exposure, males	1.2 (0.87, 1.52)	53	
	Any exposure, females	1.7 (0.33, 4.82)	3	
	Employment duration	Not reported		
	<1 yrs			
	1–4.9 yrs			
	≥5 yrs			
Biologically-monitored Danish workers				Hansen et al. ( <a href="#">2001</a> )
	Any TCE exposure, males	1.1 (0.1, 3.9)	2	
	Any TCE exposure, females		0 (0.1 exp)	
	Cumulative exposure (Ikeda)	Not reported		
	<17 ppm-yr			
	≥17 ppm-yr			
	Mean concentration (Ikeda)	Not reported		
	<4 ppm			
	4+ ppm			
	Employment duration	Not reported		
	<6.25 yr			
	≥6.25 yr			
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. ( <a href="#">1998</a> )
	TCE subcohort			
	Any exposure	Not reported		
	Males, cumulative exposure	Not reported		
	0			
	<5 ppm-yr			
	5–25 ppm-yr			
	>25 ppm-yr			
	Females, cumulative exposure	Not reported		
	0			
	<5 ppm-yr			
	5–25 ppm-yr			
	>25 ppm-yr			

**Table 4-82. Selected results from epidemiologic studies of TCE exposure and laryngeal cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
Biologically-monitored Finnish workers		Not reported		Anttila et al. ( <a href="#">1995</a> )
	Mean air-TCE (Ikeda extrapolation from U-TCA)	Not reported		
	<6 ppm			
	6+ ppm			
Biologically-monitored Swedish workers				Axelsson et al. ( <a href="#">1994</a> )
	Any TCE exposure, males	1.39 (0.17, 5.00)	2	
	Any TCE exposure, females	Not reported		
<b>Cohort and PMR-mortality</b>				
Computer manufacturing workers (IBM), New York		Not reported		Clapp and Hoffman ( <a href="#">2008</a> )
Aerospace workers (Rocketdyne)				
	Any TCE (utility or engine flush workers)	1.45 (0.18, 5.25)	2	Boice et al. ( <a href="#">2006b</a> )
	Engine flush—duration of exposure	Not reported		
	Referent			
	0 yr (utility workers with TCE exposure)			
	<4 yrs			
	≥4 yrs			
	Any exposure to TCE	Not reported		Zhao et al. ( <a href="#">2005</a> )
View-Master employees		Not reported		ATSDR ( <a href="#">2004a</a> )
	Males			
	Females			
All employees at electronic factory (Taiwan)				Chang et al. ( <a href="#">2003</a> )
	Males		0 (0.90 exp)	
	Females	0	0 (0.23 exp)	
United States uranium-processing workers (Fernald)				Ritz ( <a href="#">1999a</a> )
	Any TCE exposure	Not reported		
	Light TCE exposure, >2-yr duration	Not reported		
	Moderate TCE exposure, >2-yr duration	Not reported		
Aerospace workers (Lockheed)				Boice et al. ( <a href="#">1999</a> )
	Routine exposure	1.10 (0.30, 2.82)	4	
	Routine-intermittent exposure	Not reported		

**Table 4-82. Selected results from epidemiologic studies of TCE exposure and laryngeal cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
Aerospace workers (Hughes)				Morgan et al. ( <a href="#">1998</a> )
TCE subcohort	Not reported			
Low intensity (<50 ppm)				
High intensity (>50 ppm)				
Peak	Not reported			
No/low				
Medium/high				
Cumulative	Not reported			
Referent				
Low				
High				
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. ( <a href="#">1998</a> )
TCE subcohort	Not reported			
Males, cumulative exposure	Not reported			
0				
<5 ppm-yr				
5–25 ppm-yr				
>25 ppm-yr				
Females, cumulative exposure	Not reported			
0				
<5 ppm-yr				
5–25 ppm-yr				
>25 ppm-yr				
Cardboard manufacturing workers in Arnsburg, Germany		Not reported		Henschler et al. ( <a href="#">1995</a> )
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		Not examined		Greenland et al. ( <a href="#">1994</a> )
U.S. Coast Guard employees				Blair et al. ( <a href="#">1998</a> )
Marine inspectors	0.57 (0.01, 3.17)	1		
Noninspectors	0.58 (0.01, 3.20)	1		
Aircraft manufacturing employees (Italy)				Costa et al. ( <a href="#">1989</a> )
All employees	0.27 (0.03, 0.98)	2		
Aircraft manufacturing plant employees (San Diego, California)				Garabrant et al. ( <a href="#">1988</a> )
All subjects		0 (7.41 exp)		

In summary, studies in humans examining lung and laryngeal cancer and TCE exposure are inconclusive and do not support either a positive or a negative association between TCE exposure and lung cancer or laryngeal cancer. Raaschou-Nielsen et al. ([2003](#)), with the largest numbers of lung cancer cases of all studies, was the only one to observe a statistically

significantly elevated lung cancer risk with TCE exposure. Raaschou-Nielsen et al. ([2003](#)) also noted several factors that may have confounded or biased their results in either a positive or negative direction. This study and other cohort studies, as with almost any occupational study, were not able to control confounding by exposure to chemicals other than TCE (although no such chemical was apparent in the reports). Information available for factors related to SES status (e.g., diet, smoking, alcohol consumption) was also not available. Such information may positively confound smoking-related cancers such as lung cancer, particularly in those studies, which adopted national rates to derive expected numbers of site-specific cancer, if greater smoking rates were over-represented in blue-collar workers or residents of lower SES status. The finding of a larger risk among subjects with shortest exposure also argues against a causal interpretation for the observed association for all subjects ([NRC, 2006](#)).

Four studies reported a statistically significant deficit in lung cancer incidence ([Morgan and Cassady, 2002](#); [Boice et al., 1999](#); [Blair et al., 1998](#); [Garabrant et al., 1988](#)). Absence of smoking information in these studies would introduce a negative bias if the studied population smoked less than the referent population and may partially explain the lung cancer decrements observed in these studies. Morgan and Cassidy ([2002](#)) noted the relatively high education, high income levels, and high access to health care of subjects in this study compared to the averages for the county as a whole, likely leading to a lower smoking rate compared to their referent population. Garabrant et al. ([1988](#)) similarly attributed their observations to negative selection bias introduced when comparison is made to national mortality rates, also known as a “healthy worker effect.” The statistically significant decreasing trend in Boice et al. ([1999](#)) with exposure duration to intermittent or routine exposure may reflect a protective effect between TCE and lung cancer. The use of internal controls in this analysis reduces bias associated with use of an external population who may have different smoking patterns than an employed population. However, the exposure assessment approach in this study is limited due to inclusion of subjects identified with intermittent TCE exposure (i.e., workers who would be exposed only during particular shop runs or when assisting other workers during busy periods) ([Boice et al., 1999](#)). The Boice et al. ([1999](#)) analysis is based on twice as many lung cancer deaths (i.e., 173 lung cancer deaths) among subjects with routine or intermittent TCE exposure compared to only routinely exposed subjects (78 deaths). Subjects identified as intermittently exposed are considered as having a lower exposure potential than routinely exposed subject and their inclusion in exposure-response analyses may introduce exposure misclassification bias. Such bias is a possible explanation for the decreasing trend observation, particularly if workers with lower potential for TCE exposure have longer exposure (employment) durations.

Thus, a qualitative assessment suggests the epidemiological literature on respiratory cancer and TCE, although limited and of sufficient power to detect only large RRs, does not provide strong evidence for any association between TCE exposure and lung cancer. These

studies can only rule out risks of a magnitude of  $\geq 2.0$  for lung cancer and RRs  $>3.0$  or  $4.0$  for laryngeal cancer for exposures to studied populations.

## **4.7.2. Laboratory Animal Studies**

### **4.7.2.1. Respiratory Tract Animal Toxicity**

Limited studies are available to determine the effects of TCE exposure on the respiratory tract (summarized in Table 4-83). Many of these studies in mice have examined acute effects following i.p. administration at relatively high TCE doses. However, effects on the bronchial epithelium have been noted in mice and rats with TCE administered via gavage, with doses  $\geq 1,000$  mg/kg-day reported to cause rales and dyspnea ([Narotsky et al., 1995](#)) and pulmonary vasculitis ([NTP, 1990](#)) in rats. Mice appear to be more sensitive than rats to histopathological changes in the lung via inhalation; pulmonary effects are also seen in rats with gavage exposure. It is difficult to compare i.p. to oral and inhalation routes of exposure given the risk of peritonitis and paralytic ileus. Any inflammatory response from this route of administration can also affect the pulmonary targets of TCE exposure such as the Clara cells.

This section reviews the existing literature on TCE, and the role of the various TCE metabolites in TCE-induced lung effects. The most prominent toxic effect reported is damage to Clara cells in mouse lung. The nonciliated, columnar Clara cells comprise the majority of the bronchiolar and terminal bronchiolar epithelium in mice, and alveolar Type I and Type II cells constitute the alveolar epithelium. These cells have been proposed as a progenitor of lung adenocarcinomas in both humans and mice ([Kim et al., 2005](#)). Long-term studies have not focused on the detection of pulmonary adenoma carcinomas but have shown a consistently positive response in mice but not rats. However, chronic toxicity data on noncancer effects are very limited.

**Table 4-83. Animal toxicity studies of TCE**

Reference	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Results
Green et al. (1997b)	CD-1 mice (F)	Inhalation	450 ppm, 6 hrs/d, 5 d with 2 d break then 5 more d; sacrificed 18 hrs after 1, 5, 6, or 10 exposures	5/group	Increased vacuolation and proliferation of Clara cells caused by accumulation of chloral.
Forkert and Forkert (1994)	CD-1 mice (M)	i.p. injection	2,000 mg/kg in corn oil (0.01 mL/g body weight); sacrificed 15, 30, 60, and 90 d after single exposure	10/group	Increased fibrotic lesions, with early signs visible at 15 d postexposure.
Villaschi et al. (1991)	BC3F1 mice (M)	Single inhalation	30 min 500, 1,000, 2,000, 3,500, and 7,000 ppm; sacrificed 2 hrs, 24 hrs, 2, 5, or 7 d post exposure	3/group	Increased vacuolation and proliferation of nonciliated bronchial cells. Injury was maximal at 24 hrs with some repair occurring between 24 and 48 hrs.
Odum et al. (1992)	CD-1 mice (F)	Inhalation	6 hrs/d; separate repeated study in mice: 450 ppm for 6 hrs/d, 5 d/wk for 2 wks; sacrificed 24 hrs after exposure; repeat study sacrificed at 2, 5, 6, 8, 9, 12, or 13 d; mice: 20, 100, 200, 450, 1,000, or 2,000 ppm	4/group	Dose-dependent increase in Clara cell vacuolation in mice after a single exposure, resolved after 5 d repeated exposures but recurred following a 2-d break from exposure. Changes accompanied by decrease in CYP activity in mice. Exposure to chloral alone demonstrated similar response as TCE exposure in mice. No changes were seen in rats.
	Alpk APfSD rats (F)	Inhalation	6 hrs/d; repeat study sacrificed at 2, 5, 6, 8, 9, 12, or 13 d; rats: 500, or 1,000 ppm	4/group	
Kurasawa (1988) (translation)	Ethanol-treated (130) and nontreated (110) Wistar rats (M)	Inhalation	500, 1,000, 2,000, 4,000, and 8,000 ppm for 2 hrs; sacrificed 22 hrs after exposure	10/group	TCE exposure resulted in highly selective damage to Clara cells that occurred between 8 and 22 hrs after the highest exposure with repair by 4 wks post exposure.
Forkert et al. (2006)	CD-1 mice (M); wild-type (mixed 129/Sv and C57BL) and CYP2E1-null mice (M)	i.p. injection	500, 750, and 1,000 mg/kg in corn oil; for inhibition studies mice pretreated with 100 mg/kg diallyl sulfone; for immunoblotting, 250, 500, 750, and 1,000 mg/kg; for PNP hydroxylation, 50, 100, 250, 500, 750, and 1,000 mg/kg; sacrificed 4 hrs after exposure	4/group	TCE bioactivation by CYP2E1 and/or 2F2 correlated with bronchiolar cytotoxicity in mice.

**Table 4-83. Animal toxicity studies of TCE (continued)**

Reference	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Results
Forkert et al. (1985)	CD-1 mice (M)	i.p. injection	2,000, 2,500 or 3,000 mg/kg in mineral oil; sacrificed 24 hrs postexposure for dose response; time course sacrificed 1, 2, 12, and 24 hrs postexposure	10/group	Clara cell injury was increased following exposure at all doses tested; time course demonstrated a rapid and marked reduction in pulmonary microsomal CYP content and aryl hydrocarbon hydroxylase activity. Alveolar Type II cells were also affected.
Forkert and Birch (1989)	CD-1 mice (M)	i.p. injection	2,000 mg/kg in corn oil; sacrificed 1, 2, 4, 8, 12, and 24 hrs postexposure	10/group	Necrotic changes seen in Clara cells as soon as 1 hr postexposure; increased vacuolation was seen by 4 hrs postexposure; covalent binding of TCE to lung macromolecules peaked at 4 hrs and reached a plateau at 12 and 24 hrs post exposure.
Stewart et al. (1979); Le Mesurier et al. (1980)	Wistar Rats (F)	Inhalation (whole-body chamber)	30 min, 48.5 g/m <sup>3</sup> (9,030 ppm); sacrificed at 5 and 15 d postexposure	5/group	Decreased recovery of pulmonary surfactant (dose-dependent).
Lewis et al. (1984)	Mice	Inhalation (Pyrex bell jars)	10,000 ppm, 1–4 hrs daily for 5 consecutive d; sacrificed 24 hrs after last exposure	~28/group	Increased vacuolation and reduced activity of pulmonary mixed function oxidases.
Scott et al. (1988)	CD-1 mice (M)	i.p. injection	single injection of 2,500–3,000 mg/kg, sacrificed 24 hrs postexposure	4/group	Clara cells were damaged and exfoliated from the epithelium of the lung.
NTP (1990)	F344 rats (M,F) B6C3F <sub>1</sub> mice (M,F)	Gavage	Male rats: 0, 125, 250, 500, 1,000, and 2,000 mg/kg body weight (corn oil); female rats: 0, 62.5, 125, 250, 500 or 1,000 mg/kg body weight (corn oil); Mice: 0, 375, 750, 1,500, 3,000, and 6,000 mg/kg body weight (corn oil); dosed 5d/w for 13 wks	10/group	Increased pulmonary vasculitis in the high-dose groups of male and female rats (6/10 group as compared to 1/10 in controls). No pulmonary effects described in mice at this time point.

**Table 4-83. Animal toxicity studies of TCE (continued)**

Reference	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Results
Prendergast et al. (1967)	Sprague-Dawley or Long-Evans rats; Hartley Guinea pigs; New Zealand albino rabbits; beagle dogs; squirrel monkeys (sex not given for any species)	Inhalation	730 ppm for 8 hrs/d, 5 d/w, 6 wks or 35 ppm for 90 d constant	Rats (15); guinea pigs (15); rabbit (3); dog (2); monkey (3)	No histopathological changes observed, although rats were described to show a nasal discharge in the 6-wk study. No quantification was given.
Narotsky et al. (1995)	F344 rats (F)	Gavage	0, 1,125, or 1,500 mg/kg-d	21, 16, or 17 per group	Rales and dyspnea were observed in the TCE high-dose group; two females with dyspnea subsequently died.

#### 4.7.2.1.1. Acute and short-term effects: inhalation

Relatively high-dose single and multiple inhalation exposures to TCE result in dilation of endoplasmic reticulum and vacuolation of nonciliated (Clara) cells throughout the bronchial tree in mice. A single study in rats reported similar findings. In mice, single exposure experiments show vacuolation at all dose levels tested with the extent of damage increasing with dose. Villaschi et al. (1991) reported similar degrees of vacuolation in B6C3F<sub>1</sub> mice (3/group) at 24 hours after the start of exposure across all tested doses (500, 1,000, 2,000, 3,500, and 7,000 ppm, 30 minutes), with the percentage of the nonciliated cells remaining vacuolated at 48 hours increasing with dose. Clara cell vacuolation was reported to be resolved 7 days after single 30-minute exposures to TCE. Odum et al. (1992) reported that, when observed 24 hours after the start of 6 hours exposure, the majority of Clara cells in mice were unaffected at the lowest dose of 20 ppm exposures, while marked vacuolation was observed at 200 ppm (no quantitative measures of damage given and only three animals per group were examined).

In rats, Odum et al. (1992) reported no morphological changes in the female Alpk APfSD rat epithelium after 6 hours exposure (500 or 1,000 ppm) when observed 24 hours after the start of exposure (n = 3/group). However, Kurasawa reported pronounced dose-related morphological changes in Clara cells at the highest dose (8,000 ppm) for 2 hours in Wistar rats (n = 10 per group). At 500 and 1,000 ppm, slight dilation of the apical surface was reported, but morphological measurements (the ratio of the lengths of the apical surface to that of the base line of apical cytoplasm) were not statistically-significantly different from controls. From 2,000 to 8,000 ppm, a progressively increasing flattening of the apical surface was observed. In addition, at 2,000 ppm, slight dilation of the smooth endoplasmic reticulum was also observed, with marked dilation and possible necrosis at 8,000 ppm. Kurasawa (1988) also examined the time-course of Clara cell changes following a single 8,000-ppm exposure, reporting the greatest effects at 1 day to 1 week, repair at 2 weeks, and nearly normal morphology at 4 weeks. The only other respiratory effect that has been reported from one study in rats exposed via inhalation is a reduction in pulmonary surfactant yield following 30-minute exposures at 9,030 ppm for 5 or 15 days (Stewart et al., 1979). Therefore, single inhalation experiments (Odum et al., 1992; Villaschi et al., 1991; Kurasawa, 1988) suggest that the Clara cell is the target for TCE exposure in both rats and mice and that mice are more susceptible to these effects. However, the database is limited in its ability to discern quantitative differences in susceptibility or the nature of the dose-response after a single dose of TCE.

Other experiments examined the effects of several days of TCE inhalation exposure in mice and potential recovery. While single exposures require 1–4 weeks for complete recovery, after short-term repeated exposure, the bronchial epithelium in mice appears to either adapt to or become resistant to damage. Odum et al. (1992) and Green et al. (1997b) observed Clara cells in mice to be morphologically normal at the end of exposures 6 hours/day for 4 or 5 days. As with

single-dose experiments, the extent of recovery in multidose exposures may be dose-dependent. Using a very high dose, Lewis et al. (1984) reported vacuolation of bronchial epithelial cells after 4 hours/day, but not 1 hour/day (10,000 ppm), for 5 days in mice. In addition, Odum et al. (1992) reported that the damage to Clara cells that resolved after repeated exposures of 5 days, a sign of adaptation to TCE exposure, returned when exposure was resumed after 2 days.

In rats, only one inhalation study reported in two published articles (Le Mesurier et al., 1980; Stewart et al., 1979) using repeated exposures examined pulmonary histopathology. Interestingly, this study reported vacuolation in Type 1 alveolar cells, but not in Clara cells, after 5 days of exposure to approximately 9,030 ppm for 30 minutes/day (only dose tested). In addition, abnormalities were observed in the endothelium (bulging of thin endothelial segments into the microcirculatory lumen) and minor morphological changes in Type 2 alveolar cells. Although exposures were carried out for 5 consecutive days, histopathology was recorded up to 15 days postexposure, giving cell populations time to recover. Because earlier time points were not examined, it is not possible to discern whether the lack of reported Clara cell damage in rats following repeated exposure is due to recovery or lack of toxicity in this particular experiment.

Although recovery of individual damaged cells may occur, cell proliferation, presumed from labeling index data suggestive of increased DNA synthesis, contributes, at least in part, to the recovery of the bronchial epithelium in mice. Villaschi et al. (1991) observed a dose-dependent increase in labeling index as compared to controls in the mouse lung at 48 hours after a single TCE exposure (30 minutes; 500, 1,000, 2,000, 3,500, or 7,000 ppm), which decreased to baseline values at 7 days postexposure. Morphological analysis of cells was not performed, although the authors stated that the dividing cells had the appearance of Clara cells. Interestingly, Green et al. (1997b) reported no increase in BrdU labeling 24 hours after a single exposure (6 hours, 450 ppm), but did see increased BrdU labeling at the end of multiple exposures (1/day, 5 days) while Villaschi et al. (1991) reported increased [<sup>3</sup>H]-thymidine labeling 2, 5, and 7 days after single 30-minute exposures to 500–7,000 ppm. Therefore, the data for single exposures at 450–500 ppm may be consistent if increased cell proliferation occurred only for a short period of time around 48 hours postexposure, and was thereby effectively washed-out by the longer “averaging time” in the experiments by Green et al. (1997b). Also, these contradictory results may be due to differences in methodology. Green et al. (1997b) and Villaschi et al. (1991) reported very different control labeling indices (6 and 0%, respectively) while reporting similar absolute labeling indices at 450–500 ppm (6.5 and 5.2%, respectively). The different control values may be a result of substantially different times over which the label was incorporated: the mice in Green et al. (1997b) were given BrdU via a surgically-implanted osmotic pump over 4 days prior to sacrifice, while the mice in Villaschi et al. (1991) were given a single i.p. dose of [<sup>3</sup>H]-thymidine 1 hour prior to sacrifice. Stewart et al. (1979) observed no stimulation of thymidine incorporation after daily exposure to TCE (9,000 ppm) for up to

15 days. This study did, however, report a nonstatistically significant reduction in orotate incorporation, an indicator of RNA synthesis, after 15 days, although the data were not shown.

At the biochemical level, changes in pulmonary metabolism, particularly with respect to CYP activity, have been reported following TCE exposure via inhalation or i.p. administration in mice. Odum et al. (1992) reported reduced enzyme activity in Clara cell sonicates of ethoxycoumarin *O*-deethylase, aldrin epoxidation, and NADPH cytochrome c reductase after 6 hour exposures to 20–2,000 ppm TCE, although the reduction at 20 ppm was not statistically significant. No reduction of GST activity as determined by chlorodinitrobenzene as a substrate was detected. With repeated exposure at 450 ppm, the results were substrate-dependent, with ethoxycoumarin *O*-deethylase activity remaining reduced, while aldrin epoxidation and NADPH cytochrome c reductase activity showing some eventual recovery by 2 weeks. The results reported by Odum et al. (1992) for NADPH cytochrome c reductase were consistent with those of Lewis et al. (1984), who reported similarly reduced NADPH cytochrome c reductase activity following a much larger dose of 10,000 ppm for 1 and 4 hours/day for 5 days in mice (strain not specified). TCE exposure has also been associated with a decrease in pulmonary surfactant. Repeated exposure of female Wistar rats to TCE (9,000 ppm, 30 minutes/day) for 5 or 15 days resulted in a significant decrease in pulmonary surfactant as compared to unexposed controls (Le Mesurier et al., 1980).

#### **4.7.2.1.1.1. Acute and short-term effects: i.p. injection and gavage exposure**

As stated previously, the i.p. route of administration is not a relevant paradigm for human exposure. A number of studies used this route of exposure to study the effects of acute TCE exposure in mice. In general, similar lung targets are seen following inhalation or i.p. treatment in mice (Forkert et al., 2006; Forkert and Birch, 1989; Scott et al., 1988; Forkert et al., 1985). Inhalation studies generally reported the Clara cell as the target in mice. No lung histopathology from i.p. injection studies in rats is available. Forkert et al. (1985) and Forkert and Birch (1989) reported vacuolation of Clara cells as soon as 1 hour following i.p. administration of a single dose of 2,000 mg/kg in mice. At 2,500 mg/kg, both Forkert et al. (1985) and Scott et al. (1988) reported exfoliation of Clara cells and parenchymal changes, with morphological distortion in alveolar Type II cells and inconsistently observed minor swelling in Type I cells at 24 hours postexposure. Furthermore, at 3,000 mg/kg, Scott et al. (1988) also reported a significant (85%) decrease in intracellularly stored surfactant phospholipids at 24 hours postexposure. These data indicate that both Clara cells and alveolar Type I and II cells are targets of TCE toxicity at these doses using this route of administration. Recently, Forkert et al. (2006) reported Clara cell toxicity that showed increased severity with increased dose (pyknotic nuclei, exfoliation) at 500–1,000 mg/kg i.p. doses as soon as 4 hours postexposure in mice. Even at 500 mg/kg, a few Clara cells were reported with pyknotic nuclei that were in the process of exfoliation. Damage to alveolar Type II cells was not observed in this dose range. The study by Scott et al. (1988)

examined surfactant phospholipids and phospholipase A2 activity in male CD-1 mice exposed by i.p. injection of TCE (2,500 or 3,000 mg/kg, 24 hours). The lower concentration led to damage to and exfoliation of Clara cells from the epithelial lining into the airway lumen, while only the higher concentration led to changes in surfactant phospholipids. This study demonstrated an increase in total phospholipid content in the lamellar body fractions in the mouse lung.

The study by Narotsky et al. (1995) exposed F344 timed-pregnant rats to TCE (0, 1,125, and 1,500 mg/kg body weight) by gavage and examined both systemic toxicity and developmental effects at 14 days postexposure. Rales and dyspnea in the dams were observed in the high-dose group, with two of the animals with dyspnea subsequently dying. The developmental effects observed in this study are discussed in more detail in Section 4.8.

#### **4.7.2.1.1.2. Subchronic and chronic effects**

There are a few reports of the subchronic and chronic noncancer effects of TCE on the respiratory system from i.p. exposure in mice and from gavage exposure in rats. Forkert and Forkert (1994) reported pulmonary fibrosis in mice 90 days after i.p. administration of a single 2,000 mg/kg dose of TCE. The effects were in the lung parenchyma, not the bronchioles where Clara cell damage has been observed after acute exposure. It is possible that fibrotic responses in the alveolar region occur irrespective of where acute injury occurs. Effects upon Clara cells can also impact other areas of the lung via cytokine regulation (Elizur et al., 2008).

Alternatively, the alveolar and/or capillary components of the lung may have been affected by TCE in a manner that was not morphologically apparent in short-term experiments. In addition effects from a single or a few short-term exposures may take longer to manifest. The latter hypothesis is supported by the alveolar damage reported by Odum et al. (1992) after chloral administration by inhalation, and by the adducts reported in alveolar Type II cells by Forkert et al. (2006) after 500–1,000 mg/kg TCE i.p. administration.

As noted previously, rats have responded to short-term inhalation exposures of TCE with Clara cell and alveolar Type I and II effects. After repeated inhalation exposures over 6 weeks (8 hours/day, 5 days/week, 730 ppm) and continuous exposures over 90 days (35 ppm), Prendergast et al. (1967) noted no histopathologic changes in rats, guinea pigs, rabbits, dogs, or monkeys after TCE exposure, but did describe qualitatively observing some nasal discharge in the rats exposed for 6 weeks. The study details in Prendergast et al. (1967) are somewhat limited. Exposed animals are described as “typically” 15 Long-Evans or Sprague-Dawley rats, 15 Hartley guinea pigs, 3 squirrel monkeys, 3 New Zealand albino rabbits, and 2 beagle dogs. Controls were grouped between studies. In a 13-week NTP study in F344/N rats (n = 10/group) exposed to TCE (0–2,000 mg/kg-day 5 days/week) by gavage, pulmonary vasculitis was observed in 6/10 animals of each sex of the highest dose group (2,000 mg/kg-day), in contrast to 1/10 in controls of each sex (NTP, 1990).

#### 4.7.2.2. Respiratory Tract Cancer

Limited studies have been performed examining lung cancer following TCE exposure (summarized in Table 4-84). TCE inhalation exposure was reported to cause statistically significant increase in pulmonary tumors (i.e., pulmonary adenocarcinomas) in some studies in mice, but not in studies in rats and hamsters. Oral administration of TCE frequently resulted in elevated lung tumor incidences in mice, but not in any tested species was there a statistically significant increase. This section will describe the data regarding TCE induction of pulmonary tumors in rodent models. The next sections will consider the role of metabolism and potential modes of action for inhalation carcinogenicity, primarily in mice.

##### 4.7.2.2.1. Inhalation

There are three published inhalation studies examining the carcinogenicity of TCE at exposures from 0 to 600 ppm, two of which reported statistically significantly increased lung tumor incidence in mice at the higher concentrations ([Maltoni et al., 1988](#); [Maltoni et al., 1986](#); [Fukuda et al., 1983](#); [Henschler et al., 1980](#)). Rats and hamsters did not show an increase in lung tumors following exposure.

The inhalation studies by Fukuda et al. ([1983](#)), which involved female ICR mice and Sprague-Dawley rats, observed a threefold increase in lung tumors per mouse in those exposed to the two higher concentrations (150–450 ppm), but reported no increase in lung tumors in the rats. Maltoni et al. ([1988](#); [1986](#)) reported statistically-significantly increased pulmonary tumors in male Swiss and female B6C3F<sub>1</sub> mice at the highest dose of 600 ppm, but no significant increases in any of the other species/strains/sexes tested. Henschler et al. ([1980](#)) tested NMRI mice, Wistar rats, and Syrian hamsters of both sexes, and reported no observed increase in pulmonary tumors any of the species tested (see Appendix E for details on the conduct of these studies).

**Table 4-84. Animal carcinogenicity studies of TCE**

Reference	Animals (sex)	Exposure route	Dose/exposure concentration (stabilizers, if any)	Pulmonary tumor incidences	
				Benign + malignant	Malignant only
Fukuda et al. (1983)	ICR mice (F) Sprague-Dawley rats (F)	Inhalation, 7 hrs/d, 5 d/wk, 104 wks, hold until 107 wks	0, 50, 150, or 450 ppm (epichlorohydrin)	Mice: 6/49, 5/50, 13/50, 11/46 Rats: 0/50, 0/50, 1/47, 1/51	Mice: 1/49, 3/50, 8/50 <sup>a</sup> , 7/46 <sup>a</sup> Rats: none
Maltoni et al. (1988; 1986)	Sprague-Dawley rats (M, F) Swiss mice (M, F) B6C3F <sub>1</sub> mice (M, F)	Inhalation, 7 hrs/d, 5 d/wk, 104 wks, hold until death	0, 100, 300, or 600 ppm	Rats: 0/280, 0/260, 0/260, 0/260 Swiss Mice: M: 10/90, 11/90, 23/90 <sup>a</sup> , 27/90 <sup>b</sup> ; F: 15/90, 15/90, 13/90, 20/90 B6C3F <sub>1</sub> Mice: M: 2/90, 2/90, 3/90, 1/90; F: 4/90, 6/90, 7/90, 15/90 <sup>a</sup>	Rats: 0/280, 0/260, 0/260, 0/260 Swiss Mice: M: 0/90, 0/90, 0/90, 1/90; F: 2/90, 0/90, 0/90, 2/90 B6C3F <sub>1</sub> Mice M: 0/90, 0/90, 0/90, 0/90; F: 0/90, 1/90, 0/90, 0/90
Henschler et al. (1980)	Wistar rats (M, F) Syrian hamsters (M, F) NMRI mice	Inhalation, 6 hrs/d, 5 d/wk, 78 wks, hold until 130 wks (mice and hamsters) or 156 wks (rats)	0, 100, or 500 ppm (triethanolamine)	Rats: M: 1/29, 1/30, 1/30; F: 0/28; 1/30; 0/30 Hamsters: 0/60, 0/59, 0/60 Mice: M: 1/30, 3/29, 1/30; F: 3/29, 0/30, 1/28	Rats: M: 1/29, 1/30, 1/30; F: 0/28; 1/30; 0/30 Hamsters: 0/60, 0/59, 0/60 Mice: M: 5/30, 3/29, 1/30; F: 1/29, 3/30, 0/28
Henschler et al. (1984)	Swiss mice (M, F)	Gavage, 5/wk, 72 wks hold 104 wks	2.4 g/kg body weight (M), 1.8 g/kg body weight (F) all treatments; (control, triethanolamine, industrial, epichlorohydrin, 1,2-epoxybutane, both)	M: 18/50, 17/50, 14/50, 21/50, 15/50, 18/50; F: 12/50, 20/50, 21/50, 17/50, 18/50, 18/50	M: 8/50, 6/50, 7/50, 5/50, 7/50, 7/50; F: 5/50, 11/50, 8/50, 3/50, 7/50, 7/50
Van Duuren et al. (1979)	Swiss mice (M, F)	Gavage, 1/wk, 89 wks	0 or 0.5 mg (unknown)	0/30 for all groups	0/30 for all groups
NCI (1976)	Osborne-Mendel rats (M, F) B6C3F <sub>1</sub> mice (M, F)	Gavage, 5/wk, 78 wks, hold until 110 wks (rats) or 90 wks (mice)	Rats: TWA: 0, 549, or 1,097 mg/kg Mice: TWA: M: 0, 1,169, or 2,339 mg/kg; F: 0, 869, or 1,739 mg/kg (epoxybutane, epichlorohydrin)	Rats: M: 1/20, 0/50, 0/50; F: 0/20, 1/47, 0/50 Mice: M: 0/20, 5/50, 2/48; F: 1/20, 4/50, 7/47	Rats: M: 0/20, 0/50, 0/50; F: 0/20, 1/47, 0/50 Mice: M: 0/20, 0/50, 1/48; F: 0/20, 2/50, 2/47

**Table 4-84. Animal carcinogenicity studies of TCE (continued)**

Reference	Animals (sex)	Exposure route	Dose/exp conc (stabilizers, if any)	Pulmonary tumor incidences	
				Benign + malignant	Malignant only
NTP ( <a href="#">1988</a> )	ACI, August, Marshall, Osborne-Mendel rats	Gavage, 1/d, 5 d/wk, 103 wks	0, 500, or 1,000 mg/kg (diisopropylamine)	ACI M: 1/50, 4/47, 0/46; F: 0/49, 2/47, 2/42 August M: 1/50, 1/50, 0/49; F: 1/50, 1/50, 0/50 Marshall M: 3/49, 2/50, 2/47; F: 3/49, 3/49, 1/46 Osborne-Mendel M: 2/50, 1/50, 1/50; F: 0/50, 3/50, 2/50	ACI M: 1/50, 2/47, 0/46; F: 0/49, 1/47, 2/42 August M: 0/50, 1/50, 0/49; F: 1/50, 0/50, 0/50 Marshall M: 3/49, 2/50, 2/47; F: 3/49, 3/49, 1/46 Osborne-Mendel M: 1/50, 1/50, 0/50; F: 0/50, 3/50, 1/50
NTP ( <a href="#">1990</a> )	F344 rats (M, F) B6C3F <sub>1</sub> mice (M, F)	Gavage, 1/d, 5 d/wk, 103 wks	Mice: 0 or 1,000 mg/kg Rats: 0, 500, or 1,000 mg/kg	Mice: M: 7/49, 6/50; F: 1/48, 4/49 Rats: M: 4/50, 2/50, 3/49; F: 1/50, 1/49, 4/50	Mice: M: 3/49, 1/50; F: 1/48, 0/49 Rats: M: 3/50, 2/50, 3/49; F: 0/50, 0/49, 2/50
Maltoni et al. ( <a href="#">1988</a> ; <a href="#">1986</a> )	Sprague-Dawley rats (M, F)	Gavage, 1/d, 4–5 d/wk, 56 wks; hold until death	0, 50, or 250 mg/kg	M: 0/30, 0/30, 0/30; F: 0/30, 0/30, 0/30	M: 0/30, 0/30, 0/30; F: 0/30, 0/30, 0/30

<sup>a</sup>Statistically-significantly different from controls by Fisher's exact test ( $p < 0.05$ ).

<sup>b</sup>Statistically-significantly different from controls by Fisher's exact test ( $p < 0.01$ ).

M = males, F = females.

#### 4.7.2.2.2. Gavage

None of the six chronic gavage studies ([NTP, 1990](#); [Maltoni et al., 1988](#); [NTP, 1988](#); [Maltoni et al., 1986](#); [Henschler et al., 1984](#); [Van Duuren et al., 1979](#); [NCI, 1976](#)), which exposed multiple strains of rats and mice to 0–3,000 mg/kg TCE for at least 56 weeks, reported a statistically-significant excess in lung tumors, although nonstatistically significant increases were frequently observed in mice.

The study by Van Duuren et al. ([1979](#)) examined TCE along with 14 other halogenated compounds for carcinogenicity in both sexes of Swiss mice. While no excess tumors were observed, the dose rate of 0.5 mg once per week is equivalent to an average dose rate of approximately 2.4 mg/kg-day for a mouse weighing 30 g, which is about 400-fold smaller than that in the other gavage studies. In the NCI ([1976](#)) study, the results for Osborne-Mendel rats were considered inconclusive due to significant early mortality, but female B6C3F<sub>1</sub> mice (though not males) exhibited a nonstatistically-significant elevation in pulmonary tumor incidence. The NCI study ([1976](#)) used technical-grade TCE, which contained two known carcinogenic compounds as stabilizers (epichlorohydrin and 1,2-epoxybutane), but a later study by Henschler et al. ([1984](#)) in which mice were given TCE that was either pure, industrial, or stabilized with one or both of these stabilizers found similar pulmonary tumors regardless of the presence of stabilizers. In this study, female mice (n = 50) had elevated, but again not statistically significant, increases in pulmonary tumors. A later gavage study by NTP ([1988](#)), which used TCE stabilized with diisopropylamine, observed no pulmonary tumors, but chemical toxicity and early mortality rendered this study inadequate for determining carcinogenicity. The final NTP study ([1990](#)) in male and female F344 rats and B6C3F<sub>1</sub> mice, using epichlorohydrin-free TCE, again showed early mortality in male rats. Similar to the other gavage studies, a nonstatistically significant elevation in (malignant) pulmonary tumors was observed in mice, in this case in both sexes. These animal studies show that while there is a limited increase in lung tumors following gavage exposure to TCE in mice, the only statistically significant increase in lung tumors occurs following inhalation exposure in mice.

#### 4.7.3. Role of Metabolism in Pulmonary Toxicity

TCE oxidative metabolism has been demonstrated to play a main role in TCE pulmonary toxicity in mice. However, data are not available on the role of specific oxidative metabolites in the lung. The Clara cell is thought to be the cell type responsible for much of the CYP metabolism in the lung. Therefore, damage to this cell type would be expected to also affect metabolism. More direct measures of CYP and isozyme-specific depression following TCE exposure have been reported following i.p. administration in mice. Forkert et al. ([1985](#)) reported significant reduction in microsomal aryl hydrocarbon hydroxylase activity as well as CYP content between 1 and 24 hours after exposure (2,000–3,000 mg/kg i.p. TCE). Maximal

depression occurred between 2 and 12 hours, with aryl hydrocarbon hydroxylase activity (a function of CYP) <50% of controls and CYP content <20% of controls. While there was a trend towards recovery from 12 to 24 hours, depression was still significant at 24 hours. Forkert et al. (2005) reported decreases in immunoreactive CYP2E1, CYP2F2, and CYP2B1 in the 4 hours after TCE treatment with 750 mg/kg i.p. injection in mice. The amount and time of maximal reduction was isozyme dependent (CYP2E1: 30% of controls at 2 hours; CYP2F2: abolished at 30 minutes; CYP2B1: 43% of controls at 4 hours). Catalytic markers for CYP2E1, CYP2F2, and CYP2B enzymes showed rapid onset ( $\leq 15$  minutes after TCE administration) of decreased activity, and continued depression through 4 hours. Decrease in CYP2E1 and CYP2F2 activity (measured by PNP hydroxylase activity) was greater than that of CYP2B (measured by pentoxyresorufin *O*-dealkylase activity). Forkert et al. (2006) reported similar results in which 4 hours after treatment, immunodetectable CYP2E1 protein was virtually abolished at doses of 250–1,000 mg/kg and immunodetectable CYP2F2 protein, while still detectable, was reduced. PNP hydroxylase activity was also reduced 4 hours after treatment to 37% of controls at the lowest dose tested of 50 mg/kg, with further decreases to around 8% of control levels at doses of 500 mg/kg and higher. These results correlate with previously described increases in Clara cell cytotoxicity, as well as dichloroacetyl lysine (DAL) protein adduct formation. DAL adducts were observed in the bronchiolar epithelium of CD-1 mice and most prominent in the cellular apices of Clara cells (Forkert et al., 2006). This study also examined the effect of TCE in vitro exposure on the formation of CH in lung microsomes from male CD-1 mice and CYP2E1 knock-out mice. The rates of CH formation were the same for lysosomes from both CD-1 and CYP2E1 knockout mice from 0.25 mM to 0.75 mM, but the CH formation peaked earlier for in the wild-type lysosomes (0.75 mM) as compared to CYP2E1-null lysosomes (1 mM).

The strongest evidence for the necessary role of TCE oxidation is that pretreatment of mice with diallyl sulfone (DASO<sub>2</sub>), an inhibitor of CYP2E1 and CYP2F2, protected against TCE-induced pulmonary toxicity. In particular, following an i.p. TCE dose of 750 mg/kg, Clara cells and the bronchiolar epithelium in mice pretreated with the CYP2E1/CYP2F2 inhibitor appeared normal. In naive mice given the same dose, the epithelium was attenuated due to exfoliation and there was clear morphological distortion of Clara cells (Forkert et al., 2005). In addition, the greater susceptibility of mouse lungs relative to rat lungs is consistent with their larger capacity to oxidize TCE, as measured in vitro in lung microsomal preparations (Green et al., 1997b). Analysis by immunolocalization also found considerably higher levels of CYP2E1 in the mouse lung, heavily localized in Clara cells, as compared to rat lungs, with no detectable CYP2E1 in human lung samples (Green et al., 1997b). In addition, both Green et al. (1997b) and Forkert et al. (2006) report substantially lower metabolism of TCE in human lung microsomal preparations than either rats or mice. It is clear that CYP2E1 is not the only CYP enzyme involved in pulmonary metabolism, as lung microsomes from CYP2E1-null mice showed greater or similar rates of CH formation compared to those from wild-type mice. Recent studies have

suggested a role for CYP2F2 in TCE oxidative metabolism, although more work is needed to make definitive conclusions. In addition, there may be substantial variability in human lung oxidative metabolism, as Forkert et al. (2006) reported that in microsomal samples from eight individuals, five exhibited no detectable TCE oxidation ( $<0.05$  pmol/mg protein/20 minutes), while others exhibited levels well above the limit of detection (0.4–0.6 pmol/mg protein/minute).

In terms of direct pulmonary effects of TCE metabolites, Odum et al. (1992) reported that mice exposed to 100 ppm via inhalation of chloral for 6 hours resulted in bronchiolar lesions similar to those seen with TCE, although with a severity equivalent to 1,000 ppm TCE exposures. In addition, some alveolar necrosis, alveolar oedema, and desquamation of the epithelium were evident. In the same study, TCOH (100 and 500 ppm) also produced Clara cell damage, but with lower incidence than TCE, and without alveolar lesions, while TCA treatment produced no observable pulmonary effects. Therefore, it has been proposed that chloral is the active metabolite responsible for TCE pulmonary toxicity, and the localization of damage to Clara cells (rather than to other cell types, as seen with direct exposure to chloral) is due to the localization of oxidative metabolism in that cell type (Green, 2000; Green et al., 1997b; Odum et al., 1992). However, the recent identification by Forkert et al. (2006) of DAL adducts, also localized with Clara cells, suggests that TCE oxidation to DCAC, which is not believed to be derived from chloral, may also contribute to adverse health effects.

Due to the histological similarities between TCE- and chloral-induced pulmonary toxicity, consistent with chloral being the active moiety, it has been proposed that the limited or absent capacity for reduction of chloral (rapidly converted to CH in the presence of water) to TCOH and glucuronidation of TCOH to TCOG in mouse lungs leads to “accumulation” of chloral in Clara cells. However, the lack of TCOH glucuronidation capacity of Clara cells reported by Odum et al. (1992), while possibly an important determinant of TCOH concentrations, should have no bearing on CH concentrations, which depend on the production and clearance of CH only. While isolated mouse Clara cells form smaller amounts of TCOH relative to CH (Odum et al., 1992), the cell-type distribution of the enzymes metabolizing CH is not clear. Indeed, cytosolic fractions of mouse, rat, and human whole lungs show significant activity for CH conversion to TCOH (Green et al., 1997b). In particular, in mouse lung subcellular fractions, 1 micromole of TCE in a 1.3 mL reactival was converted to CH at a rate of 1 nmol/minute/mg microsomal protein, while 10 nmol CH in a 1.3 mL reactival was converted to TCOH at a rate of 0.24 nmol/minute/mg cytosolic protein (Green et al., 1997b). How this fourfold difference in activity would translate in vivo is uncertain given the 100-fold difference in substrate concentrations, lack of information as to the concentration-dependence of activity, and uncertain differences between cytosolic and microsomal protein content in the lung. It is unclear whether local pulmonary metabolism of chloral is the primary clearance process in vivo, as in the presence of water, chloral rapidly converts to CH, which is soluble in water and hence can rapidly diffuse to surrounding tissue and to the blood, which also has the capacity to

metabolize CH ([Lipscomb et al., 1996](#)). Nonetheless, experiments with isolated perfused lungs of rats and guinea pigs found rapid appearance of TCOH in blood following TCE inhalation exposure, with no detectable CH or TCOG ([Dalbey and Bingham, 1978](#)). Therefore, it appears likely that chloral in the lung either is rapidly metabolized to TCOH, which then diffuses to blood, or diffuses to blood as CH and is rapidly metabolized to TCOH by erythrocytes ([Lipscomb et al., 1996](#)).

This hypothesis is further supported by in vivo data. No in vivo data in rats on CH after TCE administration were located, and Fisher et al. ([1998](#)) reported CH in blood of volunteers exposed to TCE via inhalation were below detection limits. In mice, however, after both inhalation and gavage exposure to TCE, CH has been reported in whole-lung tissue at concentrations similar to or somewhat greater than that in blood ([Greenberg et al., 1999](#); [Abbas and Fisher, 1997](#)). A peak concentration (1.3 µg/g) of pulmonary CH was reported after inhalation exposure to 600 ppm—at or above exposures where Clara cell toxicity was reported in acute studies ([Green et al., 1997b](#); [Odum et al., 1992](#)). However, this was fivefold less than the reported pulmonary CH concentration (6.65 µg/g) after gavage exposures of 1,200 mg/kg. Specifically, 600- or 450-ppm exposures reported in the Maltoni et al. ([1988](#); [1986](#)) and Fukuda et al. ([1983](#)) studies result in a greater incidence in lung tumors than the 1,000–1,200 mg/kg-day exposures in the NTP ([1990](#)) and NCI ([1976](#)) bioassays. However, the peak CH levels measured in whole-lung tissues after inhalation exposure to TCE at 600 ppm were reported to be about fivefold *lower* than that at 1,200 mg/kg by gavage, therefore, showing the *opposite* pattern ([Greenberg et al., 1999](#); [Abbas and Fisher, 1997](#)). No studies of Clara cell toxicity after gavage exposures were located, but several studies in mice administered TCE via i.p. injection did show Clara cell toxicity at around a dose of 750 mg/kg ([Forkert et al., 2006](#)) or above (e.g., [Forkert and Forkert, 1994](#); [Forkert and Birch, 1989](#)). However, as noted previously, i.p. exposures are subject to an inflammatory response, confounding direct comparisons of dose via other routes of administration.

Although whole-lung CH concentrations may not precisely reflect the concentrations within specific cell types, as discussed above, the water solubility of CH suggests rapid equilibrium between cell types and between tissues and blood. Both Abbas and Fisher ([1997](#)) and Greenberg et al. ([1999](#)) were able to fit CH blood and lung levels using a PBPK model that did not include pulmonary metabolism, suggesting that lung CH levels may be derived largely by systemic delivery (i.e., from CH formed in the liver). However, a more detailed PBPK model-based analysis of this hypothesis has not been performed, as CH is not included in the PBPK model developed by Hack et al. ([2006](#)) that was updated in Section 3.5.

Two studies have reported formation of reactive metabolites in pulmonary tissues as assessed by macromolecular binding after TCE i.p. administration. Forkert and Birch ([1989](#)) reported temporal correlations between the severity of Clara cell necrosis with increased levels of covalent binding macromolecules in the lung of TCE or metabolites with a single 2,000 mg/kg

dose of [<sup>14</sup>C]-TCE. The amount of bound TCE or metabolites/g of lung tissue, DNA, or protein peaked at 4 hours and decreased progressively at 8, 12, and 24 hours. The fraction of radioactivity in lung tissue macromolecules that was covalently bound reached a plateau of about 20% from 4 to 24 hours, suggesting that clearance of total and covalently bound TCE or metabolites was similar. The amount of covalent binding in the liver was 3–10-fold higher than in the lung, although hepatic cytotoxicity was not apparent. This tissue difference could either be due to greater localization of metabolism in the lung, so that concentrations of reactive metabolites in individual Clara cells are greater than both the lung as a whole and hepatocytes, or because of greater sensitivity of Clara cells as compared to hepatocytes to reactive metabolites. More recently, Forkert et al. (2006) examined DAL adducts resulting from metabolism of TCE to DCAC as an *in vivo* marker of production of reactive metabolites. Following *i.p.* administration of 500–1,000 mg/kg TCE in CD-1 mice, the authors found localization of DAL adducts believed to be from oxidative metabolism within Clara cell apices, with dose-dependent increase in labeling with a polyclonal anti-DAL antibody that correlated with increased Clara cell damage. Dose-dependent DAL adducts were also found in alveolar Type II cells, although no morphologic changes in those cells were observed. Both Clara cell damage (as discussed above) and DAL labeling were abolished in mice pretreated with DASO<sub>2</sub>, an inhibitor of CYP2E1 and CYP2F2. However, Clara cell damage in treated CYP2E1-null mice was more severe than in CD-1 mice. Although DAL labeling was less pronounced in CYP2E1-null mice as compared to CD-1 mice, this was due in part to the greater histopathologic damage leading to attenuation of the epithelium and loss of Clara cells in the null mice. In addition, protein immunoblotting with anti-DAL, anti-CYP2E1, and anti-CYP2F2 antibodies suggested that a reactive TCE metabolite including DCAC was formed that is capable of binding to CYP2E1 and CYP2F2 and changing their protein structures. Follow-up studies are needed in the lung and other target tissues to determine the potential role of the DAL adducts in TCE-induced toxicity.

Finally, although Green (2000) and others have attributed species differences in pulmonary toxicity to differences in the capacity for oxidative metabolism in the lung, it should be noted that the concentration of the active metabolite is determined by both its production and clearance (Clewel et al., 2000). Therefore, while the maximal pulmonary capacity to produce oxidative metabolites is clearly greater in the mouse than in rats or humans, there is little quantitative information as to species differences in clearance, whether by local chemical transformation/metabolism or by diffusion to blood and subsequent systemic clearance. In addition, existing *in vitro* data on pulmonary metabolism are at millimolar TCE concentrations where metabolism is likely to be approaching saturation, so the relative species differences at lower doses has not been characterized. Studies with recombinant CYP enzymes examined species differences in the catalytic efficiencies of CYP2E1, CYP2F, and CYP2B1, but the relative contributions of each isoform to pulmonary oxidation of TCE *in vivo* remains unknown (Forkert et al., 2005). Furthermore, systemic delivery of oxidative metabolites to the lung may

contribute, as evidenced by respiratory toxicity reported with i.p. administration. Therefore, while the differences between mice and rats in metabolic capacity are correlated with their pulmonary sensitivity, it is not clear that differences in capacity alone are accurate quantitative predictors of toxic potency. Thus, while it is likely that the human lung is exposed to lower concentrations of oxidative metabolites, quantitative estimates for differential sensitivity made with currently available data and dosimetry models are highly uncertain.

In summary, it appears likely that pulmonary toxicity is dependent on in situ oxidative metabolism; however, the active agent has not been confidently identified. The similarities in histopathologic changes in Clara cells between TCE and chloral inhalation exposure, combined with the wider range of cell types affected by direct chloral administration relative to TCE, led some to hypothesize that chloral is the toxic moiety in both cases, but with that generated in situ from TCE in Clara cells “accumulating” in those cells ([Green, 2000](#)). However, chemical and toxicokinetic data suggest that such “accumulation” is unlikely for several reasons. These include the rapid conversion of chloral to CH in the presence of water, the water solubility of CH leading to rapid diffusion to other cell types and blood, the likely rapid metabolism of CH to TCOH either in pulmonary tissue or in blood erythrocytes, and in vivo data showing lack of correlation across routes of exposure between whole-lung CH concentrations and pulmonary carcinogenicity and toxicity. However, additional possibilities for the active moiety exist, such as DCAC, which is derived through a TCE oxidation pathway independent of chloral and appears to result in adducts with lysine localized in Clara cells.

#### **4.7.4. Mode of Action for Pulmonary Carcinogenicity**

A number of effects have been hypothesized to be key events in the pulmonary carcinogenicity of TCE, including cytotoxicity leading to increased cell proliferation, formation of DAL protein adducts, and mutagenicity. As stated previously, the target cell for pulmonary adenocarcinoma formation has not been established. Much of the hazard and mode-of-action information has focused on Clara cell effects from TCE, which is a target in both susceptible and nonsusceptible rodent species for lung tumors. However, the role of Clara cell susceptibility to TCE-induced lung toxicity or to other potential targets such as lung stem cells that are activated to repopulate both Clara and Type II alveolar cells after injury, has not been determined for pulmonary carcinogenicity. While all of the events described above may be plausibly involved in the mode of action for TCE pulmonary carcinogenicity, none have been directly shown to be necessary for carcinogenesis.

##### **4.7.4.1. Mutagenicity via Oxidative Metabolism**

The hypothesis is that TCE acts by a mutagenic mode of action in TCE-induced lung tumors. According to this hypothesis, the key events leading to TCE-induced lung tumor formation constitute the following: the oxidative metabolism of TCE producing chloral/CH

delivered to pulmonary tissues, causes direct alterations to DNA (e.g., mutation, DNA damage, and/or micronuclei induction). Mutagenicity is a well-established cause of carcinogenicity.

#### 4.7.4.1.1. Experimental support for the hypothesized mode of action

Pulmonary toxicity has been proposed to be dependent on in situ oxidative metabolism; however, the active agent has not been confidently identified. The similarities in histopathologic changes in Clara cells between TCE and chloral inhalation exposure, combined with the wider range of cell types affected by direct chloral administration relative to TCE, led some to hypothesize that chloral is the toxic moiety. Chloral that is formed from the metabolism of TCE is quickly converted to CH upon hydration under physiological conditions. As discussed in Section 4.2.4, CH clearly induces aneuploidy in multiple test systems, including bacterial and fungal assays in vitro ([Crebelli et al., 1991](#); [Kappas, 1989](#); [Käfer, 1986](#)), mammalian cells in vitro ([Sbrana et al., 1993](#); [Vagnarelli et al., 1990](#)), and mammalian germ-line cells in vivo ([Miller and Adler, 1992](#); [Russo et al., 1984](#)). Conflicting results were observed in in vitro and in vivo mammalian studies of micronuclei formation ([Beland, 1999](#); [Nesslany and Marzin, 1999](#); [Giller et al., 1995](#); [Russo and Levis, 1992b, a](#); [Degrassi and Tanzarella, 1988](#)) with positive results in germ-line cells ([Nutley et al., 1996](#); [Allen et al., 1994](#)). In addition, it is mutagenic in the Ames bacterial mutation assay for some strains ([Beland, 1999](#); [Giller et al., 1995](#); [Ni et al., 1994](#); [Haworth et al., 1983](#)). Structurally related chlorinated aldehydes 2-chloroacetaldehyde and 2,2-dichloroacetaldehyde are both alkylating agents, are both positive in a genotoxic assay ([Bignami et al., 1980](#)), and both interact covalently with cellular macromolecules ([Guengerich et al., 1979](#)).

As discussed in the section describing the experimental support for the mutagenic mode of action for liver carcinogenesis (see Section 4.5.7.1), it has been argued that CH mutagenicity is unlikely to be the cause of TCE carcinogenicity because the concentrations required to elicit these responses are several orders of magnitude higher than achieved in vivo ([Moore and Harrington-Brock, 2000](#)). Similar to the case of the liver, it is not clear how much of a correspondence is to be expected from concentrations in genotoxicity assays in vitro and concentrations in vivo, as reported in vivo CH concentrations are in whole-lung homogenate, while in vitro concentrations are in culture media. None of the available in vivo genotoxicity assays used the inhalation route that elicited the greatest lung tumor response under chronic exposure conditions, so direct in vivo comparisons are not possible. Finally, as discussed in Section 4.5.7.1, the use of i.p. administration in many other in vivo genotoxicity assays complicates the comparison with carcinogenicity data.

As discussed above (see Section 4.7.3), chemical and toxicokinetic data are not supportive of CH being the active agent of TCE-induced pulmonary toxicity, and directly contradict the hypothesis of chloral “accumulation.” Nonetheless, CH has been measured in the mouse lung following inhalation and gavage exposures to TCE ([Greenberg et al., 1999](#); [Abbas](#)

[and Fisher, 1997](#)), possibly the result of both in situ production and systemic delivery. Therefore, in principle, CH could cause direct alterations in DNA in pulmonary tissue. However, as discussed above, the relative amounts of CH measured in whole-lung tissue from inhalation and oral exposures do not appear to correlate with sensitivity to TCE lung tumor induction across exposure routes. While these data cannot rule out a role for mutagenicity mediated by CH due to various uncertainties, such as whether whole-lung CH concentrations accurately reflect cell-type specific concentrations and possible confounding due to strain differences between inhalation and oral chronic bioassays, they do not provide support for this mode of action.

Additional possibilities for the active moiety exist, such as DCAC, which is derived through a TCE oxidation pathway independent of chloral and which appears to result in adducts with lysine localized in Clara cells ([Forkert et al., 2006](#)). DCA, which has some genotoxic activity, is, also, presumed to be formed through this pathway (see Section 3.3). Currently, however, there are insufficient data to support a role for these oxidative metabolites in a mutagenic mode of action.

#### **4.7.4.2. Cytotoxicity Leading to Increased Cell Proliferation**

The hypothesis is that TCE acts by a cytotoxicity mode of action in TCE-induced pulmonary carcinogenesis. According to this hypothesis, the key events leading to TCE-induced lung tumor formation constitute the following: TCE oxidative metabolism in situ leads to currently unknown reactive metabolites that cause cytotoxicity, leading to compensatory cellular proliferation and subsequently increased mutations and clonal expansion of initiated cells.

##### **4.7.4.2.1. Experimental support for the hypothesized mode of action**

Evidence for the hypothesized mode of action consists primarily of: (1) the demonstration of acute cytotoxicity and transient cell proliferation following TCE exposure in laboratory mouse studies; (2) toxicokinetic data supporting oxidative metabolism being necessary for TCE pulmonary toxicity; and (3) the association of lower pulmonary oxidative metabolism and lower potency for TCE-induced cytotoxicity with the lack of observed pulmonary carcinogenicity in laboratory rats. However, there is a lack of experimental support linking TCE acute pulmonary cytotoxicity to sustained cellular proliferation of chronic exposures or clonal expansion of initiated cells.

As discussed above, a number of acute studies have shown that TCE is particularly cytotoxic to Clara cells in mice, which has been suggested to be involved in the development of mouse lung tumors ([Kim et al., 2005](#); [Buckpitt et al., 1995](#); [Forkert and Forkert, 1994](#)). In addition, studies examining cell labeling by either BrdU ([Green et al., 1997b](#)) or [<sup>3</sup>H]-thymidine incorporation ([Villaschi et al., 1991](#)) suggest increased cellular proliferation in mouse Clara cells following acute inhalation exposures to TCE. Moreover, in short-term studies, Clara cells appear

to become resistant to cytotoxicity with repeated exposure, but regain their susceptibility after 2 days without exposure. This observation led to the hypothesis that the 5 days/week inhalation dosing regime ([Maltoni et al., 1988](#); [1986](#); [Fukuda et al., 1983](#); [Henschler et al., 1980](#)) in the chronic mouse studies leads to periodic cytotoxicity in the mouse lung at the beginning of each week followed by cellular regeneration, and that the increased rate of cell division leads to increased incidence of tumors by increasing the overall mutation rate and by increasing the division rate of already initiated cells ([Green, 2000](#)). However, longer-term studies to test this hypothesis have not been carried out.

As discussed above (see Section 4.7.3), there is substantial evidence that pulmonary oxidative metabolism is necessary for TCE-induced pulmonary toxicity, although the active moiety remains unknown. In addition, the lower capacity for pulmonary oxidative metabolism in rats as compared to mice is consistent with studies in rats not reporting pulmonary cytotoxicity until exposures higher than those in the bioassays, and the lack of reported pulmonary carcinogenicity in rats at similar doses to mice. However, rats also have a lower background rate of lung tumors ([Green, 2000](#)), and so would be less sensitive to carcinogenic effects in that tissue to the extent that RRs is the important metric across species. In addition, this mode-of-action hypothesis requires a number of additional key assumptions for which there are currently no direct evidence. First, the cycle of cytotoxicity, repair, resistance to toxicity, and loss of resistance after exposure interruption, has not been documented and under the proposed mode of action should continue under chronic exposure conditions. This cycle has, thus far, only been observed in short-term (up to 13-day) studies. In addition, although Clara cells have been identified as the target of toxicity whether they or endogenous stem cells in the lung are the cells responsible for mouse lung tumors has not been established. There are currently no data as to the cell type of origin for TCE-induced lung tumors.

This hypothesized mode of action has been proposed for other compounds that induce mouse lung tumors, such as coumarin, naphthalene, and styrene (*e.g.*, [Cruzan et al., 2009](#)). Among these, only for styrene have there been studies of chronic duration linking cytotoxicity with hyperplasia, and no studies appear to provide experimental linkage to clonal expansion of initiated cells.

#### **4.7.4.3. Additional Hypothesized Modes of Action with Limited Evidence or Inadequate Experimental Support**

##### **4.7.4.3.1. Role of formation of DAL protein adducts**

As discussed above, Forkert et al. ([2006](#)) recently observed dose-dependent formation of DAL protein adducts in the Clara cells of mice exposed to TCE via i.p. injection. While adducts were highly localized in Clara cells, they were also found in alveolar Type II cells, though these cells did not show signs of cytotoxicity in this particular experimental paradigm. In terms of the mode of action for TCE-induced pulmonary carcinogenicity, these adducts may either be

causally important in and of themselves, or they may be markers of a different causal effect. For instance, it is possible that these adducts are a cause for the observed Clara cell toxicity, and Forkert et al. (2006) suggested that the lack of toxicity in alveolar Type II cells may indicate that “there may be a threshold in adduct formation and hence bioactivation at which toxicity is manifested.” In this case, they are an additional precursor event in the same causal pathway proposed above. Alternatively, these adducts may be indicative of effects related to carcinogenesis but unrelated to cytotoxicity. In this case, the Clara cell need not be the cell type of origin for mouse lung tumors.

Because of their recent discovery, there are little additional data supporting, refuting, or clarifying the potential role for DAL protein adducts in the mode of action for TCE-induced pulmonary carcinogenesis. For instance, the presence and localization of such adducts in rats has not been investigated, and could indicate the extent to which the level of adduct formation is correlated with existing data on species differences in metabolism, cytotoxicity, and carcinogenicity. In addition, the formation of these adducts has only been investigated in a single dose study using i.p. injection. As stated above, i.p. injection may involve the initiation of a systemic inflammatory response that can activate lung macrophages or affect Clara cells. Experiments with repeated exposures over chronic durations and by inhalation or oral of administration would be highly informative. Finally, the biological effects of these adducts, whether cytotoxicity or something else, have not been investigated.

#### **4.7.4.4. Conclusions About the Hypothesized Modes of Action**

##### **4.7.4.4.1. Is the hypothesized mode of action sufficiently supported in the test animals?**

###### **4.7.4.4.1.1. Mutagenicity**

CH is clearly genotoxic, as there are substantial data from multiple in vitro and in vivo assays supporting its ability induce aneuploidy, with more limited data as to other genotoxic effects, such as point mutations. CH is also clearly present in pulmonary tissues of mice following TCE exposures similar to those inducing lung tumors in chronic bioassays. However, chemical and toxicokinetic data are not supportive of CH being the predominant metabolite for TCE carcinogenicity. Such data include the water solubility of CH leading to rapid diffusion to other cell types and blood, it's likely rapid metabolism to TCOH either in pulmonary tissue or in blood erythrocytes, and in vivo data showing lack of correlation across routes of exposure between whole-lung CH concentrations and pulmonary carcinogenicity. Therefore, while a role for mutagenicity via CH in the mode of action of TCE-induced lung tumors cannot be ruled about, available evidence is inadequate to support the conclusion that direct alterations in DNA caused by CH produced in or delivered to the lung after TCE exposure constitute a mode of action for TCE-induced lung tumors.

#### **4.7.4.4.1.2. Cytotoxicity**

The mode-of-action hypothesis for TCE-induced lung tumors involving cytotoxicity is supported by relatively consistent and specific evidence for cytotoxicity at tumorigenic doses in mice. However, the majority of cytotoxicity-related key events have been investigated in studies <13 days, and none has been shown to be causally related to TCE-induced lung tumors. In addition, the cell type (or types) of origin for the observed lung tumors in mice has not been determined, so the contribution to carcinogenicity of Clara cell toxicity and subsequent regenerative cell division is not known. Similarly, the relative contribution from recently discovered dichloroacetyl-lysine protein adducts to the tumor response has not been investigated and has currently only been studied in i.p. exposure paradigms of short duration. In summary, while there are no data directly challenging the hypothesized mode of action described above, the existing support for their playing a causal role in TCE-induced lung tumors is largely associative, and based on acute or short term studies. Therefore, there are inadequate data to support a cytotoxic mode of action based on the TCE-induced cytotoxicity in Clara cells in the lungs of test animals.

#### **4.7.4.4.1.3. Additional hypothesis**

Inadequate data are available to develop a mode-of-action hypothesis based on recently discovered DAL adducts induced by TCE inhalation and i.p. exposures. It will, therefore, not be considered further in the conclusions below.

Overall, therefore, the mode of action for TCE-induced lung tumors is considered unknown at this time.

#### **4.7.4.4.2. Is the hypothesized mode of action relevant to humans?**

##### **4.7.4.4.2.1. Mutagenicity**

The evidence discussed above demonstrates that CH is mutagenic in microbial as well as test animal species. There is, therefore, the presumption that they would be mutagenic in humans. Therefore, this mode of action is considered relevant to humans.

##### **4.7.4.4.2.2. Cytotoxicity**

No data from human studies are available on the cytotoxicity of TCE and its metabolites in the lung, and no causal link between cytotoxicity and pulmonary carcinogenicity has been demonstrated in animal or human studies. Nonetheless, in terms of human relevance, no data suggest that the proposed key events are not biologically plausible in humans; therefore, qualitatively, TCE-induced lung tumors are considered relevant to humans. This conclusion that this hypothesized mode of action is qualitatively relevant has also been reached for other compounds for which the mode of action has been postulated ([Cruzan et al., 2009](#)). Information about the relative pharmacodynamic sensitivity between rodents and humans is absent, but

information on pharmacokinetic differences in lung oxidative metabolism does exist and will be considered in dose-response assessment when extrapolating between species (see Section 5.2.1.2).

#### **4.7.4.4.3. Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?**

##### **4.7.4.4.3.1. Mutagenicity**

The mutagenic mode of action is considered relevant to all populations and lifestages. According to EPA's *Guidelines for Carcinogen Risk Assessment* ([U.S. EPA, 2005b](#)) and *Supplemental Guidance for Assessing Susceptibility From Early-Life Exposure to Carcinogens* ([U.S. EPA, 2005e](#)), there may be increased susceptibility to early-life exposures for carcinogens with a mutagenic mode of action. However, because the weight of evidence is inadequate to support a mutagenic mode of action for TCE pulmonary carcinogenicity, and in the absence of chemical-specific data to evaluate differences in susceptibility, the ADAFs should not be applied, in accordance with the *Supplemental Guidance*.

##### **4.7.4.4.3.2. Cytotoxicity**

No information is available as to which populations or lifestages may be particularly susceptible to TCE-induced lung tumors. However, pharmacokinetic differences in lung oxidative metabolism among humans do exist, and because of the association between lung oxidative metabolism and toxicity, these differences will be considered in dose-response assessment when extrapolating within species.

#### **4.7.5. Summary and Conclusions**

The studies described here show pulmonary toxicity found mainly in Clara cells in mice ([Green et al., 1997b](#); [Odum et al., 1992](#); [Villaschi et al., 1991](#); [Forkert and Birch, 1989](#); [Forkert et al., 1985](#)) and rats ([Kurasawa, 1988](#)). The most convincing albeit limited data regarding this type of toxicity were demonstrated predominantly in mice exposed via inhalation, although some toxicity was shown in i.p. injection studies. Increased vacuolation of Clara cells was often seen within the first 24 hours of exposure, depending on dose, but with cellular repair occurring within days or weeks of exposure. Continued exposure led to resistance to TCE-induced Clara cell toxicity, but damage recurred if exposure was stopped after 5 days and then resumed after 2 days without exposure. However, Clara cell toxicity has only been observed in acute and short-term studies, and it is unclear whether they persist with subchronic or chronic exposure, particularly in mice, which are the more sensitive species. With respect to pulmonary carcinogenicity, statistically significantly increased incidence of lung tumors from chronic inhalation exposures to TCE was observed female ICR mice ([Fukuda et al., 1983](#)), male Swiss mice, and female B6C3F<sub>1</sub> mice ([Maltoni et al., 1988](#); [Maltoni et al., 1986](#)), though not in other

sex/strain combinations, or in rats ([Maltoni et al., 1988](#); [Maltoni et al., 1986](#); [Henschler et al., 1980](#)). However, lung toxicity and Clara cell effects have also been observed in rats. Overall, the limited carcinogenesis studies described above are consistent with TCE causing mild increases in pulmonary tumor incidence in mice, but not in other species tested such as rats and hamsters.

The epidemiologic studies are quite limited for examining the role of TCE in cancers of the respiratory system, with no studies found on TCE exposure specifically examining toxicity of the respiratory tract. The two studies found on organic solvent exposure which included TCE suggested smoking as a primary factor for observed lung function decreases among exposed workers. Animal studies have demonstrated toxicity in the respiratory tract, particularly damage to the Clara cells (nonciliated bronchial epithelial cells), as well as decreases in pulmonary surfactant following both inhalation and i.p. exposures, especially in mice. Dose-related increases in vacuolation of Clara cells have been observed in mice and rats as early as 24 hours postexposure ([2006](#); [Odum et al., 1992](#); [Forkert and Birch, 1989](#); [Kurasawa, 1988](#); [Scott et al., 1988](#); [Forkert et al., 1985](#)). Mice appear to be more sensitive to these changes, but both species show a return to normal cellular morphology at 4 weeks postexposure ([Odum et al., 1992](#)). Studies in mice have also shown an adaptation or resistance to this damage after only 4–5 days of repeated exposures ([Green et al., 1997b](#); [Odum et al., 1992](#)). The limited epidemiological literature on lung and laryngeal cancer in TCE-exposed groups is inconclusive due to study limitations (low power, null associations, CIs on RRs that include 1.0). These studies can only rule out risks of a magnitude of  $\geq 2.0$  for lung cancer and RRs  $>3.0$  or  $4.0$  for laryngeal cancer for exposures to studied populations and thus, may not detect a level of response consistent with other endpoints. Animal studies demonstrated a statistically significant increase in pulmonary tumors in mice following chronic inhalation exposure to TCE ([Maltoni et al., 1988](#); [Maltoni et al., 1986](#); [Fukuda et al., 1983](#)). These results were not seen in other species tested (rats, hamsters; [Maltoni et al., 1988](#); [Maltoni et al., 1986](#); [Fukuda et al., 1983](#); [Henschler et al., 1980](#)). By gavage, elevated, but not statistically significant, incidences of benign and/or malignant pulmonary tumors have been reported in B6C3F<sub>1</sub> mice ([NTP, 1990](#); [Henschler et al., 1984](#); [NCI, 1976](#)). No increased pulmonary tumor incidences have been reported in rats exposed to TCE by gavage ([NTP, 1990, 1988](#); [NCI, 1976](#)), although all of the studies suffered from early mortality in at least one sex of rat.

Although no epidemiologic studies on the role of metabolism of TCE in adverse pulmonary health effects have been published, animal studies have demonstrated the importance of the oxidative metabolism of TCE by CYP2E1 and/or CYP2F2 in pulmonary toxicity. Exposure to DASO<sub>2</sub>, an inhibitor of both enzymes protects against pulmonary toxicity in mice following exposure to TCE ([Forkert et al., 2005](#)). The increased susceptibility in mice correlates with the greater capacity to oxidize TCE based on increased levels of CYP2E1 in mouse lungs relative to lungs of rats and humans ([Forkert et al., 2006](#); [Green et al., 1997b](#)), but it is not clear

that these differences in capacity alone are accurate quantitative predictors of sensitivity to toxicity. In addition, available evidence argues against the previously proposed hypothesis ([e.g., Green, 2000](#)) that “accumulation” of chloral in Clara cells is responsible for pulmonary toxicity, since chloral is first converted to the water-soluble compounds, CH and TCOH, which can rapidly diffuse to surrounding tissue and blood. Furthermore, the observation of DAL protein adducts, likely derived from DCAC and not from chloral, that were localized in Clara cells suggests an alternative to chloral as the active moiety. While CH has shown substantial genotoxic activity, chemical and toxicokinetic data on CH as well as the lack of correlation across routes of exposure between in vivo measurements of CH in lung tissues and reported pulmonary carcinogenicity suggest that evidence is inadequate to conclude that a mutagenic mode of action mediated by CH is operative for TCE-induced lung tumors. Another mode of action for TCE-induced lung tumors has been plausibly hypothesized to involve cytotoxicity leading to increased cell proliferation, but the available evidence is largely associative and based on short-term studies, so a determination of whether this mode of action is operative cannot be made. The recently discovered formation of DAL protein adducts in pulmonary tissues may also play a role in the mode of action of TCE-induced lung tumors, but an adequately defined hypothesis has yet to be developed. Therefore, the mode of action for TCE-induced lung tumors is currently considered unknown, and this endpoint is thus considered relevant to humans. Moreover, none of the available data suggest that any of the currently hypothesized mechanisms would be biologically precluded in humans.

## **4.8. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY**

### **4.8.1. Reproductive Toxicity**

An assessment of the human and experimental animal data, taking into consideration the overall weight of evidence, demonstrates a concordance of adverse reproductive outcomes associated with TCE exposures. Effects on male reproductive system integrity and function are particularly notable and are discussed below. Cancers of the reproductive system in both males and females have also been identified and are discussed below.

#### **4.8.1.1. Human Reproductive Outcome Data**

A number of human studies have been conducted that examined the effects of TCE on male and female reproduction following occupational and community exposures. These are described below and summarized in Table 4-85. Epidemiological studies of female human reproduction examined infertility and menstrual cycle disturbances related to TCE exposure. Other studies of exposure to pregnant women are discussed in the section on human developmental studies (see Section 4.8.3.1). Epidemiological studies of male human reproduction examined reproductive behavior, altered sperm morphology, altered endocrine function, and infertility related to TCE exposure.

**Table 4-85. Human reproductive effects**

Subjects	Exposure	Effect	Reference
<b>Female and male combined effects</b>			
<i>Reproductive behavior</i>			
75 men and 71 women living near Rocky Mountain Arsenal, Colorado	Low: <5.0 ppb Medium: ≥5.0–<10.0 ppb High: <10.0 ppb Highest: <15 ppb	Altered libido <sup>a</sup> Low: referent Medium: OR <sub>adj</sub> : 0.67 (95% CI: 0.18–2.49) High: OR <sub>adj</sub> : 1.65 (95% CI: 0.54–5.01) Highest: OR <sub>adj</sub> : 2.46 (95% CI: 0.59–10.28)	ATSDR (2001)
<b>Female effects</b>			
<i>Infertility</i>			
197 women occupationally exposed to solvents in Finland 1973–1983	U-TCA (μmol/L) <sup>b</sup> Median: 48.1 Mean: 96.2 ± 19.2	Reduced incidence of fecundability in the high exposure group <sup>c</sup> as measured by time to pregnancy Low: IDR = 1.21 (95% CI: 0.73–2.00) High: IDR = 0.61 (95% CI: 0.28–1.33)	Sallmén et al. (1995)
71 women living near Rocky Mountain Arsenal, Colorado	Low: <5.0 ppb Medium: ≥5.0–<10.0 ppb High: <10.0 ppb	No effect on lifetime infertility <sup>a</sup> Low: referent Medium: OR <sub>adj</sub> : 0.45 (95% CI: 0.02–8.92) High: OR <sub>adj</sub> : 0.88 (95% CI: 0.13–6.22)	ATSDR (2001)
<i>Menstrual cycle disturbance</i>			
71 women living near Rocky Mountain Arsenal, Colorado	Low: <5.0 ppb Medium: ≥5.0–<10.0 ppb High: <10.0 ppb	Increase in abnormal menstrual cycle (defined as <26 d or >30 d) Low: referent Medium: OR <sub>adj</sub> : 4.17 (95% CI: 0.31–56.65) High: OR <sub>adj</sub> : 2.39 (95% CI: 0.41–13.97)	ATSDR (2001)
184 women working in a factory assembling small electrical parts in Poland	Mean indoor air TCE: 200 mg/m <sup>3</sup>	18% reporting increase in amenorrhea in exposed group (n = 140), compared to 2% increase in unexposed group (n = 44)	Zielinski (1973)
32 women working in dry cleaning or metal degreasing in Czechoslovakia <sup>d</sup>	0.28–3.4 mg/L TCE for 0.5–25 yrs	31% reporting increase in menstrual disturbances <sup>a</sup>	Bardodej and Vyskocil (1956)
20-yr-old woman occupationally exposed to TCE via inhalation	U-TTCs 3.2 ng/mL (21–25 d after exposure)	Amenorrhea, followed by irregular menstruation and lack of ovulation	Sagawa et al. (1973)
<b>Male effects</b>			
<i>Reproductive behavior</i>			
43 men working in dry cleaning or metal degreasing in Czechoslovakia	0.28–3.4 mg/L TCE for 0.5–25 yrs	30% reporting decreased potency <sup>a</sup>	Bardodej and Vyskocil (1956)
30 male workers in a money printing shop in Egypt	38–172 ppm TCE	Decreased libido reported in 10 men (33%), compared to 3 men in the control group (10%)	El Ghawabi et al. (1973)

**Table 4-85. Human reproductive effects (continued)**

Subjects	Exposure	Effect	Reference
42 yr-old male aircraft mechanic in UK	TCE exposure reported but not measured; exposure for 25 yrs	Gynaecomastia, impotence	Saihan et al. (1978)
<i>Altered sperm quality</i>			
15 men working as metal degreasers in Denmark	TCE exposure reported but not measured	Nonsignificant increase in percentage of two YFF in spermatozoa; no effect on sperm count or morphology	Rasmussen et al. (1988)
<b>85 men of Chinese descent working in an electronics factory</b>	<b>Mean personal air TCE: 29.6 ppm; Mean U-TCA: 22.4 mg/g creatinine</b>	<b>Decreased normal sperm morphology and hyperzoospermia</b>	<b>Chia et al. (1996)</b>
<i>Altered endocrine function</i>			
85 men of Chinese descent working in an electronics factory	Mean personal air TCE: 29.6 ppm; Mean U-TCA: 22.4 mg/g creatinine	Increased DHEAS and decreased FSH, SHBG and testosterone levels; dose-response observed	Chia et al. (1997)
85 men of Chinese descent working in an electronics factory	Mean personal air TCE: 29.6 ppm; Mean U-TCA: 22.4 mg/g creatinine	Decreased serum levels of testosterone and SHBG were significantly correlated with years of exposure to TCE; increased insulin levels for exposure <2 yrs	Goh et al. (1998)
<i>Infertility</i>			
282 men occupationally exposed to solvents in Finland 1973–1983	U-TCA (µmol/L): High exposure: <sup>c</sup> Mean: 45 (SD 42) Median 31 Low exposure: <sup>c</sup> Mean: 41 (SD 88) Median: 15	No effect on fecundability <sup>c</sup> (as measured by time to pregnancy) Low: FDR: 0.99 (95% CI: 0.63–1.56) Intermediate/High: FDR: <sup>c</sup> 1.03 (95% CI: 0.60–1.76)	Sallmén et al. (1998)
8 male mechanics seeking treatment for infertility in Canada	Urine (µmol/): TCA: <0.30–4.22 TCOH: <0.60–0.89 Seminal fluid (pg/extract): TCE: 20.4–5,419.0 Chloral: 61.2–1,739.0 TCOH 2.7–25.5 TCA: <100–5,504 DCA: <100–13,342	Infertility could not be associated with TCE as controls were five men also in treatment for infertility	Forkert et al. (2003)
75 men living near Rocky Mountain Arsenal, Colorado	Low: <5.0 ppb Medium: ≥5.0 to <10.0 ppb High: <10.0 ppb	No effect on lifetime infertility (not defined) Low: referent Medium: NA High: OR <sub>adj</sub> : 0.83 (95% CI: 0.11–6.37)	ATSDR (2001)

<sup>a</sup>Not defined by the authors.

<sup>b</sup>As reported in Lindbohm et al. (1990).

<sup>c</sup>Low/intermediate exposure indicated use of TCE <1 or 1–4 days/week, and biological measures indicated high exposure. High exposure indicated daily use of TCE, or if biological measures indicated high exposure.

<sup>d</sup>Number inferred from data provided in Tables 2 and 3 in Bardodej and Vyskocil (1956).

**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

DHEAS = dehydroepiandrosterone sulphate; FSH = follicle-stimulating hormone; OR<sub>adj</sub> = adjusted odds ratio; SHBG = sex-hormone binding globulin

#### **4.8.1.1.1. Female and male combined human reproductive effects**

##### ***Reproductive behavior***

A residential study of individuals living near the Rocky Mountain Arsenal in Colorado examined the reproductive outcomes in 75 men and 71 women exposed to TCE in drinking water ([ATSDR, 2001](#)). TCE exposure was classified as high (>10.0 ppb), medium ( $\geq 5.0$ –<10.0 ppb), and low (<5.0 ppb). Altered libido for men and women combined was observed in a dose-response fashion, although the results were nonsignificant. The results were not stratified by gender.

#### **4.8.1.1.2. Female human reproductive effects**

##### **4.8.1.1.2.1. Infertility**

Sallmén et al. ([1995](#)) examined maternal occupational exposure to organic solvents and time-to-pregnancy. Cases of spontaneous abortion and controls from a prior study of maternal occupational exposure to organic solvents in Finland during 1973–1983 and pregnancy outcome ([Lindbohm et al., 1990](#)) were used to study time-to-pregnancy of 197 couples. Exposure was assessed by questionnaire during the first trimester and confirmed with employment records. Biological measurements of TCA in urine in 64 women who held the same job during pregnancy and measurement (time of measurement not stated) had a median value of 48.1  $\mu\text{mol/L}$  (mean:  $96.2 \pm 19.2 \mu\text{mol/L}$ ) ([Lindbohm et al., 1990](#)). Nineteen women had low exposure to TCE (used <1 or 1–4 times/week), and 9 had high exposure to TCE (daily use). In this follow-up study, an additional questionnaire on time-to-pregnancy was answered by the mothers ([Sallmén et al., 1995](#)). The incidence density ratio (IDR) was used in this study to estimate the ratio of average incidence rate of pregnancies for exposed women compared to nonexposed women; therefore, a lower IDR indicates infertility. For TCE, a reduced incidence of fecundability was observed in the high-exposure group (IDR: 0.61, 95% CI: 0.28–1.33) but not in the low-exposure group (IDR: 1.21, 95% CI: 0.73–2.00). A similar study of paternal occupational exposure ([Sallmén et al., 1998](#)) is discussed in Section 4.8.1.1.3.4.

The residential study in Colorado discussed above did not observe an effect on lifetime infertility in the medium- ( $\text{OR}_{\text{adj}}$ : 0.45; 95% CI: 0.02–8.92) or high-exposure groups ( $\text{OR}_{\text{adj}}$ : 0.88; 95% CI: 0.13–6.22) ([ATSDR, 2001](#)). Curiously, exposed women had more pregnancies and live births than controls.

##### **4.8.1.1.2.2. Menstrual cycle disturbance**

The ATSDR ([2001](#)) study discussed above also examined effects on the menstrual cycle ([ATSDR, 2001](#)). Nonsignificant associations without a dose-response were seen for abnormal menstrual cycle in women ( $\text{OR}_{\text{adj}}$ : 2.23, 95% CI: 0.45–11.18).

Other studies have examined the effect of TCE exposure on the menstrual cycle. One study examined women working in a factory assembling small electrical parts (Zielinski, ([1973](#)),

translated). The mean concentration of TCE in indoor air was reported to be 200 mg/m<sup>3</sup>. Of the 140 exposed women, 18% suffered from amenorrhea, compared to only 2% of the 44 nonexposed workers. The other study examined 75 men and women working in dry cleaning or metal degreasing ([Bardodej and Vyskocil, 1956](#)). Exposures ranged from 0.28 to 3.4 mg/L, and length of exposure ranged from 0.5 to 25 years. This study reported that many women experienced menstrual cycle disturbances, with a trend for increasing air concentrations and increasing duration of exposure.

There is also an additional case study of a 20-year-old woman who was occupationally exposed to TCE via inhalation. The exposure was estimated to be as high as 10 mg/mL or several thousand ppm, based on urine samples 21–25 days after exposure of 3.2 ng/mL of TTCs. The primary effect was neurological, although she also experienced amenorrhea, followed by irregular menstruation and lack of ovulation as measured by basal body temperature curves ([Sagawa et al., 1973](#)).

#### **4.8.1.1.3. Male human reproductive effects**

##### **4.8.1.1.3.1. Reproductive behavior**

One study reported the effect of TCE exposure on the male reproductive behavior in 75 men working in dry cleaning or metal degreasing ([Bardodej and Vyskocil, 1956](#)). Exposures ranged from 0.28 to 3.4 mg/L, and length of exposure ranged from 0.5 to 25 years. This study found that men experienced decreased potency or sexual disturbances; the authors speculated that the effects on men could be due to the CNS effects of TCE exposure. This study also measured serial neutral 17-ketosteroid determinations, but they were found to be not statistically significant ([Bardodej and Vyskocil, 1956](#)).

In an occupational study, 30 men working in a money printing shop were exposed to TCE for <1–5 years ([El Ghawabi et al., 1973](#)). Depending on the job description, the exposures ranged from 38 to 172 ppm TCE. Ten (33%) men suffered from decreased libido, compared to three (10%) of unexposed controls. However, these results were not stratified by exposure level or duration. The authors speculated that decreased libido was likely due to the common symptoms of fatigue and sleepiness.

A case study described a 42-year-old man exposed to TCE who worked as an aircraft mechanic for approximately 25 years ([Saihan et al., 1978](#)). He suffered from a number of health complaints including gynaecomastia and impotence, along with neurotoxicity and immunotoxicity. In addition, he drank alcohol daily, which could have increased his response to TCE.

##### **4.8.1.1.3.2. Altered sperm quality**

Genotoxic effects on male reproductive function were examined in a study evaluating occupational TCE exposure in 15 male metal degreasers ([Rasmussen et al., 1988](#)). No

measurement of TCE exposure was reported. Sperm count, morphology, and spermatozoa Y-chromosomal nondisjunction during spermatogenesis were examined, along with chromosomal aberrations in cultured lymphocytes. A nonsignificant increase in percentage of two fluorescent Y-bodies (YFF) in spermatozoa were seen in the exposed group ( $p > 0.10$ ), and no difference was seen in sperm count or morphology compared to controls.

An occupational study of men using TCE for electronics degreasing ([Goh et al., 1998](#); [Chia et al., 1997](#); [Chia et al., 1996](#)) examined subjects ( $n = 85$ ) who were offered a free medical exam if they had no prior history related to endocrine function, no clinical abnormalities, and normal liver function tests; no controls were used. These participants provided urine, blood, and sperm samples. The mean urine TCA level was 22.4 mg/g creatinine (range: 0.8–136.4 mg/g creatinine). In addition, 12 participants provided personal 8-hour air samples, which resulted in a mean TCE exposure of 29.6 ppm (range: 9–131 ppm). Sperm samples were divided into two exposure groups: low for urine TCA  $<25$  mg/g creatinine and high for urine TCA  $\geq 25$  mg/g creatinine. A decreased percentage of normal sperm morphology was observed in the sperm samples in the high-exposure group ( $n = 48$ ) compared to the low-exposure group ( $n = 37$ ). However, TCE exposure had no effect on semen volume, sperm density, or sperm motility. There was also an increased prevalence of hyperzoospermia (sperm density of  $>120$  million sperm per mL ejaculate) with increasing urine TCA levels ([Chia et al., 1996](#)).

#### **4.8.1.1.3.3. Altered endocrine function**

Two studies followed up on the study by Chia et al. ([1996](#)) to examine endocrine function ([Goh et al., 1998](#); [Chia et al., 1997](#)). The first examined serum testosterone, follicle-stimulating hormone (FSH), dehydroepiandrosterone sulphate (DHEAS), and sex-hormone binding globulin (SHBG) ([Chia et al., 1997](#)). With increased number of years of exposure to TCE, increases in DHEAS levels were seen, from 255 ng/mL for  $<3$  years to 717.8 ng/mL  $\geq 7$  years of exposure. Also with increased number of years of exposure to TCE, decreased FSH, SHBG, and testosterone levels were seen. The authors speculated that these effects could be due to decreased liver function related to TCE exposure ([Chia et al., 1997](#)).

The second follow-up study of this cohort studied the hormonal effects of chronic low-dose TCE exposure in these men ([Goh et al., 1998](#)). Because urine TCE measures only indicate short-term exposure, long-term exposure was indicated by years of exposure. Hormone levels examined include androstenedione, cortisol, testosterone, aldosterone, SHBG, and insulin. Results show that a decrease in serum levels of testosterone and SHBG were significantly correlated with years of exposure to TCE, and an increase in insulin levels were seen in those exposed for  $<2$  years. Androstenedione, cortisol, and aldosterone were in normal ranges and did not change with years of exposure to TCE.

#### 4.8.1.1.3.4. Infertility

Sallmén et al. (1998) examined paternal occupational exposure and time-to-pregnancy among their wives. Cases of spontaneous abortion and controls from a prior study of pregnancy outcome (Taskinen et al., 1989) were used to study time-to-pregnancy of 282 couples. Exposure was determined by biological measurements of the father who held the same job during pregnancy and measurement (time of measurement not stated) and questionnaires answered by both the mother and father. An additional questionnaire on time-to-pregnancy was answered by the mother for this study 6 years after the original study (Sallmen et al., 1998). The level of exposure was determined by questionnaire and classified as “low/intermediate” if the chemical was used <1 or 1–4 days/week and biological measures indicated high exposure (defined as above the reference value for the general population), and “high” if used daily or if biological measures indicated high exposure. For 13 men highly exposed, mean levels of urine TCA were 45 µmol/L (SD 42 µmol/L; median 31 µmol/L); for 22 men low/intermediately exposed, mean levels of urine TCA were 41 µmol/L (SD 88 µmol/L; median 15 µmol/L). The terminology IDR was replaced by fecundability density ratio (FDR) in order to reflect that pregnancy is a desired outcome; therefore, a high FDR indicates infertility. No effect was seen on fertility in the low-exposure group (FDR: 0.99, 95% CI: 0.63–1.56) or in the intermediate-/high-exposure group (FDR: 1.03, 95% CI: 0.60–1.76). However, the exposure categories were grouped by low/intermediate vs. high, whereas the outcome categories were grouped by low vs. intermediate/high, making a dose-response association difficult.

A small occupational study reported on eight male mechanics exposed to TCE for at least 2 years who sought medical treatment for infertility (Forkert et al., 2003). The wives were determined to have normal fertility. Samples of urine from two of the eight male mechanics contained TCA and/or TCOH, demonstrating the rapid metabolism in the body. However, samples of seminal fluid taken from all eight individuals detected TCE and the metabolites CH and TCOH, with two samples detecting DCA and one sample detecting TCA. Five unexposed controls also diagnosed with infertility did not have any TCE or metabolites in samples of seminal fluid. There was no control group that did not experience infertility. Increased levels of TCE and its metabolites in the seminal fluid of exposed workers compared to lower levels found in their urine samples was explained by cumulative exposure and mobilization of TCE from adipose tissue, particularly that surrounding the epididymis. In addition, CYP2E1 was detected in the epididymis, demonstrating that metabolism of TCE can occur in the male reproductive tract. However, this study could not directly link TCE to the infertility, as both the exposed and control populations were selected due to their infertility.

The ATSDR (2001) study discussed above on the reproductive effects from TCE in drinking water of individuals living near the Rocky Mountain Arsenal in Colorado did not observe infertility or other adverse reproductive effects for the high exposure group compared to

the low exposure group (OR<sub>adj</sub>: 0.83; 95% CI: 0.11–6.37). Curiously, exposed men had more pregnancies and live births than controls.

#### 4.8.1.1.4. Summary of human reproductive toxicity

Following exposure to TCE, observed adverse effects on the female reproductive system include reduced incidence of fecundability (as measured by time-to-pregnancy) and menstrual cycle disturbances. Observed adverse effects on the male reproductive system include altered sperm morphology, hyperzoospermia, altered endocrine function, decreased sexual drive and function, and altered fertility. These are summarized in Table 4-85.

#### 4.8.1.2. Animal Reproductive Toxicity Studies

A number of animal studies have been conducted that examined the effects of TCE on reproductive organs and function following either inhalation or oral exposures. These are described below and summarized in Tables 4-86 and 4-87. Other animal studies of offspring exposed during fetal development are discussed in the section on animal developmental studies (see Section 4.8.3.2).

##### 4.8.1.2.1. Inhalation exposures

Studies in rodents exposed to TCE via inhalation are described below and summarized in Table 4-86. These studies focused on various aspects of male reproductive organ integrity, spermatogenesis, or sperm function in rats or mice. In the studies published after the year 2000, the effects of either 376 or 1,000 ppm TCE were studied following exposure durations ranging from 1 to 24 weeks, and adverse effects on male reproductive endpoints were observed.

**Table 4-86. Summary of mammalian in vivo reproductive toxicity studies—  
inhalation exposures**

Reference <sup>a</sup>	Species/strain/ sex/number	Exposure level/duration	NOAEL; LOAEL <sup>b</sup>	Effects
Forkert et al. (2002)	Mouse, CD-1, male, 6/group	0 or 1,000 ppm (5,374 mg/m <sup>3</sup> ) <sup>c</sup>  6 hrs/d, 5 d/wk, 19 d over 4 wks	LOAEL: 1,000 ppm	U-TCA and U-TCOH increased by 2 <sup>nd</sup> and 3 <sup>rd</sup> wk, respectively. CYP 2E1 and <i>p</i> -nitrophenol hydroxylation in epididymal epithelium > testicular Leydig cells. Choral also generated from TCE in epididymis > testis. Sloughing of epididymal epithelial cells after 4-wk exposure.

**Table 4-86. Summary of mammalian in vivo reproductive toxicity studies—*inhalation exposures* (continued)**

Reference <sup>a</sup>	Species/strain/ sex/number	Exposure level/duration	NOAEL; LOAEL <sup>a</sup>	Effects
Kan et al. (2007)	Mouse, CD-1, male, 4/group	0 or 1,000 ppm  6 hrs/d, 5 d/wk, 1–4 wks	LOAEL: 1,000 ppm	Light microscopy findings: degeneration and sloughing of epididymal epithelial cells as early as 1 wk into exposure; more severe by 4 wks. Ultrastructural findings: vesiculation in cytoplasm, disintegration of basolateral cell membranes, sloughing of epithelial cells. Sperm found in situ in cytoplasm of degenerated epididymal cells. Abnormalities of the head and tail in sperm located in the epididymal lumen.
Kumar et al. (2000b)	Rat, Wistar, male, 12– 13/group	0 or 376 ppm  4 hrs/d, 5 d/wk, 2–10 wks exposure, 2–8- wk rest period	LOAEL: 376 ppm	Alterations in testes histopathology (smaller, necrotic spermatogenic tubules), ↑ sperm abnormalities, and statistically significant ↑ pre- and/or postimplantation loss in litters observed in the groups with 2 or 10 wks of exposure, or 5 wks of exposure with 2-wk rest.
Kumar et al. (2000a)	Rat, Wistar, males, 12– 13/group	0 or 376 ppm  4 hrs/d, 5 d/wk, 12 and 24 wks	LOAEL: 376 ppm	Statistically significant ↓ in total epididymal sperm count and sperm motility, with statistically significant ↓ in serum testosterone, statistically significant ↑ in testes cholesterol, statistically significant ↓ of glucose 6-phosphate dehydrogenase and 17-β-hydroxy steroid dehydrogenase at 12 and 24 wks of exposure.
Kumar et al. (2001b)	Rat, Wistar, male, 6/group	0 or 376 ppm  4 hrs/d, 5 d/wk, 12 and 24 wks	LOAEL: 376 ppm	Body weight gain statistically significant ↓. Testis weight, sperm count and motility statistically significant ↓, effect stronger with exposure time. After 12 wks, numbers of spermatogenic cells and spermatids ↓, some of the spermatogenic cells appeared necrotic. After 24 wks, testes were atrophied, tubules were smaller, had Sertoli cells, and were almost devoid of spermatocytes and spermatids. Leydig cells were hyperplastic. SDH, G6PDH statistically significant ↓, GGT and β-glucuronidase statistically significant ↑; effects stronger with exposure time.
Land et al. (1981)	Mouse, C57Blx3H (F1), male, 5 or 10/group	0, 0.02%, or 0.2%  4 hrs/d, 5 d, 23- d rest	NOAEL: 0.02% LOAEL: 0.2%	Statistically significant ↑ percentage morphologically abnormal epididymal sperm.
Xu et al. (2004)	Mouse, CD-1, male, 4– 27/group	0 or 1,000 ppm (5.37 mg/L) <sup>c</sup>  6 hrs/d, 5 d/wk, 1–6 wks	LOAEL: 1,000 ppm	Statistically significant ↓ in vitro sperm-oocyte binding and in vivo fertilization

<sup>a</sup>**Bolded studies** carried forward for consideration in dose-response assessment (see Chapter 5).

<sup>b</sup>NOAEL and LOAEL are based upon reported study findings.

<sup>c</sup>Dose conversion calculations by study author(s).

G6PDH = glucose 6-p dehydrogenase

**Table 4-87. Summary of mammalian in vivo reproductive toxicity studies—oral exposures**

Reference <sup>a</sup>	Species/strain/sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL <sup>b</sup>	Effects
<b>Studies assessing male reproductive outcomes</b>					
DuTeaux et al. (2003)	Rat, Sprague-Dawley, male, 3/group	0, 0.2, or 0.4% (0, 143, or 270 mg/kg-d)	Drinking water; 3% ethoxylated castor oil vehicle	LOEL: 0.2%	TCE metabolite-protein adducts formed by a CYP-mediated pathway were detected by fluorescence immunohistochemistry in the epithelia of corpus epididymis and in efferent ducts.
DuTeaux et al. (2004a)	Rat, Sprague-Dawley, male, 3/group, or Simonson albino (UC-Davis), male, 3/group	0, 0.2, or 0.4% (0, 143, or 270 mg/kg-d) 14 d	Drinking water, 3% ethoxylated castor oil vehicle	LOAEL: 0.2%	<b>Dose-dependent ↓ in ability of sperm to fertilize oocytes collected from untreated ♀s. Oxidative damage to sperm membrane in head and mid-piece was indicated by dose-related ↑ in oxidized proteins and lipid peroxidation.</b>
Veeramachaneni et al. (2001)	Rabbit, Dutch belted, females and offspring; 7–9 offspring/group	9.5 or 28.5 ppm TCE <sup>c</sup> GD 20 through lactation, then to offspring thru postnatal wk 15	Drinking water	LOAEL: 9.5 ppm	Decreased copulatory behavior; acrosomal dysgenesis, nuclear malformations; statistically significant ↓ LH and testosterone.
Zenick et al. (1984)	Rat, Long-Evans, male, 10/group	0, 10, 100, or 1,000 mg/kg-d 6 wk, 5 d/wk; 4 wks recovery	Gavage, corn oil vehicle	NOAEL: 100 mg/kg-d LOAEL: 1,000 mg/kg-d	<b>At 1,000 mg/kg, body weight ↓, liver/body weight ratios ↑, and impaired copulatory behavior. Copulatory performance returned to normal by 5<sup>th</sup> wk of exposure. At wk 6, TCE and metabolites concentrated to a significant extent in male reproductive organs.</b>
<b>Studies assessing female reproductive outcomes</b>					
Berger and Horner (2003)	Rat, Simonson (Sprague-Dawley derived), female, (5–6); × 3/group	0 or 0.45% 2 wks	Drinking water, 3% Tween vehicle	LOAEL: 0.45%	In vitro fertilization and sperm penetration of oocytes statistically significant ↓ with sperm harvested from untreated males.
Cosby and Dukelow (1992)	Mouse, B6D2F1, female, 7–12/group	0, 24, or 240 mg/kg-d GDs 1–5, 6–10, or 11–15	Gavage, corn oil vehicle	NOAEL: 240 mg/kg-d	No treatment-related effects on in vitro fertilization in dams or offspring.

**Table 4-87. Summary of mammalian in vivo reproductive toxicity studies—oral exposures (continued)**

Reference <sup>a</sup>	Species/strain/sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL <sup>a</sup>	Effects
Manson et al. (1984)	Rat, Long-Evans, female, 23–25/group	0, 10, 100, or 1,000 mg/kg-d  6 wks: 2 wks pre mating, 1 wk mating period, GDs 1–21	Gavage, corn oil vehicle	NOAEL: 100 mg/kg-d LOAEL: 1,000 mg/kg-d	Female fertility and mating success was not affected. At 1,000 mg/kg-d group, 5/23 females died, gestation body weight gain was statistically significant ↓. After subchronic oral TCE exposure, TCE was detected in fat, adrenals, and ovaries; TCA levels in uterine tissue were high.  At 1,000 mg/kg-d, neonatal deaths (female pups) were ↑ on PNDs 1, 10, and 14. Dose-related ↑ seen in TCA in blood, liver and milk in stomach of ♀ pups, not ♂s.
Wu and Berger (2007)	Rat, Simonson (Sprague-Dawley derived), female, (number/group not reported)	0 or 0.45% (0.66 g/kg-d) <sup>d</sup>  Preovulation d 1–5, 6–10, 11–14, or 1–14	Drinking water, 3% Tween vehicle	LOAEL: 0.45%	In vitro fertilization and sperm penetration of oocytes statistically significant ↓ with sperm harvested from untreated males.
Wu and Berger (2008)	Rat, Simonson (Sprague-Dawley derived), female, (number/group not reported)	0 or 0.45% (0.66 g/kg-d) <sup>d</sup>  1 or 5 d	Drinking water, 3% Tween vehicle	NOEL: 0.45%	Ovarian mRNA expression for ALCAM and Cud21 protein were not altered.

**Table 4-87. Summary of mammalian in vivo reproductive toxicity studies—oral exposures (continued)**

Reference <sup>a</sup>	Species/strain/sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL <sup>a</sup>	Effects
<b>Studies assessing fertility and reproductive outcome in both sexes</b>					
George et al. (1985)	Mouse, CD-1, male and female, 20 pairs/treatment group; 40 controls/sex	0, 0.15, 0.30, or 0.60% <sup>c</sup> micro-encapsulated TCE  (TWA dose estimates: 0, 173, 362, or 737 mg/kg-d) <sup>d</sup>  Breeders exposed 1 wk pre mating, then for 13 wks; pregnant females throughout gestation (i.e., 18 wks total)	Dietary	Parental systemic toxicity: NOAEL: 0.30% LOAEL: 0.60%	At 0.60%, in F0: statistically significant ↑ liver weights in both sexes; statistically significant ↓ testis and seminal vesicle weight; histopathology of liver and kidney in both sexes.  At 0.60%, in F1: statistically significant ↓ body weight on PND 74, and in postpartum F1 dams; statistically significant ↑ liver, testis, and epididymis weights in males, statistically significant ↑ kidney weights in both sexes; statistically significant ↓ testis and seminal vesicle weight; histopathology of liver and kidney in both sexes.
				Parental reproductive function: LOAEL: 0.60% <sup>d</sup>	At 0.60%, in F0 and F1 males: statistically significant ↓ sperm motility.
				Offspring toxicity: NOAEL: 0.30% LOAEL: 0.60%	At 0.60%, in F1 pups: statistically significant ↓ live birth weights, statistically significant ↓ PND 4 pup body weight; perinatal mortality ↑ (PNDs 0–21).

**Table 4-87. Summary of mammalian in vivo reproductive toxicity studies—oral exposures (continued)**

Reference <sup>a</sup>	Species/strain/sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL <sup>a</sup>	Effects
George et al. (1986)	Rat, F334, males and female, 20 pairs/treatment group, 40 controls/sex	0, 0.15, 0.30, or 0.60% <sup>c</sup> micro-encapsulated TCE  Breeders exposed 1 wk pre mating, then for 13 wks; pregnant females throughout gestation (i.e., 18 wks total)	Dietary	Parental systemic toxicity: LOAEL: 0.15%	At 0.60%, in F0: statistically significant ↓ postpartum dam body weight; statistically significant ↓ term. body weight in both sexes; statistically significant ↑ liver, and kidney/adrenal weights in both sexes; statistically significant ↑ testis/epididymis weights; in F1: statistically significant ↓ testis weight.  At all doses in F1: statistically significant ↓ postpartum dam body weight; statistically significant ↓ term. body weight in both sexes, statistically significant ↑ liver weight in both sexes.  At 0.30 and 0.60%, in F1: statistically significant ↑ liver weight in females.
				Parental reproductive function: LOAEL: 0.60% <sup>c</sup>	At 0.60%, sig ↓ mating in F0 males and females (in cross-over mating trials).
				Offspring toxicity: LOAEL: 0.15%	At 0.60%, statistically significant ↓ F1 body weight on PNDs 4 and 14. At all doses, statistically significant ↓ F1 body weight on PNDs 21 and 80.  At 0.3 and 0.60%, statistically significant ↓ live F1 pups/litter. Statistically significant trend towards ↓ live litters per pair At 0.15 and 0.60%, trend toward ↓ F1 survival from PNDs 21–80.

<sup>a</sup>**Bolded studies** carried forward for consideration in dose-response assessment (see Chapter 5).

<sup>b</sup>NOAEL, LOAEL, NOEL, and LOEL are based upon reported study findings.

<sup>c</sup>Concurrent exposure to several groundwater contaminants; values given are for TCE levels in the mixture.

<sup>d</sup>Dose conversion calculations by study author(s).

<sup>e</sup>Fertility and reproduction assessment of last litter from continuous breeding phase and cross-over mating assessment (rats only) were conducted for 0 or 0.60% dose groups only.

LH = luteinizing hormone

Kumar et al. (2000b) exposed male Wistar rats in whole-body inhalation chambers to 376-ppm TCE for 4 hours/day, 5 days/week over several duration scenarios. These were 2 weeks (to observe the effect on the epididymal sperm maturation phase), 10 weeks (to observe the effect on the entire spermatogenic cycle), 5 weeks with 2 weeks of rest (to observe the effect on primary spermatocytes differentiation to sperm), 8 weeks with 5 weeks of rest (to observe effects on an intermediate stage of spermatogenesis), and 10 weeks with 8 weeks of rest (to observe the effect on spermatogonial differentiation to sperm). Control rats were exposed to ambient air. Weekly mating with untreated females was conducted. At the end of the treatment/rest periods, the animals were sacrificed; testes and cauda epididymes tissues were collected. Alterations in testes histopathology (smaller, necrotic spermatogenic tubules), increased sperm abnormalities, and significantly increased pre- and/or postimplantation loss in litters were observed in the groups with 2 or 10 weeks of exposure, or 5 weeks of exposure with 2 of weeks rest. It was hypothesized that postmeiotic cells of spermatogenesis and epididymal sperm were affected by TCE exposure, leading to reproductive impairment.

To test the hypothesis that TCE exposure adversely affects sperm function and fertilization, Xu et al. (2004) conducted a study in which male CD-1 mice were exposed by inhalation to atmospheres containing 1,000 ppm (5.37 mg/L) TCE for 1–6 weeks (6 hours/day, 5 days/week). After each TCE exposure, body weights were recorded. Following termination, the right testis and epididymis of each treated male were weighed, and sperm was collected from the left epididymis and vas deferens for assessment of the number of total sperm and motile sperm. Sperm function was evaluated in the following experiments: (1) suspensions of capacitated vas deferens/cauda epididymal sperm were examined for spontaneous acrosome reaction; (2) in vitro binding of capacitated sperm to mature eggs from female CF-1 mice (expressed as the number of sperm bound per egg) was assessed; and (3) in vivo fertilization was evaluated via mating of male mice to superovulated female CF-1 mice immediately following inhalation exposure; cumulus masses containing mature eggs were collected from the oviducts of the females, and the percentage of eggs fertilized was examined. Inhalation exposure to TCE did not result in altered body weight, testis and epididymis weights, sperm count, or sperm morphology or motility. Percentages of acrosome-intact sperm populations were similar between treated and control animals. Nevertheless, for males treated with TCE for  $\geq 2$  weeks decreases were observed in the number of sperm bound to the oocytes in vitro (significant at 2 and 6 weeks,  $p < 0.001$ ). In a follow-up assessment, control sperm were incubated for 30 minutes in buffered solutions of TCE or metabolites (CH or TCOH); while TCE-incubation had no effect on sperm-oocyte binding, decreased binding capacity was noted for the metabolite-incubated sperm. The ability for sperm from TCE-exposed males to bind to and fertilize oocytes in vivo was also found to be significantly impaired ( $p < 0.05$ ).

A study designed to investigate the role of testosterone, and of cholesterol and ascorbic acid (which are primary precursors of testosterone) in TCE-exposed rats with compromised reproductive function was conducted by Kumar et al. (2000a). Male Wistar rats (12–13/group) were exposed (whole body) to 376 ppm TCE by inhalation for 4 hours/day, 5 days/week, for either 12 or 24 weeks and then terminated. Separate ambient-air control groups were conducted for the 12- and 24-week exposure studies. Epididymal sperm count and motility were evaluated, and measures of 17- $\beta$ -hydroxy steroid dehydrogenase (17- $\beta$ -HSD), testicular total cholesterol and ascorbic acid, serum testosterone, and glucose 6-p dehydrogenase (G6PDH) in testicular homogenate were assayed. In rats exposed to TCE for either 12 or 24 weeks, total epididymal sperm count and motility, serum testosterone concentration, and specific activities of both 17- $\beta$ -HSD and G6PDH were significantly decreased ( $p < 0.05$ ), while total cholesterol content was significantly ( $p < 0.05$ ) increased. Ascorbic acid levels were not affected.

In another study, Kumar et al. (2001b) utilized the same exposure paradigm to examine cauda epididymal sperm count and motility, testicular histopathology, and testicular marker enzymes: sorbitol dehydrogenase (SDH), G6PDH, glutamyl transferase (GT), and glucuronidase, in Wistar rats (6/group). After 24 weeks of exposure, testes weights and epididymal sperm count and motility were significantly decreased ( $p < 0.05$ ). After 12 weeks of TCE exposure, histopathological examination of the testes revealed a reduced number of spermatogenic cells in the seminiferous tubules, fewer spermatids as compared to controls, and the presence of necrotic spermatogenic cells. Testicular atrophy, smaller tubules, hyperplastic Leydig cells, and a lack of spermatocytes and spermatids in the tubules were observed after 24 weeks of TCE exposure. After both 12 and 24 weeks of exposure, SDH and G6PDH were significantly ( $p < 0.05$ ) reduced, while GT and  $\beta$ -glucuronidase were significantly ( $p < 0.05$ ) increased.

In a study by Land et al. (1981), 8–10-week-old male mice (C57BlxC3H)F1 (5 or 10/group) were exposed (whole body) by inhalation to a number of anesthetic agents for 5 consecutive days at 4 hours/day and sacrificed 28 days after the first day of exposure. Chamber concentration levels for the TCE groups were 0.02 and 0.2%. The control group received ambient air. Epididymal sperm were evaluated for morphological abnormalities. At 0.2% TCE, the percentage of abnormal sperm in a sample of 1,000 was significantly ( $p < 0.01$ ) increased as compared to control mice; no treatment-related effect on sperm morphology was observed at 0.02% TCE.

Forkert et al. (2002) exposed male CD-1 mice by inhalation to 1,000-ppm TCE (6 hours/day, 5 days/week) for 4 consecutive weeks and observed sloughing of portions of the epithelium upon histopathological evaluation of testicular and epididymal tissues.

Kan et al. (2007) also demonstrated that damage to the epididymal epithelium and sperm of CD-1 mice (4/group) resulted from exposure to 0 or 1,000 ppm TCE by inhalation for 6 hours/day, 5 days/week, for 1–4 weeks. Segments of the epididymis (caput, corpus, and cauda) were examined by light and electron microscope. As early as 1 week after TCE exposure,

degeneration and sloughing of epithelial cells from all three epididymal areas were observed by light microscopy; these findings became more pronounced by 4 weeks of exposure. Vesiculation in the cytoplasm, disintegration of basolateral cell membranes, and epithelial cell sloughing were observed with electron microscopy. Sperm were found in situ in the cytoplasm of degenerated epididymal cells. A large number of sperm in the lumen of the epididymis were abnormal, including head and tail abnormalities.

#### **4.8.1.2.2. Oral exposures**

A variety of studies were conducted to assess various aspects of male and/or female reproductive capacity in laboratory animal species following oral exposures to TCE. These are described below and summarized in Table 4-87. They include studies that focused on male reproductive outcomes in rats or rabbits following gavage or drinking water exposures ([DuTeaux et al., 2004a](#); [DuTeaux et al., 2003](#); [Veeramachaneni et al., 2001](#); [Zenick et al., 1984](#)), studies that focused on female reproductive outcomes in rats following gavage or drinking water exposures ([Wu and Berger, 2008, 2007](#); [Berger and Horner, 2003](#); [Cosby and Dukelow, 1992](#); [Manson et al., 1984](#)), and studies that assessed fertility and reproductive outcome in both sexes following dietary exposures to CD-1 mice or F344 rats ([George et al., 1986](#); [George et al., 1985](#)).

##### **4.8.1.2.2.1. Studies assessing male reproductive outcomes**

Zenick et al. ([1984](#)) conducted a study in which sexually experienced Long-Evans hooded male rats were administered 0, 10, 100, or 1,000 mg/kg-day TCE by gavage in corn oil for 6 weeks. A 4-week recovery phase was also incorporated into the study design. Endpoints assessed on weeks 1 and 5 of treatment included copulatory behavior, ejaculatory plug weights, and ejaculated or epididymal sperm measures (count, motility, and morphology). Sperm measures and plug weights were not affected by treatment, nor were Week 6 plasma testosterone levels found to be altered. TCE effects on copulatory behavior (ejaculation latency, number of mounts, and number of intromissions) were observed at 1,000 mg/kg-day; these effects were recovered by 1–4 weeks posttreatment. Although the effects on male sexual behavior in this study were believed to be unrelated to narcotic effects of TCE, a later study by Nelson and Zenick ([1986](#)) showed that naltrexone (an opioid receptor antagonist, 2.0 mg/kg, i.p., administered 15 minutes prior to testing) could block the effect. Thus, it was hypothesized that the adverse effects of TCE on male copulatory behavior in the rat at 1,000 ppm may, in fact, be mediated by the endogenous opioid system at the CNS level.

In a series of experiments by DuTeaux et al. ([2004a](#); [2003](#)), adult male rats were administered 0, 0.2, or 0.4% TCE (v/v) (equivalent to 0, 2.73, or 5.46 mg/L) in a solution of 3% ethoxylated castor oil in drinking water for 14 days. These concentrations were within the range of measurements obtained in formerly contaminated drinking water wells, as reported by ATSDR ([1997b](#)). The average ingested doses of TCE (based upon animal body weight and

average daily water consumption of 28 mL) were calculated to be 143 or 270 mg/kg-day for the low- and high-dose groups, respectively ([DuTeaux et al., 2003](#)). Cauda epididymal and vas deferens sperm from treated males were incubated in culture medium with oviductal cumulus masses from untreated females to assess in vitro fertilization capability. Treatment with TCE resulted in a dose-dependent decrease in the ability of sperm to fertilize oocytes. Terminal body weights and testis/epididymal weights were similar between control and treated groups. Evaluation of sperm concentration or motility parameters did not reveal any treatment-related alterations; acrosomal stability and mitochondrial membrane potential were not affected by treatment. Although no histopathological changes were observed in the testis or in the caput, corpus, or cauda epididymis, exposure to 0.2 and 0.4% TCE resulted in slight cellular alterations in the efferent ductule epithelium.

Veeramachaneni et al. ([2001](#)) evaluated the effects of drinking water containing chemicals typical of groundwater near hazardous waste sites (including 9.5 or 28.5 ppm TCE) on male reproduction. In this study, pregnant Dutch-belted rabbits were administered treated drinking water starting on GD 20; treatment continued through the lactation period and to weaned offspring (7–9/group) through postnatal week 15. Deionized water was administered from postnatal weeks 16–61, at which time the animals were terminated. At 57–61 weeks of age, ejaculatory capability, and seminal, testicular, epididymal, and endocrine characteristics were evaluated. In both treated groups, long-term effects consisted of decreased copulatory behavior (interest, erection, and/or ejaculation), significant increases in acrosomal dysgenesis and nuclear malformations ( $p < 0.03$ ), and significant decreases in serum concentration of luteinizing hormone (LH) ( $p < 0.05$ ) and testosterone secretion after human chorionic gonadotropin administration ( $p < 0.04$ ). There were no effects on total spermatozoa per ejaculate or on daily sperm production. The contribution of individual drinking water contaminants to adverse male reproductive outcome could not be discerned in this study. Additionally, it was not designed to distinguish between adverse effects that may have resulted from exposures in late gestation (i.e., during critical period of male reproductive system development) vs. postnatal life.

#### **4.8.1.2.2.2. Studies assessing female reproductive outcomes**

In a study that evaluated postnatal growth following gestational exposures, female B6D2F1 mice (7–12/group) were administered TCE at doses of 0, 1% LD<sub>50</sub> (24 mg/kg-day), and 10% LD<sub>50</sub> (240 mg/kg-day) by gavage in corn oil on GDs 1–5, 6–10, or 11–15 (day of mating was defined as GD 1) ([Cosby and Dukelow, 1992](#)). Litters were examined for pup count, sex, weight, and crown-rump measurement until GD 21. Some offspring were retained to 6 weeks of age, at which time they were killed and the gonads were removed, weighed, and preserved. No treatment-related effects were observed in the dams or offspring. In a second series of studies conducted by ([Cosby and Dukelow](#)) and reported in the same paper, TCE and its metabolites, DCA, TCA, and TCOH, were added to culture media with capacitated sperm and cumulus

masses from B6D2F1 mice to assess effects on *in vitro* fertilization. Dose-related decreases in fertilization were observed for DCA, TCA, and TCOH at 100 and 1,000 ppm, but not with TCE. Synergistic effects were not observed with TCA and TCOH.

A study was conducted by Manson et al. ([1984](#)) to determine if subchronic oral exposure to TCE affected female reproductive performance, and if TCE or its metabolites, TCA or TCOH, accumulated in female reproductive organs or neonatal tissues. Female Long-Evans hooded rats (22–23/group) were administered 0 (corn oil vehicle), 10, 100, or 1,000 mg/kg-day of TCE by gavage for 2 weeks prior to mating, throughout mating, and to GD 21. Delivered pups were examined for gross anomalies, and body weight and survival were monitored for 31 days. Three maternal animals per group and 8–10 neonates per group (killed on GDs 3 and 31) were analyzed for TCE and metabolite levels in tissues. TCE exposure resulted in five deaths and decreased maternal body weight gain at 1,000 mg/kg-day, but did not affect estrous cycle length or female fertility at any dose level. There were no evident developmental anomalies observed at any treatment level; however, at 1,000 mg/kg-day, there was a significant increase in the number of pups (mostly female) born dead, and the cumulative neonatal survival count through PND 18 was significantly decreased as compared to control. TCE levels were uniformly high in fat, adrenal glands, and ovaries across treatment groups, and TCA levels were high in uterine tissue. TCE levels in the blood, liver, and milk contents of the stomach increased in female PND-3 neonates across treatment groups. These findings suggest that increased metabolite levels did not influence fertility, mating success, or pregnancy outcome.

In another study that examined the potential effect of TCE on female reproductive function, Berger and Horner ([2003](#)) conducted 2-week exposures of Sprague-Dawley derived female Simonson rats to tetrachloroethylene, TCE, several ethers, and 4-vinylcyclohexene diepoxide in separate groups. The TCE-treated group received 0.45% TCE in drinking water containing 3% Tween vehicle; control groups were administered either untreated water, or water containing the 3% Tween vehicle. There were 5–6 females/group, and three replicates were conducted for each group. At the end of exposure, ovulation was induced, the rats were killed, and the ovaries were removed. The zona pellucida was removed from dissected oocytes, which were then placed into culture medium and inseminated with sperm from untreated males. TCE treatment did not affect female body weight gain, the percentage of females ovulating, or the number of oocytes per ovulating female. Fertilizability of the oocytes from treated females was reduced significantly (46% for TCE-treated females vs. 56% for vehicle controls). Oocytes from TCE-treated females had reduced ability to bind sperm plasma membrane proteins compared with vehicle controls.

In subsequent studies, Wu and Berger ([Wu and Berger, 2008, 2007](#)) examined the effect of TCE on oocyte fertilizability and ovarian gene expression. TCE was administered to female Simonson rats (number of subjects not reported) in the drinking water at 0 or 0.45% (in 3% Tween vehicle); daily doses were estimated to be 0.66 g TCE/kg body weight/day. In the oocyte

fertilizability study ([Wu and Berger, 2007](#)), the female rats were treated on days 1–5, 6–10, 11–14, or 1–14 of the 2-week period preceding ovulation (on day 15). Oocytes were extracted and fertilized in vitro with sperm from a single male donor rat. With any duration of TCE exposure, fertilization (as assessed by the presence of decondensed sperm heads) was significantly ( $p < 0.05$ ) decreased as compared to controls. After exposure on days 6–10, 11–14, or 1–14, the oocytes from TCE-treated females had a significantly decreased ability to bind sperm ( $p < 0.05$ ) in comparison to oocytes from vehicle controls. Increased protein carbonyls (an indicator of oxidatively modified proteins) were detected in the granulosa cells of ovaries from females exposed to TCE for 2 weeks. The presence of oxidized protein was confirmed by Western blot analysis. Microsomal preparations demonstrated the localization of CYP 2E1 and GST (TCE-metabolizing enzymes) in the ovary. Ovarian mRNA transcription for ALCAM and Cuzd1 protein was not found to be altered after 1 or 5 days of exposure ([Wu and Berger, 2008](#)), suggesting that the posttranslational modification of proteins within the ovary may partially explain the observed reductions in oocyte fertilization.

#### **4.8.1.2.2.3. Studies assessing fertility and reproductive outcomes in both sexes**

Assessments of reproduction and fertility with continuous breeding were conducted in NTP studies in CD-1 mice ([George et al., 1985](#)) and F344 rats ([George et al., 1986](#)). TCE was administered to the mice and rats at dietary levels of 0, 0.15, 0.30, or 0.60%, based upon the results of preliminary 14-day dose-range finding toxicity studies. Actual daily intake levels for the study in mice were calculated from the results of dietary formulation analyses and body weight/food consumption data at several time points during study conduct; the most conservative were from the second week of the continuous breeding study: 0, 52.5, 266.3, and 615.0 mg/kg-day. No intake calculations were presented for the rat study. In these studies, which were designed as described by Chapin and Sloane ([1997](#)), the continuous breeding phase in F0 adults consisted of a 7-day pre-mating exposure, 98-day cohabitation period, and 28-day segregation period. In rats, a crossover mating trial (i.e., control males × control females; 0.60% TCE males × control females; control males × 0.60% TCE females) was conducted to further elucidate treatment-related adverse reproductive trends observed in the continuous breeding phase. The last litter of the continuous breeding phase was raised to sexual maturity for an assessment of fertility and reproduction in control and high-dose groups; for the rats, this included an open field behavioral assessment of F1 pups. The study protocol included terminal studies in both generations, including sperm evaluation (count, morphology, and motility) in 10 selected males per dose level, macroscopic pathology, organ weights, and histopathology of selected organs.

In the continuous breeding phase of the CD-1 mouse study ([George et al., 1985](#)), no clinical signs of toxicity were observed in the parental (F0) animals, and there were no treatment-related effects on the proportion of breeding pairs able to produce a litter, number of live pups per litter, percentage born live, proportion of pups born live, sex of pups born live, absolute live

pup weights, or adjusted female pup weights. At the high-dose level of 0.60%, a number of adverse outcomes were observed. In the parental animals, absolute and body-weight-adjusted male and female liver weight values were significantly increased ( $p < 0.01$ ), and right testis and seminal vesicle weights were decreased ( $p < 0.05$ ), but kidney/adrenal weights were not affected. Sperm motility was significantly ( $p < 0.01$ ) decreased by 45% in treated males as compared to controls. Histopathology examination revealed lesions in the liver (hypertrophy of the centrilobular liver cells) and kidneys (tubular degeneration and karyomegaly of the corticomedullary renal tubular epithelium) of F0 males and females. In the pups at 0.60%, adjusted live birth weights for males and both sexes combined were significantly decreased ( $p < 0.01$ ) as compared to control. The last control and high-dose litters of the continuous breeding assessment were raised to the age of sexual maturity for a further assessment of reproductive performance. In these F1 pups, body weights (both sexes) were significantly decreased at PND 4, and male offspring body weights were significantly ( $p < 0.05$ ) less than controls at PND 74 ( $\pm 10$ ). It was reported that perinatal mortality (PNDs 0–21) was increased, with a 61.3% mortality rate for TCE-treated pups vs. a 28.3% mortality rate for control pups. Reproductive performance was not affected by treatment, and postmortem evaluations of the F1 adult mice revealed significant findings at 0.60% TCE that were consistent with those seen in the F0 adults and additionally demonstrated renal toxicity (i.e., elevated liver and kidney/adrenal weights and hepatic and renal histopathological lesions in both sexes) elevated testis and epididymis weights in males, and decreased sperm motility (18% less than control).

The F344 rat study continuous breeding phase demonstrated no evidence of treatment-related effects on the proportion of breeding pairs able to produce a litter, percentage of pups born alive, the sex of pups born alive, or absolute or adjusted pup weights ([George et al., 1986](#)). However, the number of live pups per litter was significantly ( $p < 0.05$ ) decreased at 0.30 and 0.60% TCE, and a significant ( $p < 0.01$ ) trend toward a dose-related decrease in the number of live litters per pair was observed; individual data were reported to indicate a progressive decrease in the number of breeding pairs in each treatment group producing third, fourth, and fifth litters. The crossover mating trial conducted in order to pursue this outcome demonstrated that the proportion of detected matings was significantly depressed ( $p < 0.05$ ) in the mating pairs with TCE-treated partners compared to the control pairs. In the F0 adults at 0.60% TCE, postpartum dam body weights were significantly decreased ( $p < 0.01$  or  $0.05$ ) in the continuous breeding phase and the crossover mating trials, and terminal body weights were significantly decreased ( $p < 0.01$ ) for both male and female rats. Postmortem findings for F0 adults in the high-dose group included significantly increased absolute and body-weight-adjusted liver and kidney/adrenal weights in males, increased adjusted liver and kidney/adrenal weights in females, and significantly increased adjusted left testis/epididymal weights. Sperm assessment did not identify any effects on motility, concentration, or morphology, and histopathological examination was negative. The last control and high-dose litters of the continuous breeding

assessment were raised to the age of sexual maturity for assessment of open field behavior and reproductive performance. In these F1 pups at 0.60% TCE, body weights of male and females were significantly ( $p < 0.05$  or  $0.01$ , respectively) decreased at PNDs 4 and 14. By PND 21, pup weights in both sexes were significantly reduced in all treated groups, and this continued until termination (approximately PND 80). A tendency toward decreased postweaning survival (i.e., from PND 21 to PND  $81 \pm 10$ ) was reported for F1 pups at the 0.15 and 0.60% levels. Open field testing revealed a significant ( $p < 0.05$ ) dose-related trend toward an increase in the time required for male and female F1 weanling pups to cross the first grid in the testing device, suggesting an effect on the ability to react to a novel environment. Reproductive performance assessments conducted in this study phase were not affected by treatment. Postpartum F1 dam body weights were significantly decreased ( $p < 0.05$  or  $0.01$ ) in all of the TCE-treated groups as compared to controls, as were terminal body weights for both adult F1 males and females. Postmortem evaluations of the F1 adult rats revealed significantly ( $p < 0.01$ ) decreased left testis/epididymis weight at 0.60% TCE, and significantly ( $p < 0.05$  or  $0.01$ ) increased adjusted mean liver weight in all treated groups for males and at 0.30 and 0.60% for females. Sperm assessments for F1 males revealed a significant increase ( $p < 0.05$ ) in the percentage of abnormal sperm in the 0.30% TCE group, but no other adverse effects on sperm motility, concentration, or morphology were observed. As with the F0 adults, there were no adverse treatment-related findings revealed at histopathological assessment. The study authors concluded that the observed effects to TCE exposure in this study were primarily due to generalized toxicity and not to a specific effect on the reproductive system; however, based upon the overall toxicological profile for TCE, which demonstrates that the male reproductive system is a target for TCE exposures, this conclusion is not supported.

#### **4.8.1.3. Discussion/Synthesis of Noncancer Reproductive Toxicity Findings**

The human epidemiological findings and animal study evidence consistently indicate that TCE exposures can result in adverse reproductive outcomes. Although the epidemiological data may not always be robust or unequivocal, they demonstrate the potential for a wide range of exposure-related adverse outcomes on female and male reproduction. In animal studies, there is some evidence for female-specific reproductive toxicity; but there is strong and compelling evidence for adverse effects of TCE exposure on male reproductive system and function.

##### **4.8.1.3.1. Female reproductive toxicity**

Although few epidemiological studies have examined TCE exposure in relation to female reproductive function (see Table 4-88), the available studies provide evidence of decreased fertility, as measured by time to pregnancy ([Sallmén et al., 1995](#)) and effects on menstrual cycle patterns, including abnormal cycle length ([ATSDR, 2001](#)), amenorrhea ([Sagawa et al., 1973](#); [Zielinski, 1973](#)), and menstrual “disturbance” ([Bardodej and Vyskocil, 1956](#)). In experimental

animals, the effects on female reproduction include evidence of reduced in vitro oocyte fertilizability in rats ([Wu and Berger, 2007](#); [Berger and Horner, 2003](#)). However, in other studies that assessed reproductive outcome in female rodents ([Cosby and Dukelow, 1992](#); [George et al., 1986](#); [George et al., 1985](#); [Manson et al., 1984](#)), there was no evidence of adverse effects of TCE exposure on female reproductive function. Overall, although the data are suggestive, there are inadequate data to make conclusions as to whether adverse effects on human female reproduction are caused by TCE.

**Table 4-88. Summary of adverse female reproductive outcomes associated with TCE exposures**

Finding	Species	References
Menstrual cycle disturbance	Human	ATSDR ( <a href="#">2001</a> ) <sup>a</sup>
		Bardodej and Vyskocil ( <a href="#">1956</a> )
		Sagawa et al. ( <a href="#">1973</a> )
		Zielinski ( <a href="#">1973</a> )
Reduced fertility	Human <sup>a</sup>	Sallmén et al. ( <a href="#">1995</a> )
	Rat <sup>b</sup>	Berger and Horner ( <a href="#">2003</a> )
		Wu and Berger ( <a href="#">2007</a> )

<sup>a</sup>Not significant.

<sup>b</sup>In vitro oocyte fertilizability.

#### 4.8.1.3.2. Male reproductive toxicity

Notably, the results of a number of studies in both humans and experimental animals have suggested that exposure to TCE can result in targeted male reproductive toxicity (see Table 4-89). The adverse effects that have been observed in both male humans and male animal models include altered sperm count, morphology, or motility ([Kumar et al., 2001b](#); [Veeramachaneni et al., 2001](#); [Kumar et al., 2000a](#); [Kumar et al., 2000b](#); [Chia et al., 1996](#); [Rasmussen et al., 1988](#); [George et al., 1985](#); [Land et al., 1981](#)); decreased libido or copulatory behavior ([Veeramachaneni et al., 2001](#); [George et al., 1986](#); [Zenick et al., 1984](#); [Saihan et al., 1978](#); [El Ghawabi et al., 1973](#); [Bardodej and Vyskocil, 1956](#)); alterations in serum hormone levels ([Veeramachaneni et al., 2001](#); [Kumar et al., 2000a](#); [Goh et al., 1998](#); [Chia et al., 1997](#)); and reduced fertility ([George et al., 1986](#)). However, other studies in humans did not see evidence of altered sperm count or morphology ([Rasmussen et al., 1988](#)) or reduced fertility ([Forkert et al., 2003](#); [Sallmen et al., 1998](#)), and some animal studies also did not identify altered sperm measures ([Xu et al., 2004](#); [Cosby and Dukelow, 1992](#); [George et al., 1986](#); [Zenick et al., 1984](#)). Additional adverse effects observed in animals include histopathological lesions of the testes ([Kumar et al., 2001b](#); [Kumar et al., 2000b](#); [George et al., 1986](#)) or epididymides ([Kan et al., 2007](#); [Forkert et al., 2002](#)) and altered in vitro sperm-oocyte binding and/or in vivo fertilization for TCE and/or its metabolites ([DuTeaux et al., 2004a](#); [Xu et al., 2004](#)).

**Table 4-89. Summary of adverse male reproductive outcomes associated with TCE exposures**

<b>Finding</b>	<b>Species</b>	<b>References</b>
Testicular toxicity/pathology	Rat	George et al. ( <a href="#">1986</a> )
		Kumar et al. ( <a href="#">2000b</a> )
		Kumar et al. ( <a href="#">2001b</a> )
	Mouse	Kan et al. ( <a href="#">2007</a> )
Epididymal toxicity/pathology	Mouse	Forkert et al. ( <a href="#">2002</a> )
Decreased sperm quantity/quality	Human	Chia et al. ( <a href="#">1996</a> )
		Rasmussen et al. ( <a href="#">1988</a> ) <sup>a</sup>
	Rat	Kumar et al. ( <a href="#">2001b</a> ; <a href="#">2000a</a> ; <a href="#">2000b</a> )
	Mouse	George et al. ( <a href="#">1985</a> )
		Land et al. ( <a href="#">1981</a> )
Rabbit	Veeramachaneni et al. ( <a href="#">2001</a> )	
Altered in vitro sperm-oocyte binding or in vivo fertilization	Rat	DuTeaux et al. ( <a href="#">2004a</a> )
	Mouse	Cosby and Dukelow ( <a href="#">1992</a> ) <sup>b</sup>
		Xu et al. ( <a href="#">2004</a> ) <sup>b</sup>
Altered sexual drive or function	Human	El Ghawabi et al. ( <a href="#">1973</a> )
		Saihan et al. ( <a href="#">1978</a> ) <sup>c</sup>
		Bardodej and Vyskocil ( <a href="#">1956</a> )
	Rat	George et al. ( <a href="#">1986</a> )
		Zenick et al. ( <a href="#">1984</a> )
	Rabbit	Veeramachaneni et al. ( <a href="#">2001</a> )
Altered serum testosterone levels	Human	Chia et al. ( <a href="#">1997</a> ) <sup>d</sup>
		Goh et al. ( <a href="#">1998</a> ) <sup>e</sup>
	Rat	Kumar et al. ( <a href="#">2000a</a> )
	Rabbit	Veeramachaneni et al. ( <a href="#">2001</a> )
Reduced fertility	Rat	George et al. ( <a href="#">1986</a> )
Gynaecomastia	Human	Saihan et al. ( <a href="#">1978</a> ) <sup>c</sup>

<sup>a</sup>Nonsignificant increase in percentage of two YFF in spermatozoa; no effect on sperm count or morphology.

<sup>b</sup>Observed with metabolite(s) of TCE only.

<sup>c</sup>Case study of one individual.

<sup>d</sup>Also observed altered levels of DHEAS, FSH, and SHBG.

<sup>e</sup>Also observed altered levels of SHBG.

In spite of the preponderance of studies demonstrating effects on sperm parameters, there is an absence of overwhelming evidence in the database of adverse effects of TCE on overall fertility in the rodent studies. That is not surprising, however, given the redundancy and efficiency of rodent reproductive capabilities. Nevertheless, the continuous breeding reproductive toxicity study in rats ([George et al., 1986](#)) did demonstrate a trend towards reproductive compromise (i.e., a progressive decrease in the number of breeding pairs producing third, fourth, and fifth litters).

It is noted that in the studies by George et al. ([1986](#); [George et al., 1985](#)), adverse reproductive outcomes in male rats and mice were observed at the highest dose level tested (0.060% TCE in diet), which was also systemically toxic (i.e., demonstrating kidney toxicity and liver enzyme induction and toxicity, sometimes in conjunction with body weight deficits). Because of this, the study authors concluded that the observed reproductive toxicity was a secondary effect of generalized systemic toxicity; however, this conclusion is not supported by the overall toxicological profile of TCE, which provides significant evidence indicating that TCE is a reproductive toxicant.

#### **4.8.1.3.2.1. The role of metabolism in male reproductive toxicity**

There has been particular focus on evidence of exposure to male reproductive organs by TCE and/or its metabolites, as well as the role of TCE metabolites in the observed toxic effects.

In humans, a few studies demonstrating male reproductive toxicity have measured levels of TCE in the body. U-TCA was measured in men employed in an electronics factory, and adverse effects observed included abnormal sperm morphology and hyperzoospermia and altered serum hormone levels ([Goh et al., 1998](#); [Chia et al., 1997](#); [Chia et al., 1996](#)). U-TCA was also measured as a marker of exposure to TCE in men occupationally exposed to solvents, although this study did not report any adverse effects on fertility ([Sallmen et al., 1998](#)).

In the study in Long-Evans male rats by Zenick et al. ([1984](#)), blood and tissue levels of TCE, TCA, and TCOH were measured in three rats/group following 6 weeks of gavage treatment at 0, 10, 100, and 1,000 mg/kg-day. Additionally, the levels of TCE and metabolites were measured in seminal plugs recovered following copulation at week 5. Marked increases in TCE levels were observed only at 1,000 mg/kg-day, in blood, muscle, adrenals, and seminal plugs. It was reported that dose-related increases in TCA and TCOH concentrations were observed in the organs evaluated, notably including the reproductive organs (epididymis, vas deferens, testis, prostate, and seminal vesicle), thus creating a potential for interference with reproductive function.

This potential was explored further in a study by Forkert et al. ([2002](#)), in which male CD-1 mice were exposed by inhalation to 1,000 ppm TCE (6 hours/day, 5 days/week) for 4 consecutive weeks. Urine was obtained on days 4, 9, 14, and 19 of exposure and analyzed for concentrations of TCE and TCOH. Microsomal preparations from the liver, testis, and

epididymis were used for immunoblotting, determining *p*-nitrophenol hydroxylase and CYP2E1 activities, and evaluating the microsomal metabolism of TCE.

Subsequent studies conducted by the same laboratory ([Forkert et al., 2003](#)) evaluated the potential of the male reproductive tract to accumulate TCE and its metabolites including chloral, TCOH, TCA, and DCA. Human seminal fluid and urine samples from eight mechanics diagnosed with clinical infertility and exposed to TCE occupationally were analyzed. Urine samples from two of the eight subjects contained TCA and/or TCOH, suggesting that TCE exposure and/or metabolism was low during the time just prior to sample collection. TCE, chloral, and TCOH were detected in seminal fluid samples from all eight subjects, while TCA was found in one subject, and DCA was found in two subjects. Additionally, TCE and its metabolites were assessed in the epididymis and testis of CD-1 mice (4/group) exposed by inhalation (6 hours/day, 5 days/week) to 1,000 ppm TCE for 1, 2, and 4 weeks. TCE, chloral, and TCOH were found in the epididymis at all timepoints, although TCOH levels were increased significantly (tripled) at 4 weeks of exposure. This study showed that the metabolic disposition of TCE in humans is similar to that in mice, indicating that the murine model is appropriate for investigating the effects of TCE-induced toxicity in the male reproductive system. These studies provide support for the premise that TCE is metabolized in the human reproductive tract, mainly in the epididymis, resulting in the production of metabolites that cause damage to the epididymal epithelium and affect the normal development of sperm.

Immunohistochemical experiments ([Forkert et al., 2002](#)) confirmed the presence of CYP2E1 in the epididymis and testis of mice; it was found to be localized in the testicular Leydig cells and the epididymal epithelium. Similar results were obtained with the immunohistochemical evaluation of human and primate tissue samples. CYP2E1 has been previously shown by Lipscomb et al. ([1998a](#)) to be the predominant CYP enzyme catalyzing the hepatic metabolism of TCE in both animals and rodents. These findings support the role of CYP2E1 in TCE metabolism in the male reproductive tract of humans, primates, and mice.

#### **4.8.1.3.2.2. Mode of action for male reproductive toxicity**

A number of studies have been conducted to attempt to characterize various aspects of the mode of action for observed male reproductive outcomes.

Studies by Kumar et al. ([2001b](#); [2000a](#)) suggest that perturbation of testosterone biosynthesis may have some role in testicular toxicity and altered sperm measures. Significant decreases in the activity of G6PDH and accumulation of cholesterol are suggestive of an alteration in testicular steroid biosynthesis. Increased testicular lipids, including cholesterol, have been noted for other testicular toxicants such as lead ([Saxena et al., 1987](#)), triethylenemelamine ([Johnson et al., 1967](#)), and quinalphos ([Ray et al., 1987](#)), in association with testicular degeneration and impaired spermatogenesis. Since testosterone has been shown to be essential for the progression of spermatogenesis ([O'Donnell et al., 1994](#)), alterations in

testosterone production could be a key event in male reproductive dysfunction following TCE exposure. Additionally, the observed TCE-related reduction of 17- $\beta$ -HSD, which is involved in the conversion of androstenedione to testosterone, has also been associated with male reproductive insufficiency following exposure to phthalate esters ([Srivastava, 1991](#)), quinalphos ([Ray et al., 1987](#)), and lead ([Saxena et al., 1987](#)). Reductions in SDH, which are primarily associated with the pachytene spermatocyte maturation of germinal epithelium, have been shown to be associated with depletion of germ cells ([Chapin et al., 1982](#); [Mills and Means, 1972](#)), and the activity of G6PDH is greatest in premeiotic germ cells and Leydig cells of the interstitium ([Blackshaw, 1970](#)). The increased GT and glucuronidase observed following TCE exposures appear to be indicative of impaired Sertoli cell function ([Sherins and Hodgen, 1976](#); [Hodgen and Sherins, 1973](#)). Based upon the conclusions of these studies, Kumar et al. ([2001b](#)) hypothesized that the reduced activity of G6PDH and SDH in testes of TCE-exposed male rats is indicative of the depletion of germ cells, spermatogenic arrest, and impaired function of the Sertoli cells and Leydig cells of the interstitium.

In the series of experiments by DuTeaux et al. ([2004a](#); [2003](#)), protein dichloroacetyl adducts were found in the corpus epididymis and in the efferent ducts of rats administered TCE; this effect was also demonstrated following in vitro exposure of reproductive tissues to TCE. Oxidized proteins were detected on the surface of spermatozoa from TCE-treated rats in a dose-response pattern; this was confirmed using a Western blotting technique. Soluble (but not mitochondrial) cysteine-conjugate  $\beta$ -lyase was detected in the epididymis and efferent ducts of treated rats. Following a single i.p. injection of DCVC, no dichloroacetylated protein adducts were detected in the epididymis and efferent ducts. The presence of CYP2E1 was found in epididymis and efferent ducts, suggesting a role of CYP-dependent metabolism in adduct formation. An in vitro assay was used to demonstrate that epididymal and efferent duct microsomes are capable of metabolizing TCE; TCE metabolism in the efferent ducts was found to be inhibited by anti-CYP2E1 antibody. Lipid peroxidation in sperm, presumably initiated by free radicals, was increased in a significant ( $p < 0.005$ ) dose-dependent manner after TCE exposure.

Overall, it has been suggested ([DuTeaux et al., 2004a](#)) that reproductive organ toxicities observed following TCE exposure are initiated by metabolic bioactivation, leading to subsequent protein adduct formation. It has been hypothesized that epoxide hydrolases in the rat epididymis may play a role in the biological activation of metabolites ([DuTeaux et al., 2004b](#)). Disruption of colony stimulating factor and of macrophage development may also play a role in sperm production ([Cohen et al., 1999](#)), and thus, may be another route through which immune-related effects of TCE may operate. In addition, the potential for epigenetic changes, through which heritable changes in gene mutations occur without changes in DNA sequencing, should also be considered in the evaluation of transgenerational effects ([Guerrero-Bosagna and Skinner, 2009](#)).

#### **4.8.1.3.3. Summary of noncancer reproductive toxicity**

The toxicological database for TCE includes a number of studies that demonstrate adverse effects on the integrity and function of the reproductive system in females and males. Both the epidemiological and animal toxicology databases provide suggestive, but limited, evidence of adverse outcomes to female reproductive outcomes. However, much more extensive evidence exists in support of an association between TCE exposures and male reproductive toxicity. The available epidemiological data and case reports that associate TCE with adverse effects on male reproductive function are limited in size and provide little quantitative dose data ([Lamb and Hentz, 2006](#)). However, the animal data provide extensive evidence of TCE-related male reproductive toxicity. Strengths of the database include the presence of both functional and structural outcomes, similarities in adverse treatment-related effects observed in multiple species, and evidence that metabolism of TCE in male reproductive tract tissues is associated with adverse effects on sperm measures in both humans and animals (suggesting that the murine model is appropriate for extrapolation to human health risk assessment). Additionally, some aspects of a putative mode of action (e.g., perturbations in testosterone biosynthesis) appear to have some commonalities between humans and animals.

#### **4.8.2. Cancers of the Reproductive System**

The effects of TCE on cancers of the reproductive system have been examined for males and females in both epidemiological and experimental animal studies. The epidemiological literature includes data on prostate in males and cancers of the breast and cervix in females. The experimental animal literature includes data on prostate and testes in male rodents; and uterus, ovary, mammary gland, vulva, and genital tract in female rodents. The evidence for these cancers is generally not robust.

##### **4.8.2.1. Human Data**

The epidemiologic evidence on TCE and cancer of the prostate, breast, and cervix is from cohort and geographic-based studies. Two additional case-control studies of prostate cancer in males are nested within cohorts ([Krishnadasan et al., 2007](#); [Greenland et al., 1994](#)). The nested case-control studies are identified in Tables 4-90 through 4-92 with cohort studies given their source population for case and control identification. One population-based, case-control study examined on TCE exposure and prostate ([Siemiatycki, 1991](#)); however, no population case-control studies on breast or cervical cancers and TCE exposure were found in the peer-reviewed literature.

**Table 4-90. Summary of human studies on TCE exposure and prostate cancer**

Studies	Exposure group	RR (95% CI)	Number of observable events	Reference
<b>Cohort studies—incidence</b>				
Aerospace workers (Rocketdyne)				Krishnadasan et al. (2007)
	Low/moderate TCE score	1.3 (0.81, 2.1) <sup>a,b</sup>	90	
	High TCE score	2.1 (1.2, 3.9) <sup>a,b</sup>	45	
	<i>p</i> for trend	0.02		
	Low/moderate TCE score	1.3 (0.81, 2.1) <sup>a,c</sup>		
	High TCE score	2.4 (1.3, 4.4) <sup>a,c</sup>		
	<i>p</i> for trend	0.01		
All employees at electronics factory (Taiwan)		0.14 (0.00, 0.76) <sup>d</sup>	1	Chang et al. (2005)
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al. (2003)
	Any exposure	0.9 (0.79, 1.08)	163	
Biologically-monitored Danish workers				Hansen et al. (2001)
	Any TCE exposure, females	0.6 (0.2, 1.3)	6	
Aircraft maintenance workers (Hill Air Force Base, Utah)				
	TCE subcohort	Not reported	158	Blair et al. (1998)
	Cumulative exposure			
	0	1.0 <sup>e</sup>		
	<5 ppm-yr	1.1 (0.7, 1.6)	64	
	5–25 ppm-yr	1.0 (0.6, 1.6)	38	
	>25 ppm-yr	1.2 (0.8, 1.8)	56	
	TCE subcohort	1.2 (0.92, 1.76)	116	Radican et al. (2008)
	Cumulative exposure			
	0	1.0 <sup>e</sup>		
	<5 ppm-yr	1.03 (0.65, 1.62)	41	
	5–25 ppm-yr	1.33 (0.82, 2.15)	42	
	>25 ppm-yr	1.31 (0.84, 2.06)	43	
Biologically-monitored Finnish workers		1.38 (0.73, 2.35)	13	Anttila et al. (1995)
	Mean air-TCE (Ikeda extrapolation)			
	<6 ppm	1.43 (0.62, 2.82)	8	
	6+ ppm	0.68 (0.08, 2.44)	2	

**Table 4-90. Summary of human studies on TCE exposure and prostate cancer (continued)**

Studies	Exposure group	RR (95% CI)	Number of observable events	Reference
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al. ( <a href="#">1995</a> )
	Exposed workers	Not reported		
Biologically-monitored Swedish workers		1.25 (0.84, 1.84)	26	Axelson et al. ( <a href="#">1994</a> )
Cardboard manufacturing workers, Atlanta area, Georgia		Not reported		Sinks et al. ( <a href="#">1992</a> )
<b>Cohort and PMR-mortality</b>				
Aerospace workers (Rocketdyne)				Boice et al. ( <a href="#">2006b</a> )
	Any TCE (utility/eng flush)	0.82 (0.36, 1.62)	8	
View-Master employees		1.69 (0.68, 3.48) <sup>f</sup>	8	ATSDR ( <a href="#">2004a</a> )
All employees at electronics factory (Taiwan)		Not reported	0	Chang et al. ( <a href="#">2003</a> )
Fernald workers				Ritz ( <a href="#">1999a</a> )
	Any TCE exposure	Not reported		
	Light TCE exposure, >2-yr duration	0.91 (0.38, 2.18) <sup>e,g</sup>	10	
	Moderate TCE exposure, >2-yr duration	1.44 (0.19, 11.4) <sup>e,g</sup>	1	
Aerospace workers (Lockheed)				Boice et al. ( <a href="#">1999</a> )
	Routine exposure to TCE	1.31 (0.52, 2.69)	7	
	Routine-intermittent	Not reported		
Aerospace workers (Hughes)				Morgan et al. ( <a href="#">2000</a> , <a href="#">1998</a> )
	TCE subcohort	1.18 (0.73, 1.80)	21	
	Low intensity (<50 ppm)	1.03 (0.51, 1.84)	7	
	High intensity (>50 ppm)	0.47 (0.15, 1.11)	14	
	TCE subcohort (Cox Analysis)			
	Never exposed	1.00 <sup>e</sup>		
	Ever exposed	1.58 (0.96, 2.62) <sup>h</sup>		
	Peak			
	No/low	1.00 <sup>e</sup>		
	Medium/high	1.39 (0.80, 2.41) <sup>h</sup>		
	Cumulative			
	Referent	1.00 <sup>e</sup>		
	Low	1.72 (0.78, 3.80) <sup>h</sup>		
	High	1.53 (0.85, 2.75) <sup>h</sup>		

**Table 4-90. Summary of human studies on TCE exposure and prostate cancer (continued)**

Studies	Exposure group	RR (95% CI)	Number of observable events	Reference
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. (1998)
	TCE subcohort	1.1 (0.6, 1.8)	54	
	Cumulative exposure			
	0	1.0 <sup>e</sup>		
	<5 ppm-yr	0.9 (0.5, 1.8)	19	
	>25 ppm-yr	1.3 (0.7, 2.4)	22	
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al. (1995)
	TCE exposed workers	Not reported		
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		0.82 (0.46, 1.46) <sup>a</sup>	58	Greenland et al. (1994)
Cardboard manufacturing workers, Atlanta area, Georgia		Not reported	0	Sinks et al. (1992)
U.S. Coast Guard employee				Blair et al. (1989)
	Marine inspectors	1.06 (0.51, 1.95)	10	
	Noninspectors	0.57 (0.15, 1.45)	7	
Aircraft manufacturing plant employees (Italy)				Costa et al. (1989)
Aircraft manufacturing plant employees (San Diego, California)		0.93 (0.60, 1.37)	25	Garabrant et al. (1988)
Lamp manufacturing workers (GE)		1.56 (0.63, 3.22)	7	Shannon et al. (1988)
Rubber workers				Wilcosky et al. (1984)
	Any TCE exposure	0.62 (not reported)	3	
<b>Case-control studies</b>				
Population of Montreal, Canada				Siemiatycki (1991)
	Any TCE exposure	1.1 (0.6, 2.1) <sup>i</sup>	11	
	Substantial TCE exposure	1.8 (0.8, 4.0) <sup>i</sup>	7	
<b>Geographic-based studies</b>				
Residents in two study areas in Endicott, New York		1.05 (0.75, 1.43)	40	ATSDR (2006a)
Residents of 13 census tracts in Redlands, California		1.11 (0.98, 1.25) <sup>j</sup>	483	Morgan and Cassady (2002)
Finnish residents				Vartiainen et al. (1993)
	Residents of Hausjarvi	Not reported		
	Residents of Huttula	Not reported		

<sup>a</sup>OR from nested case-control study.

<sup>b</sup>OR, zero lag.

<sup>c</sup>OR, 20-year lag.

<sup>d</sup>Chang et al. (2005) presents SIRs for a category site of all cancers of male genital organs.

<sup>e</sup>Internal referents, workers without TCE exposure.

<sup>f</sup>PMR.

<sup>g</sup>Analysis for >2 years exposure duration and a lagged TCE exposure period of 15 years.

<sup>h</sup>Risk ratio from Cox Proportional Hazard Analysis, stratified by age and sex, from Environmental Health Strategies (1997) Final Report to Hughes Corporation (Communication from Paul A. Cammer, President, Trichloroethylene Issues Group to Cheryl Siegel Scott, U.S. EPA, December 22, 1997).

<sup>i</sup>90% CI.

<sup>j</sup>99% CI.

**Table 4-91. Summary of human studies on TCE exposure and breast cancer**

Studies	Exposure group	RR (95% CI)	Number of observable events	Reference
<b>Cohort studies—incidence</b>				
Aerospace workers (Rocketdyne)				Zhao et al. ( <a href="#">2005</a> )
	Any TCE exposure	Not reported		
	Low cumulative TCE score			
	Medium cumulative TCE score			
	High TCE score			
	<i>p</i> for trend			
All employees at electronics factory (Taiwan)				
	Females	1.09 (0.96, 1.22) <sup>a</sup>	286	Sung et al. ( <a href="#">2007</a> )
	Females	1.19 (1.03, 1.36)	215	Chang et al. ( <a href="#">2005</a> )
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al. ( <a href="#">2003</a> )
	Any exposure, males	0.5 (0.06, 1.90)	2	
	Any exposure, females	1.1 (0.89, 1.24)	145	
Biologically-monitored Danish workers				Hansen et al. ( <a href="#">2001</a> )
	Any TCE exposure, males		0 (0.2 exp)	
	Any TCE exposure, females	0.9 (0.2, 2.3)	4	
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. ( <a href="#">1998</a> )
	TCE subcohort	Not reported	34	
	Females, cumulative exposure			
	0	1.0 <sup>b</sup>		
	<5 ppm-yr	0.3 (0.1, 1.4)	20	
	5–25 ppm-yr	0.4 (0.1, 2.9)	11	
	>25 ppm-yr	0.4 (0.4, 1.2)	3	
Biologically-monitored Finnish workers		Not reported		Anttila et al. ( <a href="#">1995</a> )
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al. ( <a href="#">1995</a> )
	Exposed workers	Not reported		
Biologically-monitored Swedish workers		Not reported		Axelsson et al. ( <a href="#">1994</a> )
Cardboard manufacturing workers, Atlanta area, Georgia		Not reported		Sinks et al. ( <a href="#">1992</a> )

**Table 4-91. Summary of human studies on TCE exposure and breast cancer (continued)**

Studies	Exposure group	RR (95% CI)	Number of observable events	Reference	
<b>Cohort and PMR-mortality</b>					
Aerospace workers (Rocketdyne)					
	Any TCE (utility/eng flush)	Not reported		Boice et al. (2006b)	
	Any exposure to TCE	Not reported		Zhao et al. (2005)	
	Low cumulative TCE score	Not reported			
	Medium cumulative TCE score	Not reported			
	High TCE score	Not reported			
	<i>p</i> for trend				
View-Master employees					
	Males		0 (0.05 exp)	ATSDR (2004a)	
	Females	1.02 (0.67, 1.49) <sup>c</sup>	27		
Fernald workers					
	Any TCE exposure	Not reported		Ritz (1999a)	
	Light TCE exposure, >2-yr duration	Not reported			
	Moderate TCE exposure, >2-yr duration	Not reported			
Aerospace workers (Lockheed)					
	Routine exposure to TCE	1.31 (0.52, 2.69) <sup>d</sup>	7	Boice et al. (1999)	
	Routine-intermittent <sup>a</sup>	Not reported			
Aerospace workers (Hughes)					
	TCE subcohort	0.75 (0.43, 1.22) <sup>d</sup>	16	Morgan et al. (1998)	
	Low intensity (<50 ppm)	1.03 (0.51, 1.84) <sup>d</sup>	11		
	High intensity (>50 ppm)	0.47 (0.15, 1.11) <sup>d</sup>	5		
TCE subcohort (Cox Analysis)					
	Never exposed	1.00 <sup>d</sup>	NR		
	Ever exposed	0.94 (0.51, 1.75) <sup>d,e</sup>	NR		
Peak					
	No/low	1.00 <sup>d</sup>			
	Medium/high	1.14 (0.48, 2.70) <sup>d,e</sup>	NR		
Cumulative					
	Referent	1.00 <sup>b</sup>			
	Low	1.20 (0.60, 2.40) <sup>d,e</sup>	NR		
	High	0.65 (0.25, 1.69) <sup>d,e</sup>	NR		

**Table 4-91. Summary of human studies on TCE exposure and breast cancer (continued)**

Studies	Exposure group	RR (95% CI)	Number of observable events	Reference
Aircraft maintenance workers (Hill Air Force Base, Utah)				
	TCE subcohort (females)	2.0 (0.9, 4.6)	20	Blair et al. (1998)
	Females, cumulative exposure			
	0	1.0 <sup>b</sup>		
	<5 ppm-yr	2.4 (1.1, 5.2)	10	
	5–25 ppm-yr	1.2 (0.3, 5.4)	21	
	>25 ppm-yr	1.4 (0.6, 3.2)	8	
	Low level intermittent exposure	3.1 (1.5, 6.2)	15	
	Low level continuous exposure	3.4 (1.4, 8.0)	8	
	Frequent peaks	1.4 (0.7, 3.2)	10	
	TCE subcohort (females)	1.23 (0.73, 2.06)	26	Radican et al. (2008)
	Females, cumulative exposure			
	0	1.0 <sup>b</sup>		
	<5 ppm-yr	1.57 (0.81, 3.04)	12	
	5–25 ppm-yr	1.01 (0.31, 3.30)	3	
	>25 ppm-yr	1.05 (0.53, 2.07)	11	
	Low level intermittent exposure	1.92 (1.08, 3.43)	18	
	Low level continuous exposure	1.71 (0.79, 3.71)	8	
	Frequent peaks	1.08 (0.57, 2.02)	14	
Cardboard manufacturing workers in Arnsburg, Germany				
	TCE exposed workers	Not examined		Henschler et al. (1995)
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		Not reported		Greenland et al. (1994)
Cardboard manufacturing workers, Atlanta area, Georgia		Not reported	0	Sinks et al. (1992)
U.S. Coast Guard employees				
	Marine inspectors	Not reported		Blair et al. (1989)
	Noninspectors	Not reported		
Aircraft manufacturing plant employees (Italy)		Not reported <sup>f</sup>		Costa et al. (1989)
Aircraft manufacturing plant employees (San Diego, California)				
	All subjects, females	0.81 (0.52, 1.48) <sup>d</sup>	16	Garabrant et al. (1988)
Lamp manufacturing workers (GE)				
	Coil/wire drawing	2.04 (0.88, 4.02)	8	Shannon et al. (1988)
	Other areas	0.97 (0.57, 1.66)	13	
<b>Case-control studies</b>				
Population of Montreal, Canada				Siemiatycki (1991)
	Any TCE exposure	Not reported		
	Substantial TCE exposure	Not reported		

**Table 4-91. Summary of human studies on TCE exposure and breast cancer (continued)**

Studies	Exposure group	RR (95% CI)	Number of observable events	Reference
<b>Geographic-based studies</b>				
	Residents in two study areas in Endicott, New York	0.88 (0.65, 1.18)	46	ATSDR ( <a href="#">2006a</a> )
	Residents of 13 census tracts in Redlands, California	1.09 (0.97, 1.21)	536	Morgan and Cassady ( <a href="#">2002</a> )
	Finnish residents			Vartiainen et al. ( <a href="#">1993</a> )
	Residents of Hausjarvi	Not reported		
	Residents of Huttula	Not reported		

<sup>a</sup>15-year lag.

<sup>b</sup>Internal referents, workers not exposed to TCE.

<sup>c</sup>PMR.

<sup>d</sup>In Garabrant et al. ([1988](#)), Morgan et al. ([1998](#)), and Boice et al. ([1999](#)), breast cancer risk is for males and females combined (ICD-9, 174, 175).

<sup>e</sup>Risk ratio from Cox Proportional Hazard Analysis, stratified by age and sex, from Environmental Health Strategies ([1997](#)) Final Report to Hughes Corporation c,c.CEa.

<sup>f</sup>The cohort of Blair et al. ([1989](#)) and Costa et al. ([1989](#)) are composed of males only.

NR = not reported

**Table 4-92. Summary of human studies on TCE exposure and cervical cancer**

Exposure group		RR (95% CI)	Number of observable events	Reference
<b>Cohort studies—incidence</b>				
Aerospace workers (Rocketdyne)				Zhao et al. (2005)
	Any exposure to TCE	Not reported		
	Low cumulative TCE score	Not reported		
	Medium cumulative TCE score			
	High TCE score			
	<i>p</i> for trend			
All employees at electronics factory (Taiwan)		0.96 (0.86, 1.22) <sup>a</sup>	337	Sung et al. (2007)
Danish blue-collar worker w/TCE exposure				Raaschou-Nielsen et al. (2003)
	Any exposure	1.9 (1.42, 2.37)	62	
	Exposure lag time			
	20 yrs	1.5 (0.7, 2.9)	9	
	Employment duration			
	<1 yr	2.5 (1.7, 3.5)	30	
	1–4.9 yrs	1.6 (1.0, 2.4)	22	
	≥5 yrs	1.3 (0.6, 2.4)	10	
Biologically-monitored Danish workers				Hansen et al. (2001)
	Any TCE exposure	3.8 (1.0, 9.8)	4	
	Cumulative exposure (Ikeda)			
	<17 ppm-yr	2.9 (0.04, 16)	1	
	≥17 ppm-yr	2.6 (0.03, 14)	1	
	Mean concentration (Ikeda)			
	<4 ppm	3.4 (0.4, 12)	2	
	4+ ppm	4.3 (0.5, 16)	2	
	Employment duration			
	<6.25 yrs	3.8 (0.1, 21)	1	
	≥6.25 yrs	2.1 (0.03, 12)	1	
Aircraft maintenance workers from Hill Air Force Base, Utah				Blair et al. (1998)
	TCE subcohort	Not reported		
	Cumulative exposure	Not reported		

**Table 4-92. Summary of human studies on TCE exposure and cervical cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
Biologically-monitored Finnish workers				Anttila et al. ( <a href="#">1995</a> )
All subjects		2.42 (1.05, 4.77)	8	
Mean air-TCE (Ikeda extrapolation)				
<6 ppm	1.86 (0.38, 5.45)	3		
6+ ppm	4.35 (1.41, 10.1)	5		
Cardboard manufacturing workers in Arnzburg, Germany				Henschler et al. ( <a href="#">1995</a> )
Exposed workers		Not reported		
Biologically-monitored Swedish workers				Axelson et al. ( <a href="#">1994</a> )
Any TCE exposure		Not reported		
Cardboard manufacturing workers, Atlanta area, Georgia				Sinks et al. ( <a href="#">1992</a> )
All subjects		Not reported		
<b>Cohort studies-mortality</b>				
Aerospace workers (Rocketdyne)				
Any TCE (utility/eng flush)		Not reported		Boice et al. ( <a href="#">2006b</a> )
Any exposure to TCE		Not reported		
View-Master employees				ATSDR ( <a href="#">2004a</a> )
Females		1.77 (0.57, 4.12) <sup>b</sup>	5	
United States uranium-processing workers (Fernald, Ohio)				Ritz ( <a href="#">1999a</a> )
Any TCE exposure		Not reported		
Light TCE exposure, >2-yr duration		Not reported		
Moderate TCE exposure, >2-yr duration		Not reported		
Aerospace workers (Lockheed)				Boice et al. ( <a href="#">1999</a> )
Routine exposure		-- (0.00, 5.47)	0	
Routine-intermittent		Not reported		
Aerospace workers (Hughes)				Morgan et al. ( <a href="#">1998</a> )
TCE subcohort		(0.00, 1.07)	0 (3.5 exp)	
Low intensity (<50 ppm)			0 (1.91 exp)	
High intensity (>50 ppm)			0 (1.54 exp)	

**Table 4-92. Summary of human studies on TCE exposure and cervical cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
Aircraft maintenance workers (Hill Air Force Base, Utah)				
TCE subcohort	TCE subcohort	1.8 (0.5, 6.5) <sup>c</sup>	5	Blair et al. (1998)
	Cumulative exposure			
	0	1.0 <sup>c</sup>		
	<5 ppm-yr	0.9 (0.1, 8.3)	1	
	5–25 ppm-yr		0	
	>25 ppm-yr	3.0 (0.8, 11.7)	4	
TCE subcohort	TCE subcohort	1.67 (0.54, 5.22)	6	Radican et al. (2008)
	Cumulative exposure			
	0	1.0 <sup>c</sup>		
	<5 ppm-yr	0.76 (0.09, 6.35)	1	
	5–25 ppm-yr		0	
	>25 ppm-yr	2.83 (0.86, 9.33)	5	
Cardboard manufacturing workers in Arnsburg, Germany				
	TCE exposed workers	Not reported		Henschler et al. (1995)
	Unexposed workers	Not reported		
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		Not examined <sup>d</sup>		Greenland et al. (1994)
Cardboard manufacturing workers, Atlanta area, Georgia		Not reported		Sinks et al. (1992)
U.S. Coast Guard employees		Not reported <sup>e</sup>		Blair et al. (1989)
Aircraft manufacturing plant employees (Italy)		Not reported <sup>e</sup>		Costa et al. (1989)
Aircraft manufacturing plant employees (San Diego, California)				
	All subjects	0.61 (0.25, 1.26) <sup>f</sup>	7	Garabrant et al. (1988)
Lamp manufacturing workers (GE)				
	Coil/wire drawing	1.05 (0.03, 5.86)	1	Shannon et al. (1988)
	Other areas	1.16 (0.32, 2.97)	4	

**Table 4-92. Summary of human studies on TCE exposure and cervical cancer (continued)**

Exposure group	RR (95% CI)	Number of observable events	Reference
<b>Case-control studies</b>			
<b>Geographic-based studies</b>			
Residents in two study areas in Endicott, New York	1.06 (0.29, 2.71)	<6	ATSDR (2006a)
Residents in Texas			Coyle et al. (2005)
Counties reporting any air TCE release	66.4 <sup>g</sup>		
Countries not reporting any air TCE release	60.8 <sup>g</sup>		
Residents of 13 census tracts in Redlands, California	0.65 (0.38, 1.02)	29	Morgan and Cassady (2002)
Finnish residents			Vartiainen et al. (1993)
Residents of Hausjarvi	Not reported		
Residents of Huttula	Not reported		

<sup>a</sup>SIR for females in Sung et al. (2007) reflects a 15-year lag period.

<sup>b</sup>PMR.

<sup>c</sup>Internal referents, workers not exposed to TCE.

<sup>d</sup>Nested case-control analysis.

<sup>e</sup>Males only in cohort.

<sup>f</sup>SMR is for cancer of the genital organs (cervix, uterus, endometrium, etc.).

<sup>g</sup>Median annual age-adjusted breast cancer rate (1995–2000).

#### 4.8.2.1.1. Prostate cancer

Sixteen cohort or PMR studies, two nested case-control, one population case-control, and two geographic-based studies present RR estimates for prostate cancer (Radican et al., 2008; Krishnadasan et al., 2007; ATSDR, 2006a; Boice et al., 2006b; Chang et al., 2005; ATSDR, 2004a; Chang et al., 2003; Raaschou-Nielsen et al., 2003; Morgan and Cassady, 2002; Hansen et al., 2001; Boice et al., 1999; Ritz, 1999a; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994; Siemiatycki, 1991; Blair et al., 1989; Garabrant et al., 1988; Shannon et al., 1988; Wilcosky et al., 1984). Three small cohort studies (Henschler et al., 1995; Sinks et al., 1992; Costa et al., 1989), one multiple-site population case-control (Siemiatycki, 1991), and one geographic-based study (Vartiainen et al., 1993) do not report estimates for prostate cancer in their published papers. Twelve of the 19 studies with prostate cancer RR estimates had high likelihood of TCE exposure in individual study subjects and were judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis (Radican et al., 2008; Krishnadasan et al., 2007; Boice et al., 2006b; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Morgan et al., 2000; Boice et al., 1999; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994; Siemiatycki, 1991). Krishnadasan et al. (2007), in their nested case-control study of prostate cancer, observed a twofold OR estimate with high cumulative TCE exposure score (2.4, 95% CI: 1.3, 4.4, 20-year lagged exposure) and an increasing positive relationship between prostate

cancer incidence and TCE cumulative exposure score ( $p = 0.02$ ). TCE exposure was positively correlated with several other occupational exposures, and Krishnadasan et al. (2007) adjusted for possible confounding from all other chemical exposures as well as age at diagnosis, occupational physical activity, and SES status in statistical analyses. RR estimates in studies other than Krishnadasan et al. (2007) were  $>1.0$  for overall TCE exposure [1.8, 95% CI: 0.8, 4.0 (Siemiatycki, 1991); 1.1, 95% CI: 0.6, 1.8 (Blair et al., 1998) and 1.20, 95% CI: 0.92, 1.76, with an additional 10-year follow-up (Radican et al., 2008); 1.58, 95% CI: 0.96, 2.62 (Morgan et al., 2000, 1998; EHS, 1997); 1.3, 95% CI: 0.52, 2.69 (Boice et al., 1999); 1.38, 95% CI: 0.73, 2.35 (Anttila et al., 1995)] and prostate cancer risks did not appear to increase with increasing exposure. Four studies observed RR estimates below 1.0 for overall TCE exposure (0.93, 95% CI: 0.60, 1.37 (Garabrant et al., 1988); 0.6, 95% CI: 0.2, 1.3 (Hansen et al., 2001); 0.9, 95% CI: 0.79, 1.08 (Raaschou-Nielsen et al., 2003); 0.82, 95% CI: 0.36, 1.62 (Boice et al., 2006b), and are not considered inconsistent because alternative explanations are possible and included observations are based on few subjects, lowering statistical power, or to poorer exposure assessment approaches that may result in a higher likelihood of exposure misclassification.

Seven other cohort, PMR, and geographic-based studies were given less weight in the analysis because of their lesser likelihood of TCE exposure and other study design limitations that would decrease statistical power and study sensitivity (ATSDR, 2006a; Chang et al., 2005; ATSDR, 2004a; Morgan and Cassady, 2002; Blair et al., 1989; Shannon et al., 1988; Wilcosky et al., 1984). Chang et al. (2005) observed a statistically significant deficit in prostate cancer risk, based on one case, and an insensitive exposure assessment (0.14, 95% CI: 0.00, 0.76). Relative risks in the other five studies ranged from 0.62 (CI not presented in paper) (Wilcosky et al., 1984) to 1.11 (95% CI: 0.98, 1.25) (Morgan and Cassady, 2002).

Risk factors for prostate cancer include age, family history of prostate cancer, and ethnicity as causal with inadequate evidence for a relationship with smoking or alcohol (Wigle et al., 2008). All studies except Krishnadasan et al. (2007) were not able to adjust for possible confounding from other chemical exposures in the work environment. None of the studies including Krishnadasan et al. (2007) accounted for other well-established nonoccupational risk factors for prostate cancer such as race, prostate cancer screening, and family history. There is limited evidence that physical activity may provide a protective effect for prostate cancer (Wigle et al., 2008). Krishnadasan et al. (2008) examined the effect of physical activity in the Rocketdyne aerospace cohort (Krishnadasan et al., 2007; Zhao et al., 2005). Their finding of a protective effect with high physical activity (0.55, 95% CI: 0.32, 0.95,  $p$  trend = 0.04) after control for TCE exposure provides additional evidence (Krishnadasan et al., 2008) and suggests that underlying risk may be obscured in studies lacking adjustment for physical activity.

#### 4.8.2.1.2. Breast cancer

Fifteen studies of TCE exposure reported findings on breast cancer in males and females combined ([Boice et al., 1999](#); [Greenland et al., 1994](#); [Garabrant et al., 1988](#)), in males and females, separately ([Clapp and Hoffman, 2008](#); [ATSDR, 2004a](#); [Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#)), or in females only ([Radican et al., 2008](#); [Sung et al., 2007](#); [ATSDR, 2006a](#); [Chang et al., 2005](#); [Coyle et al., 2005](#); [Blair et al., 1998](#); [Morgan et al., 1998](#); [Shannon et al., 1988](#)). Six studies have high likelihood of TCE exposure in individual study subjects and met, to a sufficient degree, the standards of epidemiologic design and analysis ([Radican et al., 2008](#); [Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#); [Boice et al., 1999](#); [Blair et al., 1998](#); [Morgan et al., 1998](#)). Four studies with risk estimates for other cancer sites did not report risk estimates for breast cancer ([Boice et al., 2006b](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#); [Siemiatycki, 1991](#)). No case-control studies were found on TCE exposure, although several studies examined occupational title or organic solvent as a class ([Ji et al., 2008](#); [Rennix et al., 2005](#); [Band et al., 2000](#); [Weiderpass et al., 1999](#)). While association is seen with occupational title or industry and breast cancer [employment in aircraft and aircraft part industry, 2.48, 95% CI: 1.14, 5.39 ([Band et al., 2000](#)); solvent user: 1.48, 95% CI: 1.03, 2.12 ([Rennix et al., 2005](#))], TCE exposure is not uniquely identified. The two studies suggest that an association between organic solvents and female breast cancer needs further investigation of possible risk factors.

Relative risk estimates in the five studies in which there is a high likelihood of TCE exposure in individual study subjects and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review ranged from 0.75 (0.43, 1.22) (females and males; ([Morgan et al., 1998](#))) to 2.0 (0.9, 4.6) (mortality in females; ([Blair et al., 1998](#))). Blair et al. ([1998](#)) additionally observed stronger risk estimates for breast cancer mortality among females with low-level, intermittent (3.1, 95% CI: 1.5, 6.2) and low-level, continuous (3.4, 95% CI: 1.4, 8.0) TCE exposures, but not with frequent peaks (1.4, 95% CI: 0.7, 3.2). A similar pattern of risks was also observed by Radican et al. ([2008](#)) who studied mortality in this cohort and adding 10 years of follow-up, although the magnitude of breast cancer risk in females was lower than that observed in Blair et al. ([1998](#)). Risk estimates did not appear to increase with increasing cumulative exposure in the two studies that included exposure-response analyses ([Blair et al., 1998](#); [Morgan et al., 1998](#)). None of these five studies reported a statistically significant deficit in breast cancer and CIs on RRs estimates included 1.0 (no risk). Few female subjects in these studies appear to have high TCE exposure. For example, Blair et al. ([1998](#)) identified 8 of the 28 breast cancer deaths and 3 of the 34 breast cancer cases with high cumulative exposure.

Relative risk estimates in six studies of lower likelihood TCE exposure and other design deficiencies ranged from 0.81 (95% CI: 0.52, 1.48) ([Garabrant et al., 1988](#)) to 1.19 (1.03, 1.36) ([Chang et al., 2005](#)). These studies lack a quantitative surrogate for TCE exposure to individual subjects and instead classify all subjects as “potentially exposed,” with resulting large dilution of

actual risk and decreased sensitivity ([Sung et al., 2007](#); ATSDR, 2006a; [NRC, 2006](#); [Chang et al., 2005](#); [Morgan and Cassady, 2002](#); [Garabrant et al., 1988](#); [Shannon et al., 1988](#)).

Four studies reported on male breast cancer separately ([Clapp and Hoffman, 2008](#); [ATSDR, 2004a](#); [Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#)) and a total of three cases were observed. Breast cancer in men is a rare disease and is best studied using a case-control approach ([Weiss et al., 2005](#)). Reports exist of male breast cancer among former residents of Camp Lejuene ([ATSDR, 2010, 2007b](#)). Further assessment of TCE exposure and male breast cancer is warranted.

Overall, the epidemiologic studies on TCE exposure and breast cancer are quite limited in statistical power; observations are based on few breast cancer cases or on inferior TCE exposure assessment in studies with large numbers of observed cases. Additionally, adjustment for nonoccupational breast cancer risk factors is less likely in cohort and geographic-based studies given their use of employment and public records. Breast cancer mortality observations in Blair et al. ([1998](#)) and further follow-up of this cohort by Radican et al. ([2008](#)) of an elevated risk with overall TCE exposure, particularly low-level intermittent and continuous TCE exposure, provide evidence of an association with TCE. No other study with high likelihood of TCE exposure in individual study subjects reported a statistically significant association with breast cancer, although few observed cases leading to lower statistical power or examination of risk for males and females combined are alternative explanations for the null observations in these studies. Both Chang et al. ([2005](#)) and Sung et al. ([2007](#)), two overlapping studies of female electronics workers exposed to TCE, perchloroethylene, and mixed solvents, reported association with breast cancer incidence, with breast cancer risk in Chang et al. ([2005](#)) appearing to increase with employment duration. Both studies, in addition to association provided by studies of exposure to broader category of organic solvents ([Rennix et al., 2005](#); [Band et al., 2000](#)), support Blair et al. ([1998](#)) and Radican et al. ([2008](#)), although the lack of exposure assessment is an uncertainty. The epidemiologic evidence is limited for examining TCE and breast cancer, and while these studies do not provide any strong evidence for association with TCE exposure, they in turn do not provide evidence of an absence of association.

#### **4.8.2.1.3. Cervical cancer**

Eleven cohort or PMR studies and two geographic-based studies present RR estimates ([Radican et al., 2008](#); [Sung et al., 2007](#); ATSDR, 2006a, [2004a](#); [Raaschou-Nielsen et al., 2003](#); [Morgan and Cassady, 2002](#); [Hansen et al., 2001](#); [Boice et al., 1999](#); [Blair et al., 1998](#); [Morgan et al., 1998](#); [Anttila et al., 1995](#); [Garabrant et al., 1988](#); [Shannon et al., 1988](#)). Seven of these studies had high likelihood of TCE exposure in individual study subjects and were judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis ([Radican et al., 2008](#); [Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#); [Boice et al., 1999](#); [Blair et al., 1998](#); [Morgan et al., 1998](#); [Anttila et al., 1995](#)). Three small cohort studies ([Henschler et al.,](#)

[1995](#); [Sinks et al., 1992](#); [Costa et al., 1989](#)) as well as three studies with high likelihood of TCE exposure in individual study subjects ([Boice et al., 2006b](#); [Zhao et al., 2005](#); [Axelson et al., 1994](#)) did not present RR estimates for cervical cancer. Additionally, one population case-control and one geographic study of several site-specific cancers did not present information on cervical cancer ([Vartiainen et al., 1993](#); [Siemiatycki, 1991](#)).

Five studies with high likelihood of TCE exposure in individual study subjects and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review observed elevated risk for cervical cancer and overall TCE exposure [2.42, 95% CI: 1.05, 4.77 ([Anttila et al., 1995](#)); 1.8, 95% CI: 0.5, 6.5 ([Blair et al., 1998](#)) that changed little with an additional 10 years follow-up, 1.67, 95% CI: 0.54, 5.22 ([Radican et al., 2008](#)); 3.8, 95% CI: 1.0, 9.8 ([Hansen et al., 2001](#)); 1.9, 95% CI: 1.42, 2.37 ([Raaschou-Nielsen et al., 2003](#))]. The observations of a three- to fourfold elevated cervical cancer risk with high mean TCE exposure compared to subjects in the low exposure category [6+ ppm: 4.35, 95% CI: 1.41, 10.1 ([Anttila et al., 1995](#)); 4+ ppm: 4.3, 95% CI: 0.5, 16 ([Hansen et al., 2001](#))] or with high cumulative TCE exposure (0.25-ppm year: 3.0, 95% CI: 0.8, 11.7 ([Blair et al., 1998](#)), 2.83, 95% CI: 0.86, 9.33 ([Radican et al., 2008](#))) provide additional support for association with TCE. Cervical cancer risk was lowest for subjects in the high-exposure duration category ([Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#)); however, duration of employment is a poor exposure metric given that subjects may have differing exposure intensity with similar exposure duration ([NRC, 2006](#)). No deaths due to cervical cancer were observed in two other studies ([Boice et al., 1999](#); [Morgan et al., 1998](#)); less than four deaths were expected, suggesting that these cohorts contained few female subjects with TCE exposure.

Human papilloma virus and low SES status are known risk factors for cervical cancer ([American Cancer Society, 2008](#)). Subjects in Raaschou-Nielsen et al. (2003) are blue-collar workers and low SES status likely explains observed associations in this and the other studies. The use of internal controls in Blair et al. (1998) who are similar in SES status as TCE subjects is believed to partly account for possible confounder related to SES status; however, direct information on individual subjects is lacking.

Six other cohort, PMR, and geographic-based studies were given less weight in the analysis because of their lesser likelihood of TCE exposure and other study design limitations that would decrease statistical power and study sensitivity ([Sung et al., 2007](#); ATSDR, 2006a, 2004a; [Morgan and Cassady, 2002](#); [Garabrant et al., 1988](#); [Shannon et al., 1988](#)). Cervical cancer risk estimates in these studies ranged between 0.65 (95% CI: 0.38, 1.02) ([Morgan and Cassady, 2002](#)) and 1.77 [PMR; 95% CI: 0.57, 4.12 ([ATSDR, 2004a](#))]. No study reported a statistically significant deficit in cervical cancer risk.

#### 4.8.2.2. Animal Studies

Histopathology findings have been noted in reproductive organs in various cancer bioassay studies conducted with TCE. A number of these findings (summarized in Table 4-93) do not demonstrate a treatment-related profile.

**Table 4-93. Histopathology findings in reproductive organs**

<b>Tumor incidence in mice after 18 mo inhalation exposure<sup>a</sup></b>								
	<b>Tissue</b>	<b>Finding</b>	<b>Control</b>	<b>100 ppm</b>	<b>500 ppm</b>			
Males	Number examined:		30	29	30			
	Prostate	Myoma	1	0	0			
	Testis	Carcinoma	0	0	1			
		Cyst	0	0	1			
Females	Number examined:		29	30	28			
	Uterus	Adenocarcinoma	1	0	0			
	Ovary	Adenocarcinoma	1	0	0			
		Adenoma	3	1	3			
		Carcinoma	0	2	2			
		Granulosa cell tumor	4	0	2			
<b>Tumor incidence in rats after 18 mo inhalation exposure<sup>a</sup></b>								
	<b>Tissue</b>	<b>Finding</b>	<b>Control</b>	<b>100 ppm</b>	<b>500 ppm</b>			
Males	Number examined:		29	30	30			
	Testis	Interstitial cell tumors	4	0	3			
Females	Number examined:		28	30	30			
	Mammary	Fibroadenoma	2	0	0			
		Adenocarcinoma	3	2	2			
	Uterus	Adenocarcinoma	3	1	4			
	Ovary	Carcinoma	4	0	1			
		Granulosa cell tumor	1	0	0			
	Genital tract	Squamous cell carcinoma	0	2	0			
<b>Tumor incidence in hamsters after 18 mo inhalation exposure<sup>a</sup></b>								
	<b>Tissue</b>	<b>Finding</b>	<b>Control</b>	<b>100 ppm</b>	<b>500 ppm</b>			
Females	Number examined:		30	29	30			
	Ovary	Cystadenoma	1	0	0			
<b>Tumor incidence in mice after 18 mo gavage administration<sup>b</sup></b>								
	<b>Tissue</b>	<b>Finding</b>	<b>Control</b>	<b>TCE Pure</b>	<b>TCE Industrial</b>	<b>TCE + EPC</b>	<b>TCE + BO</b>	<b>TCE + EPC + BO</b>
Females	Number examined:		50	50	50	50	48	50
	Mammary	Carcinoma	1	2	0	0	0	0
	Ovary	Granulosa cell tumor	0	1	0	0	0	0
	Vulva	Squamous cell carcinoma	0	0	0	0	1	1

<sup>a</sup>Henschler et al. (1980).

<sup>b</sup>Henschler et al. (1984).

EPC = epichlorohydrin; BO = 1,2-epoxybutane

Cancers of the reproductive system that are associated with TCE exposure and observed in animal studies are comprised of testicular tumors (interstitial cell and Leydig cell). A summary of the incidences of testicular tumors observed in male rats is presented in Table 4-94.

**Table 4-94. Testicular tumors in male rats exposed to TCE, adjusted for reduced survival<sup>a</sup>**

<b>Interstitial cell tumors after 103-wk gavage exposure, beginning at 6.5–8 wks of age (NTP, 1988, 1990)</b>				
<b>Administered dose (mg/kg-d)</b>	<b>Untreated control</b>	<b>Vehicle control</b>	<b>500</b>	<b>1,000</b>
Male ACI rats	38/45 (84%)	36/44 (82%)	23/26 (88%)	17/19 (89%)
Male August rats	36/46 (78%)	34/46 (74%)	30/34 (88%)	26/30 (87%)
Male Marshall rats <sup>b</sup>	16/46 (35%)	17/46 (37%)	21/33 (64%)	32/39 (82%)
Male Osborne-Mendel rats	1/30 (3%)	0/28 (0%)	0/25 (0%)	1/19 (5%)
Male F344/N rats	44/47 (94%)	47/48 (98%)	47/48 (98%)	32/44 (73%)
<b>Leydig cell tumors after 104-wk inhalation exposure, beginning at 12 wks of age (Maltoni et al., 1986)</b>				
<b>Administered daily concentration (mg/m<sup>3</sup>)<sup>c</sup></b>	<b>Control</b>	<b>112.5</b>	<b>337.5</b>	<b>675</b>
Male Sprague-Dawley rats <sup>b</sup>	6/114 (5%)	16/105 (15%)	30/107 (28%)	31/113 (27%)

<sup>a</sup>ACI rats alive at week 70, August rats at week 65, Marshall rats at week 32, Osborne-Mendel rats at week 97, F344/N rats at week 32, Sprague-Dawley rats at week 81 (except BT304) or week 62 (except BT304 bis).

<sup>b</sup>Equivalent to 100, 300, or 600 ppm (100 ppm = 540 mg/m<sup>3</sup>), adjusted for 7 hours/day, 5 days/week exposure.

<sup>c</sup>Statistically significant by Cochran-Armitage trend test ( $p < 0.05$ ).

Sources: NTP (1988) Tables A2, C2, E2, G2; NTP (1990) Table A3; Maltoni et al. (1986) IV/IV Table 21, IV/V Table 21.

#### 4.8.2.3. Mode of Action for Testicular Tumors

The database for TCE does not include an extensive characterization of the mode of action for Leydig cell tumorigenesis in the rat, although data exist that are suggestive of hormonal disruption in male rats. A study by Kumar et al. (2000a) found significant decreases in serum testosterone concentration and in 17-β-HSD, G6PDH, and total cholesterol and ascorbic acid levels in testicular homogenate from male rats that had been exposed via inhalation to 376 ppm TCE for 12 or 24 weeks. In a follow-up study, Kumar et al. (2001b) also identified decreases in SDH in the testes of TCE-treated rats. These changes are markers of disruption to testosterone biosynthesis. Evidence of testicular atrophy, observed in the 2001 study by Kumar et al., as well as the multiple in vivo and in vitro studies that observed alterations in spermatogenesis and/or sperm function, could also be consistent with alterations in testosterone levels. Therefore, while the available data are suggestive of a mode of action involving hormonal disruption for TCE-induced testicular tumors, the evidence is inadequate to specify and test a hypothesized sequence of key events.

Leydig cell tumors can be chemically induced by alterations of steroid hormone levels, through mechanisms such as agonism of estrogen, gonadotropin releasing hormone, or dopamine receptors; antagonism of androgen receptors; and inhibition of 5 $\alpha$ -reductase, testosterone biosynthesis, or aromatase ([Cook et al., 1999](#)). For those plausible mechanisms that involve disruption of the hypothalamic-pituitary-testis (HPT) axis, decreased testosterone or estradiol levels or recognition is involved, and increased LH levels are commonly observed. Although there is evidence to suggest that humans are quantitatively less sensitive than rats in their proliferative response to LH, evidence of treatment-related Leydig cell tumors in rats that are induced via HPT disruption is considered to represent a potential risk to humans (with the possible exception of GnRh or dopamine agonists), since the pathways for regulation of the HPT axis are similar in rats and humans ([Clegg et al., 1997](#)).

### **4.8.3. Developmental Toxicity**

An evaluation of the human and experimental animal data for developmental toxicity, considering the overall weight and strength of the evidence, suggests a potential for adverse outcomes associated with pre- and/or postnatal TCE exposures.

#### **4.8.3.1. Human Developmental Data**

Epidemiological developmental studies (summarized in Table 4-95) examined the relationship between TCE exposure and prenatal developmental outcomes including spontaneous abortion and perinatal death; decreased birth weight, small for gestational age (SGA), and postnatal growth; congenital malformations; and other adverse birth outcomes. Postnatal developmental outcomes examined include developmental neurotoxicity, developmental immunotoxicity, other developmental outcomes, and childhood cancer.

##### **4.8.3.1.1. Adverse fetal/birth outcomes**

###### **4.8.3.1.1.1. Spontaneous abortion and perinatal death**

Spontaneous abortion or miscarriage is defined as nonmedically induced premature delivery of a fetus prior to 20 weeks of gestation. Perinatal death is defined as stillbirths and deaths before 7 days after birth. Available data comes from several studies of occupational exposures in Finland and Santa Clara, California, and by geographic-based studies in areas with known contamination of water supplies in Woburn, Massachusetts; Tucson Valley, Arizona; Rocky Mountain Arsenal, Colorado; Endicott, New York; and New Jersey.

**Table 4-95. Developmental studies in humans**

Subjects	Exposure	Effect	Reference
<b>Adverse fetal/birth outcomes</b>			
Spontaneous abortion and perinatal death			
371 men occupationally exposed to solvents in Finland 1973–1983	Questionnaire Low/rare used <1 d/wk; Intermediate used 1–4 d/wk or intermediate/low TCA urine levels; High/frequent used daily or high TCA urine levels	No risk of spontaneous abortion after paternal exposure, based on 17 cases and 35 controls exposed to TCE OR: 1.0, 95% CI: 0.6–2.0	Taskinen et al. (1989)
535 women occupationally exposed to solvents in Finland 1973–1986	Questionnaire Rare used 1–2 d/wk; Frequent used ≥3 d/wk	Increased risk of spontaneous abortion among frequently-exposed women, based on 7 cases and 9 controls exposed to TCE OR: 1.6, 95% CI: 0.5–4.8	Taskinen et al. (1994)
3,265 women occupationally exposed to organic solvents in Finland 1973–1983	Questionnaire U-TCA: median: 48.1 µmol/L; mean 96.2 ± 19.2 µmol/L	No increased risk of spontaneous abortion based on 3 cases and 13 controls exposed to TCE OR: 0.6, 95% CI: 0.2–2.3	Lindbohm et al. (1990)
361 women occupationally and residentially exposed to solvents in Santa Clara County, California June 1986–February 1987 (735 controls)	Questionnaire	Increased risk of spontaneous abortion based on 6 cases and 4 controls exposed to TCE <sup>a</sup> OR: 3.1, 95% CI: 0.92–10.4	Windham et al. (1991)
4,396 pregnancies among residents of Woburn, Massachusetts 1960–1982	TCE: 267 µg/L Tetrachloroethylene: 21 µg/L Chloroform: 12 µg/L	Increased risk of perinatal death (n = 67) after 1970 (p = 0.55) but not before 1970 (OR: 10, p = 0.003) No increased risk of spontaneous abortion (n = 520; p = 0.66)	Lagakos et al. (1986)
707 parents of children with congenital heart disease in Tucson Valley, Arizona 1969–1987	6–239 ppb TCE, along with DCA and chromium	No increased risk of fetal death (not quantified) based on 246 exposed and 461 unexposed cases	Goldberg et al. (1990)
75 men and 71 women living near Rocky Mountain Arsenal, Colorado 1981–1986	Low: <5.0 ppb Medium: ≥5.0–<10.0 ppb High: <10.0 ppb	Increased risk of miscarriage OR <sub>adj</sub> : 4.44, 95% CI: 0.76–26.12 Increased risk of no live birth OR <sub>adj</sub> : 2.46, 95% CI: 0.24–24.95	ATSDR (2001)

**Table 4-95. Developmental studies in humans (continued)**

Subjects	Exposure	Effect	Reference
1,440 pregnancies among residents of Endicott, New York 1978–2002	Indoor air from soil vapor: 0.18–140 mg/m <sup>3</sup>	No increase in spontaneous fetal death SIR: 0.66, 95% CI: 0.22–1.55	ATSDR (2008b, 2006a)
81,532 pregnancies among residents of 75 New Jersey towns 1985–1988 (3 control groups)	55 ppb TCE, along with many other compounds	No increased risk of fetal death for >10 ppb OR: 1.12	Bove (1996); Bove et al. (1995)
Decreased birth weight, SGA, and postnatal growth			
361 women occupationally and residentially exposed to solvents in Santa Clara County, California June 1986–February 1987 (735 controls)	Questionnaire	Increased risk of IUGR based on one case exposed to both TCE and tetrachloroethylene OR: 12.5	Windham et al. (1991)
3,462 births in Woburn, Massachusetts 1960–1982	267 µg/L TCE in drinking water, along with tetrachloroethylene and chloroform	No increase in low birth weight ( <i>p</i> = 0.77)	Lagakos et al. (1986)
1,099 singleton births <sup>b</sup> to residents of three census tracts near Tucson International Airport 1979–1981 (877 controls)	<5–107 µg/L	No increase in full-term low birth weight (OR: 0.81) No increase in low birth weight (OR: 0.9) Increase in very low birth weight OR: 3.3, 95% CI: 0.53–20.6	Rodenbeck et al. (2000)
1,440 births <sup>c</sup> to residents of Endicott, New York 1978–2002	Indoor air from soil vapor: 0.18–140 mg/m <sup>3</sup>	Small increase in low birth weight OR: 1.26, 95% CI: 1.00–1.59 Small increase in SGA OR: 1.22, 95% CI: 1.02–1.45 Increase in full-term low birth weight OR: 1.41, 95% CI: 1.01–1.95	ATSDR (2008b, 2006a)
6,289 pregnancies among women residing at Camp Lejeune, North Carolina 1968–1985 (141 short-term and 31 long-term TCE-exposed, 5,681 unexposed controls) <sup>d</sup>	Tarrawa Terrace: TCE: 8 ppb 1,2-DCE: 12 ppb Perchloroethylene: 215 ppb Hadnot Point: TCE: 1,400 ppb 1,2-DCE: 407 ppb	Change in mean birth weight Long-term total: –139 g, 90% CI: –277, –1 Long-term males: –312 g, 90% CI: –540, –85 Short term total: +70g, 90% CI: –6, 146 Increase in SGA Long-term total: OR: 1.5, 90% CI: 0.5, 3.8 Long-term males: OR: 3.9, 90% CI: 1.1–11.9 Short term total: OR: 1.1, 90% CI: 0.2–1.1	ATSDR (1998a)

**Table 4-95. Developmental studies in humans (continued)**

Subjects	Exposure	Effect	Reference
81,532 pregnancies <sup>c</sup> among residents of 75 New Jersey towns 1985–1988	55 ppb TCE, along with many other compounds	Decreased birth weight at >5 ppb by 17.9g No increase in prematurity at >10 ppb: OR: 1.02 Increase in low birth weight, term >10 ppb: OR: 1.23, 50% CI: 1.09–1.39 No risk for very low birth weight	Bove (1996); Bove et al. (1995)
Congenital malformations			
1,148 men and 969 women occupationally exposed to TCE in Finland 1963–1976	U-TCA: <10 to >500 mg/L	No congenital malformations reported	Tola et al. (1980)
371 men occupationally exposed to solvents in Finland 1973–1983	Low/rare used <1 d/wk; Intermediate used 1–4 d/wk or if biological measures indicated high exposure; High/frequent used daily or if biological measures indicated high exposure	No increase in congenital malformations based on 17 cases and 35 controls exposed to TCE OR: 0.6, 95% CI: 0.2–2.0	Taskinen et al. (1989)
100 babies with oral cleft defects born to women occupationally exposed in Europe 1989–1992	Questionnaire	Increase in cleft lip based on 2 of 4 TCE-exposed women OR <sub>adj</sub> : 3.21, 95% CI: 0.49–20.9 Increase in cleft palate based on 2 of 4 TCE-exposed women OR <sub>adj</sub> : 4.47, 95% CI: 1.02–40.9	Lorente et al. (2000)
4,396 pregnancies among residents of Woburn, Massachusetts 1960–1982	TCE: 267 µg/L Tetrachloroethylene: 21 µg/L Chloroform: 12 µg/L	Increase in eye/ear birth anomalies: OR: 14.9, <i>p</i> < 0.0001 Increase in CNS/chromosomal/oral cleft anomalies: OR: 4.5, <i>p</i> = 0.01 Increase in kidney/urinary tract disorders: OR: 1.35, <i>p</i> = 0.02 Small increase in lung/respiratory tract disorders: OR: 1.16, <i>p</i> = 0.05 No increase in cardiovascular anomalies (n = 5): <i>p</i> = 0.91	Lagakos et al. (1986)
707 children with congenital heart disease in Tucson Valley, Arizona 1969–1987 (246 exposed, 461 unexposed)	Wells contaminated with TCE (range: 6–239 ppb), along with DCA and chromium	Increase in congenital heart disease <1981: OR: ≈3 ( <i>p</i> < 0.005) >1981: OR: ≈1 Increased prevalence after maternal exposure during first trimester ( <i>p</i> < 0.001, 95% CI: 1.14–4.14)	Goldberg et al. (1990)
75 men, 71 women living near Rocky Mountain Arsenal, Colorado 1981–1986	Low: <5.0 ppb Medium: ≥5.0–<10.0 ppb High: <10.0 ppb	Increase in total birth defects (n = 9) OR: 5.87, 95% CI: 0.59–58.81	ATSDR (2001)

**Table 4-95. Developmental studies in humans (continued)**

Subjects	Exposure	Effect	Reference
Births to residents of Endicott, New York 1983–2000 <sup>f</sup>	Indoor air from soil vapor: 0.18–140 mg/m <sup>3</sup>	No increase in total birth defects: RR: 1.08, 95% CI: 0.82–1.42 Increase in total cardiac defects: RR: 1.94, 95% CI: 1.21–3.12 Increase in major cardiac defects: RR: 2.52, 95% CI: 1.2–5.29 Increase in conotruncal heart defects: RR: 4.83, 95% CI: 1.81–12.89	ATSDR (2008b, 2006a)
81,532 pregnancies among residents of 75 New Jersey towns 1985–1988	55 ppb TCE, along with many other compounds	No increase in total birth defects: >10 ppb: OR: 1.12 Increase in total CNS defects at high dose >1–5 ppb: OR: 0.93, 90% CI: 0.47–1.77 >10 ppb: OR: 1.68, 90% CI: 0.76–3.52 Increase in neural tube defects >1–5 ppb: OR: 1.58, 90% CI: 0.69–3.40 >10 ppb: OR: 2.53, 90% CI: 0.91–6.37 Increase in oral clefts: >5 ppb: OR: 2.24, 95% CI: 1.16–4.20 Increase in major cardiac defects: >10 ppb: OR: 1.24, 50% CI: 0.75–1.94 Increase in ventricular septal defects >5ppb: OR: 1.30, 95% CI: 0.88–1.87	Bove (1996); Bove et al. (1995)
1,623 children <20 yrs old dying from congenital anomalies in Maricopa County, Arizona 1966–1986	8.9 and 29 ppb TCE in drinking water	Increase in deaths due to congenital anomalies in East Central Phoenix 1966–1969: RR: 1.4, 95% CI: 1.1–1.7 1970–1981: RR: 1.5, 95% CI: 1.3–1.7 1982–1986: RR: 2.0, 95% CI: 1.5–2.5	AZ DHS (Flood, 1988)
4,025 infants born with congenital heart defects in Milwaukee, Wisconsin 1997–1999	Maternal residence within 1.32 miles from at least one TCE emissions source	Increase in congenital heart defects for mothers ≥38 yrs old Exposed: OR: 6.2, 95% CI: 2.6–14.5 Unexposed: OR: 1.9, 95% CI: 1.1–3.5 No increase in congenital heart defects for exposed mothers <38 yrs old: OR: 0.9, 95% CI: 0.6–1.2	Yauck et al. (2004)
12 children exposed to TCE in well water in Michigan	5–10 yrs to 8–14 ppm	One born with multiple birth defects	Bernad et al. (1987), abstract
Other adverse birth outcomes			
34 live births for which inhalation of TCE for anesthesia was used in Japan 1962–1967	2–8 mL (mean 4.3 mL) for 2–98 min (mean: 34.7 min)	One case of asphyxia; 3 “sleepy babies” with Apgar scores of 5–9; delayed appearance of newborn reflexes	Beppu (1968)

**Table 4-95. Developmental studies in humans (continued)**

Subjects	Exposure	Effect	Reference
51 U.K. women whose fetus was considered to be at risk for hypoxia during labor administered TCE as an analgesic (50 controls)	Amount and route of exposure not reported	TCE caused fetal pH to fall more, base deficit increased more, and PO <sub>2</sub> fell more than the control group by fourfold or more compared to other analgesics used	Phillips and Macdonald ( <a href="#">1971</a> )
<b>Postnatal developmental outcomes</b>			
Developmental neurotoxicity			
54 individuals from 3 residential cohorts in the United States exposed to TCE in drinking water	Woburn, Massachusetts 63–400 ppb for <1–12 yrs Alpha, Ohio 3.3–330 ppb for 5–17 yrs Twin Cities, Minnesota 261–2,440 ppb for 0.25–25 yrs	Woburn, Massachusetts Verbal naming/language impairment in 6/13 children (46%) Alpha, Ohio Verbal naming/language impairment in 1/2 children (50%) Twin Cities, Minnesota Verbal naming/language impairment in 4/4 children (100%) Memory impairment in 4/4 children (100%) Academic impairment in 4/4 children (100%) Moderate encephalopathy in 4/4 children (100%) Poor performance on reading/spelling test in 3/4 children (75%) Poor performance on information test in 3/4 children (75%)	White et al. ( <a href="#">1997</a> )
284 cases of ASD diagnosed <9 yrs old and 657 controls born in the San Francisco Bay Area 1994	Births geocoded to census tracts, and linked to hazardous air pollutants data	Increase in ASD upper 3 <sup>rd</sup> quartile: OR: 1.37, 95% CI: 0.96–1.95 upper 4 <sup>th</sup> quartile: OR: 1.47, 95% CI: 1.03–2.08	Windham et al. ( <a href="#">2006</a> )
948 children (<18 yrs) in the TCE Subregistry	0.4–>5,000 ppb TCE	Increase in speech impairment: 0–9 yrs old: RR: 2.45, 99% CI: 1.31–4.58 10–17 yrs old: RR: 1.14, 99% CI: 0.46–2.85 Increase in hearing impairment: 0–9 yrs old: RR: 2.13, 99% CI: 1.12–4.07 10–17 yrs old: RR: 1.12, 99% CI: 0.52–2.24	ATSDR ( <a href="#">2002</a> ); Burg et al. ( <a href="#">1995</a> ); Burg and Gist ( <a href="#">1999</a> )
12 children exposed to TCE in well water in Michigan	5–10 yrs to 8–14 ppm	9 of 12 children (75%) had poor learning ability, aggressive behavior, and low attention span	Bernad et al. ( <a href="#">1987</a> ), abstract

**Table 4-95. Developmental studies in humans (continued)**

Subjects	Exposure	Effect	Reference
Developmental immunotoxicity			
200 children aged 36 mo old born prematurely <sup>g</sup> and at risk of atopy <sup>h</sup> in Leipzig, Germany 1995–1996	Median air level in child's bedroom: 0.42 µg/m <sup>3</sup>	No association with allergic sensitization to egg white and milk, or to cytokine producing peripheral T-cells	Lehmann et al. (2001)
85 healthy <sup>i</sup> full-term neonates born in Leipzig, Germany 1997–1999	Median air level in child's bedroom 3–4 wks after birth: 0.6 µg/m <sup>3</sup>	Significant reduction of Th1 IL-2 producing T-cells	Lehmann et al. (2002)
Other developmental outcomes			
55 children (6 mo to 10 yrs old) were anesthetized for operations to repair developmental defects of the jaw and face in Poland 1964	≥10 mL TCE	Reports of bradycardia, accelerated heart rate, and respiratory acceleration observed; no arrhythmia was observed	Jasinka (1965), translation
Childhood cancer			
98 children (<10 yrs old) diagnosed with brain tumors in Los Angeles County 1972–1977	Questionnaire of parental occupational exposures	Two cases were reported for TCE exposure, one with methyl ethyl ketone	Peters and Preston-Martin (1981)
22 children (<19 yrs old) diagnosed with neuroblastoma in United States and Canada 1992–1994 (12 controls)	Questionnaire of parental occupational exposures	Increase in neuroblastoma after paternal exposure OR: 1.4, 95% CI: 0.7–2.9 Maternal exposure not reported	De Roos et al. (2001)
61 boys and 62 girls (<10 yrs old) diagnosed with leukemia and 123 controls in Los Angeles County 1980–1984	Questionnaire of parents for occupational exposure	Increase in leukemia after paternal exposure Preconception (1 yr): OR: 2.0, <i>p</i> = 0.16 Prenatal: OR: 2.0, <i>p</i> = 0.16 Postnatal: OR: 2.7, <i>p</i> = 0.7 Maternal exposure not reported	Lowengart et al. (1987)
1,842 children (<15 yrs old) diagnosed with ALL in United States and Canada 1989–1993 (1986 controls)	Questionnaire of parents for occupational exposure	Increase in ALL after maternal exposure Preconception: OR: 1.8, 95% CI: 0.6–5.2 Pregnancy: OR: 1.8, 95% CI: 0.5–6.4 Postnatal: OR: 1.4, 95% CI: 0.5–4.1 Anytime: OR: 1.8, 95% CI: 0.8–4.1 No increase in ALL after paternal exposure Anytime: OR: 1.1, 95% CI: 0.8–1.5	Shu et al. (1999)

**Table 4-95. Developmental studies in humans (continued)**

Subjects	Exposure	Effect	Reference
109 children (<15 yrs old) born in the U.K. 1974–1988 (218 controls)	Questionnaire of parents for occupational exposure	Increase in leukemia and NHL after paternal exposure Preconception: OR: 2.27, 95% CI: 0.84–6.16 Prenatal: OR: 4.40, 95% CI: 1.15–21.01 Postnatal: OR: 2.66, 95% CI: 0.82–9.19 No increase in leukemia and NHL after maternal exposure Preconception: OR: 1.16, 95% CI: 0.13–7.91	McKinney et al. (1991)
22 children (<15 yrs old) diagnosed with childhood cancer in California 1988–1998	0.09–97 ppb TCE in drinking water	No increase in total cancer: SIR: 0.83, 99% CI: 0.44–1.40 No increase in CNS cancer: SIR: 1.05, 99% CI: 0.24–2.70 No increase in leukemia: SIR: 1.09, 99% CI: 0.38–2.31	Morgan and Cassady (2002)
1,190 children (<20 yrs old) diagnosed with leukemia in 4 counties in New Jersey 1979–1987	0–67 ppb TCE in drinking water	Increase in ALL in girls with >5 ppb exposure <20 yrs old: RR: 3.36, 95% CI: 1.29–8.28 <5 yrs old: RR: 4.54, 95% CI: 1.47–10.6	Cohn et al. (1994b)
24 children (<15 yrs old) diagnosed with leukemia in Woburn, Massachusetts 1969–1997	267 µg/L TCE in drinking water, along with tetrachloroethylene, arsenic, and chloroform	Increase in childhood leukemia Preconception: OR <sub>adj</sub> : 2.61, 95% CI: 0.47–14.97 Pregnancy: OR <sub>adj</sub> : 8.33, 95% CI: 0.73–94.67 Postnatal: OR <sub>adj</sub> : 1.18, 95% CI: 0.28–5.05 Ever: OR <sub>adj</sub> : 2.39, 95% CI: 0.54–10.59	Costas et al. (2002); Cutler et al. (1986); Lagakos et al. (1986); MDPH (1997c) <sup>j</sup>
347 children (<20 yrs old) diagnosed with cancer in Endicott, New York 1980–2001	indoor air from soil vapor: 0.18–140 mg/m <sup>3</sup>	No increase in cancer (<6 cases, similar to expected)	ATSDR (2008b, 2006a)
189 children (<20 yrs old) diagnosed with cancer in Maricopa County, Arizona 1965–1990	8.9 and 29 ppb TCE in drinking water	Increase in leukemia: 1965–1986: SIR: 1.67, 95% CI: 1.20–2.27 1982–1986: SIR: 1.91, 95% CI: 1.11–3.12 No increase in total childhood cancers, lymphoma, brain/CNS, or other cancers	AZ DHS (Flood, 1997a; Flood, 1988) (1990) <sup>k</sup>
16 children (<20 yrs old) diagnosed with cancer in East Phoenix, Arizona 1965–1986	TCE, TCA, and other contaminants in drinking water	No increase in leukemia: SIR: 0.85, 95% CI: 0.50–1.35	AZ DHS (Kioski et al., 1990b)

**Table 4-95. Developmental studies in humans (continued)**

Subjects	Exposure	Effect	Reference
37 children (<20 yrs old) diagnosed with cancer in Pima County, Arizona 1970–1986	1.1–239 ppb TCE, along with 1,1-DCE, chloroform and chromium in drinking water	Increase in leukemia (n = 11): SIR: 1.50, 95% CI: 0.76–2.70 No increase in testicular cancer (n = 6): SIR: 0.78, 95% CI: 0.32–1.59 No increase in lymphoma (n = 2): SIR: 0.63, 95% CI: 0.13–1.80 No increase in CNS/brain cancer (n = 3): SIR: 0.84, 95% CI: 0.23–2.16 Increase in other cancer (n = 15): SIR: 1.40, 95% CI: 0.79–2.30	AZ DHS ( <a href="#">Kioski et al., 1990a</a> )

<sup>a</sup>Of those exposed to TCE, four were also exposed to tetrachloroethylene and one was also exposed to paint strippers and thinners.

<sup>b</sup>Full term defined as between 35 and 46 weeks gestation, low birth weight as <2,501 g, and very low birth weight as <1,501 g.

<sup>c</sup>Low birth weight defined as <2,500, moderately low birth weight (1,500–<2,500 g), term low birth weight (≥37 weeks gestation and <25,000 g).

<sup>d</sup>Unexposed residents resided at locations not classified for long-term or short-term TCE exposure. Long-term TCE exposed mothers resided at Hospital Point during 1968–1985 for at least 1 week prior to birth. Short-term TCE exposed mothers resided at Berkeley Manor, Midway Park, Paradise Point, and Wakins Village at the time of birth and at least 1 week during January 27 to February 7, 1985. In addition, the mother’s last menstrual period occurred on or before January 31, 1985 and the birth occurred after February 2, 1985.

<sup>e</sup>Low birth weight defined as <2,500 g, very low birth weight as <1,500 g.

<sup>f</sup>1,440 births reported for years 1978–2002, but number not reported for years 1983–2000.

<sup>g</sup>Premature defined as 1,500–2,500 g at birth.

<sup>h</sup>Risk of atopy defined as cord blood IgE >0.9 kU/L; double positive family atopy history.

<sup>i</sup>Healthy birth defined as ≥2,500 g and ≥37 weeks gestation.

<sup>j</sup>Only results from Costas et al. ([2002](#)) are reported in the table.

<sup>k</sup>Only results from AZ DHS ([1990](#)) are reported in the table.

ALL = acute lymphoblastic leukemia; IUGR = intrauterine growth restriction

#### 4.8.3.1.1.1. Occupational studies

The risks of spontaneous abortion and congenital malformations among offspring of men occupationally exposed to TCE and other organic solvents were examined by Taskinen et al. (1989). This nested case-control study was conducted in Finland from 1973 to 1983. Exposure was determined by biological measurements of the father and questionnaires answered by both the mother and father. The level of exposure was classified as “low/rare” if the chemical was used <1 day/week, “intermediate” if used 1–4 days/week or if TCA urine measurements indicated intermediate/low exposure, and “high/frequent” if used daily or if TCA urine measurements indicated clear occupational exposure (defined as above the reference value for the general population). There was no risk of spontaneous abortion from paternal TCE exposure (OR: 1.0, 95% CI: 0.6–2.0), although there was a significant increase for paternal organic solvent exposure (OR: 2.7, 95% CI: 1.3–5.6) and a nonsignificant increase for maternal organic solvent exposure (OR: 1.4, 95% CI: 0.6–3.0). (Also see section below for results from this study for congenital malformations.)

Another case-control study in Finland examined pregnancy outcomes in 1973–1986 among female laboratory technicians aged 20–34 years (Taskinen et al., 1994). Exposure was reported via questionnaire, and was classified as “rare” if the chemical was used 1–2 days/week, and “frequent” if used at least 3 days/week. Cases of spontaneous abortion ( $n = 206$ ) were compared with controls who had delivered a baby and did not report prior spontaneous abortions ( $n = 329$ ). A nonstatistically significant increased risk was seen between spontaneous abortion and TCE use at least 3 days/week (OR: 1.6, 95% CI: 0.5–4.8).

The association between maternal exposure to organic solvents and spontaneous abortion was examined in Finland for births 1973–1983 (Lindbohm et al., 1990). Exposure was assessed by questionnaire and confirmed with employment records, and the level of exposure was either high, low, or none based on the frequency of use and known information about typical levels of exposure for job type. Biological measurements of TCA in urine were also taken on 64 women, with a median value of 48.1  $\mu\text{mol/L}$  (mean:  $96.2 \pm 19.2 \mu\text{mol/L}$ ). Three cases and 13 controls were exposed to TCE, with no increased risk seen for spontaneous abortion (OR: 0.6, 95% CI: 0.2–2.3,  $p = 0.45$ ).

A case-control study in Santa Clara County, California, examined the association between solvents and adverse pregnancy outcomes in women  $\geq 18$  years old (Windham et al., 1991). For pregnancies occurring between June 1986 and February 1987, 361 cases of spontaneous abortion were compared to 735 women who had a live birth during this time period. Telephone interviews included detailed questions on occupational solvent exposure, as well as additional questions on residential solvent use. For TCE exposure, 6 cases of spontaneous abortion were compared to 4 controls of live births; of these 10 TCE-exposed individuals, 4 reported exposure to tetrachloroethylene, and 1 reported exposure to paint strippers and thinners. An increased risk of spontaneous abortions was seen with TCE exposure (OR: 3.1,

95% CI: 0.92–10.4), with a statistically significant increased risk for those exposed  $\geq 0.5$  hours/week (OR: 7.7, 95% CI: 1.3–47.4). An increased risk for spontaneous abortion was also seen for those reporting a more “intense” exposure based primarily on odor, as well as skin contact or other symptoms (OR: 3.9,  $p = 0.04$ ). (Also see section below from this study on low birth weight.)

#### 4.8.3.1.1.1.2. Geographic-based studies

A community in Woburn, Massachusetts with contaminated well water experienced an increased incidence of adverse birth outcomes and childhood leukemia ([Lagakos et al., 1986](#)). In 1979, the wells supplying drinking water were found to be contaminated with 267 ppb TCE, 21 ppb tetrachloroethylene, and 12 ppb chloroform, and were subsequently closed. Pregnancy and childhood outcomes were examined from 4,396 pregnancies among residents ([Lagakos et al., 1986](#)). No association between water access and incidence of spontaneous abortion ( $n = 520$ ) was observed ( $p = 0.66$ ). The town’s water distribution system was divided into five zones, which was reorganized in 1970. Prior to 1970, no association was observed between water access and incidence of perinatal deaths ( $n = 46$  stillbirths and 21 deaths before 7 days) ( $p = 0.55$ ). However, after 1970, a statistically significant positive association between access to contaminated water and perinatal deaths was observed (OR: 10.0,  $p = 0.003$ ). The authors could not explain why this discrepancy was observed, but speculated that contaminants were either not present prior to 1970, or were increased after 1970. (Also see sections below on decreased birth weight, congenital malformations, and childhood cancer for additional results from this cohort.)

A community in Tucson Valley, Arizona with contaminated well water had a number of reported cases of congenital heart disease. The wells were found to be contaminated with TCE (range = 6–239 ppb), along with DCE and chromium ([Goldberg et al., 1990](#)). This study identified 707 children born with congenital heart disease during the years 1969–1987. Of the study participants, 246 families had parental residential and occupational exposure during 1 month prior to conception and during the first trimester of pregnancy, and 461 families had no exposure before the end of the first trimester. In addition to this control group, two others were used: (1) those that had contact with the contaminated water area, and (2) those that had contact with the contaminated water area and matched with cases for education, ethnicity, and occupation. Among these cases of congenital heart disease, no significant difference was seen for fetal death (not quantified) for exposed cases compared to unexposed cases. (Also see section below on congenital malformations for additional results from this cohort.)

A residential study of individuals living near the Rocky Mountain Arsenal in Colorado examined the outcomes in offspring of 75 men and 71 women exposed to TCE in drinking water ([ATSDR, 2001](#)). TCE exposure was stratified by high ( $>10.0$  ppb), medium ( $\geq 5.0$ – $<10.0$  ppb), and low ( $<5.0$  ppb). Among women with  $>5$  ppb exposure experiencing miscarriage ( $n = 22/57$ ) compared to unexposed women experiencing miscarriage ( $n = 2/13$ ) an elevated nonsignificant

association was observed (OR<sub>adj</sub>: 4.44, 95% CI: 0.76–26.12). For lifetime number of miscarriages reported by men and women, results were increased but without dose-response for women (medium: OR<sub>adj</sub>: 8.56, 95% CI: 0.69–105.99; high: OR<sub>adj</sub>: 4.16, 95% CI: 0.61–25.99), but less for men (medium: OR<sub>adj</sub>: 1.68, 95% CI: 0.26–10.77; high: OR<sub>adj</sub>: 0.65, 95% CI: 0.12–3.48). Among women with >5 ppb exposure experiencing no live birth (n = 9/57) compared to unexposed women experiencing no live birth (n = 1/13) an elevated nonsignificant association was observed (OR<sub>adj</sub>: 2.46, 95% CI: 0.24–24.95). (Also see below for results from this study on birth defects.)

NYS DOH and ATSDR conducted a study in Endicott, New York to examine childhood cancer and birth outcomes in an area contaminated by a number of VOCs, including “thousands of gallons” of TCE ([ATSDR, 2006a](#)). Soil vapor levels tested ranged from 0.18 to 140 mg/m<sup>3</sup> in indoor air. A follow-up study by ATSDR ([2008b](#)) reported that during the years 1978–1993 only five spontaneous fetal deaths occurring ≥20 weeks gestation were reported when 7.5 were expected (SIR: 0.66, 95% CI: 0.22–1.55). (See sections on low birth weight, congenital malformations, and childhood cancer for additional results from this cohort.)

Women were exposed to contaminated drinking water while pregnant and living in 75 New Jersey towns during the years 1985–1988 ([Bove, 1996](#); [Bove et al., 1995](#)). The water contained multiple trihalomethanes, including an average of 55 ppb TCE, along with tetrachloroethylene, 1,1,1-trichloroethane, carbon tetrachloride, 1,2-dichloroethane, and benzene. A number of birth outcomes were examined for 81,532 pregnancies, which resulted in 80,938 live births and 594 fetal deaths. No association was seen for exposure to >10 ppb TCE and fetal death (OR<sub>adj</sub>: 1.12). (See below for results from this study on decreased birth weight and congenital malformations.)

#### **4.8.3.1.1.2. Decreased birth weight, SGA, and postnatal growth**

Available data pertaining to birth weight and other growth-related outcomes come from the case-control study in Santa Clara, California (discussed above), and by geographic-based studies as well as geographic areas with known contamination of water supplies areas in Woburn, Massachusetts; Tucson, Arizona, Endicott, New York; Camp Lejeune, North Carolina; and New Jersey.

##### **4.8.3.1.1.2.1. Occupational studies**

The case-control study of the relationship between solvents and adverse pregnancy outcomes discussed above ([Windham et al., 1991](#)) also examined intrauterine growth restriction (IUGR). Telephone interviews included detailed questions on occupational solvent exposure, as well as additional questions on residential solvent use. An increased risk of IUGR was observed (OR: 12.5), although this was based only on one case that was exposed to both TCE and tetrachloroethylene (also see section above on spontaneous abortion).

#### 4.8.3.1.1.2.2. Geographic-based studies

The study of Woburn, Massachusetts with contaminated well water discussed above ([Lagakos et al., 1986](#)) examined birth weight. Of 3,462 live births surviving to 7 days, 220 were <6 pounds at birth (6.4%). No association was observed between water access and low birth weight ( $p = 0.77$ ). (See section on spontaneous abortion for study details, and see sections on spontaneous abortion, congenital malformations, and childhood cancer for additional results from this cohort.)

An ecological analysis of well water contaminated with TCE in Tucson and birth-weight was conducted by Rodenbeck et al. ([2000](#)). The source of the exposure was a U.S. Air Force plant and the Tucson International Airport. The wells were taken out of service in 1981 after concentrations of TCE were measured in the range of <5–107  $\mu\text{g/L}$ . The study population consisted of 1,099 babies born within census tracts between 1979 and 1981, and the comparison population consisted of 877 babies from nearby unexposed census tracts. There was a nonsignificant increased risk for maternal exposure to TCE in drinking water and very-low-birth-weight (<1,501 g) (OR: 3.3, 95% CI: 0.53–20.6). No increases were observed in the low-birth-weight (<2,501 g) (OR: 0.9) or full-term (>35–<46-week gestation) low-birth-weight (OR: 0.81).

The study of VOC exposure in Endicott, New York reported data on low birth weight and SGA ([ATSDR, 2006a](#)), see section on spontaneous abortion for study details). For births occurring during the years 1978–2002, low birth weight was slightly but statistically elevated (OR: 1.26, 95% CI: 1.00–1.59), as was SGA (OR: 1.22, 95% CI: 1.02–1.45), and full-term low birth weight (OR: 1.41, 95% CI: 1.01–1.95). (Also see sections on spontaneous abortion, congenital malformations, and childhood cancer for additional results from this cohort.)

Well water at the U.S. Marine Corps Base in Camp Lejeune, North Carolina was identified to be contaminated with TCE, tetrachloroethylene, and 1,2-dichloroethane in April, 1982 and the wells were closed in December, 1984. ATSDR examined pregnancy outcomes among women living on the base during the years 1968–1985 ([ATSDR, 1998a](#)). Compared to unexposed residents<sup>12</sup> ( $n = 5,681$ ), babies exposed to TCE long-term<sup>13</sup> ( $n = 31$ ) had a lower mean birth weight after adjustment for gestational age (-139 g, 90% CL = -277, -1), and babies exposed short-term<sup>14</sup> ( $n = 141$ ) had a slightly higher mean birth weight (+70 g, 90% CL = -6, 146). For the long-term group, no effect was seen for very low birth weight (<1,500 g) or prematurity (>5 ppb, OR: 1.05). No preterm births were reported in the long-term group and those ( $n = 8$ ) in the short-term group did not have an increased risk (OR: 0.7, 90% CI: 0.3–1.2).

---

<sup>12</sup>Unexposed residents resided at locations not classified for long- or short-term TCE exposure.

<sup>13</sup>Long-term TCE exposed mothers resided at Hospital Point during 1968–1985 for at least 1 week prior to birth.

<sup>14</sup>Short-term TCE exposed mothers resided at Berkeley Manor, Midway Park, Paradise Point, and Wakins Village at the time of birth and at least 1 week during January 27 to February 7, 1985. In addition, the mother's last menstrual period occurred on or before January 31, 1985 and the birth occurred after February 2, 1985.

A higher prevalence of SGA<sup>15</sup> was seen in the long-term exposed group (n = 3; OR 1.5, 90% CL: 0.5, 3.8) compared to the short-term exposed group (OR: 1.1, 90% CI: 0.2–1.1). When the long-term group was stratified by gender, male offspring were at more risk for both reduced birth weight (-312 g, 90% CL = -632, -102) and SGA (OR: 3.9, 90% CL: 1.1–11.8). This study is limited due to the mixture of chemicals in the water, as well as its small sample size. ATSDR is currently reanalyzing the findings because of an error in the exposure assessment related to the start-up date of a water treatment plant ([ATSDR, 2009, 2007a](#); [U.S. GAO, 2007a, b](#))

Pregnancy outcomes among women who were exposed to contaminated drinking water while pregnant and living in 75 New Jersey towns during the years 1985–1988 were examined by Bove et al. ([Bove, 1996](#); [Bove et al., 1995](#)). The water contained multiple trihalomethanes, including an average of 55 ppb TCE, along with tetrachloroethylene, 1,1,1-trichloroethane, carbon tetrachloride, 1,2-dichloroethane, and benzene. A number of birth outcomes were examined for 81,532 pregnancies, which resulted in 80,938 live births and 594 fetal deaths. A slight decrease of 17.9 g in birth weight was seen for exposure >5 ppb, with a slight increase in risk for exposure >10 ppb (OR: 1.23), but no effect was seen for very low birth weight or SGA/prematurity (>5 ppb, OR: 1.05). However, due to the multiple contaminants in the water, it is difficult to attribute the results solely to TCE exposure. (See below for results from this study on congenital malformations.)

#### **4.8.3.1.1.3. Congenital malformations**

Three studies focusing on occupational solvent exposure and congenital malformations from Europe provide data pertaining to TCE. Analyses of risk of congenital malformations were also included in the studies in the four geographic areas described above (Woburn, Massachusetts; Tucson, Arizona; Rocky Mountain Arsenal, Colorado; Endicott, New York; and New Jersey), as well as additional sites in Phoenix, Arizona; and Milwaukee, WI. Specific categories of malformations examined include cardiac defects, as well as cleft lip or cleft palate.

##### **4.8.3.1.1.3.1. Occupational studies**

A study of 1,148 men and 969 women occupationally exposed to TCE in Finland from 1963 to 1976 examined congenital malformations of offspring ([Toila et al., 1980](#)). U-TCA measurements available for 2,004 employees ranged from <10 to >500 mg/L, although 91% of the samples were <100 mg/L. No congenital malformations were seen in the offspring of women between the ages of 15–49 years, although 3 were expected based on the national incidence. Expected number of cases for the cohort could not be estimated because the number of pregnancies was unknown.

---

<sup>15</sup>The criteria for SGA being singleton births less than the 10<sup>th</sup> percentile of published sex-specific growth curves.

Men from Finland occupationally exposed to organic solvents including TCE did not observe a risk of congenital malformations from paternal organic solvent exposure based on 17 cases and 35 controls exposed to TCE (OR: 0.6, 95% CI: 0.2–2.0) ([Taskinen et al., 1989](#)). (Also see section above on spontaneous abortion for study details and additional results from this cohort.)

An occupational study of 100 women who gave birth to babies born with oral cleft defects and 751 control women with normal births were examined for exposure to a number of agents including TCE during the first trimester of pregnancy ([Lorente et al., 2000](#)). All women were participants in a multicenter European case-referent study whose children were born between 1989 and 1992. Four women were exposed to TCE, resulting in two cases of cleft lip (OR<sub>adj</sub>: 3.21, 95% CI: 0.49–20.9), and two cases of cleft palate (OR<sub>adj</sub>: 4.47, 95% CI: 1.02–40.9). Using logistic regression, the increased risk of cleft palate remained high (OR: 6.7, 95% CI: 0.9–49.7), even when controlling for tobacco and alcohol consumption (OR: 7.8, 95% CI: 0.8–71.8). However, the number of cases was small, and exposure levels were not known.

#### **4.8.3.1.1.3.2. Geographic-based studies**

A community in Woburn, Massachusetts with contaminated well water experienced an increased incidence of adverse birth outcomes and childhood leukemia ([Lagakos et al., 1986, see section on spontaneous abortion for study details](#)). Statistically significant positive association between access to contaminated water and eye/ear birth anomalies (OR: 14.9,  $p < 0.0001$ ), CNS/chromosomal/oral cleft anomalies (OR: 4.5,  $p = 0.01$ ), kidney/urinary tract disorders (OR: 1.35,  $p = 0.02$ ) and lung/respiratory tract disorders (OR: 1.16,  $p = 0.05$ ) were observed. There were also five cases of cardiovascular anomalies, but there was not a significant association with TCE ( $p = 0.91$ ). However, since organogenesis occurs during gestational weeks 3–5 in humans, some of these effects could have been missed if fetal loss occurred. (Also see sections on spontaneous abortion, perinatal death, decreased birth weight, and childhood cancer for additional results from this cohort.)

A high prevalence of congenital heart disease was found within an area of Tucson Valley, Arizona ([Goldberg et al., 1990, see section on spontaneous abortion for study details and additional results](#)). Of the total 707 case families included, 246 (35%) were exposed to wells providing drinking water found to be contaminated with TCE (range = 6–239 ppb), along with DCE and chromium. Before the wells were closed after the contamination was discovered in 1981, the OR of congenital heart disease was 3 times higher for those exposed to contaminated drinking water compared to those not exposed; after the wells were closed, there was no difference seen. This study observed 18 exposed cases of congenital heart disease when 16.4 would be expected (RR: 1.1). Prevalence of congenital heart disease in offspring after maternal exposure during the first trimester (6.8 in 1,000 live births) was significantly increased compared to nonexposed families (2.64 in 1,000 live births) ( $p < 0.001$ , 95% CI: 1.14–4.14). No

difference in prevalence was seen if paternal data were included, and there was no difference in prevalence by ethnicity. In addition, no significant difference was seen for cardiac lesions.

A residential study of individuals living near the Rocky Mountain Arsenal in Colorado examined the outcomes in offspring of 75 men and 71 women exposed to TCE in drinking water ([ATSDR, 2001](#)). The risk was elevated for the nine birth defects observed (OR: 5.87, 95% CI: 0.59–58.81), including one nervous system defect, one heart defect, and one incidence of cerebral palsy. The remaining cases were classified as “other,” and the authors speculate these may be based on inaccurate reports. (See above for study details and results on spontaneous abortion.)

The study of VOC exposure in Endicott, New York examined a number of birth defects during the years 1983–2000 ([ATSDR, 2006a](#)), see section on spontaneous for study details). These include total reportable birth defects, structural birth defects, surveillance birth defects, total cardiac defects, major cardiac defects, cleft lip/cleft palate, neural tube defects, and choanal atresia (blocked nasal cavities). There were 56 expected cases of all birth defects and 61 were observed resulting in no elevation of risk (rate ratio: 1.08, 95% CI: 0.82–1.42). There were no cases of cleft lip/cleft palate, neural tube defects, or choanal atresia. Both total cardiac defects (n = 15; rate ratio: 1.94, 95% CI: 1.21–3.12) and major cardiac defects (n = 6; rate ratio: 2.52, 95% CI: 1.2–5.29) were statistically increased. A follow-up study by ATSDR ([2008b](#)) reported that conotruncal heart malformations were particularly elevated (n = 4; rate ratio: 4.83, 95% CI: 1.81–12.89). The results remained significantly elevated (rate ratio: 3.74; 95% CI: 1.21–11.62) when infants with Down syndrome were excluded from the analysis. (Also see sections on spontaneous abortion, decreased birth weight, and childhood cancer for additional results from this cohort.)

In the New Jersey study described previously, the prevalence of birth defects reported by surveillance systems was examined among the women exposed to TCE and other contaminants in water while pregnant between 1985 and 1988 ([Bove, 1996](#); [Bove et al., 1995](#)). For exposure >10 ppb (n = 1,372), an increased risk, with relatively wide CIs, was seen for all birth defects (OR: 2.53, 95% CI: 0.77–7.34). An increased risk was also seen for CNS defects (>10 ppb: OR: 1.68), specifically 56 cases of neural tube defects (<1–5 ppb: 1.58, 95% CI: 0.61–3.85; >10 ppb: OR: 2.53, 95% CI: 0.77–7.34). A slight increase was seen in major cardiac defects (>10 ppb: OR: 1.24, 50% CI: 0.75–1.94), including ventricular septal defects (>5 ppb: OR: 1.30, 95% CI: 0.88–1.87). An elevated risk was seen for nine cases of oral clefts (<5 ppb: OR: 2.24, 95% CI: 1.04–4.66), although no dose-response was seen (>10 ppb, OR: 1.30). However, due to the multiple contaminants in the water, it is difficult to attribute the results solely to TCE exposure. (See above for results from this study on fetal death and decreased birth weight.)

Arizona Department of Health Services (AZ DHS) conducted studies of contaminated drinking water and congenital malformations (<20 years old) in Maricopa County, which encompasses Phoenix and the surrounding area ([Flood, 1988](#)). TCE contamination was

associated with elevated levels of deaths in children <20 years old due to total congenital anomalies in East Central Phoenix from 1966 to 1969 (RR: 1.4, 95% CI: 1.1–1.7), from 1970 to 1981 (RR: 1.5, 95% CI: 1.3–1.7), and from 1982 to 1986 (RR: 2.0, 95% CI: 1.5–2.5), as well as in other areas of the county. (See below for results from this study on childhood leukemia.)

A study was conducted of children born in 1997–1999 with congenital heart defects in Milwaukee, Wisconsin ([Yauck et al., 2004](#)). TCE emissions data were ascertained from state and EPA databases, and distance between maternal residence and the emission source was determined using a GIS. Exposure was defined as those within 1.32 miles from at least one site. Results showed that an increased risk of congenital heart defects was seen for the offspring of exposed mothers  $\geq 38$  years old (OR: 6.2, 95% CI: 2.6–14.5), although an increased risk was also seen for offspring of unexposed mothers  $\geq 38$  years old (OR: 1.9, 95% CI: 1.1–3.5), and no risk was seen for offspring of exposed mothers <38 years old (OR: 0.9, 95% CI: 0.6–1.2). The authors speculate that studies that did not find a risk only examined younger mothers. The authors also note that statistically significant increased risk was seen for mothers with preexisting diabetes, chronic hypertension, or alcohol use during pregnancy.

An abstract reported that 28 people living in a Michigan town were exposed for 5–10 years to 8–14 ppm TCE in well water ([Bernad et al., 1987, abstract](#)). One child was born with multiple birth defects, with no further details.

#### **4.8.3.1.1.4. Other adverse birth outcomes**

TCE was previously used as a general anesthetic during pregnancy. One study measured the levels of TCE in maternal and newborn blood after use during 34 vaginal childbirths ([Beppu, 1968](#)). TCE was administered through a vaporizer from two to 98 minutes (mean 34.7 minutes) at volumes of 2 to 8 mL (mean 4.3 mL). Mean blood TCE concentrations were  $2.80 \pm 1.14$  mg/dL in maternal femoral arteries;  $2.36 \pm 1.17$  mg/dL in maternal cubital veins;  $1.83 \pm 1.08$  mg/dL in umbilical vein; and  $1.91 \pm 0.95$  mg/dL in the umbilical arteries. A significant correlation was seen for maternal arterial blood and infants' venous blood, and the concentration of the fetal blood was lower than that of the mother. Of these newborns, one had asphyxia and three "sleepy babies" had Apgar scores of 5–9; however, these results could not be correlated to length of inhalation and there was no difference in the TCE levels in the mother or newborn blood compared to those without adverse effects. Discussion included delayed newborn reflexes (raising the head and buttocks, bending the spine, and sound reflex), blood pressure, jaundice, and body weight gain; however, the results were compared to newborns exposed to other compounds, not to an unexposed population. This study also examined the concentration of TCE in one mother at 22-weeks gestation exposed for four minutes, after which the fetus was "artificially delivered." Maternal blood concentration was 3.0 mg/dL, and 0.9 mg/dL of TCE was found in the fetal heart, but not in other organs.

Another study of TCE administered during childbirth to the mother as an analgesic examined perinatal measures, including fetal pH, fetal partial pressure carbon dioxide (PCO<sub>2</sub>) fetal base deficit, fetal partial pressure oxygen (PO<sub>2</sub>), Apgar scores, and neonatal capillary blood ([Phillips and Macdonald, 1971](#)). The study consisted of 152 women whose fetus was considered to be at risk for hypoxia during labor. Out of this group, 51 received TCE (amount and route of exposure not reported). TCE caused fetal pH to fall more, base deficit increased more, and PO<sub>2</sub> fell more than the control group by fourfold or more compared to other analgesics used.

#### **4.8.3.1.2. Postnatal developmental outcomes**

##### **4.8.3.1.2.1. Developmental neurotoxicity**

The studies examining neurotoxic effects from TCE exposure are discussed in Section 4.3, and the human developmental neurotoxic effects are reiterated here.

##### **4.8.3.1.2.1.1. Occupational studies**

An occupational study examined the neurodevelopment of the offspring of 32 women exposed to various organic solvents during pregnancy ([Laslo-Baker et al., 2004](#); [Till et al., 2001a](#)). Three of these women were exposed to TCE; however, no levels were measured and the results for examined outcomes are for total organic solvent exposure, and are not specific to TCE.

##### **4.8.3.1.2.1.2. Geographic-based studies**

A study of three residential cohorts (Woburn, Massachusetts; Alpha, Ohio; and Twin Cities, Minnesota) examined the neurological effects of TCE exposure in drinking water ([White et al., 1997](#)). For Woburn, Massachusetts, 28 individuals ranging from 9 to 55 years old were assessed, with exposure from a tanning factor and chemical plant at levels of 63–400 ppb for <1–12 years; the time between exposure and neurological examination was about 5 years. In this cohort, 6/13 children (46%) had impairments in the verbal naming/language domain. For Alpha, Ohio, 12 individuals ranging from 12 to 68 years old were assessed, with exposure from degreasing used at a manufacturing operation at levels of 3.3–330 ppb for 5–17 years; the time between exposure and neurological examination was 5–17 years. In this cohort, one of two children (50%) had impairments in the verbal naming/language domain. For Twin Cities, Minnesota, 14 individuals ranging from 8 to 62 years old were assessed, with exposure from an army ammunition plant at levels of 261–2,440 ppb for 0.25–25 years; the time between exposure and neurological examination was 4–22 years. In this cohort, four of four children (100%) had impairments in the verbal naming/language, memory, and academic domains and were diagnosed with moderate encephalopathy; and three of four children (75%) performed poorly on the WRAT-R Reading and Spelling and WAIS-R Information tests.

A case-control study was conducted to examine the relationship between multiple environmental agents and ASD ([Windham et al., 2006](#)). Cases (n = 284) and controls (n = 657) were born in 1994 in the San Francisco Bay Area. Cases were diagnosed before age 9 years. Exposure was determined by geocoding births to census tracts, and linking to hazardous air pollutants data. An elevated risk was seen for TCE in the upper 3<sup>rd</sup> quartile (OR: 1.37, 95% CI: 0.96–1.95), and a statistically significant elevated risk was seen for the upper 4<sup>th</sup> quartile (OR: 1.47, 95% CI: 1.03–2.08).

The TCE Subregistry ([Burg and Gist, 1999](#); [Burg et al., 1995](#)), including 948 children <18 years old from 13 sites located in 3 states, was examined for any association of ingestion of drinking water contaminated with TCE and various health effects ([ATSDR, 2003b](#); [Burg and Gist, 1999](#); [Burg et al., 1995](#)). Exposure groups included: (1) maximum TCE exposure; (2) cumulative TCE exposure; (3) cumulative chemical exposure; and (4) duration of exposure. Exposed children 0–9 years old had statistically increased hearing impairment compared to controls (RR: 2.13, 99% CI: 1.12–4.07), with children <5 years old having a 5.2-fold increase over controls. Exposed children 0–9 years old also had statistically increased speech impairment (RR: 2.45, 99% CI: 1.31–4.58). In addition, anemia and other blood disorders were statistically higher for males 0–9 years old. The authors noted that exposure could have occurred prenatally or postnatally. There was further analysis on the 116 exposed children and 182 controls who were under 10 years old at the time that the baseline study was conducted by ATSDR. This analysis did not find a continued association with speech and hearing impairment in these children; however, the absence of acoustic reflexes (contraction of the middle ear muscles in response to sound) remained significant ([ATSDR, 2003b](#)). No differences were seen when stratified by prenatal and postnatal exposure.

Twenty-eight people living in a Michigan town were exposed for 5–10 years to 8–14 ppm TCE in well water ([Bernad et al., 1987, abstract](#)). Ten adults and 12 children completed a questionnaire on neurotoxic endpoints. Nine of the 12 children had poor learning ability, aggressive behavior, and low attention span.

#### **4.8.3.1.2.2. Developmental immunotoxicity**

The studies examining human immunotoxic effects from TCE exposure are discussed in Section 4.6.1. The studies reporting developmental effects are reiterated briefly here.

Two studies focused on immunological development in children after maternal exposure to VOCs ([Lehmann et al., 2002](#); [Lehmann et al., 2001](#)). The first examined premature neonates (1,500–2,500 g) and neonates at risk of atopy (cord blood IgE >0.9 kU/L; double positive family atopy history) at 36 months of age ([Lehmann et al., 2001](#)). The median air level in children's bedrooms measured 0.42 µg/m<sup>3</sup>. There was no association with allergic sensitization to egg white and milk, or to cytokine producing peripheral T-cells. The second examined healthy, full-term neonates (≥2,500 g; ≥37 weeks gestation) born in Leipzig, Germany ([Lehmann et al., 2002](#)).

The median air level in the children's bedrooms 3–4 weeks after birth measured  $0.6 \mu\text{g}/\text{m}^3$ . A significant reduction of Th1 IL-2 producing T-cells was observed.

Byers et al. (1988) observed altered immune response in family members of children diagnosed with leukemia in Woburn, Massachusetts (Lagakos et al., 1986, see below for results of this study). The family members included 13 siblings under 19 years old at the time of exposure; however, an analysis looking at only these children was not done. This study is discussed in further detail in Section 4.6.1.

#### **4.8.3.1.2.3. Other developmental outcomes**

A study demonstrated the adverse effects of TCE used as an anesthetic in children during operations during 1964 in Poland to repair developmental defects of the jaw and face (Jasińska, 1965, translation). Fifty-five children ranging from 6 months to 10 years old were anesthetized with at least 10 mL TCE placed into an evaporator. Bradycardia occurred in two children, an accelerated heart rate of 20–25 beats per minute occurred in seven children, no arrhythmia was observed, and arterial blood pressure remained steady or dropped by 10 mmHg only. Respiratory acceleration was observed in 25 of the children, and was seen more in infants and younger children.

#### **4.8.3.1.2.4. Childhood cancer**

Several studies of parental occupational exposure were conducted in North America and the United Kingdom to determine an association with childhood cancer. A number of geographic-based studies were conducted in California; New Jersey; Woburn, Massachusetts; Endicott, New York; Phoenix, Arizona; and Tucson, Arizona. Specific categories of childhood cancers examined include leukemia, NHL, and CNS tumors.

##### **4.8.3.1.2.4.1. Occupational studies**

Brain tumors were observed in 98 children >10 years old at diagnosis from 1972–1977 in Los Angeles County (Peters et al., 1985; Peters et al., 1981). Exposure was determined by questionnaire. Two cases whose father had TCE exposure were reported: one case of oligodendroglioma in an 8-year-old whose father was a machinist, and astrocytoma in a 7-year-old whose father was an inspector for production scheduling and parts also exposed to methyl ethyl ketone (Peters et al., 1981). Peters et al. (1985) also briefly mentioned five cases of brain tumors in the offspring and no controls of paternal exposure to TCE (resulting in an inability to calculate an OR), but without providing any additional data.

A case-control study was conducted to assess an association between parental occupational exposure and neuroblastoma diagnosed in offspring <19 years old in the United States and Canada from May 1992 to April 1994 (De Roos et al., 2001). Paternal self-reported exposure to TCE was reported in 22 cases and 12 controls, resulting in an elevated risk of

neuroblastoma in the offspring (OR: 1.4, 95% CI: 0.7–2.9). Maternal exposure to TCE was not reported.

A case-control study of parental occupational exposure and childhood leukemia was conducted in Los Angeles County ([Lowengart et al., 1987](#)). Children (61 boys and 62 girls) diagnosed at <10 years old (mean age 4 years) from 1980 to 1984 were included in the analysis. Paternal occupation exposure to TCE was elevated for 1 year preconception (OR: 2.0,  $p = 0.16$ ), prenatal (OR: 2.0,  $p = 0.16$ ), and postnatal (OR: 2.7,  $p = 0.7$ ) exposure periods. Maternal exposure to TCE was not reported.

A case-control study children diagnosed with acute lymphoblastic leukemia (ALL) examined parental occupational exposure to hydrocarbons in the United States and Canada ([Shu et al., 1999](#)). Children were under the age of 15 years at diagnosis during the years 1989–1993. Cases were confirmed with a bone marrow sample. Questionnaires on maternal and paternal exposures were given to 1,842 case-control pairs, resulting in 15 cases and 9 controls maternally exposed and 136 cases and 104 controls paternally exposed to TCE. There was an increased but nonsignificant risk for maternal exposure to TCE during preconception (OR: 1.8, 95% CI: 0.6–5.2), pregnancy (OR: 1.8, 95% CI: 0.5–6.4), postnatally (OR: 1.4, 95% CI: 0.5–4.1), or any of these periods (OR: 1.8, 95% CI: 0.8–4.1). However, there was no increased risk for paternal exposure to TCE.

Occupational exposure in communities in the United Kingdom was examined to determine an association with leukemia and NHL diagnosed in the offspring ([McKinney et al., 1991](#)). Paternal occupational exposure was elevated for exposure occurring during preconception (OR: 2.27, 95% CI: 0.84–6.16), prenatal (OR: 4.40, 95% CI: 1.15–21.01), and postnatal (OR: 2.66, 95% CI: 0.82–9.19) exposure periods. Risk from maternal preconception exposure was not elevated (OR: 1.16, 95% CI: 0.13–7.91). However, the number of cases examined in this study was low, particularly for maternal exposure.

#### **4.8.3.1.2.4.2. Geographic-based studies**

A California community exposed to TCE (0.09–97 ppb) in drinking water from contaminated wells was examined for cancer ([Morgan and Cassady, 2002](#)). A specific emphasis was placed on the examination of 22 cases of childhood cancer diagnosed before 15 years old. However, the incidence did not exceed those expected for the community for total cancer (SIR: 0.83, 99% CI: 0.44–1.40), CNS cancer (SIR: 1.05, 99% CI: 0.24–2.70), or leukemia (SIR: 1.09, 99% CI: 0.38–2.31).

An examination of drinking water was conducted in four New Jersey counties to determine an association with leukemia and NHL ([Cohn et al., 1994b](#)). A number of contaminants were reported, including VOCs and trihalomethanes. TCE was found as high as 67 ppb, and exposure categories were assigned to be >0.1, 0.1–5, and >5 ppb. A significantly elevated dose-response risk for ALL was observed for girls diagnosed before 20 years old (RR:

3.36, 95% CI: 1.29–8.28), which was increased among girls diagnosed before 5 years old (RR:4.54, 95% CI: 1.47–10.6). A significantly elevated dose-response risk for girls was also observed for total leukemia (RR: 1.43, 95% CI: 1.07–1.98).

The Woburn, Massachusetts community with contaminated well water experienced an increase in the incidence of childhood leukemia ([Costas et al., 2002](#); [MDPH, 1997b](#); [Cutler et al., 1986](#); [Lagakos et al., 1986](#)). An initial study examined 12 cases of childhood leukemia diagnosed in children <15 years old between 1969 and 1979, when 5.2 cases were expected, and a higher risk was observed in boys compared to girls; however, no factors were observed to account for this increase ([Cutler et al., 1986](#)). Another study observed statistically significant positive association between access to contaminated water; 20 cases of childhood cancer were observed for both cumulative exposure metric (OR: 1.39,  $p = 0.03$ ), and none vs. some exposure metric (OR: 3.03,  $p = 0.02$ ) ([Lagakos et al., 1986](#)). Massachusetts Department of Public Health ([MDPH, 1997b](#)) conducted a case-control study of children <20 years old living in Woburn and diagnosed with leukemia between 1969 and 1989 ( $n = 21$ ) and observed that consumption of drinking water increased the risk of leukemia (OR: 3.03, 95% CI: 0.82–11.28), with the highest risk from exposure during fetal development (OR: 8.33, 95% CI: 0.73–94.67). This study found that paternal occupational exposure to TCE was not related to leukemia in the offspring ([MDPH, 1997b](#)). In the most recent update, Costas et al. ([2002](#)) reported that between the years 1969 and 1997, 24 cases of childhood leukemia were observed when 11 were expected. Risk was calculated for cumulative exposure to contaminated drinking water 2 years prior to conception (OR<sub>adj</sub>: 2.61, 95% CI: 0.47–14.97), during pregnancy (OR<sub>adj</sub>: 8.33, 95% CI: 0.73–94.67), postnatal (OR<sub>adj</sub>: 1.18, 95% CI: 0.28–5.05), and any of these time periods (OR<sub>adj</sub>: 2.39, 95% CI: 0.54–10.59). A dose-response was observed during pregnancy only. Cases were more likely to be male (76%), <9 years old at diagnosis (62%), breast-fed (OR: 10.17, 95% CI: 1.22–84.50), and exposed during pregnancy (adjusted OR [OR<sub>adj</sub>]: 8.33, 95% CI: 0.73–94.67). A dose-response was seen during the pregnancy exposure period, with the most exposed having an OR<sub>adj</sub> of 14.30 (95% CI: 0.92–224.52). Other elevated risks observed included maternal alcohol intake during pregnancy (OR: 1.50, 95% CI: 0.54–4.20), having a paternal grandfather diagnosed with cancer (OR: 2.01, 95% CI: 0.73–5.58), father employed in a high risk industry (OR: 2.55, 95% CI: 0.78–8.30), and public water being the subject's primary beverage (OR: 3.03, 95% CI: 0.82–11.28). (Also see sections on spontaneous abortion, perinatal death, decreased birth weight, and congenital malformations for additional results from this cohort.)

The study of VOC exposure in Endicott, New York discussed above observed fewer than six cases of cancer that were diagnosed between 1980 and 2001 in children <20 years old, and did not exceed expected cases or types ([ATSDR, 2006a](#)). (See section on spontaneous abortion for study details, and sections on spontaneous abortion, decreased birth weight, and congenital malformations for additional results from this cohort.)

The AZ DHS conducted a number of studies of contaminated drinking water and 189 cases of childhood cancer (<20 years old) ([Flood, 1997a](#); [ADHS, 1990](#); [Kioski et al., 1990a](#); [Kioski et al., 1990b](#); [Flood, 1988](#)). In Maricopa County, which encompasses Phoenix and the surrounding area, TCE contamination (8.9 and 29 ppb in two wells) was associated with elevated levels of childhood leukemia (n = 67) in west central Phoenix during 1965–1986 (SIR: 1.67, 95% CI: 1.20–2.27) and 1982–1986 (SIR: 1.91, 95% CI: 1.11–3.12), but did not observe a significant increase in total childhood cancers, lymphoma, brain/CNS, or other cancers during these time periods ([ADHS, 1990](#)). (See above for results from this study on congenital anomalies.) A follow-up study retrospectively asked parents about exposures and found that residence within 2 miles of wells contaminated with TCE was not a risk factor for childhood leukemia, but identified a number of other risk factors ([Flood, 1997a](#)). A further study of East Phoenix, reported on TCE contamination found along with 1,1,1-trichloroethane and 25 other contaminants in well water (levels not reported) and found no increase in incidence of childhood leukemia (SIR: 0.85, 95% CI: 0.50–1.35) based on 16 cases ([Kioski et al., 1990b](#)). There were also 16 cases of other types of childhood cancer, but were too few to be analyzed separately. In Pima County, which encompasses Tucson and the surrounding area, TCE was found in drinking wells (1.1–239 ppb), along with 1,1-DCE, chloroform, and chromium and found a nonstatistically elevated risk of leukemia was observed (SIR: 1.50, 95% CI: 0.76–2.70), but no risk was observed for testicular cancer, lymphoma, or CNS/brain cancer ([Kioski et al., 1990a](#)).

#### **4.8.3.1.3. Summary of human developmental toxicity**

Epidemiological developmental studies examined the association between TCE exposure and a number of prenatal and postnatal developmental outcomes. Prenatal developmental outcomes examined include spontaneous abortion and perinatal death; decreased birth weight, SGA, and postnatal growth; congenital malformations; and other adverse birth outcomes. Postnatal developmental outcomes examined include developmental neurotoxicity, developmental immunotoxicity, other developmental outcomes, and childhood cancer related to TCE exposure.

More information on developmental outcomes is expected. A follow-up study of the Camp Lejeune cohort ([ATSDR, 1998a](#)) for birth defects and childhood cancers was initiated in 1999 ([ATSDR, 2003a](#)) and expected to be completed soon ([ATSDR, 2009](#); [U.S. GAO, 2007a, b](#)). Out of a total of 106 potential cases of either birth defects or childhood cancer, 57 have been confirmed and will constitute the cases. These will be compared 548 control offspring of mothers who also lived at Camp Lejeune during their pregnancy from 1968 to 1985. As part of this study, a drinking water model was developed to determine a more accurate level and duration of exposure to these pregnant women ([ATSDR, 2007a](#)). Additional health studies have been suggested, including adverse neurological or behavioral effects or pregnancy loss.

#### 4.8.3.2. Animal Developmental Toxicology Studies

A number of animal studies have been conducted to assess the potential for developmental toxicity of TCE. These include studies conducted in rodents by prenatal inhalation or oral exposures (summarized in Tables 4-96 and 4-97), as well as assessments in nonmammalian species (e.g., avian, amphibian, and invertebrate species) exposed to TCE during development. Studies have been conducted that provide information on the potential for effects on specific organ systems, including the developing nervous, immune, and pulmonary systems. Additionally, a number of research efforts have focused on further characterization of the mode of action for cardiac malformations that have been reported to be associated with TCE exposure.

**Table 4-96. Summary of mammalian in vivo developmental toxicity studies—inhale exposures**

Reference <sup>a</sup>	Species/strain/sex/number	Exposure level/duration	NOAEL; LOAEL <sup>b</sup>	Effects
Carney et al. (2006)	Rat, Sprague-Dawley, females, 27 dams/group	0, 50, 150, or 600 ppm (600 ppm = 3.2 mg/L) <sup>c</sup>  6 hrs/d; GDs 6–20	Maternal NOAEL: 150 ppm Maternal LOAEL: 600 ppm	↓ Body weight gain (22% less than control) on GDs 6–9 at 600 ppm.
			Developmental NOAEL: 600 ppm	No evidence of developmental toxicity, including heart defects.
Dorfmueller et al. (1979)	Rat, Long-Evans, females, 30 dams/group	0 or 1,800 ± 200 ppm (9,674 ± 1,075 mg/m <sup>3</sup> ) <sup>c</sup>  2 wks, 6 hrs/d, 5 d/wk; prior to mating and/or on GDs 0–20	Maternal NOAEL: 1,800 ± 200 ppm	No maternal abnormalities.
			Developmental LOAEL: 1,800 ± 200 ppm	Statistically significant ↑ skeletal and soft tissue anomalies in fetuses from dams exposed during pregnancy only. No statistically significant treatment effects on behavior of offspring 10, 20, or 100 d postpartum. Body weight gains statistically significant ↓ in pups from dams with pregestational exposure.
Hardin et al. (1981)	Rat, Sprague-Dawley, female, nominal 30/group	0 or 500 ppm  6–7 hrs/d; GDs 1–19	Maternal NOAEL: 500 ppm	No maternal toxicity.
			Developmental NOAEL: 500 ppm	No embryonic or fetal toxicity.
	Rabbit, New Zealand white, female, nominal 20/group	0 or 500 ppm  6–7 hrs/d; GDs 1–24	Maternal NOAEL: 500 ppm	No maternal toxicity.
			Developmental LOAEL: 500 ppm	Hydrocephaly observed in two fetuses of two litters, considered equivocal evidence of teratogenic potential.
Healy et al. (1982)	Rat, Wistar, females, 31–32 dams/group	0 or 100 ppm  4 hrs/d; GDs 8–21	Maternal NOAEL: 100 ppm	No maternal abnormalities.
			Developmental LOAEL: 100 ppm	Litters with total resorptions statistically significant ↑. Statistically significant ↓ fetal weight, and ↑ bipartite or absent skeletal ossification centers.

**Table 4-96. Summary of mammalian in vivo developmental toxicity studies—*inhalation exposures***

Reference <sup>a</sup>	Species/strain/ sex/number	Exposure level/ duration	NOAEL; LOAEL <sup>a</sup>	Effects
Schwetz et al. (1975)	Rat, Sprague-Dawley, female, 20–35/group Mouse, Swiss-Webster, females, 30–40 dams/group	0 or 300 ppm  7 hrs/d; GDs 6–15	Maternal LOAEL: 300 ppm	4–5% ↓ maternal body weight
			Developmental NOAEL: 300 ppm	No embryonic or fetal toxicity; not teratogenic.
Westergren et al. (1984)	Mouse, NMRI, male and female, 6–12 offspring/group	0 or 150 ppm  24 hrs/d; 30 d (during 7 d of mating and until GD 22)	Developmental LOAEL <sup>d</sup> : 150 ppm <sup>c</sup>	Specific gravity of brains statistically significant ↓ at PNDs 0, 10, and 20–22. Similar effects at PNDs 20–22 in occipital cortex and cerebellum. No effects at 1 mo of age.

<sup>a</sup>**Bolded studies** carried forward for consideration in dose-response assessment (see Chapter 5).

<sup>b</sup>NOAEL and LOAEL are based upon reported study findings.

<sup>c</sup>Dose conversions provided by study author(s).

<sup>d</sup>Parental observations not reported.

**Table 4-97. Ocular defects observed (Narotsky et al., 1995)**

Dose TCE (mg/kg-d)	Incidence (number affected pups/total number pups) <sup>a</sup>	Percentage of pups with eye defects
0	1/197	0.51
10.1	0/71	0.00
32	0/85	0.00
101	3/68	4.41
320	3/82	3.66
475	6/100	6.00
633	6/100	6.00
844	7/58	12.07
1,125	12/44	27.27

<sup>a</sup>Reported in Barton and Das (1996).

#### 4.8.3.2.1. Mammalian studies

Studies that have examined the effects of TCE on mammalian development following either inhalation or oral exposures are described below and summarized in Tables 4-96 and 4-98, respectively.

**Table 4-98. Summary of mammalian in vivo developmental toxicity studies—oral exposures**

Reference <sup>a</sup>	Species/strain/sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL <sup>b</sup>	Effects
Blossom and Doss (2007)	Mouse, MRL +/+, dams and both sexes offspring, 3 litters/group, 8–12 offspring/group	0, 0.5, or 2.5 mg/mL  Parental mice and/or offspring exposed from GD 0 to 7–8 mo of age	Drinking water	Developmental LOAEL = 0.5 mg/mL <sup>c</sup>	At 0.5 mg/mL: statistically significant ↓ postweaning weight; statistically significant ↑ IFN $\gamma$ produced by splenic CD4+ cells at 5–6 wks; statistically significant ↓ splenic CD8+ and B220+ lymphocytes; statistically significant ↑ IgG2a and histone; statistically significant altered CD4–/CD8– and CD4+/CD8+ thymocyte profile. At 2.5 mg/mL: statistically significant ↓ postweaning weight; statistically significant ↑ IFN $\gamma$ produced by splenic CD4+ and CD8+ cells at 4–5 and 5–6 wks; statistically significant ↓ splenic CD4+, CD8+, and B220+ lymphocytes; statistically significant altered CD4+/CD8+ thymocyte profile.
Blossom et al. (2008)	Mouse, MRL +/+, dams and both sexes offspring, 8 litters/group, 3–8 offspring/group	0 or 0.1 mg/mL (maternal dose = 25.7 mg/kg-d; offspring PNDs 24–42 dose— 31.0 mg/kg-d  Parental mice and/or offspring exposed from GD 0 to PND 42	Drinking water	Developmental LOAEL = 1,400 ppb <sup>c</sup>	At 0.1 mg/mL: at PND 20, statistically significant ↑ thymocyte cellularity and distribution, associated with statistically significant ↑ in thymocyte subset distribution; statistically significant ↑ reactive oxygen species generation in total thymocytes; statistically significant ↑ in splenic CD4+ T-cell production of IFN- $\gamma$ and IL-2 in females and TNF- $\alpha$ in males at PND 42. Significantly impaired nest-building behaviors at PND 35. Increased aggressive activities, and increased oxidative stress and impaired thiol status in the cerebellar tissue of male offspring at PND 40.

**Table 4-98. Summary of mammalian in vivo developmental toxicity studies—oral exposures (continued)**

Reference <sup>a</sup>	Species/strain/sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL <sup>a</sup>	Effects
Collier et al. (2003)	Rat, Sprague-Dawley, female, number dams/group not reported	0, 0.11, or 1.1 mg/mL  (0, 830, or 8,300 µgM) <sup>d</sup>  GDs 0–11	Drinking water	Developmental LOEL: 0.11 mg/mL	Embryos collected between GDs 10.5 and 11. Gene expression at 1.1 mg/mL TCE: 8 housekeeping genes ↑, and one gene ↓; 3 stress response genes ↑, IL-10 ↓; 2 cyto-skeletal/cell adhesion/blood related genes ↑, 3 genes ↓; 2 heart-specific genes ↑. Effects at 0.11 mg/mL reduced considerably. Two possible markers for fetal TCE exposure identified as Serca-2 Ca <sup>+2</sup> ATPase and GPI-p137.
Cosby and Dukelow (1992)	Mouse, B6D2F1, female, 28–62 dams/group	0, 24, or 240 mg/kg-d  GDs 1–5, 6–10, or 11–15	Gavage in corn oil	Maternal NOAEL: 240 mg/kg-d	No maternal toxicity.
				Developmental NOAEL: 240 mg/kg-d	No effects on embryonic or fetal development.
Dawson et al. (1993)	Rat, Sprague-Dawley, 116 females allocated to 11 groups	0, 1.5, or 1,100 ppm  2 mo before mating and/or during gestation	Drinking water	Maternal NOAEL: 1,100 ppm	No maternal toxicity.
				Developmental LOAEL: 1.5 ppm	Statistically significant ↑ in heart defects, primarily atrial septal defects, found at both dose levels in groups exposed prior to pregnancy and during pregnancy, as well as in group exposed to 1,100 ppm dose during pregnancy only. No statistically significant ↑ in congenital heart defects in groups exposed prior to pregnancy only.
Fisher et al. (2001); Warren et al. (2006)	Rat, Sprague-Dawley, female, 20–25 dams/group	0 or 500 mg/kg-d  GDs 6–15	Gavage in soybean oil	Maternal NOAEL: 500 mg/kg-d	No maternal toxicity.
				Developmental NOAEL: 500 mg/kg-d	No developmental toxicity. The incidence of heart malformations for fetuses from TCE-treated dams (3–5%) did not differ from negative controls. No eye defects observed.

**Table 4-98. Summary of mammalian in vivo developmental toxicity studies—oral exposures (continued)**

Reference <sup>a</sup>	Species/strain/ sex/number	Dose level/ exposure duration	Route/ vehicle	NOAEL; LOAEL <sup>a</sup>	Effects
Fredriksson et al. (1993)	Mouse, NMRI, male pups, 12 pups from 3–4 different litters/group	0, 50, or 290 mg/kg-d  PNDs 10–16	Gavage in a 20% fat emulsion prepared from egg lecithin and peanut oil	Developmental LOAEL: 50 mg/kg-d	Rearing activity statistically significant ↓ at both dose levels on PND 60.
George et al. (1986)	Rat, F334, male and female, 20 pairs/treatment group, 40 controls/sex	0, 0.15, 0.30, or 0.60% micro-encapsulated TCE  Breeders exposed 1 wk pre mating, then for 13 wks; pregnant ♀s throughout pregnancy (i.e., 18 wks total)	Dietary	LOAEL: 0.15%	Open field testing in pups: a statistically significant dose-related trend toward ↑ time required for male and female pups to cross the first grid in the test device.
Isaacson and Taylor (1989)	Rat, Sprague-Dawley, females, 6 dams/group	0, 312, or 625 mg/L (0, 4.0, or 8.1 mg/d) <sup>d</sup>  Dams (and pups) exposed from 14 d prior to mating until end of lactation.	Drinking water	Developmental LOAEL: 312 mg/L <sup>b</sup>	Statistically significant ↓ myelinated fibers in the stratum lacunosum-moleculare of pups. Reduction in myelin in the hippocampus.
Johnson et al. (2003)	Rat, Sprague-Dawley, female, 9–13/group, 55 in control group	0, 2.5, 250, 1.5, or 1,100 ppm  (0, 0.00045, 0.048, 0.218, or 129 mg/kg-d) <sup>d</sup>  GDs 0–22	Drinking water	Developmental NOAEL: 2.5 ppb Developmental LOAEL: 250 ppb <sup>b</sup>	Statistically significant ↑ in percentage of abnormal hearts and the percentage of litters with abnormal hearts at ≥250 ppb.
Narotsky et al. (1995)	Rat, F344, females, 8–12 dams/group	0, 10.1, 32, 101, 320, 475, 633, 844, or 1,125 mg/kg-d  GDs 6–15	Gavage in corn oil	Maternal LOAEL: 475 mg/kg-d	Statistically significant dose-related ↓ dam body weight gain at all dose levels on GDs 6–8 and 6–20. Delayed parturition at ≥475 mg/kg-d; ataxia at ≥633 mg/kg-d; mortality at 1,125 mg/kg-d.

**Table 4-98. Summary of mammalian in vivo developmental toxicity studies—oral exposures (continued)**

Reference <sup>a</sup>	Species/strain/ sex/number	Dose level/ exposure duration	Route/ vehicle	NOAEL; LOAEL <sup>a</sup>	Effects
Narotsky et al. (1995) (continued)				Developmental NOAEL: 32 mg/kg-d Developmental LOAEL: 101 mg/kg-d	↑ full litter resorption and postnatal mortality at ≥425 mg/kg-d. Statistically significant prenatal loss at 1,125 mg/kg-d. Pup body weight ↓ (not statistically significant) on PNDs 1 and 6. Statistically significant ↑ in pups with eye defects at 1,125 mg/kg-d. Dose-related (not statistically significant) ↑ in pups with eye defects at ≥101 mg/kg-d.
Narotsky and Kavlock (1995)	Rat, F344, females, 16–21 dams/group	0, 1,125, or 1,500 mg/kg-d  GDs 6–19	Gavage in corn oil	Maternal LOAEL: 1,125 mg/kg-d	Ataxia, ↓ activity, piloerection; dose-related ↓ body weight gain.
				Developmental LOAEL: 1,125 mg/kg-d	Statistically significant ↑ full litter resorptions, ↓ live pups/litter; statistically significant ↓ pup body weight on PND 1; statistically significant ↑ incidences of microphthalmia and anophthalmia.
Noland-Gerbec et al. (1986)	Rat, Sprague-Dawley, females, 9–11 dams/group	0 or 312 mg/L (Average total intake of dams: 825 mg TCE over 61 d) <sup>d</sup>  Dams (and pups) exposed from 14 d prior to mating until end of lactation	Drinking water	Developmental LOEL: 312 mg/L <sup>b</sup>	Statistically significant ↓ uptake of [ <sup>3</sup> H]-2-DG in whole brains and cerebella (no effect in hippocampus) of exposed pups at 7, 11, and 16 d, but returned to control levels by 21 d.

**Table 4-98. Summary of mammalian in vivo developmental toxicity studies—oral exposures (continued)**

Reference <sup>a</sup>	Species/strain/sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL <sup>a</sup>	Effects
Peden-Adams et al. (2006)	Mouse, B6C3F <sub>1</sub> , dams and both sexes offspring, 5 dams/group; 5–7 pups/group at 3 wks; 4–5 pups/sex/group at 8 wks	0, 1,400, or 14,000 ppb  Parental mice and/or offspring exposed during mating, and from GD 0 thru 3 or 8 wks of age	Drinking water	Developmental LOAEL: 1,400 ppb <sup>c</sup>	At 1,400 ppb: Suppressed PFC responses in males at 3 and 8 wks of age and in females at 8 wks of age. Delayed hypersensitivity response increased at 8 wks of age in females. At 14,000 ppb: Suppressed PFC responses in males and females at 3 and 8 wks of age. Splenic cell population decreased in 3-wk-old pups. Increased thymic T-cells at 8 wks of age. Delayed hypersensitivity response increased at 8 wks of age in males and females.
Peden-Adams et al. (2008)	Mouse, MRL +/+, dams and both sexes offspring, unknown number litters/group, 6–10 offspring/sex/group	0, 1,400, or 14,000 ppb (vehicle = 1% Emulphor)  Parental mice and/or offspring exposed from GD 0 to 12 mo of age	Drinking water	Developmental LOAEL = 1,400 ppb <sup>c</sup>	At 1,400 ppb: splenic CD4–/CD8– cells statistically significant ↑ in females; thymic CD4+/CD8+ cells statistically significant ↓ in males; 18% ↑ in male kidney weight. At 14,000 ppb: thymic T-cell subpopulations (CD8+, CD4/CD8–, CD4+) statistically significant ↓ in males.
Taylor et al. (1985)	Rat, Sprague-Dawley, females, number dams/group not reported	0, 312, 625, or 1,250 mg/L  Dams (and pups) exposed from 14 d prior to mating until end of lactation	Drinking water	Developmental LOAEL: 312 mg/L <sup>c</sup>	Exploratory behavior statistically significant ↑ in 60- and 90-d old male rats at all treatment levels. Locomotor activity was higher in rats from dams exposed to 1,250 ppm TCE.

<sup>a</sup>**Bolded studies** carried forward for consideration in dose-response assessment (see Chapter 5).

<sup>b</sup>NOAEL, LOAEL, and LOEL are based upon reported study findings.

<sup>c</sup>Dose conversions provided by study author(s).

<sup>d</sup>Maternal observations not reported.

#### 4.8.3.2.1.1. Inhalation exposures

Dorfmueller et al. (1979) conducted a study in which TCE was administered by inhalation exposure to groups of approximately 30 female Long-Evans hooded rats at a concentration of 1,800 ± 200 ppm before mating only, during gestation only, or throughout the pre-mating and gestation periods. Half of the dams were killed at the end of gestation and half

were allowed to deliver. There were no effects on body weight change or relative liver weight in the dams. The number of corpora lutea, implantation sites, live fetuses, fetal body weight, resorptions, and sex ratio were not affected by treatment. In the group exposed only during gestation, a significant increase in four specific sternebral, vertebral, and rib findings, and a significant increase in displaced right ovary were observed upon fetal skeletal and soft tissue evaluation. Mixed function oxidase enzymes (ethoxycoumarin and ethoxyresorbin), which are indicative of CYP and P448 activities, respectively, were measured in the livers of dams and fetuses, but no treatment-related findings were identified. Postnatal growth was significantly ( $p < 0.05$ ) decreased in the group with gestation-only exposures. Postnatal behavioral studies, consisting of an automated assessment of ambulatory response in a novel environment on GDs 10, 20, and 100, did not identify any effect on general motor activity of offspring following in utero exposure to TCE.

In a study by Schwetz et al. (1975), pregnant Sprague-Dawley rats and Swiss Webster mice (30–40 dams/group) were exposed to TCE via inhalation at a concentration of 300 ppm for 7 hours/day on GDs 6–15. The only adverse finding reported was a statistically significant 4–5% decrease in maternal rat body weight. There were no treatment-related effects on pre- and postimplantation loss, litter size, fetal body weight, crown-rump length, or external, soft tissue, or skeletal findings.

Hardin et al. (1981) summarized the results of inhalation developmental toxicology studies conducted in pregnant Sprague-Dawley rats and New Zealand white rabbits for a number of industrial chemicals, including TCE. Exposure concentrations of 0 or 500 ppm TCE were administered for 6–7 hours/day, on GDs 1–19 (rats) or 1–24 (rabbits), and cesarean sections were conducted on GDs 21 or 30, respectively. There were no adverse findings in maternal animals. No statistically significant increase in the incidence of malformations was reported for either species; however, the presence of hydrocephaly in two fetuses of two TCE-treated rabbit litters was interpreted as a possible indicator of teratogenic potential.

Healy et al. (1982) did not identify any treatment-related fetal malformations following inhalation exposure of pregnant inbred Wistar rats to 0 or 100 ppm (535 mg/m<sup>3</sup>) on GDs 8–21. In this study, significant differences between control and treated litters were observed as an increased incidence of total litter loss ( $p < 0.05$ ), decreased mean fetal weight ( $p < 0.05$ ), and increased incidence of minor ossification variations ( $p = 0.003$ ) (absent or bipartite centers of ossification).

Carney et al. (2006) investigated the effects of whole-body inhalation exposures to pregnant Sprague-Dawley rats at nominal (and actual) chamber concentrations of 0, 50, 150, or 600 ppm TCE for 6 hours/day, 7 days/week, on GDs 6–20. This study was conducted under Good Laboratory Practice regulations according to current EPA and Organisation for Economic Co-operation and Development (OECD) regulatory testing guidelines (i.e., OPPTS 870.3700 and OECD GD 414). Maternal toxicity consisted of a statistically significant decrease (22%) in body

weight gain during the first 3 days of exposure to 600-ppm TCE, establishing a no-observed-effect concentration (NOEC) of 150 ppm for dams. No significant difference between control and TCE-treated groups was noted for pregnancy rates, number of corpora lutea, implantations, viable fetuses per litter, percentage pre- and postimplantation loss, resorption rates, fetal sex ratios, or gravid uterine weights. External, soft tissue, and skeletal evaluation of fetal specimens did not identify any treatment-related effects. No cardiac malformations were identified in treated fetuses. The fetal NOEC for this study was established at 600 ppm.

Westergren et al. (1984) examined brain specific gravity of NMRI mice pups following developmental exposures to TCE. Male and female mice were separately exposed 24 hours/day (except for limited periods of animal husbandry activities) to 0 or 150 ppm TCE for 30 days and mated during exposure for 7 days. Exposure of the females was continued throughout gestation, until the first litter was born. Offspring (6–12/group; litter origin not provided in report) were terminated on PNDs 1, 10, 21–22, or 30. The specific gravity of the brain frontal cortex, cortex, occipital cortex, and cerebellum were measured. The cortex specific gravity was significantly decreased at PND 1 ( $p < 0.001$ ) and 10 ( $p < 0.01$ ) in pups from exposed mice. There were also significant differences ( $p < 0.05$ ) in the occipital cortex and cerebellum at PNDs 20–22. This was considered suggestive of delayed maturation. No significant differences between control and treated pups were observed at 1 month of age.

#### **4.8.3.2.1.2. Oral exposures**

A screening study conducted by Narotsky and Kavlock (1995) assessed the developmental toxicity potential of a number of pesticides and solvents, including TCE. In this study, F344 rats were administered TCE by gavage at 0, 1,125, and 1,500 mg/kg-day on GDs 6–19, and litters were examined on GDs 1, 3, and 6. TCE-related increased incidences of full-litter resorptions, decreased litter sizes, and decreased mean pup birth weights were observed at both treatment levels. Additionally, TCE treatment was reported to be associated with increased incidences of eye abnormalities (microphthalmia or anophthalmia). Increased incidences of fetal loss and percentage of pups with eye abnormalities were confirmed by Narotsky et al. (1995) in a preliminary dose-setting study that treated F344 rats with TCE by gavage doses of 475, 633, 844, or 1,125 mg/kg-day on GDs 6–15, and then in a  $5 \times 5 \times 5$  mixtures study that used TCE doses of 0, 10.1, 32, 101, and 320 mg/kg-day on GDs 6–15. In both studies, dams were allowed to deliver, and pups were examined postnatally. The incidence of ocular defects observed across all TCE treatment levels tested is presented in Table 4-97.

Other developmental findings in this study included increased full litter resorption at 475, 844, and 1,125 mg/kg-day; increased postnatal mortality at 425 mg/kg-day. Pup body weights were decreased (not significantly) on PNDs 1 and 6 at 1,125 mg/kg-day. In both the Narotsky and Kavlock (1995) and Narotsky et al. (1995) studies, significantly decreased maternal body weight gain was observed at the same treatment levels at which full litter resorption was noted.

Additionally, in Narotsky et al. (1995), maternal observations included delayed parturition at 475, 844, and 1,125 mg/kg-day, ataxia at 633 mg/kg-day, and mortality at 1,125 mg/kg-day.

Cosby and Dukelow (1992) administered TCE in corn oil by gavage to female B6D2F1 mice (28–62/group) on GDs 1–5, 6–10, or 11–15 (where mating = GD 1). Dose levels were 0, 1/100, and 1/10 of the oral LD<sub>50</sub> (i.e., 0, 24.02, and 240.2 mg/kg body weight). Dams were allowed to deliver; litters were evaluated for pup count sex, weight, and crown-rump length until weaning (PND 21). Some litters were retained until 6 weeks of age, at which time gonads (from a minimum of 2 litters/group) were removed, weighed, and examined. No treatment-related reproductive or developmental abnormalities were observed.

A single dose of TCE was administered by gavage to pregnant CD-1 mice (9–19/group) at doses of 0, 0.1, or 1.0 µg/kg in distilled water, or 0, 48.3, or 483 mg/kg in olive oil, 24 hours after pre-mating human chorionic gonadotropin (hCG) injection (Coberly et al., 1992). At 53 hours after the hCG-injection, the dams were terminated, and the embryos were flushed from excised oviducts. Chimera embryos were constructed, cultured, and examined. Calculated proliferation ratios did not identify any differences between control and treated blastomeres. A lack of treatment-related adverse outcome was also noted when the TCE was administered by i.p. injection to pregnant mice (16–39/group) at 24 and 48 hours post-hCG at doses of 0, 0.01, 0.02, or 10 µg/kg body weight.

In a study intended to confirm or refute the cardiac teratogenicity of TCE that had been previously observed in chick embryos, Dawson et al. (1990) continuously infused the gravid uterine horns of Sprague-Dawley rats with solutions of 0, 15, or 1,500 ppm TCE (or 1.5 or 150-ppm DCE) on GDs 7–22. At terminal cesarean section on GD 22, the uterine contents were examined, and fetal hearts were removed and prepared for further dissection and examination under a light microscope. Cardiac malformations were observed in 3% of control fetuses, 9% of the 15-ppm TCE fetuses ( $p = 0.18$ ), and 14% of the 1,500-ppm TCE fetuses. ( $p = 0.03$ ). There was a >60% increase in the percentage of defects with a 100-fold increase in dose. No individual malformation or combination of abnormalities was found to be selectively induced by treatment.

To further examine these TCE-induced cardiac malformations in rats, Dawson et al. (1993) administered 0, 1.5 or 1,100-ppm TCE in drinking water to female Sprague-Dawley rats. Experimental treatment regimens were: (1) a period of approximately 2 months prior to pregnancy plus the full duration of pregnancy; (2) the full duration of pregnancy only; or (3) an average of 3 months before pregnancy only. The average total daily doses of TCE consumed for each exposure group at both dose levels were

	1.5 ppm	1,100 ppm
Group 1	23.5 µL	1,206 µL
Group 2	0.78 µL	261 µL
Group 3	3.97 µL	1,185 µL

The study also evaluated 0, 0.15, or 110 ppm DCE in drinking water, with treatment administered: (1) 2 months prior to pregnancy plus the full duration of pregnancy, or (2) an average of 2 months before pregnancy only. At terminal cesarean section, uterine contents were examined, fetuses were evaluated for external defects, and the heart of each fetus was removed for gross histologic examination under a dissecting microscope, conducted without knowledge of treatment group. There were no differences between TCE-treated and control group relative to percentage of live births, implants, and resorptions. The percentage of cardiac defects in TCE-treated groups ranged from 8.2 to 13.0%, and was statistically significant as compared to the control incidence of 3%. The dose-response was relatively flat, even in spite of the extensive difference between the treatment levels. There was a broad representation of various types of cardiac abnormalities identified, notably including multiple transposition, great artery, septal, and valve defects (see Table 4-99). No particular combination of defects or syndrome predominated. Exposure before pregnancy did not appear to be a significant factor in the incidence of cardiac defects.

**Table 4-99. Types of congenital cardiac defects observed in TCE-exposed fetuses**

Cardiac abnormalities	Control	TCE concentrations					
		Premating		Premating/gestation		Gestation only	
		1,100 ppm	1.5 ppm	1,100 ppm	1.5 ppm	1,100 ppm	1.5 ppm
d-Transposition (right chest)	2						
l-Transposition (left chest)					2		1
Great artery defects				1	2		1
Atrial septal defects	1	7	3	19	5	7	4
Mitral valve defects				5	8		
Tricuspid valve defects		1		1	2		
Ventricular septal defects							
Subaortic	1			4	1	1	2
Membranous				2			
Muscular	2	1	1	4		4	1
Endocardial cushion defect	1					1	
Pulmonary valve defects			3	2	1		1
Aortic valve defects			1	2	2	2	
Situs inversus				1			
Total abnormalities	7	9	8	41	23	15	10
Total abnormal hearts	7	9	8	40	23	11	9

Source: ([Dawson et al., 1993, Table 3](#)).

In an attempt to determine a threshold for cardiac anomalies following TCE exposures, Johnson et al. ([Johnson et al., 2005, 2003](#)) compiled and reanalyzed data from five studies

conducted from 1989 to 1995. In these studies, TCE was administered in drinking water to Sprague-Dawley rats throughout gestation (i.e., a total of 22 days) at levels of 2.5 ppb (0.0025 ppm), 250 ppb (0.25 ppm), 1.5, or 1,100 ppm. The dams were terminated on the last day of pregnancy and fetuses were evaluated for abnormalities of the heart and great vessels. The control data from the five studies were combined prior to statistical comparison to the individual treated groups, which were conducted separately. The study author reported that significant increases in the percentage of abnormal hearts and the percentage of litters with abnormal hearts were observed in a generally dose-responsive manner at  $\geq 250$  ppb (see Table 4-100).

**Table 4-100. Types of heart malformations per 100 fetuses**

Type of defect/100 fetuses	Control	TCE dose group			
		1,100 ppm	1.5 ppm	250 ppb	2.5 ppb
Abnormal looping	0.33		1		
Coronary artery/sinus				1.82	
Aortic hypoplasia			0.55		
Pulmonary artery hypoplasia			0.55		
Atrial septal defect	1.16	6.67	2.21	0.91	
Mitral valve defect	0.17			0.91	
Tricuspid valve defect				0.91	
Ventricular septal defect					
Perimembranous (subaortic)	0.33	2.86	1.66		
Muscular	0.33	0.95	0.55		
Atriventricular septal defect	0.17	0.95			
Pulmonary valve defect					
Aortic valve defects		1.9		0.91	
Fetuses with abnormal hearts (n)	13	11	9	5	0
Total fetuses (n)	606	105	181	110	144
Litters with fetuses with abnormal hearts/litter (n)	9/55	6/9	5/13	4/9	0/12
Litter with fetuses with abnormal hearts/number litters (%)	16.4	66.7	38.5	44.4	0.0

Source: ([Johnson et al., 2003, Table 2, p. 290](#)).

In a study by Fisher et al. ([2001](#)), pregnant Sprague-Dawley rats were administered daily gavage doses on GDs 6–15 of TCE (500 mg/kg-day), TCA (300 mg/kg-day), or DCA (300 mg/kg-day). Cesarean delivery of fetuses was conducted on GD 21. Water and soybean oil negative control groups, and a retinoic acid positive control group were also conducted simultaneously. Maternal body weight gain was not significantly different from control for any of the treated groups. No significant differences were observed for number of implantations, resorptions, or litter size. Mean fetal body weight was reduced by treatment with TCA and DCA. The incidence of heart malformations was not significantly increased in treated groups as

compared to controls. The fetal rate of cardiac malformations ranged from 3 to 5% across the TCE, TCA, and DCA dose groups and from 6.5 to 2.9% for the soybean and water control dose groups, respectively. It was suggested that the apparent differences between the results of this study and the Dawson et al. (1993) study may be related to factors such as differences in purity of test substances or in the rat strains, or differences in experimental design (e.g., gavage vs. drinking water, exposure only during the period of organogenesis versus during the entire gestation period, or the use of a staining procedure). The rats from this study were also examined for eye malformations to follow-up on the findings of Narotsky (1995). As reported in Warren et al. (2006), gross evaluation of the fetuses as well as computerized morphometry conducted on preserved and sectioned heads revealed no ocular anomalies in the groups treated with TCE. This technique allowed for quantification of the lens area, globe area, medial canthus, distance, and interocular distance. DCA treatment was associated with statistically significant reductions in the lens area, globe area, and interocular distance. All four measures were reduced in the TCA-treated group, but not significantly. The sensitivity of the assay was demonstrated successfully with the use of a positive control group that was dosed on GDs 6–15 with a known ocular teratogen, retinoic acid (15 mg/kg-day).

Johnson et al. (1998b; 1998a) conducted a series of studies to determine whether specific metabolites of TCE or DCE were responsible for the cardiac malformations observed in rats following administration during the period of organogenesis. Several metabolites of the two chemicals were administered in drinking water to Sprague-Dawley rats from GDs 1 to 22. These included carboxy methylcystine, dichloroacetaldehyde, dichlorovinyl cystine, monochloroacetic acid, TCA, trichloroacetaldehyde, and TCOH. DCA, a primary common metabolite of TCE and DCE, was not included in these studies. The level of each metabolite administered in the water was based upon the dosage equivalent expected if 1,100 ppm (the limit of solubility) TCE broke down completely into that metabolite. Cesarean sections were performed on GD 22, uterine contents were examined, and fetuses were processed and evaluated for heart defects according to the procedures used by Dawson et al. (1993). No treatment-related maternal toxicity was observed for any metabolite group. Adverse fetal outcomes were limited to significantly increased incidences of fetuses with abnormal hearts (see Table 4-101). Significant increases in fetuses with cardiac defects (on a per-fetus and per-litter basis) were observed for only one of the metabolites evaluated (i.e., TCA [2,730 ppm, equivalent to a dose of 291 mg/kg-day]). Notably, significant increases in fetuses with cardiac malformations were also observed with 1.5 or 1,100 ppm TCE (0.218 or 129 mg/kg-day), or with 0.15 or 110 ppm DCE (0.015 or 10.64 mg/kg-day), but in each case, only with pre-pregnancy-plus-pregnancy treatment regimens. The cardiac abnormalities observed were diverse and did not segregate to any particular anomaly or grouping. Dose related increases in response were observed for the overall number of fetuses with any cardiac malformation for both TCE and DCE; however, no dose-related increase occurred for any specific cardiac anomaly (Johnson et al., 1998a).

**Table 4-101. Congenital cardiac malformations**

Heart abnormalities	Treatment group													
	Normal water	TCE p+p 1,100 ppm	TCE p+p 1.5 ppm	TCE p 1,100 ppm	DCE p+p 110 ppm	DCE p+p 0.15 ppm	TCA p 2,730 ppm	MCA p 1,570 ppm	TCEth p 1,249 ppm	TCAlid p 1,232 ppm	DCAld p 174 ppm	CMC p 473 ppm	DCVC p 50 ppm	
Abnormal looping	2	–	2	–	–	–	–	–	–	–	–	–	–	
Aortic hypoplasia	–	1	1	–	1	–	1	–	1	–	1	–	1	
Pulmonary artery hypoplasia	–	–	1	–	–	–	2	1	–	–	2	–	–	
Atrial septal defects	7	19	5	7	11	7	3	3	–	2	–	–	1	
Mitral valve defects, hypoplasia or ectasia	1	5	8	–	4	3	1	–	1	2	–	–	1	
Tricuspid valve defects, hypoplasia or ectasia	–	1	1	–	1	–	–	–	1	–	–	–	–	
Ventricular septal defects														
Perimembranous <sup>a</sup>	2	6	2	1	4	1	4	–	–	3	–	1	–	
Muscular	2	4	–	4	2	1	1	–	1	–	–	2	2	
Atrioventricular septal defects	1	–	–	1	1	–	–	–	–	–	–	–	–	
Pulmonary valve defects	–	2	1	–	1	–	1	3	1	1	–	–	–	
Aortic valve defects	–	2	2	2	2	3	–	–	1	–	–	1	–	
Situs inversus	–	1	–	–	–	–	–	–	–	–	–	–	–	
Total														
Abnormal hearts	15	41	23	15	25	15	13	7	6	8	3	4	5	
Fetuses with abnormal hearts	13	40 <sup>b</sup>	22 <sup>b</sup>	11 <sup>b</sup>	24 <sup>b</sup>	14 <sup>b</sup>	12 <sup>b</sup>	6	5	8	3	4	5	
Fetuses	605	434	255	105	184	121	114	132	121	248	101	85	140	

<sup>a</sup>Subaortic.

<sup>b</sup>Per-fetus statistical significance (Fisher's exact test).

p = pregnancy; p+p = pregnancy and prepregnancy

Source: ([Johnson et al., 1998b, Table 2, p. 997](#)).

The TCE metabolites TCA and DCA were also studied by Smith et al. ([1992](#); [1989](#)). Doses of 0, 330, 800, 1,200, or 1,800 mg/kg TCA were administered daily by gavage to Long-Evan hooded rats on GDs 6–15. Similarly, DCA was administered daily by gavage to Long-Evans rats on GDs 6–15 in two separate studies, at 0, 900, 1,400, 1,900, or 2,400 mg/kg-day and 0, 14, 140, or 400 mg/kg-day. Embryo lethality and statistically or biologically significant incidences of orbital anomalies (combined soft tissue and skeletal findings) were observed for TCA at  $\geq 800$  mg/kg-day, and for DCA at  $\geq 900$  mg/kg-day. Fetal growth (body weight and crown-rump length) was affected at  $\geq 330$  mg/kg-day for TCE and at  $\geq 400$  mg/kg-day for DCA. For TCA, the most common cardiac malformations observed were levocardia at  $\geq 330$  mg/kg-day and interventricular septal defect at  $\geq 800$  mg/kg-day. For DCA, levocardia was observed at  $\geq 900$  mg/kg-day, interventricular septal defect was observed at  $\geq 1,400$  mg/kg-day, and a defect between the ascending aorta and right ventricle was observed in all treated groups (i.e.,  $\geq 14$  mg/kg-day, although the authors appeared to discount the single fetal finding at the lowest dose tested). Thus, NOAELs were not definitively established for either metabolite, although it appears that TCA was generally more potent than DCA in inducing cardiac abnormalities.

These findings were followed up by a series of studies on DCA reported by Epstein et al. ([1992](#)), which were designed to determine the most sensitive period of development and further characterize the heart defects. In these studies, Long-Evans hooded rats were dosed by gavage with a single dose of 2,400 mg/kg-day on selected GDs (6–8, 9–11, or 12–15); with a single dose of 2,400 mg/kg on days 10, 11, 12, or 13; or with a single dose of 3,500 mg/kg on days 9, 10, 11, 12, or 13. The heart defects observed in these studies were diagnosed as high interventricular septal defects rather than membranous type interventricular septal defects. The authors hypothesized that high intraventricular septal defects are a specific type of defect produced by a failure of proliferating interventricular septal tissue to fuse with the right tubercle of the atrioventricular cushion tissue. This study identified GDs 9 through 12 as a particularly sensitive period for eliciting high interventricular septal defects. It was postulated that DCA interferes with the closure of the tertiary interventricular foramen, allowing the aorta to retain its embryonic connection with the right ventricle. Further, it was suggested that the selectivity of DCA in inducing cardiac malformations may be due to the disruption of a discrete cell population.

TCE, DCE, and TCA were administered in drinking water to pregnant Sprague-Dawley rats on GDs 0–11 ([Collier et al., 2003](#)). Treatment levels were 0, 110, or 1,100 ppm (i.e., 0, 830, or 8,300  $\mu\text{gM}$ ) TCE; 0, 11, or 110 ppm (i.e., 0, 110, or 1,100  $\mu\text{gM}$ ) DCE; 0, 2.75, or 27.3 mg/mL (i.e., 0, 10, or 100 mM) TCA. Embryos (including hearts) were harvested between embryonic days 10.5–11, since this is the stage at which the developmental processes of myoblast differentiation, cardiac looping, atrioventricular valve formation, and trabeculation would typically be occurring. A PCR-based subtraction scheme was used to identify genes that were differentially regulated with TCE or metabolite exposure. Numerous differentially regulated

gene sequences were identified. Upregulated transcripts included genes associated with stress response (Hsp 70) and homeostasis (several ribosomal proteins). Downregulated transcripts included extracellular matrix components (GPI-p137 and vimentin) and Ca<sup>2+</sup> responsive proteins (Serca-2 Ca<sup>2+</sup>-ATPase and β-catenin). Serca-2 Ca<sup>2+</sup> and GPI-p137 were identified as two possible markers for fetal TCE exposure. Differential regulation of expression of these markers by TCE was confirmed by dot blot analysis and semiquantitative real time PCR with decreased expression seen at levels of TCE exposure between 100 and 250 ppb (0.76 and 1.9 μM).

#### **4.8.3.2.1.2.1. Developmental neurotoxicity and developmental immunotoxicity**

Several studies were conducted that included assessments of the effects of TCE oral exposure on the developing nervous system ([Blossom et al., 2008](#); [Fredriksson et al., 1993](#); [Isaacson and Taylor, 1989](#); [George et al., 1986](#); [Noland-Gerbec et al., 1986](#); [Dorfmueller et al., 1979](#)) or immune system ([Blossom et al., 2008](#); [Peden-Adams et al., 2008](#); [Blossom and Doss, 2007](#); [Peden-Adams et al., 2006](#)). These studies, summarized below, are addressed in additional detail in Sections 4.3 (nervous system) and 4.6.2.1.2 (immune system).

#### **4.8.3.2.1.2.2. Developmental neurotoxicity**

Fredriksson et al. ([1993](#)) conducted a study in male NMRI weanling mice (12/group, selected from 3–4 litters), which were exposed to TCE by gavage at doses of 0 (vehicle), 50, or 290 mg/kg-day TCE in a fat emulsion vehicle, on PNDs 10–16. Locomotor behavior (horizontal movement, rearing, and total activity) were assessed over three 20-minute time periods at GDs 17 and 60. There were no effects of treatment in locomotor activity at PND 17. At PND 60, the mice treated with 50 and 290 mg/kg-day TCE showed a significant ( $p < 0.01$ ) decrease in rearing behavior at the 0–20- and 20–40-minute time points, but not at the 40–60 minute time point. Mean rearing counts were decreased by over 50% in treated groups as compared to control. Horizontal activity and total activity were not affected by treatment.

Open field testing was conducted in control and high-dose F1 weanling F344 rat pups in an NTP reproduction and fertility study with continuous breeding ([George et al., 1986](#)). In this study, TCE was administered at dietary levels of 0, 0.15, 0.30, or 0.60%. The open field testing revealed a significant ( $p < 0.05$ ) dose-related trend toward an increase in the time required for male and female pups to cross the first grid in the testing device, suggesting an effect on the ability to react to a novel environment.

Taylor et al. ([1985](#)) administered TCE in drinking water (0, 312, 625, or 1,250 ppm) to female Sprague-Dawley rats for 14 days prior to breeding, and from GD 0 through PND 21. The number of litters/group was not reported, nor did the study state how many pups per litter were evaluated for behavioral parameters. Exploratory behavior was measured in the pups in an automated apparatus during a 15-minute sampling period on PNDs 28, 60, and 90. Additionally, wheel-running, feeding, and drinking behavior was monitored 24 hours/day on PNDs 55–60.

The number of exploratory events was significantly increased by approximately 25–50% in 60- and 90-day old male TCE-treated rats at all dose levels, with the largest effect observed at the highest dose level tested, although there were no effects of treatment on the number of infrared beam-breaks. No difference between control and treated rats was noted for pups tested on PND 28. Wheel-running activity was increased approximately 40% in 60-day-old males exposed to 1,250-ppm TCE as compared to controls. It is notable that adverse outcomes reported in the developmentally-exposed offspring on this study were observed long after treatment ceased.

Using a similar treatment protocol, the effects of TCE on development of myelinated axons in the hippocampus was evaluated by Isaacson and Taylor (1989) in Sprague-Dawley rats. Female rats (6/group) were exposed in the drinking water from 14 days prior to breeding and through the mating period; the dams and their pups were then exposed throughout the prenatal period and until PND 21, when they were sacrificed. The dams received 0, 312, or 625 ppm (0, 4, or 8.1 mg/day) TCE in the drinking water. Myelinated fibers were counted in the hippocampus of 2–3 pups per treatment group at PND 21, revealing a decrease of approximately 40% in myelinated fibers in the CA1 area of the hippocampus of pups from dams at both treatment levels, with no dose-response relationship. There was no effect of TCE treatment on myelination in several other brain regions including the internal capsule, optic tract, or fornix.

A study by Noland-Gerbec et al. (1986) examined the effect of pre- and perinatal exposure to TCE on 2-deoxyglucose (2-DG) uptake in the cerebellum, hippocampus, and whole brain of neonatal rats. Sprague-Dawley female rats (9–11/group) were exposed via drinking water to 0 or 312 mg TCE/L distilled water from 14 days prior to mating until their pups were euthanized at GD 21. The total TCE dose received by the dams was 825 mg over the 61-day exposure period. Pairs of male neonates were euthanized on PNDs 7, 11, 16, and 21. There was no significant impairment in neonatal weight or brain weight attributable to treatment, nor were other overt effects observed. 2-DG uptake was significantly reduced from control values in neonatal whole brain (9–11%) and cerebellum (8–16%) from treated rats at all ages studied, and hippocampal 2-DG uptake was significantly reduced (7–21% from control) in treated rats at all ages except at PND 21.

In a study by Blossom et al. (2008), MRL +/+ mice were treated in the drinking water with 0 or 0.1 mg/mL TCE from maternal GD 0 through offspring PND 42. Based on drinking water consumption data, average maternal doses of TCE were 25.7 mg/kg-day, and average offspring (PNDs 24–42) doses of TCE were 31.0 mg/kg-day. In this study, a subset of offspring (three randomly selected neonates from each litter) was evaluated for righting reflex on PNDs 6, 8, and 10; bar-holding ability on PNDs 15 and 17; and negative geotaxis on PNDs 15 and 17; none of these were impaired by treatment. In an assessment of offspring nest building on PND 35, there was a significant association between impaired nest quality and TCE exposure; however, TCE exposure did not have an effect on the ability of the mice to detect social and

nonsocial odors on PND 29 using olfactory habituation and dishabituation methods. Resident intruder testing conducted on PND 40 to evaluate social behaviors identified significantly more aggressive activities (i.e., wrestling and biting) in TCE-exposed juvenile male mice as compared to controls. Cerebellar tissue homogenates from the male TCE-treated mice had significantly lower GSH levels and GSH:GSSG ratios, indicating increased oxidative stress and impaired thiol status; these have been previously reported to be associated with aggressive behaviors ([Franco et al., 2006](#)). Qualitative histopathological examination of the brain did not identify alterations indicative of neuronal damage or inflammation. Although the study author attempted to link the treatment-related alterations in social behaviors to the potential for developmental exposures to TCE to result in autism in humans, this association is not supported by data and is considered speculative at this time.

As previously noted, postnatal behavioral studies conducted by Dorfmueller et al. ([1979](#)) did not identify any changes in general motor activity measurements of rat offspring on PNDs 10, 20, and 100 following maternal gestational inhalation exposure to TCE at 1,800 ± 200 ppm.

#### **4.8.3.2.1.2.3. Developmental immunotoxicity**

Peden-Adams et al. ([2006](#)) assessed the potential for developmental immunotoxicity following TCE exposures. In this study, B6C3F<sub>1</sub> mice (5/sex/group) were administered TCE via drinking water at dose levels of 0, 1,400 or 14,000 ppb from maternal GD 0 to either PND 3 or 8, when offspring lymphocyte proliferation, NK cell activity, SRBC-specific IgM production (PFC response), splenic B220+ cells, and thymus and spleen T-cell immunophenotypes were assessed. (A total of 5–7 pups per group were evaluated at week 3, and the remainder were evaluated at week 8.) Observed positive responses consisted of suppressed PFC responses in males at both ages and both TCE treatment levels, and in females at both ages at 14,000 ppb and at 8 weeks of age at 1,400 ppb. Spleen numbers of B220+ cells were decreased in 3-week-old pups at 14,000 ppb. Pronounced increases in all thymus T-cell subpopulations (CD4+, CD8+, CD4+/CD8+, and CD4-/CD8-) were observed at 8 weeks of age. Delayed hypersensitivity response, assessed in offspring at 8 weeks of age, was increased in females at both treatment levels and in males at 14,000 ppb only. No treatment-related increase in serum anti-dsDNA antibody levels was found in the offspring at 8 weeks of age.

In a study by Blossom and Doss ([2007](#)), TCE was administered to groups of pregnant MRL +/+ mice in drinking water at levels of 0, 0.5, or 2.5 mg/mL. TCE was continuously administered to the offspring until young adulthood (i.e., 7–8 weeks of age). Offspring postweaning body weights were significantly decreased in both treated groups. Decreased spleen cellularity and reduced numbers of CD4+, CD8+, and B220+ lymphocyte subpopulations were observed in the postweaning offspring. Thymocyte development was altered by TCE exposures (significant alterations in the proportions of double-negative subpopulations and

inhibition of in vitro apoptosis in immature thymocytes). A dose-dependent increase in CD4+ and CD8+ T-lymphocyte IFN $\gamma$  was observed in peripheral blood by 4–5 weeks of age, although these effects were no longer observed at 7–8 weeks of age. Serum antihistone autoantibodies and total IgG<sub>2a</sub> were significantly increased in treated offspring; however, no histopathological signs of autoimmunity were observed in the liver and kidneys at sacrifice.

Blossom et al. (2008) administered TCE to MRL +/+ mice (8 dams/group) in the drinking water at levels of 0 or 0.1 mg/mL from GD 0 through PND 42. Average maternal doses of TCE were 25.7 mg/kg-day, and average offspring (PNDs 24–42) doses of TCE were 31.0 mg/kg-day. Subsets of offspring were sacrificed at PNDs 10 and 20, and thymus endpoints (i.e., total cellularity, CD4+/CD8+ ratios, CD24 differentiation markers, and double-negative subpopulation counts) were evaluated. Evaluation of the thymus identified a significant treatment-related increase in cellularity, accompanied by alterations in thymocyte subset distribution, at PND 20 (sexes combined). TCE treatment also appeared to promote T-cell differentiation and maturation at PND 42. Indicators of oxidative stress were measured in the thymus at PNDs 10 and 20, and in the brain at PND 42, and ex vivo evaluation of cultured thymocytes indicated increased reactive oxygen species generation. Mitogen-induced intracellular cytokine production by splenic CD4+ and CD8+ T-cells was evaluated in juvenile mice and brain tissue was examined at PND 42 for evidence of inflammation. Evaluation of peripheral blood indicated that splenic CD4+ T-cells from TCE-exposed PND 42 mice produced significantly greater levels of IFN- $\gamma$  and IL-2 in males and TNF- $\alpha$  in both sexes. There was no effect on cytokine production on PND 10 or 20.

Peden-Adams et al. (2008) administered TCE to MRL+/+ mice (unspecified number of dams/group) in drinking water at levels of 0, 1,400, or 14,000 ppb from GD 0 and continuing until the offspring were 12 months of age. At 12 months of age, final body weight; spleen, thymus, and kidney weights; spleen and thymus lymphocyte immunophenotyping (CD4 or CD8); splenic B-cell counts; mitogen-induced splenic lymphocyte proliferation; serum levels of autoantibodies to dsDNA and GA, periodically measured from 4 to 12 months of age; and urinary protein measures were recorded. Reported sample sizes for the offspring measurements varied from 6 to 10 per sex per group; the number of source litters represented within each sample was not specified. The only organ weight alteration was an 18% increase in kidney weight in the 1,400 ppb males. Splenic CD4-/CD8- cells were altered in female mice (but not males) at 1,400 ppm only. Splenic T-cell populations, numbers of B220+ cells, and lymphocyte proliferation were not affected by treatment. Populations of thymic T-cell subpopulations (CD8+, CD4-/CD8-, and CD4+) were significantly decreased in male but not female mice following exposure to 14,000 ppb TCE, and CD4+/CD8+ cells were significantly reduced in males by treatment with both TCE concentrations. Autoantibody levels (anti-dsDNA and anti-GA) were not increased in the offspring over the course of the study.

Although all of the developmental immunotoxicity studies with TCE (Peden-Adams et al., (2006), (2008); Blossom and Doss, (2007); Blossom et al., (2008)) exposed the offspring during critical periods of pre- and postnatal immune system development, they were not designed to assess issues such as posttreatment recovery, latent outcomes, or differences in severity of response that might be attributed to the early life exposures.

#### **4.8.3.2.1.3. i.p. exposures**

The effect of TCE on pulmonary development was evaluated in a study by Das and Scott (1994). Pregnant Swiss-Webster mice (5/group) were administered a single i.p. injection of TCE in peanut oil at doses of 0 or 3,000 mg/kg on GD 17 (where mating = day 1). Lungs from GDs 18 and 19 fetuses and from neonates on PNDs 1, 5, and 10 were evaluated for phospholipid content, DNA, and microscopic pathology. Fetal and neonatal (PND 1) mortality was significantly increased ( $p < 0.01$ ) in the treated group. Pup body weight and absolute lung weight were significantly decreased ( $p < 0.05$ ) on PND 1, and mean absolute and relative (to body weight) lung weights were significantly decreased on GDs 18 and 19. Total DNA content ( $\mu\text{g}/\text{mg}$  lung) was similar between control and treated mice, but lung phospholipid was significantly ( $p < 0.05$ ) reduced on GD 19 and significantly increased ( $p < 0.05$ ) on PND 10 in the TCE-treated group. Microscopic examination revealed delays in progressive lung morphological development in treated offspring, first observed at GD 19 and continuing at least through PND 5.

#### **4.8.3.2.2. Studies in nonmammalian species**

##### **4.8.3.2.2.1. Avian**

Injection of White Leghorn chick embryos with 1, 5, 10, or 25  $\mu\text{mol}$  TCE per egg on days 1 and 2 of embryogenesis demonstrated mortality, growth defects, and morphological anomalies at evaluation on day 14 (Bross et al., 1983). These findings were consistent with a previous study that had been conducted by Elovaara et al. (1979). Up to 67% mortality was observed in the treated groups, and most of the surviving embryos were malformed (as compared to a complete absence of malformed chicks in the untreated and mineral-oil-treated control groups). Reported anomalies included subcutaneous edema, evisceration (gastroschisis), light dermal pigmentation, beak malformations, club foot, and patchy feathering. Retarded growth was observed as significantly ( $p < 0.05$ ) reduced crown-rump, leg, wing, toe, and beak lengths as compared to untreated controls. This study did not identify any liver damage or cardiac anomalies.

In a study by Loeber et al. (1988), 5, 10, 15, 20, or 25  $\mu\text{mol}$  TCE was injected into the air space of White Longhorn eggs at embryonic stages 6, 12, 18, or 23. Embryo cardiac development was examined in surviving chicks in a double-blinded manner at stages 29, 34, or 44. Cardiac malformations were found in 7.3% of TCE-treated hearts, compared to 2.3% of

saline controls and 1.5% of mineral oil controls. The observed defects included septal defects, cor biloculare, conotruncal abnormalities, atrioventricular canal (AVC) defects, and abnormal cardiac muscle.

Drake et al. (2006b) injected embryonated White Leghorn chicken eggs (Babcock or Bovan strains) with 0, 0.4, 8, or 400 ppb TCE per egg during the period of cardiac valvuloseptal morphogenesis (i.e., 2–3.3 days incubation). The injections were administered in four aliquots at Hamberger and Hamilton (HH) stages 13, 15, 17, and 20, which spanned the major events of cardiac cushion formation, from induction through mesenchyme transformation and migration. Embryos were harvested 22 hours after the last injection (i.e., HH 24 or HH 30) and evaluated for embryonic survival, apoptosis, cellularity and proliferation, or cardiac function. Survival was significantly reduced for embryos at 8 and 400 ppb TCE at HH 30. Cellular morphology of cushion mesenchyme, cardiomyocytes, and endocardocytes was not affected by TCE treatment; however, the proliferative index was significantly increased in the AVC cushions at both treatment levels and in the outflow tract (OFT) cushions at 8 ppb. This resulted in significant cushion hypercellularity for both the OFT and AVC of TCE-treated embryos. Similar outcomes were observed in embryos when TCA or TCOH was administered, and the effects of TCA were more severe than for TCE. Doppler ultrasound assessment of cardiac hemodynamics revealed no effects of TCE exposure on cardiac cycle length or heart rate; however, there was a reduction in dorsal aortic blood flow, which was attributed to a 30.5% reduction in the active component of atrioventricular blood flow. Additionally the passive-to-active atrioventricular blood flow was significantly increased in treated embryos, and there was a trend toward lower stroke volume. The overall conclusion was that exposure to 8 ppb TCE during cushion morphogenesis reduced the cardiac output of the embryos in this study. The findings of cardiac malformations and/or mortality following in ovo exposure to chick embryos with 8 ppb TCE during the period of valvuloseptal morphogenesis has also been confirmed by Rufer et al. (2010; 2008).

In a follow-up study, Drake et al. (2006a) injected embryonated White Leghorn chicken eggs with TCE or TCA during the critical window of avian heart development, beginning at HH stage 3+ when the primary heart field is specified in the primitive streak and ending approximately 50 hours later at HH stage 17, at the onset of chambering. Total dosages of 0, 0.2, 2, 4, 20, or 200 nmol (equivalent to 0, 0.4, 4, 8, 40, or 400 ppb) were injected in four aliquots into each egg yolk during this window (i.e., at stages 3+, 6, 13, and 17: hours 16, 24, 46, and 68). Embryos were harvested at 72 hours, 3.5 days, 4 days or 4.25 days (HH stages 18, 21, 23, or 24, respectively) and evaluated for embryonic survival, cardiac function, or cellular parameters. Doppler ultrasound technology was utilized to assess cardiovascular effects at HH 18, 21, and 23. In contrast to the results of Drake et al. (2006b), all of the functional parameters assessed (i.e., cardiac cycle length, heart rate, stroke volume, and dorsal aortic and atrioventricular blood flow) were similar between control and TCE- or TCA-treated embryos. The authors attributed this difference in response between studies to dependence upon developmental stage at the time

of exposure. In this case, the chick embryo was relatively resistant to TCE when exposure occurred during early cardiogenic stages, but was extremely vulnerable when TCE exposure occurred during valvuloseptal morphogenesis. It was opined that this could explain why some researchers have observed no developmental cardiac effects after TCE exposure to mammalian models, while others have reported positive associations.

#### **4.8.3.2.2.2. Amphibian**

The developmental toxicity of TCE was evaluated in the *Frog Embryo Teratogenesis Assay: Xenopus* by Fort et al. (1993; 1991). Late *Xenopus laevis* blastulae were exposed to TCE, with and without exogenous metabolic activation systems, or to TCE metabolites (DCA, TCA, TCOH, or oxalic acid), and developmental toxicity ensued. Findings included alterations in embryo growth, and increased types and severity of induced malformations. Findings included cardiac malformations that were reportedly similar to those that had been observed in avian studies. It was suggested that a mixed function oxidase-mediated reactive epoxide intermediate (i.e., TCE-oxide) may play a significant role in observed developmental toxicity in in vitro tests.

Likewise, McDaniel et al. (2004) observed dose-dependent increases in developmental abnormalities in embryos of four North American amphibian species (wood frogs, green frogs, American toads, and spotted salamanders) following 96-hour exposures to TCE. The median effective concentration (EC<sub>50</sub>) for malformations was 40 mg/L for TCE in green frogs, while American toads were less sensitive (with no EC<sub>50</sub> at the highest concentration tested—85 mg/L). Although significant mortality was not observed, the types of malformations noted would be expected to compromise survival in an environmental context.

#### **4.8.3.2.2.3. Invertebrate**

The response of the daphnid *Ceriodaphnia dubia* to six industrial chemicals, including TCE, was evaluated by Niederlehner et al. (1998). Exposures were conducted for 6–7 days, according to standard EPA testing guidelines. Lethality, impairment of reproduction, and behavioral changes, such as narcosis and abnormal movement, were observed with TCE exposures. The reproductive sublethal effect concentration value for TCE was found to be 82 µM.

#### **4.8.3.2.3. In vitro studies**

Rat whole embryo cultures were used by Saillenfait et al. (1995) to evaluate the embryotoxicity of TCE, tetrachloroethylene, and four metabolites (TCA, DCA, CH, and trichloroacetyl chloride). In this study, explanted embryos of Sprague-Dawley rats were cultured in the presence of the test chemicals for 46 hours and subsequently evaluated. Concentration-dependant decreases in growth and differentiation, and increases in the incidence of morphologically abnormal embryos were observed for TCE at ≥5 mM.

Whole embryo cultures were also utilized by Hunter et al. (1996) in evaluating the embryotoxic potential of a number of disinfection byproducts, including the TCE metabolites DCA and TCA. CD-1 mouse conceptuses (GD 9; 3–6 somites) were cultured for 24–26 hours in treated medium. DCA levels assessed were 0, 734, 1,468, 4,403, 5,871, 7,339, 11,010, or 14,680  $\mu\text{M}$ ; TCA levels assessed were 0, 500, 1,000, 2,000, 3,000, 4,000, and 5,000  $\mu\text{M}$ . For DCA, neural tube defects were observed at levels  $\geq 5,871 \mu\text{M}$ , heart defects were observed at  $\geq 7,339 \mu\text{M}$ , and eye defects were observed at levels  $\geq 11,010 \mu\text{M}$ . For TCA, neural tube defects were observed at levels  $\geq 2,000 \mu\text{M}$ , heart and eye defects were observed at  $\geq 3,000 \mu\text{M}$ . The heart defects for TCA were reported to include incomplete looping, a reduction in the length of the heart beyond the bulboventricular fold, and a marked reduction in the caliber of the heart tube lumen. Overall benchmark concentrations (i.e., the lower limit of the 95% CI required to produce a 5% increase in the number of embryos with neural tube defects) were 2,451.9  $\mu\text{M}$  for DCA and 1,335.8  $\mu\text{M}$  for TCA (Richard and Hunter, 1996).

Boyer et al. (2000) used an in vitro chick-AVC culture to test the hypothesis that TCE might cause cardiac valve and septal defects by specifically perturbing epithelial-mesenchymal cell transformation of endothelial cells in the AVC and outflow tract areas of the heart. AV explants from Stage 16 White Leghorn chick embryos were placed in hydrated collagen gels, with medium and TCE concentrations of 0, 50, 100, 150, 200, or 250 ppm. TCE was found to block the endothelial cell-cell separation process that is associated with endothelial activation as well as to inhibit mesenchymal cell formation across all TCE concentrations tested. TCE did not, however, have an effect on the cell migration rate of fully formed mesenchymal cells. TCE-treatment was also found to inhibit the expression of transformation factor Mox-1 and extracellular matrix protein fibrillin 2, two protein markers of epithelial-mesenchyme cell transformation.

#### **4.8.3.3. Discussion/Synthesis of Developmental Data**

In summary, an overall review of the weight of evidence in humans and experimental animals is suggestive of the potential for developmental toxicity with TCE exposure. A number of developmental outcomes have been observed in the animal toxicity and the epidemiological data, as discussed below. These include adverse fetal/birth outcomes including death (spontaneous abortion, perinatal death, pre- or postimplantation loss, resorptions), decreased growth (low birth weight, SGA, IUGR, decreased postnatal growth), and congenital malformations, in particular cardiac defects. Postnatal developmental outcomes include developmental neurotoxicity, developmental immunotoxicity, and childhood cancer.

##### **4.8.3.3.1. Adverse fetal and early neonatal outcomes**

Studies that demonstrate adverse fetal or early neonatal outcomes are summarized in Table 4-102. In human studies of prenatal TCE exposure, increased risk of spontaneous abortion

was observed in some studies ([ATSDR, 2001](#); [Taskinen et al., 1994](#); [Windham et al., 1991](#)), but not in others ([ATSDR, 2008b, 2001](#); [Goldberg et al., 1990](#); [Lindbohm et al., 1990](#); [Taskinen et al., 1989](#); [Lagakos et al., 1986](#)). In addition, perinatal deaths were observed after 1970, but not before 1970 ([Lagakos et al., 1986](#)). In rodent studies that examined offspring viability and survival, there was an indication that TCE exposure may have resulted in increased pre-and/or postimplantation loss ([Kumar et al., 2000b](#); [Narotsky and Kavlock, 1995](#); [Healy et al., 1982](#)), and in reductions in live pups born as well as in postnatal and postweaning survival ([George et al., 1986](#); [George et al., 1985](#)).

**Table 4-102. Summary of adverse fetal and early neonatal outcomes associated with TCE exposures**

Positive finding	Species	Reference
Spontaneous abortion, miscarriage, pre-and/or postimplantation loss	Human	ATSDR (2001) <sup>a</sup> ; Taskinen et al. (1994) <sup>a</sup> ; Windham et al. (1991)
	Rat	Kumar et al. (2000b); Healy et al. (1982); Narotsky and Kavlock (1995); Narotsky et al. (1995)
Perinatal death, reduction in live births	Human	Lagakos et al. (1986) <sup>b</sup>
	Mouse	George et al. (1985)
	Rat	George et al. (1986)
Postnatal and postweaning survival	Mouse	George et al. (1985)
	Rat	George et al. (1986)
Decreased birth weight, SGA, postnatal growth	Human	ATSDR (1998a); ATSDR (2006a); Rodenbeck et al. (2000) <sup>c</sup> ; Windham et al. (1991)
	Mouse	George et al. (1985)
	Rat	George et al. (1986); Healy et al. (1982); Narotsky and Kavlock (1995); Narotsky et al. (1995)

<sup>a</sup>Not significant.

<sup>b</sup>Observed for exposures after 1970, but not before.

<sup>c</sup>Increased risk for very low birth weight but not low birth weight or full-term low birth weight.

Decreased birth weight and SGA was observed ([ATSDR, 2006a](#); [Rodenbeck et al., 2000](#); [ATSDR, 1998a](#); [Windham et al., 1991](#)); however, no association was observed in other studies ([Bove, 1996](#); [Bove et al., 1995](#); [Lagakos et al., 1986](#)). While comprising both occupational and environmental exposures, these human studies are, overall, not highly informative due to their small numbers of cases and limited exposure characterization or to the fact that exposures to mixed solvents were involved. However, decreased fetal weight, live birth weights and postnatal growth were also observed in rodents, ([Narotsky and Kavlock, 1995](#); [George et al., 1986](#); [George et al., 1985](#); [Healy et al., 1982](#)), adding to the weight of evidence for this endpoint. It is noted that the rat studies reporting effects on fetal or neonatal viability and growth used F344 or Wistar

rats, while several other studies, which used Sprague-Dawley rats, reported no increased risk in these developmental measures ([Carney et al., 2006](#); [Hardin et al., 1981](#); [Schwetz et al., 1975](#)).

Overall, based on weakly suggestive epidemiologic data and fairly consistent laboratory animal data, it can be concluded that TCE exposure poses a potential hazard for prenatal losses and decreased growth or birth weight of offspring.

#### 4.8.3.3.2. Cardiac malformations

A discrete number of epidemiological studies and studies in laboratory animal models have identified an association between TCE exposures and cardiac defects in developing embryos and/or fetuses. These are listed in Table 4-103. Additionally, a number of avian and rodent in vivo studies and in vitro assays have examined various aspects of the induction of cardiac malformations.

**Table 4-103. Summary of studies that identified cardiac malformations associated with TCE exposures**

Finding	Species	References
Cardiac defects	Human	ATSDR ( <a href="#">2008b</a> , <a href="#">2006a</a> ); Yauck et al. ( <a href="#">2004</a> )
	Rat	Dawson et al. ( <a href="#">1993</a> , <a href="#">1990</a> ); Johnson et al. ( <a href="#">2003</a> ); Johnson et al. ( <a href="#">2005</a> ); Johnson et al. ( <a href="#">1998b</a> ; <a href="#">1998a</a> ) <sup>a</sup> ; Smith et al. ( <a href="#">1989</a> ), ( <a href="#">1992</a> ) <sup>a</sup> ; Epstein et al. ( <a href="#">1992</a> ) <sup>a</sup>
	Chicken	Bross et al. ( <a href="#">1983</a> ); Boyer et al. ( <a href="#">2000</a> ); Loeber et al. ( <a href="#">1988</a> ); Drake et al. ( <a href="#">2006a</a> ; <a href="#">2006b</a> ); Mishima et al. ( <a href="#">2006</a> ); Rufer et al. ( <a href="#">2010</a> ; <a href="#">2008</a> )
Altered heart rate	Human	Jasinka ( <a href="#">1965, translation</a> )

<sup>a</sup>Metabolites of TCE.

In humans, an increased risk of cardiac defects has been observed after exposure to TCE in studies reported by ATSDR ([2008b](#), [2006a](#)) and Yauck et al. ([2004](#)), although others saw no significant effect ([Bove, 1996](#); [Bove et al., 1995](#); [Goldberg et al., 1990](#); [Lagakos et al., 1986](#)), possibly due to a small number of cases. In addition, altered heart rate was seen in one study ([Jasińska, 1965, translation](#)). A cohort of water contamination in Santa Clara County, California is often cited as a study of TCE exposure and cardiac defects; however, the chemical of exposure is in fact trichloroethane, not TCE ([Deane et al., 1989](#); [Swan et al., 1989](#)).

In laboratory animal models, avian studies were the first to identify adverse effects of TCE exposure on cardiac development. As described in Section 4.8.3.2.2.1, cardiac malformations have been reported in chick embryos exposed to TCE ([Rufer et al., 2008](#); [Drake et al., 2006a](#); [Drake et al., 2006b](#); [Mishima et al., 2006](#); [Boyer et al., 2000](#); [Loeber et al., 1988](#); [Bross et al., 1983](#)). Additionally, a number of studies were conducted in rodents in which

cardiac malformations were observed in fetuses following the oral administration of TCE to maternal animals during gestation ([Johnson et al., 2005, 2003](#); [Dawson et al., 1993, 1990](#)) (see Section 4.8.3.2.1.2). Cardiac defects were also observed in rats following oral gestational treatment with metabolites of TCE ([Johnson et al., 1998b](#); [Johnson et al., 1998a](#); [Epstein et al., 1992](#); [Smith et al., 1992](#); [Smith et al., 1989](#)).

However, cardiac malformations were not observed in a number of other studies in laboratory animals in which TCE was administered during the period of cardiac organogenesis and fetal visceral findings were assessed. These included inhalation studies in rats ([Carney et al., 2006](#); [Healy et al., 1982](#); [Hardin et al., 1981](#); [Dorfmueller et al., 1979](#); [Schwetz et al., 1975](#)) and rabbits ([Hardin et al., 1981](#)), and gavage studies in rats ([Fisher et al., 2001](#); [Narotsky and Kavlock, 1995](#); [Narotsky et al., 1995](#)) and mice ([Cosby and Dukelow, 1992](#)).

It is generally recognized that response variability among developmental bioassays conducted with the same chemical agent may be related to factors such as the study design (e.g., the species and strain of laboratory animal model used, the day(s) or time of day of dose administration in relation to critical developmental windows, the route of exposure, the vehicle used, the day of study termination), or the study methodologies (e.g., how fetuses were processed, fixed, and examined; what standard procedures were used in the evaluation of morphological landmarks or anomalies; and whether there was consistency in the fetal evaluations that were conducted). In the case of studies that addressed cardiac malformations, there is additional concern as to whether detailed visceral observations were conducted and whether or not cardiac evaluation was conducted using standardized dissection procedures (e.g., with the use of a dissection microscope or including confirmation by histopathological evaluation, and whether the examinations were conducted by technicians who were trained and familiar with fetal cardiac anatomy). Furthermore, interpretation of the findings can be influenced by the analytical approaches applied to the data as well as by biological considerations such as the historical incidence data for the species and strain of interest. These issues have been critically examined in the case of the TCE developmental toxicity studies ([Watson et al., 2006](#); [Hardin et al., 2005](#)).

In the available animal developmental studies with TCE, differences were noted in the procedures used to evaluate fetal cardiac morphology following TCE gestational exposures across studies, and some of these differences may have resulted in inconsistent fetal outcomes and/or the inability to detect cardiac malformations. Most of the studies that did not identify cardiac anomalies used a traditional free-hand sectioning technique ([as described in Wilson, 1965](#)) on fixed fetal specimens ([Healy et al., 1982](#); [Hardin et al., 1981](#); [Dorfmueller et al., 1979](#); [Schwetz et al., 1975](#)). Detection of cardiac anomalies can be enhanced through the use of a fresh dissection technique as described by Staples ([1974](#)) and Stuckhardt and Poppe ([1984](#)); a significant increase in treatment-related cardiac heart defects was observed by Dawson et al. ([1990](#)) when this technique was used. Further refinement of this fresh dissection technique was

employed by Dawson and colleagues at the University of Arizona (UA), resulting in several additional studies that reported cardiac malformations ([Johnson et al., 2005, 2003](#); [Dawson et al., 1993](#)). However, two studies conducted in an attempt to verify the teratogenic outcomes of the UA laboratory studies used the same or similar enhanced fresh dissection techniques and were unable to detect cardiac anomalies ([Carney et al., 2006](#); [Fisher et al., 2001](#)). Although the Carney et al. (2006) study was administered via inhalation (a route that has not previously been shown to produce positive outcomes), the Fisher et al. (2001) study was administered orally and included collaboration between industry and UA scientists. It was suggested that the apparent differences between the results of the Fisher et al. (2001) study and the Dawson et al. (1993) and Johnson et al. (2003) studies may be related to factors such as differences in purity of test substances or in the rat strains, or differences in experimental design (e.g., gavage vs. drinking water, exposure only during the period of organogenesis versus during the entire gestation period, or the use of a staining procedure).

It is notable that all studies that identified cardiac anomalies following gestational exposure to TCE or its metabolites were: (1) conducted in rats and (2) dosed by an oral route of exposure (gavage or drinking water). Cross-species and route-specific differences in fetal response may be due in part to toxicokinetic factors. Although a strong accumulation and retention of TCA was found in the amniotic fluid of pregnant mice following inhalation exposures to TCE ([Ghantous et al., 1986](#)), other toxicokinetic factors may be critical. The consideration of toxicokinetics in determining the relevance of murine developmental data for human risk assessment is briefly discussed by Watson et al. (2006). There are differences in the metabolism of TCE between rodent and humans in that TCE is metabolized more efficiently in rats and mice than humans, and a greater proportion of TCE is metabolized to DCA in rodents versus to TCA in humans. Studies that examined the induction of cardiac malformations with gestational exposures of rodents to various metabolites of TCE identified TCA and DCA as putative cardiac teratogens. Johnson et al. (1998b; 1998a) and Smith et al. (1989) reported increased incidences of cardiac defects with gestational TCA exposures, while Smith et al. (1992) and Epstein et al. (1992) reported increased incidences following DCA exposures.

In all studies that observed increased cardiac defects, either TCE or its metabolites were administered during critical windows of in utero cardiac development, primarily during the entire duration of gestation, or during the period of major organogenesis (e.g., GDs 6–15 in the rat). The study by Epstein et al. (1992) used dosing with DCA on discrete days of gestation and had identified GDs 9 through 12 as a particularly sensitive period for eliciting high interventricular septal defects associated with exposures to TCE or its metabolites.

In the oral studies that identified increased incidences of cardiac malformations following gestational exposure to TCE, there was a broad range of administered doses at which effects were observed. In drinking water studies, Dawson et al. (1993) observed cardiac anomalies at 1.5 and 1,100 ppm (with no NOAEL) and Johnson et al. (2005, 2003) reported effects at 250 ppb

(with a NOAEL of 2.5 ppb). One concern is the lack of a clear dose-response for the incidence of any specific cardiac anomaly or combination of anomalies was not identified, a disparity for which no reasonable explanation for this disparity has been put forth.

The analysis of the incidence data for cardiac defects observed in the Dawson et al. (1993, 1990) and Johnson et al. (2005, 2003) studies has been critiqued (Watson et al., 2006). Issues of concern that have been raised include the statistical analyses of findings on a per-fetus (rather than the more appropriate per-litter) basis (Benson, 2004). Johnson et al. was further criticized for the use of nonconcurrent control data in the analysis (Hardin et al., 2004). In response, the study author has further explained procedures used (Johnson et al., 2004) and has provided individual litter incidence data to the EPA for independent statistical analysis (P. Johnson, personal communication, 2008) (see Section 5.1.2.8). In sum, while the studies by Dawson et al. (1993, 1990) and Johnson et al. (2005, 2003), have significant limitations, there is insufficient reason to dismiss their findings.

#### **4.8.3.3.2.1. Mode of action for cardiac malformations**

A number of in vitro studies have been conducted to further characterize the potential for alterations in cardiac development that have been attributed to exposures with TCE and/or its metabolites. It was noted that many of the cardiac defects observed in humans and laboratory species (primarily rats and chickens) involved septal and valvular structures.

During early cardiac morphogenesis, outflow tract and AV endothelial cells differentiate into mesenchymal cells. These mesenchymal cells have characteristics of smooth muscle-like myofibroblasts and form endocardial cushion tissue, which is the primordia of septa and valves in the adult heart. Events that take place in cardiac valve formation in mammals and birds are summarized by NRC (2006) and reproduced in Table 4-104.

**Table 4-104. Events in cardiac valve formation in mammals and birds<sup>a</sup>**

Stage and event	Structural description <sup>b</sup>
Early cardiac development	The heart is a hollow, linear, tube-like structure with two cell layers. The outer surface is a myocardial cell layer, and the inner luminal surface is an endothelial layer. Extracellular matrix is between the two cell layers.
Epithelial-mesenchymal cell transformation	A subpopulation of endothelial cells lining the AVC detaches from adjacent cells and invades the underlying extracellular matrix. Three events occur: <ul style="list-style-type: none"> <li>➤ Endothelial cell activation (avian stage 14)</li> <li>➤ Mesenchymal cell formation (avian stage 16)</li> <li>➤ Mesenchymal cell migration into the extracellular matrix (avian stages 17 and 18).</li> </ul>
Mesenchymal cell migration and proliferation	Endothelial-derived mesenchymal cells migrate toward the surrounding myocardium and proliferate to populate the AVC extracellular matrix.
Development of septa and valvular structures	Cardiac mesenchyme provides cellular constituents for: <ul style="list-style-type: none"> <li>➤ Septum intermedium</li> <li>➤ Valvular leaflets of the mitral and tricuspid AV valves.</li> </ul> The septum intermedium subsequently contributes to: <ul style="list-style-type: none"> <li>➤ Lower portion of the interatrial septum</li> <li>➤ Membranous portion of the interventricular septum.</li> </ul>

<sup>a</sup>As summarized in NRC (2006).

<sup>b</sup>Markwald et al. (1996; 1984); Boyer et al. (2000).

Methods have been developed to extract the chick stage 16 AVC from the embryo and culture it on a hydrated collagen gel for 24–48 hours, allowing evaluation of the described stages of cardiac development and their response to chemical treatment. Factors that have been shown to influence the induction of endocardial cushion tissue include molecular components such as fibronectin, laminin, and galactosyltransferase (Loeber and Runyan, 1990; Mjaatvedt et al., 1987), components of the extracellular matrix (Mjaatvedt et al., 1991), and smooth muscle  $\alpha$ -actin and transforming growth factor  $\beta$ 3 (Nakajima et al., 1997; Ramsdell and Markwald, 1997).

Boyer et al. (2000) utilized the in vitro chick AVC culture system to examine the molecular mechanism of TCE effects on cardiac morphogenesis. AVC explants from stage 16 chick embryos (15/treatment level) were placed onto collagen gels and treated with 0, 50, 100, 150, 200, or 250 ppm TCE and incubated for a total of 54 hours. Epithelial-mesenchymal transformation, endothelial cell density, cell migration, and immunohistochemistry were evaluated. TCE treatment was found to inhibit endothelial cell activation and normal mesenchymal cell transformation, endothelial cell-cell separation, and protein marker expression (i.e., transcription factor Mox-1 and extracellular matrix protein fibrillin 2). Mesenchymal cell migration was not affected, nor was the expression of smooth muscle  $\alpha$ -actin. The study authors proposed that TCE may cause cardiac valvular and septal malformations by inhibiting endothelial separation and early events of mesenchymal cell formation. Hoffman et al. (2004) proposed alternatively that TCE may be affecting the adhesive properties of the endocardial cells. No experimental data are currently available that address the levels of TCE in cardiac

tissue in vivo, resulting in some questions ([Dugard, 2000](#)) regarding the relevance of these mechanistic findings to human health risk assessment.

In a study by Mishima et al. ([2006](#)), White Leghorn chick whole embryo cultures (stage 13 and 14) were used to assess the susceptibility of endocardial epithelial-mesenchymal transformation in the early chick heart to TCE at analytically determined concentrations of 0, 10, 20, 40, or 80 ppm. This methodology maintained the anatomical relationships of developing tissues and organs, while exposing precisely staged embryos to quantifiable levels of TCE and facilitating direct monitoring of developmental morphology. Following 24 hours of incubation, the numbers of mesenchymal cells in the inferior and superior AV cushions were counted. TCE treatment significantly reduced the number of mesenchymal cells in both the superior and inferior AV cushions at 80 ppm.

Ou et al. ([2003](#)) examined the possible role of endothelial nitric oxide synthase (which generates nitric oxide that has an important role in normal endothelial cell proliferation and hence normal blood vessel growth and development) in TCE-mediated toxicity. Cultured proliferating bovine coronary endothelial cells were treated with TCE at 0–100  $\mu\text{M}$  and stimulated with a calcium ionophore to determine changes in endothelial cells and the generation of endothelial nitric oxide synthase, nitric oxide, and superoxide anion. TCE was shown to alter heat shock protein interactions with endothelial nitric oxide synthase and induce endothelial nitric oxide synthase to shift nitric oxide to superoxide-anion generation. These findings provide insight into how TCE impairs endothelial proliferation.

Several studies have also identified a TCE-related perturbation of several proteins involved in regulation of intracellular  $\text{Ca}^{2+}$ . After 12 days of maternal exposure to TCE in drinking water, *Serca2a* (sarcoendoplasmic reticulum  $\text{Ca}^{2+}$  ATPase) mRNA expression was reduced in rat embryo cardiac tissues ([Collier et al., 2003](#)). Selmin et al. ([2008](#)) conducted a microarray analysis of a P19 mouse stem cell line exposed to 1-ppm TCE in vitro, identifying altered expression of *Ryr2* (ryanodine receptor isoform 2), a  $\text{Ca}^{2+}$  release channel that is important in normal rhythmic heart activity ([Gyorke and Terentyev, 2008](#)). Alterations in  $\text{Ca}^{2+}$  cycling and resulting contractile dysfunction is a recognized pathogenic mechanism of cardiac arrhythmias and sudden cardiac death ([Lehnart et al., 2008](#); [Yano et al., 2008](#); [Leandri et al., 1995](#)). Caldwell et al. ([2008c](#)) used real-time PCR and digital imaging microscopy to characterize the effects of various doses of TCE on gene expression and  $\text{Ca}^{2+}$  response to vasopressin in rat cardiac myocytes (H9c2). *Serca2a* and *Ryr2* expression were reduced at 12 and 48 hours following exposure to TCE. Additionally,  $\text{Ca}^{2+}$  response to vasopressin was altered following TCE treatment. Makwana et al. ([2010](#)) dosed chick embryos in ovo with 8 or 800 ppb TCE; real time-PCR analysis of RNA isolated during specific windows of cardiac development demonstrated effects on the expression of genes associated with reduced blood flow. Although it has been hypothesized that TCE might interfere with the folic acid/methylation pathway in liver and kidney and alter gene regulation by epigenetic

mechanisms, Caldwell et al. (2010) found that the effects of TCE exposure on normal gene expression in rat embryonic hearts was not altered by the administration of exogenous folate. Overall, these data suggest that TCE may disrupt the ability to regulate cellular  $\text{Ca}^{2+}$  fluxes, altering blood flow and leading to morphogenic consequences in the developing heart. This remains an open area of research.

Thus, in summary, a number of studies have been conducted in an attempt to characterize the mode of action for TCE-induced cardiac defects. A major research focus has been on disruptions in cardiac valve formation, using avian in ovo and in vitro studies. These studies demonstrated treatment-related alterations in endothelial cushion development that could plausibly be associated with defects involving septal and valvular morphogenesis in rodents and chickens. However, a broad array of cardiac malformations has been observed in animal models following TCE exposures (Johnson et al., 2005, 2003; Dawson et al., 1993), and other evidence of molecular disruption of  $\text{Ca}^{2+}$  during cardiac development has been examined (Caldwell et al., 2008c; Selmin et al., 2008; Collier et al., 2003), suggesting the possible existence of multiple modes of action. The observation of defective myocardial development in a mouse model deficient for gp130, a signal transducer receptor for IL-6 (Yoshida et al., 1996), suggests the potential involvement of immune-mediated effects.

#### **4.8.3.3.2.2. Association of PPAR $\alpha$ with developmental outcomes**

The PPARs are ligand activated receptors that belong to the nuclear hormone receptor family. Three isotypes have been identified (PPAR $\alpha$ , PPAR $\delta$  [also known as PPAR $\beta$ ], and PPAR $\gamma$ ). These receptors, upon binding to an activator, stimulate the expression of target genes implicated in important metabolic pathways. In rodents, all three isotypes show specific time- and tissue-dependent patterns of expression during fetal development and in adult animals. In development, they have been especially implicated in several aspects of tissue differentiation (e.g., of the adipose tissue, brain, placenta, and skin). Epidermal differentiation has been linked strongly with PPAR $\alpha$  and PPAR $\delta$  (Michalik et al., 2002). PPAR $\alpha$  starts late in development, with increasing levels in organs such as liver, kidney, intestine, and pancreas; it is also transiently expressed in fetal epidermis and CNS (Braissant and Wahli, 1998) and has been linked to phthalate-induced developmental and testicular toxicity (Corton and Lapinskas, 2005). Liver, kidney, and heart are the sites of highest PPAR $\alpha$  expression (Toth et al., 2007). PPAR $\delta$  and PPAR $\gamma$  have been linked to placental development and function, with PPAR $\gamma$  found to be crucial for vascularization of the chorioallantoic placenta in rodents (Wendling et al., 1999), and placental anomalies mediated by PPAR $\gamma$  have been linked to rodent cardiac defects (Barak et al., 2008). While it might be hypothesized that there is some correlation between PPAR signaling, fetal deaths, and/or cardiac defects observed following TCE exposures in rodents, no definitive data have been generated that elucidate a possible PPAR-mediated mode of action for these outcomes.

#### **4.8.3.3.2.3. Summary of the weight of evidence on cardiac malformations**

The evidence for an association between TCE exposures in the human population and the occurrence of congenital cardiac defects is not particularly strong. Many of the epidemiological study designs were not sufficiently robust to detect exposure-related birth defects with a high degree of confidence. However, two well-conducted studies by ATSDR ([2008b](#), [2006a](#)) clearly demonstrated an elevation in cardiac defects. It could be surmised that the identified cardiac defects were detected because they were severe, and that additional cases with less severe cardiac anomalies may have gone undetected.

The animal data provide strong, but not unequivocal, evidence of the potential for TCE-induced cardiac malformations following oral exposures during gestation. Strengths of the evidence are the duplication of the adverse response in several studies from the same laboratory group, detection of treatment-related cardiac defects in both mammalian and avian species (i.e., rat and chicken), general cross-study consistency in the positive association of increased cardiac malformations with test species (i.e., rat), route of administration (i.e., oral), and the methodologies used in cardiac morphological evaluation (i.e., fresh dissection of fetal hearts). Furthermore, when differences in response are observed across studies, they can generally be attributed to obvious methodological differences, and a number of *in ovo* and *in vitro* studies demonstrate a consistent and biologically plausible mode of action for one type of malformation observed. Weaknesses in the evidence include lack of a clear dose-related response in the incidence of cardiac defects, and the broad variety of cardiac defects observed, such that they cannot all be grouped easily by type or etiology.

Taken together, the epidemiological and animal study evidence raise sufficient concern regarding the potential for developmental toxicity (increased incidence of cardiac defects) with *in utero* TCE exposures.

#### **4.8.3.3.3. Other structural developmental outcomes**

A summary of other structural developmental outcomes that have been associated with TCE exposures is presented in Table 4-105.

**Table 4-105. Summary of other structural developmental outcomes associated with TCE exposures**

Finding	Species	References
Eye/ear birth anomalies	Human	Lagakos et al. (1986)
	Rat	Narotsky (1995); Narotsky and Kavlock (1995)
Oral cleft defects	Human	Bove (1996); Bove et al. (1995); Lagakos et al. (1986); Lorente et al. (2000)
Kidney/urinary tract disorders	Human	Lagakos et al. (1986)
Musculoskeletal birth anomalies	Human	Lagakos et al. (1986)
Anemia/blood disorders	Human	Burg and Gist (1999)
Lung/respiratory tract disorders	Human	Lagakos et al. (1986)
	Mouse	Das and Scott (1994)
Skeletal	Rat	Healy et al. (1982)
Other <sup>a</sup>	Human	ATSDR (2001)

<sup>a</sup>As reported by the authors.

In humans, a variety of birth defects other than cardiac have been observed. These include total birth defects (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Flood, 1988) CNS birth defects (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986), eye/ear birth anomalies (Lagakos et al., 1986); oral cleft defects (Lorente et al., 2000; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986); kidney/urinary tract disorders (Lagakos et al., 1986); musculoskeletal birth anomalies (Lagakos et al., 1986); anemia/blood disorders (Burg and Gist, 1999); and lung/respiratory tract disorders (Lagakos et al., 1986). While some of these results were statistically significant, they have not been reported elsewhere. Occupational cohort studies, while not reporting positive results, are generally limited by the small number of observed or expected cases of birth defects (Lorente et al., 2000; Taskinen et al., 1989; Tola et al., 1980).

In experimental animals, a statistically significant increase in the incidence of fetal eye defects, primarily microphthalmia and anophthalmia, manifested as reduced or absent eye bulge, was observed in rats following gavage administration of 1,125 mg/kg-day TCE during the period of organogenesis (Narotsky and Kavlock, 1995; Narotsky et al., 1995). Dose-related nonsignificant increases in the incidence of F344 rat pups with eye defects were also observed at lower dose levels (101, 320, 475, 633, and 844 mg/kg-day) in the Narotsky et al. (1995) study (also reported in Barton and Das, 1996). However, no other developmental or reproductive toxicity studies identified abnormalities of eye development following TCE exposures. For example, in a study reported by Warren et al. (2006), extensive computerized morphometric ocular evaluation was conducted in Sprague-Dawley rat fetuses that had been examined for cardiac defects by Fisher et al. (2001); the dams had been administered TCE (500 mg/kg-day),

DCA (300 mg/kg-day), or TCA (300 mg/kg-day) during GDs 6–15. No ocular defects were found with TCE exposures; however, significant reductions in the lens area, globe area, and interocular distance were observed with DCA exposures, and nonsignificant decreases in these measures as well as the medial canthus distance were noted with TCA exposures.

Developmental toxicity studies conducted by Smith et al. (1992; 1989) also identified orbital defects (combined soft tissue and skeletal abnormalities) in Long-Evans rat fetuses following GD 6–15 exposures with TCA and DCA (statistically or biologically significant at  $\geq 800$  and  $\geq 900$  mg/kg-day, respectively). Overall, the study evidence indicates that TCE and its oxidative metabolites can disrupt ocular development in rats. In addition to the evidence of alteration to the normal development of ocular structure, these findings may also be an indicator of disruptions to nervous system development. It has been suggested by Warren et al. (2006) and Williams and DeSesso (2008) that the effects of concern (defined as statistically significant outcomes) are observed only at high dose levels and are not relevant to risk assessment for environmental exposures. On the other hand, Barton and Das (1996) point out that BMD modeling of the quantal eye defect incidence data provides a reasonable approach to the development of oral toxicity values for TCE human health risk assessment. It is also noted that concerns may exist not only for risks related to low level environmental exposures, but also for risks resulting from acute or short-term occupational or accidental exposures, which may be associated with much higher inadvertent doses.

It was also notable that a study using a single i.p. dose of 3,000 mg/kg TCE to mice during late gestation (GD 17) identified apparent delays in lung development and increased neonatal mortality (Das and Scott, 1994). No further evaluation of this outcome has been identified in the literature.

Healy et al. (1982) did not identify any treatment-related fetal malformations following inhalation exposure of pregnant inbred Wistar rats to 0 or 100 ppm (535 mg/m<sup>3</sup>) on GDs 8–21. In this study, significant differences between control and treated litters were observed as an increased incidence of minor ossification variations ( $p = 0.003$ ) (absent or bipartite centers of ossification).

#### **4.8.3.3.4. Developmental neurotoxicity**

Studies that address effects of TCE on the developing nervous system are discussed in detail in Section 4.3, addressed above in the sections on human developmental toxicity (see Section 4.8.3) and on mammalian studies (see Section 4.8.3.2.1) by route of exposure, and summarized in Table 4-106. The available data collectively suggest that the developing brain is susceptible to TCE exposures.

**Table 4-106. Summary of developmental neurotoxicity associated with TCE exposures**

Positive findings	Species	References
CNS defects, neural tube defects	Human	ATSDR (2001)
		Bove (1996); Bove et al. (1995)
		Lagakos et al. (1986)
Eye defects	Rat	Narotsky (1995); Narotsky and Kavlock (1995)
Delayed newborn reflexes	Human	Beppu (1968)
Impaired learning or memory	Human	Bernad et al. (1987), abstract
		White et al. (1997)
Aggressive behavior	Human	Bernad et al. (1987), abstract
	Rat	Blossom et al. (2008)
Hearing impairment	Human	ATSDR (2003b); Burg et al. (1995); Burg and Gist (1999)
		Beppu (1968)
Speech impairment	Human	ATSDR (2003b); Burg et al. (1995); Burg and Gist (1999)
		White et al. (1997)
Encephalopathy	Human	White et al. (1997)
Impaired executive function	Human	White et al. (1997)
Impaired motor function	Human	White et al. (1997)
Attention deficit	Human	Bernad et al. (1987), abstract
ASD	Human	Windham et al. (2006)
Delayed or altered biomarkers of CNS development	Rat	Isaacson and Taylor (1989); Noland-Gerbec et al. (1986); Westergren et al. (1984)
Behavioral alterations	Mice	Blossom et al. (2008); Fredriksson et al. (1993)
	Rat	George et al. (1986); Taylor et al. (1985)

In humans, CNS birth defects were observed in a few studies ([ATSDR, 2001](#); [Bove, 1996](#); [Bove et al., 1995](#); [Lagakos et al., 1986](#)). Postnatally, observed adverse effects in humans include delayed newborn reflexes following use of TCE during childbirth ([Beppu, 1968](#)), impaired learning or memory ([White et al., 1997](#); [Bernad et al., 1987, abstract](#)); aggressive behavior ([Bernad et al., 1987, abstract](#)); hearing impairment ([ATSDR, 2003b](#); [Burg and Gist, 1999](#); [Burg et al., 1995](#); [Beppu, 1968](#)); speech impairment ([Burg and Gist, 1999](#); [White et al., 1997](#); [Burg et al., 1995](#)); encephalopathy ([White et al., 1997](#)); impaired executive and motor function ([White et al., 1997](#)); attention deficit ([White et al., 1997](#); [Bernad et al., 1987, abstract](#)), and ASD ([Windham et al., 2006](#)). While there are broad developmental neurotoxic effects that have been associated with TCE exposure, there are many limitations in the studies.

More compelling evidence for the adverse effect of TCE exposure on the developing nervous system is found in the animal study data, although a rigorous evaluation of potential

outcomes has not been conducted. For example, there has not been an assessment of cognitive function (i.e., learning and memory) following developmental exposures to TCE, nor have most of the available studies characterized the pre- or postnatal exposure of the offspring to TCE or its metabolites. Nevertheless, there is evidence of treatment-related alterations in brain development and in behavioral parameters (e.g., spontaneous motor activity and social behaviors) associated with exposures during neurological development. The animal study database includes the following information: following inhalation exposures of 150 ppm to mice during mating and gestation, the specific gravity of offspring brains were significantly decreased at postnatal time points through the age of weaning; however, this effect did not persist to 1 month of age ([Westergren et al., 1984](#)). In studies reported by Taylor et al. ([1985](#)), Isaacson and Taylor ([1989](#)), and Noland-Gerbec et al. ([1986](#)), 312 mg/L exposures in drinking water that were initiated 2 weeks prior to mating and continued to the end of lactation resulted, respectively, in: (1) significant increases in exploratory behavior at GDs 60 and 90; (2) reductions in myelination in the brains of offspring at weaning; and (3) significantly decreased uptake of 2-deoxyglucose in the neonatal rat brain (suggesting decreased neuronal activity). Ocular malformations in rats observed by Narotsky ([1995](#)) and Narotsky and Kavlock ([1995](#)) following maternal gavage doses of 1,125 mg/kg-day during gestation may also be indicative of alterations of nervous system development. Gestational exposures to mice ([Fredriksson et al., 1993](#)) resulted in significantly decreased rearing activity on GD 60, and dietary exposures during the course of a continuous breeding study in rats ([George et al., 1986](#)) found a significant trend toward increased time to cross the first grid in open field testing. In a study by Blossom et al. ([2008](#)), alterations in social behaviors (deficits in nest-building quality and increased aggression in males) were observed in pubertal-age MRL +/+ mice that had been exposed to 0.1 mg/mL TCE via drinking water during prenatal and postnatal development (until PND 42). Dorfmueller et al. ([1979](#)) was the only study that assessed neurobehavioral endpoints following in utero exposure (maternal inhalation exposures of 1,800 ± 200 ppm during gestation) and found no adverse effects that could be attributed to TCE exposure. Specifically, an automated assessment of ambulatory response in a novel environment on GDs 10, 20 and 100, did not identify any effect on general motor activity of offspring.

#### **4.8.3.3.5. Developmental immunotoxicity**

Studies that address the developmental immunotoxic effects of TCE are discussed in detail in Section 4.6, addressed above in the sections on human developmental toxicity (see Section 4.8.3) and on mammalian studies (see Section 4.8.3.2.1) by route of exposure, and summarized in Table 4-107.

**Table 4-107. Summary of developmental immunotoxicity associated with TCE exposures**

Finding	Species (strain)	References
Significant reduction in Th1 IL-2 producing cells	Human	Lehmann et al. (2002)
Altered immune response	Human	Byers et al. (1988)
Suppression of PFC responses, increased T-cell subpopulations, decreased spleen cellularity, and increased hypersensitivity response	Mouse (B6C3F <sub>1</sub> )	Peden-Adams et al. (2006)
Altered splenic and thymic T-cell subpopulations	Mouse (MRL +/+)	Peden-Adams et al. (2008)
Altered thymic T-cell subpopulations; transient increased proinflammatory cytokine production by T-cells; increased autoantibody levels and IgG	Mouse (MRL +/+)	Blossom and Doss (2007)
Increased proinflammatory cytokine production by T-cells	Mouse (MRL +/+)	Blossom et al. (2008)

Two epidemiological studies that addressed potential immunological perturbations in children that were exposed to TCE were reported by Lehmann et al. (2002; 2001). In the 2001 study, no association was observed between TCE and allergic sensitization to egg white and milk, or to cytokine producing peripheral T-cells, in premature neonates and 36-month-old neonates that were at risk of atopy. In the 2002 study, there was a significant reduction in Th1 IL-2 producing cells. Another study observed altered immune response in family members of those diagnosed with childhood leukemia, including 13 siblings under 19 years old at the time of exposure, but an analysis looking at only these children was not done (Byers et al., 1988).

Several studies were identified (Blossom et al., 2008; Peden-Adams et al., 2008; Blossom and Doss, 2007; Peden-Adams et al., 2006) that assessed the potential for developmental immunotoxicity in mice following oral (drinking water) TCE exposures during critical pre- and postnatal stages of immune system development. Peden-Adams et al. (2006) noted evidence of immune system perturbation (suppression of PFC responses, increased T-cell subpopulations, decreased spleen cellularity, and increased hypersensitivity response) in B6C3F<sub>1</sub> offspring following in utero and 8 weeks of postnatal exposures to TCE. Evidence of autoimmune response was not observed in the offspring of this nonautoimmune-prone strain of mice. However, in a study by Peden-Adams et al. (2008) MRL +/+ mice, which are autoimmune-prone, were exposed from conception until 12 months of age. Consistent with the Peden-Adams et al. (2006) study, no evidence of increased autoantibody levels was observed in the offspring. In two other studies focused on autoimmune responses following drinking water exposures of MRL +/+ mice to TCE during in utero development and continuing until the time of sexual maturation, Blossom and Doss (2007) and Blossom et al. (2008) reported some peripheral blood changes that were indicative of treatment-related autoimmune responses in offspring. Positive response levels were 0.5 and 2.5 mg/mL for Blossom and Doss (2007) and 0.1 mg/mL for Blossom et al. (2008). None of these studies were designed to extensively evaluate recovery, latent outcomes, or differences in severity of response that might be attributed to the early life

exposures. Consistency in response in these animal studies was difficult to ascertain due to the variations in study design (e.g., animal strain used, duration of exposure, treatment levels evaluated, timing of assessments, and endpoints evaluated). Likewise, the endpoints assessed in the few epidemiological studies that evaluated immunological outcomes following developmental exposures to TCE were dissimilar from those evaluated in the animal models, and so provided no clear cross-species correlation. The most sensitive immune system response noted in the studies that exposed developing animals were the decreased PFC and increased hypersensitivity observed by Peden-Adams et al. (2006); treatment-related outcomes were noted in mice exposed in the drinking water at a concentration of 1,400 ppb. None of the other studies that treated mice during immune system development assessed these same endpoints; therefore, direct confirmation of these findings across studies was not possible. It is noted, however, that similar responses were not observed in studies in which adult animals were administered TCE (e.g., Woolhiser et al., 2006), suggesting increased susceptibility in the young. Differential lifestage-related responses have been observed with other diverse chemicals (e.g., diethylstilbestrol; diazepam; lead; 2,3,7,8-tetrachlorobenzo-*p* dioxin; and tributyltin oxide) in which immune system perturbations were observed at lower doses and/or with greater persistence when tested in developing animals as compared to adults (Luebke et al., 2006). Thus, such an adverse response with TCE exposure is considered biologically plausible and an issue of concern for human health risk assessment.

#### 4.8.3.3.6. Childhood cancers

A summary of childhood cancers that have been associated with TCE exposures discussed above is presented in Table 4-108. A summary of studies that observed childhood leukemia is also discussed in detail in Sections 4.6.1.2 and 4.8.3.1.2.4 contains details of epidemiologic studies on childhood brain cancer.

**Table 4-108. Summary of childhood cancers associated with TCE exposures**

Finding	Species	References
Leukemia	Human	AZ DHS ( <a href="#">ADHS, 1990</a> ; <a href="#">Flood, 1988</a> )
		AZ DHS ( <a href="#">Kioski et al., 1990a</a> )
		Cohn et al. ( <a href="#">1994b</a> )
		Cutler et al. ( <a href="#">1986</a> ); Costas et al. ( <a href="#">2002</a> ); Lagakos et al. ( <a href="#">1986</a> ); MDPH ( <a href="#">1997c</a> )
		Lowengart et al. ( <a href="#">1987</a> )
		McKinney et al. ( <a href="#">1991</a> )
		Shu et al. ( <a href="#">1999</a> )
Neuroblastoma	Human	De Roos et al. ( <a href="#">2001</a> )
		Peters et al. ( <a href="#">1985</a> ; <a href="#">1981</a> )

A nonsignificant increased risk of leukemia diagnosed during childhood has been observed in a number of studies examining TCE exposure ([Costas et al., 2002](#); [Shu et al., 1999](#); [MDPH, 1997b](#); [Cohn et al., 1994b](#); [McKinney et al., 1991](#); [ADHS, 1990](#); [Kioski et al., 1990a](#); [Flood, 1988](#); [Lowengart et al., 1987](#); [Lagakos et al., 1986](#)). However, other studies did not observe an increased risk for childhood leukemia after TCE exposure ([Morgan and Cassady, 2002](#); [Flood, 1997b](#); [Kioski et al., 1990b](#)), possibly due to the limited number of cases or the analysis based on multiple solvents.

CNS cancers during childhood have been reported on in a few studies. Neuroblastomas were not statistically elevated in one study observing parental exposure to multiple chemicals, including TCE ([De Roos et al., 2001](#)). Brain tumors were observed in another study, but the OR could not be determined ([Peters et al., 1985](#); [Peters et al., 1981](#)). CNS cancers were not elevated in other studies ([Morgan and Cassady, 2002](#); [Kioski et al., 1990a](#)). Other studies did not see an excess risk of total childhood cancers (ATSDR, 2006a; [Morgan and Cassady, 2002](#)).

A follow-up study of the Camp Lejeune cohort that will examine childhood cancers (along with birth defects) was initiated in 1999 ([ATSDR, 2003a](#)), is expected to be completed soon ([ATSDR, 2009](#); [U.S. GAO, 2007b, a](#)), and may provide additional insight.

No studies of cancers in experimental animals in early lifestages have been identified.

## **4.9. OTHER SITE-SPECIFIC CANCERS**

### **4.9.1. Esophageal Cancer**

Increasing esophageal cancer incidence has been observed in males, but not females in the United States between 1975 and 2002, a result of increasing incidence of esophageal adenocarcinoma ([Ward et al., 2006](#)). Males also have higher age-adjusted incidence and mortality rates (incidence, 7.8 per 100,000; mortality, 7.8 per 100,000) than females (incidence, 2.0 per 100,000; mortality, 1.7 per 100,000) ([Ries et al., 2008](#)). Survival for esophageal cancer remains poor, and age-adjusted mortality rates are just slightly lower than incidence rates. Major risk factors associated with esophageal cancer are smoking and alcohol for squamous cell carcinoma, typically found in the upper third of the esophagus, and obesity, gastroesophageal reflux, and Barrett's esophagus for adenocarcinoma that generally occurs in the lower esophagus ([Ward et al., 2006](#)).

Seventeen epidemiologic studies on TCE exposure reported RRs for esophageal cancer ([Clapp and Hoffman, 2008](#); [Radican et al., 2008](#); [Sung et al., 2007](#); [ATSDR, 2006a](#); [Boice et al., 2006b](#); [Zhao et al., 2005](#); [ATSDR, 2004a](#); [Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#); [Boice et al., 1999](#); [Ritz, 1999a](#); [Blair et al., 1998](#); [Greenland et al., 1994](#); [Siemiatycki, 1991](#); [Blair et al., 1989](#); [Costa et al., 1989](#); [Garabrant et al., 1988](#)). Ten studies had high likelihood of TCE exposure in individual study subjects and were judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis ([Radican et al., 2008](#); [Boice et al., 2006b](#); [Zhao et al., 2005](#); [Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#); [Boice et al., 1999](#); [Ritz,](#)

[1999a](#); [Blair et al., 1998](#); [Greenland et al., 1994](#); [Siemiatycki, 1991](#)). Four studies with TCE exposure potential assigned to individual subjects ([Blair et al., 1998 \[Incidence\]](#); [Morgan et al., 1998](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#)) did not present RR estimates for esophageal cancer and TCE exposure nor did two other studies, which carry less weight in the analysis because of design limitations ([Henschler et al., 1995](#); [Sinks et al., 1992](#)). Only Raaschou-Nielsen et al. ([2003](#)) examined esophageal cancer histologic type, an important consideration given differences between suspected risk factors for adenocarcinoma and those for squamous cell carcinoma. Appendix B identifies these studies' design and exposure assessment characteristics.

Several population case-control studies ([Ramanakumar et al., 2008](#); [Santibanez et al., 2008](#); [Weiderpass et al., 2003](#); [Engel et al., 2002](#); [Parent et al., 2000b](#); [Gustavsson et al., 1998](#); [Yu et al., 1988](#)) examined esophageal cancer and organic solvents or occupational job titles with past TCE use documented ([Bakke et al., 2007](#)). RR estimates in case-control studies that examine metal occupations or job titles, or solvent exposures are found in Table 4-109. The lack of exposure assessment to TCE, low prevalence of exposure to chlorinated hydrocarbon solvents, or few exposed cases and controls in those studies lowers their sensitivity for informing evaluations of TCE and esophageal cancer.

**Table 4-109. Selected observations from case-control studies of TCE exposure and esophageal cancer**

Study population	Exposure group	All esophageal cancers		Squamous cell cancer		Adenocarcinoma		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Population of regions in Eastern Spain								Santibañez et al. (2008)
	Metal molders, welders, etc.	0.94 (0.14, 6.16)	3	0.40 (0.05, 3.18)	2	3.55 (0.28, 44.70)	1	
	Metal-processing plant operators	1.14 (0.29, 4.44)	5	1.23 (0.23, 6.51)	4	0.86 (0.08, 8.63)	1	
	Chlorinated hydrocarbon solvents							
	Low exposure	1.05 (0.15, 7.17)	2		0	4.92 (0.69, 34.66)	2	
	High exposure	1.76 (0.40, 7.74)	6	2.18 (0.41, 11.57)	5	3.03 (0.28, 32.15)	1	
Population of Montreal, Canada								Ramanakumar et al. (2008); Parent et al. (2000b)
	Painter, Metal coatings							
	Any exposure	1.3 (0.4, 4.2)	6					
	Substantial exposure	4.2 (1.1, 17.0)	4					
	Solvents							
	Any exposure	1.1 (0.7, 1.7)	39	1.4 (0.8, 2.5)	30			
	Nonsubstantial exposure	1.0 (0.5, 1.9)	16	1.3 (0.6, 2.6)	12			
	Substantial exposure	1.1 (0.6, 1.9)	39	1.4 (0.8, 2.5)	30			
Population of Sweden								Jansson et al., (2006; 2005)
	Organic solvents							
	No exposure			1.0	145	1.0	128	
	Moderate exposure			0.7 (0.4, 1.5)	15	1.2 (0.6, 2.3)	14	
	High exposure			1.3 (0.7, 2.3)	21	1.4 (0.7, 2.5)	18	
	Test for trend							
	No exposure			1.0		1.0		
	Moderate exposure			0.5 (0.1, 3.9) <sup>a</sup>	1	0.4 (0.1, 1.5) <sup>a</sup>	2	
	High exposure			0.4 (0.1, 1.8) <sup>a</sup>	2	0.9 (0.5, 1.6) <sup>a</sup>	12	
	Test for trend							
				$p = 0.44$		$p = 0.36$		

**Table 4-109. Selected observations from case-control studies of TCE exposure and esophageal cancer (continued)**

Study population	Exposure group	All esophageal cancers		Squamous cell cancer		Adenocarcinoma		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Population of Finland (Females)								Weiderpass et al. ( <a href="#">2003</a> )
	Chlorinated hydrocarbon solvents							
	Low level exposure	0.95 (0.54, 1.66)	Not reported					
	High level exposure	0.62 (0.34, 1.13)	Not reported					
Population of New Jersey, Connecticut, Washington State								Engel et al. ( <a href="#">2002</a> )
	Precision metal workers	Not reported		0.7 (0.3, 1.5)	12	1.4 (0.8, 2.3)	25	
	Metal product manufacturing	Not reported		0.8 (0.3, 1.8)	15	1.3 (0.8, 2.3)	26	

<sup>a</sup>Jansson et al. ([2006](#)) is a registry-based study of the Swedish Construction Worker Cohort. RRs are incidence rate ratios from Cox regression analysis using calendar time and adjustment for attained age, calendar period at entry into the cohort, tobacco smoking status at entry into the cohort and BMI at entry into the cohort.

Table 4-110 presents risk estimates for TCE exposure and esophageal cancer observed in cohort, PMR, case-control, and geographic-based studies. Ten studies in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) reported risk estimates for esophageal cancer ([Radican et al., 2008](#); [Boice et al., 2006b](#); [Zhao et al., 2005](#); [Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#); [Boice et al., 1999](#); [Ritz, 1999a](#); [Blair et al., 1998](#); [Greenland et al., 1994](#); [Siemiatycki, 1991](#)). Some evidence for association with esophageal cancer and overall TCE exposure comes from studies with high likelihood of TCE exposure (5.6, 95% CI: 0.7, 44.5 ([Blair et al., 1998](#)) and 1.88, 95% CI: 0.61, 5.79 [[Radican et al. \(2008\)](#), which was an update of [Blair et al. \(1998\)](#) with an additional 10 years of follow-up]; 4.2, 95% CI: 1.5, 9.2, ([Hansen et al., 2001](#)); 1.2, 95% CI: 0.84, 1.57 ([Raaschou-Nielsen et al., 2003](#))]. Two studies support an association with adenocarcinoma histologic type of esophageal cancer and TCE exposure [five of the six observed esophageal cancers were adenocarcinomas [ $<1$  expected; [Hansen et al. \(2001\)](#)]; 1.8, 95% CI: 1.2, 2.7 ([Raaschou-Nielsen et al., 2003](#))]. Risk estimates in other studies are based on few deaths, low statistical power to detect a doubling of esophageal cancer risk, and CIs that include a risk estimate of 1.0 (no increased risk).

**Table 4-110. Summary of human studies on TCE exposure and esophageal cancer**

Exposure group		RR (95% CI)	Number of observable events	Reference
<b>Cohort studies—incidence</b>				
Aerospace workers (Rocketdyne)				Zhao et al. (2005)
	Any exposure to TCE	Not reported		
	Low cumulative TCE score	1.00 <sup>a</sup>	9	
	Med cumulative TCE score	1.66 (0.62, 4.41) <sup>b</sup>	8	
	High TCE score	0.82 (0.17, 3.95) <sup>b</sup>	2	
	<i>p</i> for trend	<i>p</i> = 0.974		
All employees at electronics factory (Taiwan)				Sung et al. (2007)
	Males	Not reported		
	Females	1.16 (0.0.14, 4.20) <sup>c</sup>	2	
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al. (2003)
	Any exposure, all subjects	1.2 (0.84, 1.57)	44	
	Any exposure, males	1.1 (0.81, 1.53)	40	
	Any exposure, females	2.0 (0.54, 5.16)	4	
	Any exposure, males	1.8 (1.15, 2.73) <sup>d</sup>	23	
	Any exposure, females		0 (0.4 exp) <sup>d</sup>	
Exposure lag time				
	20 yrs	1.7 (0.8, 3.0) <sup>d</sup>	10	
Employment duration				
	<1 yr	1.7 (0.6, 3.6) <sup>d</sup>	6	
	1–4.9 yrs	1.9 (0.9, 3.6) <sup>d</sup>	9	
	≥5 yrs	1.9 (0.8, 3.7) <sup>d</sup>	8	
Subcohort with higher exposure				
	Any TCE exposure	1.7 (0.9, 2.9) <sup>d</sup>	13	
Employment duration				
	1–4.9 yrs	1.6 (0.6, 3.4) <sup>d</sup>	6	
	≥5 yrs	1.9 (0.8, 3.8) <sup>d</sup>	7	

**Table 4-110. Summary of human studies on TCE exposure and esophageal cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
Biologically-monitored Danish workers		4.0 (1.5, 8.72)	6	Hansen et al. ( <a href="#">2001</a> )
Any TCE exposure, males		4.2 (1.5, 9.2)	6	
Adenocarcinoma histologic type		3.6 (1.2, 8.3) <sup>e</sup>	5	
Any TCE exposure, females			0 (0.1 exp)	
Cumulative exposure (Ikeda)				
<17 ppm-yr		6.5 (1.3, 19)	3	
≥17 ppm-yr		4.2 (1.5, 9.2)	3	
Mean concentration (Ikeda)				
<4 ppm		8.0 (2.6, 19)	5	
4+ ppm		1.3 (0.02, 7.0)	1	
Employment duration				
<6.25 yr		4.4 (0.5, 16)	2	
≥6.25 yr		6.6 (1.8, 17)	4	
Aircraft maintenance workers from Hill Air Force Base				Blair et al. ( <a href="#">1998</a> )
TCE subcohort		Not reported		
Males, cumulative exposure				
0		1.0 <sup>a</sup>		
<5 ppm-yr		Not reported		
5–25 ppm-yr		Not reported		
>25 ppm-yr		Not reported		
Females, cumulative exposure				
0		1.0 <sup>a</sup>		
<5 ppm-yr		Not reported		
5–25 ppm-yr		Not reported		
>25 ppm-yr		Not reported		
Biologically-monitored Finnish workers				Anttila et al. ( <a href="#">1995</a> )
All subjects		Not reported		
Mean air-TCE (Ikeda extrapolation)				
<6 ppm		Not reported		
6+ ppm		Not reported		

**Table 4-110. Summary of human studies on TCE exposure and esophageal cancer (continued)**

Exposure group	RR (95% CI)	Number of observable events	Reference
Cardboard manufacturing workers in Arnsburg, Germany			Henschler et al. (1995)
Exposed workers	Not reported		
Biologically-monitored Swedish workers			Axelson et al. (1994)
Any TCE exposure, males	Not reported		
Any TCE exposure, females	Not reported		
Cardboard manufacturing workers, Atlanta area, Georgia			Sinks et al. (1992)
All subjects	Not reported		
<b>Cohort and PMR studies-mortality</b>			
Computer manufacturing workers (IBM), New York			Clapp and Hoffman (2008)
Males	1.12 (0.30, 2.86) <sup>f</sup>		
	5.24 (0.13, 29.2) <sup>f</sup>		
Aerospace workers (Rocketdyne)			
Any TCE (utility/eng flush)	0.88 (0.18, 2.58)	3	Boice et al. (2006b)
Any exposure to TCE	Not reported		Zhao et al. (2005)
Low cumulative TCE score	1.00 <sup>a</sup>	18	
Medium cumulative TCE score	1.40 (0.70, 2.82) <sup>b</sup>	15	
High TCE score	1.27 (0.52, 3.13) <sup>b</sup>	7	
<i>p</i> for trend	<i>p</i> = 0.535		
View-Master employees			ATSDR (2004a)
Males	0.62 (0.02, 3.45) <sup>f</sup>	1	
Females		0 (1.45 exp) <sup>f</sup>	
All employees at electronics factory (Taiwan)			Chang et al. (2003)
Males		0 (3.34 exp)	
Females		0 (0.83 exp)	
United States uranium-processing workers (Fernald)			Ritz (1999a)
Any TCE exposure	Not reported		
Light TCE exposure, >2-yr duration	2.61 (0.99, 6.88) <sup>g</sup>	12	
Moderate TCE exposure, >2-yr duration		0	

**Table 4-110. Summary of human studies on TCE exposure and esophageal cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
Aerospace workers (Lockheed)				Boice et al. (1999)
Routine exposure	0.83 (0.34, 1.72)	7		
Routine-intermittent <sup>a</sup>	Not presented	11		
Duration of exposure				
0 yr	1.0 <sup>a</sup>	28		
<1 yr	0.23 (0.05, 0.99)	2		
1–4 yrs	0.57 (0.20, 1.67)	4		
≥5 yrs	0.91 (0.38, 2.22)	7		
<i>p</i> for trend	<i>p</i> > 0.20			
Aerospace workers (Hughes)				Morgan et al. (1998)
TCE subcohort	Not reported			
Low intensity (<50 ppm)				
High intensity (>50 ppm)				
TCE subcohort (Cox Analysis)	Not reported			
Never exposed				
Ever exposed				
Peak	Not reported			
No/Low				
Medium/high				
Cumulative	Not reported			
Referent				
Low				
High				
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. (1998)
TCE subcohort	5.6 (0.7, 44.5) <sup>a</sup>	10		
Males, cumulative exposure				
0	1.0 <sup>a</sup>			
<5 ppm-yr	Not reported <sup>h</sup>	3		
5–25 ppm-yr	Not reported <sup>h</sup>	2		
>25 ppm-yr	Not reported <sup>h</sup>	4		

**Table 4-110. Summary of human studies on TCE exposure and esophageal cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference	
Females, cumulative exposure				Blair et al. (1998) (continued)	
	0	1.0 <sup>a</sup>			
	<5 ppm-yr	3.6 (0.2, 58)	1		
	5–25 ppm-yr		0		
	>25 ppm-yr		0		
TCE subcohort		1.88 (0.61, 5.79)	17	Radican et al. (2008)	
Males, cumulative exposure		1.66 (0.48, 5.74)	15		
	0	1.0 <sup>a</sup>			
	<5 ppm-yr	1.84 (0.48, 7.14)	7		
	5–25 ppm-yr	1.33 (0.27, 6.59)	3		
	>25 ppm-yr	1.67 (0.40, 7.00)	5		
Females, cumulative exposure		2.81 (0.25, 31.10)	2		
	0	1.0 <sup>a</sup>			
	<5 ppm-yr	3.99 (0.25, 63.94)	1		
	5–25 ppm-yr	9.59 (0.60, 154.14)	1		
	>25 ppm-yr		0		
Cardboard manufacturing workers in Arnsburg, Germany					Henschler et al. (1995)
	TCE exposed workers	Not reported			
	Unexposed workers	Not reported			
Deaths reported to among GE pension fund (Pittsfield, Massachusetts)		0.95 (0.1, 3.17) <sup>i</sup>	13	Greenland et al. (1994)	
Cardboard manufacturing workers, Atlanta area, Georgia		Not reported		Sinks et al. (1992)	
U.S. Coast Guard employees				Blair et al. (1989)	
	Marine inspectors	0.72 (0.09, 2.62)	2		
	Noninspectors	0.74 (0.09, 2.68)	2		
Aircraft manufacturing plant employees (Italy)				Costa et al. (1989)	
	All subjects	0.21 (0.01, 1.17)	1		
Rubber Workers		Not reported <sup>i</sup>		Wilcosky et al. (1984)	
Aircraft manufacturing plant employees (San Diego, California)				Garabrant et al. (1988)	
	All subjects	1.14 (0.62, 1.92)	14		

**Table 4-110. Summary of human studies on TCE exposure and esophageal cancer (continued)**

Exposure group	RR (95% CI)	Number of observable events	Reference
<b>Case-control studies</b>			
Population of Montreal, Canada			Siemiatycki et al. (1991); Parent et al. (2000b)
Any TCE exposure	0.5 (0.1, 2.5) <sup>j</sup>	1	
Substantial TCE exposure	0.8 (0.1, 4.6) <sup>j</sup>	1	
<b>Geographic-based studies</b>			
Residents in two study areas in Endicott, New York	0.78 (0.29, 1.70)	6	ATSDR (2006a)
Residents of 13 census tracts in Redlands, California	Not reported		Morgan and Cassady (2002)
Finnish residents			Vartiainen et al. (1993)
Residents of Hausjarvi	Not reported		
Residents of Huttula	Not reported		

<sup>a</sup>Internal referents, workers not exposed to TCE.

<sup>b</sup>Ritz (1999a) and Zhao et al. (2005) reported RRs for the combined site of esophagus and stomach.

<sup>c</sup>Sung et al. (2007) and Chang et al. (2005)—SIR for females and reflects a 10-yr lag period.

<sup>d</sup>SIR for adenocarcinoma of the esophagus.

<sup>e</sup>The SIR for adenocarcinoma histologic type cannot be calculated because Hansen et al. (2001) do not present expected numbers for adenocarcinoma histologic type of esophageal cancer. An approximation of the SIR for adenocarcinoma histologic type is presented using the expected number of total number of expected esophageal cancers for males ( $n = 1.4$ ). The expected numbers of esophageal adenocarcinomas in males will be lower; Hansen et al. (2001) noted the proportion of adenocarcinomas among the comparable Danish male population during the later period of the study (1990–1996) as 38%. A rough approximation of the expected number of esophageal carcinomas would be 0.5 expected cases and an approximated SIR of 9.4 (3.1, 22).

<sup>f</sup>PMR.

<sup>g</sup>Adjusted RRs for >2-year exposure duration and 15-year lag from 1<sup>st</sup> exposure.

<sup>h</sup>No esophageal cancer deaths occurred in the referent population in Blair et al. (1998) and RR could not be calculated for this reason.

<sup>i</sup>OR from nested case-control analysis.

<sup>j</sup>90% CI.

Seven other studies (Clapp and Hoffman, 2008; Sung et al., 2007; ATSDR, 2006a, 2004a; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988) with lower likelihood for TCE exposure, in addition to limited statistical power and other design limitations, observed RR estimates between 0.21 (95% CI: 0.001, 1.17) (Costa et al., 1989) and 1.14 (95% CI: 0.62, 1.92) (Garabrant et al., 1988). For these reasons, esophageal cancer observations in these studies are not inconsistent with Blair et al. (1998) and its update Radican et al. (2008), Hansen et al. (2001), or Raaschou-Nielsen et al. (2003). No study reported a statistically significant deficit in the esophageal cancer risk estimate and overall of TCE exposure. Of those studies with exposure-response analyses, a pattern of increasing esophageal cancer RR with increasing exposure metric is not generally noted (Radican et al., 2008; Zhao et al., 2005; Boice et al., 1999; Blair et al., 1998; Siemiatycki, 1991) except for Hansen et al. (2001) and Raaschou-Nielsen et al. (2003). In

these last two studies, esophageal cancer RR estimates associated with long employment duration were slightly higher [SIR: 6.6, 95% CI: 1.8, 17 ([Hansen et al., 2001](#)); SIR: 1.9, 95% CI: 0.8, 3.7 ([Raaschou-Nielsen et al., 2003](#))] than those for short employment duration [SIR: 4.4, 95% CI: 0.5, 16 ([Hansen et al., 2001](#)); SIR: 1.7, 95% CI: 0.6, 3.6 ([Raaschou-Nielsen et al., 2003](#))]. Hansen et al. ([2001](#)) also reported risk for two other TCE exposure surrogates, average intensity and cumulative exposure, and in both cases, observed lower risk estimates with the higher exposure surrogate.

Meta-analysis is not adopted as a tool for examining the body of epidemiologic evidence on esophageal cancer and TCE exposure given the absence of reported RR estimates in several of the studies in which there is a high likelihood of TCE exposure in individual study subjects and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review ([Morgan et al., 1998](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#)).

Overall, three cohort studies in which there is a high likelihood of TCE exposure in individual study subjects and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review provide some evidence of association for esophageal cancer and TCE exposure. The finding in two of these studies of esophageal risk estimates among subjects with long employment duration were higher than those associated with low employment duration provides additional evidence ([Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#)). The cohort studies are unable to directly examine possible confounding due to suspected risk factors for esophageal cancer such as smoking, obesity, and alcohol. The use of an internal referent group, similar in SES status as exposed subjects, is believed to minimize but may not completely control for possible confounding related to smoking and health status (Blair et al., ([1998](#)); Radican et al., ([2008](#)); Zhao et al., ([2005](#)); Boice et al., ([2006b](#))). Observation of a higher risk for adenocarcinoma histologic type than for a combined category of esophageal cancer in Raaschou-Nielsen et al. ([2003](#)) also suggests minimal confounding from smoking. Smoking is not identified as a possible risk factor for the adenocarcinoma histologic type of esophageal cancer, but is believed to be a risk factor for squamous cell histologic type. Furthermore, the magnitude of lung cancer risk in Raaschou-Nielsen et al. ([2003](#)) suggests that a high smoking rate is unlikely. The lack of association with overall TCE exposure and the absence of exposure-response patterns in the other studies of TCE exposure may reflect limitations in statistical power, the possibility of exposure misclassification, and differences in measurement methods. These studies do not provide evidence against an association between TCE exposure and esophageal cancer.

#### **4.9.2. Bladder Cancer**

Twenty-five epidemiologic studies present risk estimates for bladder cancer ([Radican et al., 2008](#); [Sung et al., 2007](#); ATSDR, 2006a; [Boice et al., 2006b](#); [Chang et al., 2005](#); [Zhao et al., 2005](#); [ATSDR, 2004a, b](#); [Chang et al., 2003](#); [Raaschou-Nielsen et al., 2003](#); [Morgan and](#)

[Cassady, 2002](#); [Hansen et al., 2001](#); [Pesch et al., 2000a](#); [Boice et al., 1999](#); [Blair et al., 1998](#); [Morgan et al., 1998](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#); [Greenland et al., 1994](#); [Sinks et al., 1992](#); [Siemiatycki, 1991](#); [Mallin, 1990](#); [Blair et al., 1989](#); [Costa et al., 1989](#); [Garabrant et al., 1988](#); [Shannon et al., 1988](#)). Table 4-111 presents risk estimates for TCE exposure and bladder cancer observed in cohort, case-control, and geographic-based studies. Thirteen studies, all either cohort or case-control studies, which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) or which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review, reported RR estimates for bladder or urothelial cancer between 0.6 ([Siemiatycki, 1991](#)) and 1.7 ([Boice et al., 2006b](#)) and overall TCE exposure. RR estimates were generally based on small numbers of cases or deaths, except for one study ([Raaschou-Nielsen et al., 2003](#)), with the result of wide CIs on the estimates. Of these studies, two reported statistically significant elevated bladder or urothelial cancer risks with the highest cumulative TCE exposure category (2.71, 95% CI: 1.10, 6.65 ([Morgan et al., 1998](#)); 1.8, 95% CI: 1.2, 2.7 ([Pesch et al., 2000a](#)) and five presented risk estimates and categories of increasing cumulative TCE exposure ([Radican et al., 2008](#); [Zhao et al., 2005](#); [Pesch et al., 2000a](#); [Blair et al., 1998](#); [Morgan et al., 1998](#)). Risk estimates in Morgan et al. ([1998](#)), Pesch et al. ([2000a](#)), and Zhao et al. ([2005](#)) appeared to increase with increasing cumulative TCE exposure with the *p*-value for trend of 0.07 in Zhao et al. ([2005](#)), the only study to present a formal statistical test for linear trend. Risk estimates did not appear to either increase or decrease with increasing cumulative TCE exposure in Blair et al. ([1998](#)) or its update Radican et al. ([2008](#)), which added another 10 years of follow-up. Twelve additional studies were given less weight because of their lesser likelihood of TCE exposure and other design limitations that would decrease statistical power and study sensitivity ([Sung et al., 2007](#); ATSDR, 2006a; [Chang et al., 2005](#); ATSDR, 2004a; [Chang et al., 2003](#); [Morgan and Cassady, 2002](#); [Sinks et al., 1992](#); [Mallin, 1990](#); [Blair et al., 1989](#); [Costa et al., 1989](#); [Garabrant et al., 1988](#); [Shannon et al., 1988](#)).

**Table 4-111. Summary of human studies on TCE exposure and bladder cancer**

Exposure group		RR (95% CI)	Number of observabl e events	Reference
<b>Cohort studies—incidence</b>				
Aerospace workers (Rocketdyne)				Zhao et al. ( <a href="#">2005</a> )
	Any exposure to TCE	Not reported		
	Low cumulative TCE score	1.00 <sup>a</sup>	20	
	Medium cumulative TCE score	1.54 (0.81, 2.92) <sup>b</sup>	19	
	High TCE score	1.98 (0.93, 4.22) <sup>b</sup>	11	
	<i>p</i> for trend	<i>p</i> = 0.069		
TCE, 20-yr exposure lag				
	Low cumulative TCE score	1.00 <sup>a</sup>	20	
	Medium cumulative TCE score	1.76 (0.61, 5.10) <sup>c</sup>	20	
	High TCE score	3.68 (0.87, 15.5) <sup>c</sup>	10	
	<i>p</i> for trend	<i>p</i> = 0.064		
All employees at electronics factory (Taiwan)				
	Males	Not reported		Sung et al. ( <a href="#">2007</a> )
	Females	0.34 (0.07, 1.00)	10	
	Males	1.06 (0.45, 2.08) <sup>d</sup>	8	Chang et al. ( <a href="#">2005</a> )
	Females	1.09 (0.56, 1.91) <sup>d</sup>	12	
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al. ( <a href="#">2003</a> )
	Any exposure, all subjects	1.1 (0.92, 1.21)	220	
	Any exposure, males	1.0 (0.89, 1.18)	203	
	Any exposure, females	1.6 (0.93, 2.57)	17	
Biologically-monitored Danish workers		1.0 (0.48, 1.86)	10	Hansen et al. ( <a href="#">2001</a> )
	Any TCE exposure, males	1.1 (0.50, 2.0)	10	
	Any TCE exposure, females	0.5 expected	0	
Aircraft maintenance workers from Hill Air Force Base				Blair et al. ( <a href="#">1998</a> )
	TCE subcohort	Not reported		
Males, cumulative exposure				
	0	1.0 <sup>a</sup>		
	<5 ppm-yr	1.7 (0.6, 4.4)	13	
	5–25 ppm-yr	1.7 (0.6, 4.9)	9	
	>25 ppm-yr	1.4 (0.5, 4.1)	9	

**Table 4-111. Summary of human studies on TCE exposure and bladder cancer (continued)**

Exposure group		RR (95% CI)	Number of observabl e events	Reference
Females, cumulative exposure				Blair et al. ( <a href="#">1998</a> ) (continued)
0		1.0 <sup>a</sup>		
<5 ppm-yr		1.1 (0.1, 10.8)	1	
5–25 ppm-yr			0	
>25 ppm-yr		1.0 (0.1, 9.1)	1	
Biologically-monitored Finnish workers				Anttila et al. ( <a href="#">1995</a> )
All subjects		0.82 (0.27, 1.90)	5	
Biologically-monitored Swedish workers				Axelson et al. ( <a href="#">1994</a> )
Any TCE exposure, males		1.02 (0.44, 2.00)	8	
Any TCE exposure, females		Not reported		
<b>Cohort and PMR studies-mortality</b>				
Aerospace workers (Rocketdyne)				
Any TCE (utility/eng flush)		1.66 (0.54, 3.87)	5	Boice et al. ( <a href="#">2006b</a> )
Any exposure to TCE		Not reported		Zhao et al. ( <a href="#">2005</a> )
Low cumulative TCE score		1.00 <sup>a</sup>	8	
Med cumulative TCE score		1.27 (0.43, 3.73) <sup>b</sup>	6	
High TCE score		1.15 (0.29, 4.51) <sup>b</sup>	3	
<i>p</i> for trend		<i>p</i> = 0.809		
TCE, 20-yr exposure lag				
Low cumulative TCE score		1.00 <sup>a</sup>	8	
Medium cumulative TCE score		0.95 (0.15, 6.02) <sup>c</sup>	7	
High TCE score		1.85 (0.12, 27.7) <sup>c</sup>	2	
<i>p</i> for trend		<i>p</i> = 0.533		
View-Master employees				ATSDR ( <a href="#">2004a</a> )
Males		1.22 (0.15, 4.40)		
Females		0.78 (0.09, 2.82)		
United States uranium-processing workers (Fernald)				Ritz ( <a href="#">1999a</a> )
Any TCE exposure		Not reported		
Light TCE exposure, >2-yr duration		Not reported		
Moderate TCE exposure, >2-yr duration		Not reported		
Aerospace workers (Lockheed)				Boice et al. ( <a href="#">1999</a> )
Routine exposure		0.55 (0.18, 1.28)	5	
Routine-intermittent <sup>a</sup>		Not reported		

**Table 4-111. Summary of human studies on TCE exposure and bladder cancer (continued)**

Exposure group		RR (95% CI)	Number of observabl e events	Reference
Aerospace workers (Hughes)				Morgan et al. ( <a href="#">1998</a> )
TCE subcohort		1.36 (0.59, 2.68)	8	
Low intensity (<50 ppm)		0.51 (0.01, 2.83)	1	
High intensity (>50 ppm)		1.79 (0.72, 3.69)	7	
TCE subcohort (Cox Analysis)				
Never exposed		1.0 <sup>a</sup>		
Ever exposed		2.05 (0.86, 4.85) <sup>c</sup>	8	
Peak				
No/low		1.0 <sup>a</sup>		
Medium/high		1.41 (0.52, 3.81)	5	
Cumulative				
Referent		1.0 <sup>a</sup>		
Low		0.69 (0.09, 5.36)	1	
High		2.71 (1.10, 6.65)	7	
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. ( <a href="#">1998</a> )
TCE subcohort		1.2 (0.5, 2.9) <sup>a</sup>	17	
Males, cumulative exposure				
0		1.0 <sup>a</sup>		
<5 ppm-yr		1.8 (0.5, 6.2)	7	
5–25 ppm-yr		2.1 (0.6, 8.0)	5	
>25 ppm-yr		1.0 (0.2, 5.1)	3	
Females, cumulative exposure				
0		1.0 <sup>a</sup>		
<5 ppm-yr			0	
5–25 ppm-yr			0	
>25 ppm-yr		0.8 (0.1, 7.5)	1	
TCE subcohort		0.80 (0.41, 1.58)	25	
Males, cumulative exposure				
0		1.0 <sup>a</sup>		
<5 ppm-yr		0.96 (0.37, 2.51)	9	
5–25 ppm-yr		1.77 (0.70, 4.52)	10	
>25 ppm-yr		0.67 (0.15, 2.95)	5	
				Radican et al. ( <a href="#">2008</a> )

**Table 4-111. Summary of human studies on TCE exposure and bladder cancer (continued)**

Exposure group		RR (95% CI)	Number of observabl e events	Reference
	Females, cumulative exposure	0.22 (0.03, 1.83)	1	Radican et al. (2008) (continued)
	0	1.0 <sup>a</sup>		
	<5 ppm-yr		0	
	5–25 ppm-yr	2.86 (0.27, 29.85)	1	
	>25 ppm-yr		0	
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al. (1995)
	TCE exposed workers	Not reported		
	Unexposed workers	Not reported		
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		0.85 (0.32, 2.23) <sup>f</sup>	20	Greenland et al. (1994)
Cardboard manufacturing workers, Atlanta area, Georgia				Sinks et al. (1992)
		0.3 (0.0, 1.6)	1	
U.S. Coast Guard employees				Blair et al. (1989)
	Marine inspectors	0.50 (0.06, 1.79)	2	
	Noninspectors	0.90 (0.18, 2.62)	3	
Aircraft manufacturing plant employees (Italy)				Costa et al. (1989)
	All subjects	0.74 (0.30, 1.53)	7	
Aircraft manufacturing plant employees (San Diego, California)				Garabrant et al. (1988)
	All subjects	1.26 (0.74, 2.03)	17	
Lamp manufacturing workers (GE)		0.93 (0.19, 2.72)	3	Shannon et al. (1988)
<b>Case-control studies</b>				
Population of five regions in Germany				Pesch et al. (2000a)
	Any TCE exposure	Not reported		
	Males	Not reported		
	Females	Not reported		
	Males			
	Medium	0.8 (0.6, 1.2) <sup>g</sup>	47	
	High	1.3 (0.8, 1.7) <sup>g</sup>	74	
	Substantial	1.8 (1.2, 2.7) <sup>g</sup>	36	
Population of Montreal, Canada				Siemiatycki (1991); Siemiatycki et al. (1994)
	Any TCE exposure	0.6 (0.3, 1.2)	8	
	Substantial TCE exposure	0.7 (0.3, 1.6)	5	
<b>Geographic-based studies</b>				
Residents in two study areas in Endicott, New York				ATSDR (2006a)
		0.71 (0.38, 1.21)	13	
Residents of 13 census tracts in Redlands, California				Morgan and Cassady (2002)
		0.98 (0.71, 1.29) <sup>h</sup>	82	
Finnish residents				Vartiainen et al. (1993)
	Residents of Hausjarvi	Not reported		

**Table 4-111. Summary of human studies on TCE exposure and bladder cancer (continued)**

Exposure group		RR (95% CI)	Number of observabl e events	Reference
	Residents of Huttula	Not reported		
Residents of 9 county area in Northwestern Illinois				Mallin ( <a href="#">1990</a> )
All zip codes in study area				
	Males	1.4 (1.1, 1.9)	47	
	Females	1.8 (1.2, 2.7)	21	
Cluster community				
	Males	1.7 (1.1, 2.6)	21	
	Females	2.6 (1.2, 4.7)	10	
Adjacent community				
	Males	1.2 (0.6, 2.0)	12	
	Females	1.6 (0.5, 3.8)	5	
Remainder of zip code areas				
	Males	1.4 (0.8, 2.2)	14	
	Females	1.4 (0.5, 3.0)	6	

<sup>a</sup>Internal referents, workers not exposed to TCE.

<sup>b</sup>RR estimates for TCE exposure after adjustment for 1<sup>st</sup> employment, SES status, and age at event.

<sup>c</sup>RR estimates for TCE exposure after adjustment for 1<sup>st</sup> employment, SES status, age at event, and all other carcinogen exposures, including hydrazine.

<sup>d</sup>Chang et al. ([2005](#)) and Costa et al. ([1989](#)) report estimated risks for a combined site of all urinary organ cancers.

<sup>e</sup>Risk ratio from Cox Proportional Hazard Analysis, stratified by age, sex and decade ([EHS, 1997](#)).

<sup>f</sup>OR from nested case-control analysis.

<sup>g</sup>OR for urothelial cancer, a category of bladder, ureter, and renal pelvis cancers) and cumulative TCE exposure, as assigned using a JTEM approach ([Pesch et al., 2000a](#)).

<sup>h</sup>99% CI.

Meta-analysis is not adopted as a tool for examining the body of epidemiologic evidence on bladder cancer and TCE.

Overall, three cohort or case-control studies in which there is a high likelihood of TCE exposure in individual study subjects and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review provide some evidence of association for bladder or urothelial cancer and high cumulative TCE exposure ([Zhao et al., 2005](#); [Pesch et al., 2000a](#); [Morgan et al., 1998](#)). The case-control study of Pesch et al. ([2000a](#)) adjusted for age, study center, and cigarette smoking, with a finding of a statistically significant risk estimate between urothelial cancer and the highest TCE exposure category. Cancer cases in this study are of several sites (bladder, ureter, and renal pelvis), and grouping different site-specific cancers with possible etiologic heterogeneity may introduce misclassification bias. The cohort studies are unable to directly examine possible confounding due to suspected risk factors for esophageal cancer such as smoking, obesity, and alcohol. The use of an internal referent group, similar in

SES status as exposed subjects, by Morgan et al. ([1998](#)) and Zhao et al. ([2005](#)) is believed to minimize but may not completely control for possible confounding related to smoking and health status. The lack of association with overall TCE exposure in other studies and the absence of exposure-response patterns with TCE exposure in Blair et al. ([1998](#)) and Radican et al. ([2008](#)) may reflect limitations in statistical power, the possibility of exposure misclassification, and differences in measurement methods. These studies do not provide evidence against an association between TCE exposure and bladder cancer.

#### **4.9.3. CNS and Brain Cancers**

Brain cancer is examined in most cohort studies and in one case-control study ([Clapp and Hoffman, 2008](#); [Radican et al., 2008](#); [Sung et al., 2007](#); [Boice et al., 2006b](#); [Chang et al., 2005](#); [Zhao et al., 2005](#); [Chang et al., 2003](#); [Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#); [Boice et al., 1999](#); [Ritz, 1999a](#); [Blair et al., 1998](#); [Morgan et al., 1998](#); [Anttila et al., 1995](#); [Henschler et al., 1995](#); [Greenland et al., 1994](#); [Heineman et al., 1994](#); [Blair et al., 1989](#); [Costa et al., 1989](#); [Garabrant et al., 1988](#)). Overall, these epidemiologic studies do not provide strong evidence for or against association between TCE and brain cancer in adults (see Table 4-112). RR estimates in well-designed and -conducted cohort studies, Axelson et al. ([1994](#)), Anttila et al. ([1995](#)), Blair et al. ([1998](#)), its follow-up reported in Radican et al. ([2008](#)), Morgan et al. ([1998](#)), Boice et al. ([1999](#)), Zhao et al. ([2005](#)), and Boice et al. ([2006b](#)), are near a risk of 1.0 and imprecise, CIs all include a risk estimate of 1.0. All studies except Raaschou-Nielsen et al. ([2003](#)), observations are based on few events and lowered statistical power. Bias resulting from exposure misclassification is likely in these studies, although of a lower magnitude compared to other cohort studies identified in Table 4-112, and may partly explain observations. Exposure misclassification is also likely in the case-control study of occupational exposure of Heineman et al. ([1994](#)) who do not report association with TCE exposure.

**Table 4-112. Summary of human studies on TCE exposure and brain cancer**

Exposure group		RR (95% CI)	Number of observable events	Reference
<b>Cohort studies—incidence</b>				
Aerospace workers (Rocketdyne)				Zhao et al. (2005)
Any exposure to TCE		Not reported		
Low cumulative TCE score		1.00 <sup>a</sup>	7	
Medium cumulative TCE score		0.46 (0.09, 2.25) <sup>b</sup>	2	
High TCE score		0.47 (0.06, 3.95) <sup>b</sup>	1	
<i>p</i> for trend		<i>p</i> = 0.382		
All employees at electronics factory (Taiwan)				
Males		Not reported		Sung et al. (2007)
Females		1.07 (0.59, 1.80) <sup>c</sup>		
Males		0.40 (0.05, 1.46)	2	Chang et al. (2005)
Females		0.97 (0.54, 1.61)	15	
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al. (2003)
Any exposure, all subjects		1.0 (0.84, 1.24)	104	
Any exposure, males		1.0 (0.76, 1.18)	85	
Any exposure, females		1.1 (0.67, 1.74)	19	
Biologically-monitored Danish workers				Hansen et al. (2001)
Any TCE exposure, males		0.4 (0.01, 2.1)	1	
Any TCE exposure, females		0.5 expected	0	
Aircraft maintenance workers from Hill Air Force Base				Blair et al. (1998)
TCE subcohort		Not reported		
Males, cumulative exposure				
0		1.0 <sup>a</sup>		
<5 ppm-yr		2.0 (0.2, 19.7)	3	
5–25 ppm-yr		3.9 (0.4, 34.9)	4	
>25 ppm-yr		0.8 (0.1, 13.2)	1	
Females, cumulative exposure				
0		1.0 <sup>a</sup>		
<5 ppm-yr			0	
5–25 ppm-yr			0	
>25 ppm-yr			0	
Biologically-monitored Finnish workers				Anttila et al. (1995)
All subjects		1.09 (0.50, 2.07)	9	
Mean air-TCE (Ikeda extrapolation)				
<6 ppm		1.52 (0.61, 3.13)	7	
6+ ppm		0.76 (0.01, 2.74)	2	
Biologically-monitored Swedish workers				Axelson et al. (1994)
Any TCE exposure, males		Not reported		
Any TCE exposure, females		Not reported		

**Table 4-112. Summary of human studies on TCE exposure and brain cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
<b>Cohort and PMR studies-mortality</b>				
Computer manufacturing workers (IBM), New York				Clapp and Hoffman ( <a href="#">2008</a> )
	Males	1.90 (0.52, 4.85)	4	
	Females		0	
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	0.81 (0.17, 2.36)	3	Boice et al. ( <a href="#">2006b</a> ) Zhao et al. ( <a href="#">2005</a> )
	Any exposure to TCE	Not reported		
	Low cumulative TCE score	1.00 <sup>a</sup>	12	
	Medium cumulative TCE score	0.42 (0.12, 1.50)	3	
	High TCE score	0.83 (0.23, 3.08)	3	
	<i>p</i> for trend	<i>p</i> = 0.613		
View-Master employees				
	Males	Not reported		ATSDR ( <a href="#">2004a</a> )
	Females	Not reported		
All employees at electronics factory (Taiwan)				
	Males	0.96 (0.01, 5.36)	1	Chang et al. ( <a href="#">2003</a> )
	Females	0.96 (0.01, 5.33)	1	
United States uranium-processing workers (Fernald)				
	Any TCE exposure	Not reported		Ritz ( <a href="#">1999a</a> )
	Light TCE exposure, >2-yr duration, 0 lag	1.81 (0.49, 6.71) <sup>d</sup>	6	
	Moderate TCE exposure, >2-yr duration, 0 lag	3.26 (0.37, 28.9) <sup>d</sup>	1	
	Light TCE exposure, >5-yr duration, 15-yr lag	5.41 (0.87, 33.9) <sup>d</sup>	3	
	Moderate TCE exposure, >5-yr duration, 15-yr lag	14.4 (1.24, 167) <sup>d</sup>	1	
Aerospace workers (Lockheed)				
	Routine exposure	0.54 (0.15, 1.37)	4	Boice et al. ( <a href="#">1999</a> )
	Routine-intermittent <sup>a</sup>	Not presented		
Aerospace workers (Hughes)				
	TCE subcohort	0.99 (0.64, 1.47)	4	Morgan et al. ( <a href="#">1998</a> )
	Low intensity (<50 ppm) <sup>d</sup>	0.73 (0.09, 2.64)	2	
	High intensity (>50 ppm) <sup>d</sup>	0.44 (0.05, 1.58)	2	
Aircraft maintenance workers (Hill Air Force Base, Utah)				
	TCE subcohort	0.8 (0.2, 2.2) <sup>a</sup>	11	Blair et al. ( <a href="#">1998</a> )

**Table 4-112. Summary of human studies on TCE exposure and brain cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
Males, cumulative exposure				Blair et al. (1998) (continued)
0	1.0 <sup>a</sup>			
<5 ppm-yr	0.7 (0.7, 3.3)	3		
5–25 ppm-yr	2.0 (0.5, 8.4)	5		
>25 ppm-yr	0.9 (0.2, 4.4)	2		
Females, cumulative exposure				
0	1.0 <sup>a</sup>			
<5 ppm-yr		0		
5–25 ppm-yr		0		
>25 ppm-yr		0		
TCE subcohort		1.02 (0.39, 2.67)	17	Radican et al. (2008)
Males, cumulative exposure		1.26 (0.43, 3.75)	17	
0	1.0 <sup>a</sup>			
<5 ppm-yr	1.46 (0.44, 4.86)	8		
5–25 ppm-yr	1.74 (0.49, 6.16)	6		
>25 ppm-yr	0.66 (0.15, 2.95)	3		
Females, cumulative exposure			0	
0				
<5 ppm-yr				
5–25 ppm-yr				
>25 ppm-yr				
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al. (1995)
TCE exposed workers	3.70 (0.09, 20.64)	1		
Unexposed workers	9.38 (1.93, 27.27)	3		
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		0.93 (0.32, 2.69) <sup>e</sup>	16	Greenland et al. (1994)
Cardboard manufacturing workers, Atlanta area, Georgia				Sinks et al. (1992)
	Not reported			
U.S. Coast Guard employees				Blair et al. (1989)
Marine inspectors	1.70 (0.55, 3.95)	5		
Noninspectors	1.36 (0.44, 3.17)	5		
Aircraft manufacturing plant employees (Italy)				Costa et al. (1989)
All subjects	0.79 (0.16, 2.31)	3		
Aircraft manufacturing plant employees (San Diego, California)				Garabrant et al. (1988)
All subjects	0.78 (0.42, 1.34)	16		

**Table 4-112. Summary of human studies on TCE exposure and brain cancer (continued)**

Exposure group		RR (95% CI)	Number of observable events	Reference
<b>Case-control studies</b>				
Children's Cancer Group/Pediatric Oncology Group				De Roos et al. ( <a href="#">2001</a> )
Any TCE exposure		1.64 (0.95, 2.84)	37	
<b>Neuroblastoma, ≤15 yrs of age</b>				
Paternal TCE exposure				
Self-reported exposure		1.4 (0.7, 2.9)	22	
IH assignment of probable exposure		0.9 (0.3, 2.5)	9	
Population of So. LA, NJ, Philadelphia, PA				Heineman et al. ( <a href="#">1994</a> )
Any TCE exposure		1.1 (0.8, 1.6)	128	
Low exposure		1.1 (0.7, 1.7)	27	
Medium exposure		1.1 (0.6, 1.8)	42	
High exposure		1.1 (0.5, 2.8)	12	
<i>p</i> for trend		0.45		
<b>Geographic-based studies</b>				
Residents in two study areas in Endicott, New York				ATSDR ( <a href="#">2006a</a> )
<b>Brain/CNS, ≤19 yrs of age</b>		Not reported	<6	
Residents of 13 census tracts in Redlands, California				Morgan and Cassady ( <a href="#">2002</a> )
<b>Brain/CNS, &lt;15 yrs of age</b>		1.05 (0.24, 2.70) <sup>f</sup>	6	
Resident of Tucson Airport Area, Arizona				AZ DHS ( <a href="#">1995</a> , <a href="#">1990</a> )
<b>Brain/CNS, ≤19 yrs of age</b>				
1970–1986		0.84 (0.23, 2.16)	3	
1987–1991		0.78 (0.26, 2.39)	2	

<sup>a</sup>Internal referents, workers not exposed to TCE.

<sup>b</sup>RRs for TCE exposure after adjustment for 1<sup>st</sup> employment, SES status, and age at event.

<sup>c</sup>SIR from analyses lagging exposure 10 years prior to end of follow-up or date of incident cancer.

<sup>d</sup>RRs for TCE exposure after adjustment for time since 1<sup>st</sup> hired, external and internal radiation dose, and same chemical at a different level.

<sup>e</sup>OR from nested case-control analysis.

<sup>f</sup>99% CI.

Three geographic-based studies and one case-control study examined childhood brain cancer (ATSDR, 2006a; [Morgan and Cassady, 2002](#); [De Roos et al., 2001](#); [ADHS, 1995, 1990](#)). The strongest study, De Roos et al. ([2001](#)), a population case-control study that examined paternal exposure, used expert judgment to evaluate the probability of TCE exposure from self-reported information in an attempt to reduce exposure misclassification bias. The OR estimate in this study was 0.9 (95% CI: 0.3, 2.5). Like many population case-control studies, a low prevalence of TCE exposure was found, and only nine fathers were identified with probable TCE exposure by the industrial hygiene review, which greatly impacted statistical power. There is some concern for childhood brain cancer and organic solvent exposure based on Peters et al.

(1981) whose case-control study of childhood brain cancer reported to the Los Angeles County Cancer Surveillance Program observed a high OR estimate for paternal employment in the aircraft industry (OR:  $\infty$ ,  $p < 0.001$ ). This study does not present an OR for TCE exposure only although it did identify two of the 14 case and control fathers with previous employment in the aircraft industry reported exposure to TCE.

#### **4.10. SUSCEPTIBLE LIFESTAGES AND POPULATIONS**

Variation in response among segments of the population may be due to age, genetics, and ethnicity, as well as to differences in lifestyle, nutrition, and disease status. These could be potential risk factors that play an important role in determining an individual's susceptibility and sensitivity to chemical exposures. Available studies on TCE toxicity in relation to some of these risk factors including lifestage, gender, genetics, race/ethnicity, preexisting health status, and lifestyle are discussed below. However, there is a general lack of data demonstrating the modulation of health effects from TCE exposure based on these factors. Additional data examining these factors would provide further understanding of the populations that may be more susceptible to the health effects from TCE exposure. Others have also reviewed factors related to human variability and their potential for susceptibility to TCE ([NRC, 2006](#); [Clewett et al., 2000](#); [Pastino et al., 2000](#); [ATSDR, 1998b, 1997c](#); [Barton et al., 1996](#); [Davidson and Beliles, 1991](#)).

##### **4.10.1. Lifestages**

Individuals of different lifestages are physiologically, anatomically, and biochemically different. Early (infants and children) and later (the elderly) lifestages differ greatly from adulthood in body composition, organ function, and many other physiological parameters that can influence the toxicokinetics of chemicals and their metabolites in the body ([Guzelian et al., 1992](#)). The limited data on TCE exposure among these segments of the population—particularly individuals in early lifestages—suggest they may have greater susceptibility than does the general population. This section presents and evaluates the pertinent published literature available to assess how individuals of differing lifestages may respond differently to TCE.

###### **4.10.1.1. Early Lifestages**

###### **4.10.1.1.1. Early lifestage-specific exposures**

Section 2.4 describes the various exposure pathways of concern for TCE. For all postnatal lifestages, the primary exposure routes of concern include inhalation and contaminated drinking water. In addition, there are exposure pathways to TCE are unique to early lifestages. Fetal and infant exposure to TCE can occur through placental transfer and breast milk consumption if the mother has been exposed, and could potentially increase overall TCE exposure. Placental transfer of TCE has been demonstrated in humans ([Laham, 1970](#); [Beppu,](#)

1968), rats ([Withey and Karpinski, 1985](#)), mice ([Ghantous et al., 1986](#)), rabbits ([Beppu, 1968](#)), and sheep and goats ([Helliwell and Hutton, 1950](#)). Similarly, TCE has been found in breast milk in humans ([Fisher et al., 1997](#); [Pellizzari et al., 1982](#)), goats ([Hamada and Tanaka, 1995](#)), and rats ([Fisher et al., 1990](#)). Pellizzari et al. (1982) conducted a survey of environmental contaminants in human milk, using samples from cities in the northeastern region of the United States and one in the southern region and detected TCE in 8 milk samples taken from 42 lactating women. No details of when the samples were taken postpartum, milk lipid content, or TCE concentration in milk or blood were reported. Fisher et al. (1997) predicted that a nursing infant would consume 0.496 mg TCE during a 24-hour period. In lactating rats exposed to 600 ppm (3,225 mg/m<sup>3</sup>) TCE for 4 hours resulted in concentrations of TCE in milk of 110 µg/mL immediately following the cessation of exposure ([Fisher et al., 1990](#)).

Direct childhood exposures to TCE from oral exposures may also occur. A contamination of infant formula resulted in levels of 13 ppb ([Fan, 1988](#)). Children consume high levels of dairy products, and TCE has been found in butter and cheese ([Wu and Schaum, 2000](#)). In addition, TCE has been found in food and beverages containing fats such as margarine ([Wallace et al., 1984](#)), grains, and peanut butter ([Wu and Schaum, 2000](#)), all of which children consume in high amounts. A number of studies have examined the potential adverse effects of prenatal or postnatal exposure to drinking water contaminated with TCE ([ATSDR, 2001](#); [Sonnenfeld et al., 2001](#); [Rodenbeck et al., 2000](#); [Burg and Gist, 1999](#); [ATSDR, 1998b](#); [White et al., 1997](#); see Section 4.10.2.1; [Bove, 1996](#); [Bove et al., 1995](#); [Goldberg et al., 1990](#); [Bernad et al., 1987, abstract](#); [Lagakos et al., 1986](#)). TCE in residential water may also be a source of dermal or inhalation exposure during bathing and showering ([Franco et al., 2007](#); [Lee et al., 2002](#); [Wu and Schaum, 2000](#); [Giardino and Andelman, 1996](#); [Weisel and Jo, 1996](#); [Fan, 1988](#)); it has been estimated that showering and bathing scenarios in water containing 3 ppm TCE, a child of 22 kg receives a higher dose (about 1.5 times) on a mg/kg basis than a 70 kg adult ([Fan, 1988](#)).

Direct childhood inhalation exposure to TCE have been documented in both urban and rural settings. A study of VOCs measured personal, indoor, and outdoor TCE in 284 homes, with 72 children providing personal measures and time-activity diaries ([Adgate et al., 2004b](#)). The intensive-phase of the study found a mean personal level of 0.8 µg/m<sup>3</sup> and mean indoor and outdoor levels of 0.6 µg/m<sup>3</sup>, with urban homes have significantly higher indoor levels of TCE than nonurban homes ( $t = 2.3, p = 0.024$ ) ([Adgate et al., 2004b](#)). A similar study of personal, indoor, and outdoor TCE was conducted in two inner-city elementary schools as well as in the homes of 113 children along with time-activity diaries, and found a median a median personal level of 0.3 µg/m<sup>3</sup>, a median school indoor level of 0.2 µg/m<sup>3</sup>, a median home indoor level of 0.3 µg/m<sup>3</sup>, and a median outdoor level of 0.3 µg/m<sup>3</sup> in the winter, with slightly lower levels in the spring ([Adgate et al., 2004a](#)). Studies from Leipzig, Germany measured the median air level of TCE in children's bedrooms to be 0.42 µg/m<sup>3</sup> ([Lehmann et al., 2001](#)) and 0.6 µg/m<sup>3</sup>

([Lehmann et al., 2002](#)). A study of VOCs in Hong Kong measured air levels in schools, including an 8-hour average of 1.28  $\mu\text{g}/\text{m}^3$ , which was associated with the lowest risk of cancer in the study ([Guo et al., 2004](#)). Another found air TCE levels to be highest in school/work settings, followed by outside, in home, in other, and in transit settings ([Sexton et al., 2007](#)). Measured indoor air levels ranged from 0.18 to 140  $\mu\text{g}/\text{m}^3$  for children exposed through vapor intrusion from soil vapor ([ATSDR, 2006a](#)). Contaminated soil may be a source of either dermal or ingestion exposure of TCE for children ([Wu and Schaum, 2000](#)).

Additional TCE exposure has also been documented to have occurred during medical procedures. TCE was used in the past as an anesthetic during childbirth ([Phillips and Macdonald, 1971](#); [Beppu, 1968](#)) and surgery during childhood ([Jasińska, 1965](#)). These studies are discussed in more detail in Section 4.8.3.1.1. In addition, the TCE metabolite, CH, has been used as an anesthetic for children for CAT scans ([Steinberg, 1993](#)).

Dose received per body weight for 3 ppm TCE via oral, dermal, dermal plus inhalation, and bathing scenarios was estimated for a 10-kg infant, a 22-kg child, and a 70-kg adult ([Fan, 1988](#)) (see Table 4-113). For the oral route (drinking water), an infant would receive a higher daily dose than a child, and the child more than the adult. For the dermal and dermal plus inhalation route, the child would receive more than the adult. For the bathing scenario, the infant and child would receive comparable amounts, more than the adult.

**Table 4-113. Estimated lifestage-specific daily doses for TCE in water<sup>a</sup>**

	Body weight		
	Infant (10 kg)	Child (22 kg)	Adult (70 kg)
Drinking water	0.3 mg/kg	0.204 mg/kg	0.086 mg/kg
Showering—dermal	–	0.1 mg/kg	0.064 mg/kg
Showering—dermal and inhalation	–	0.129 mg/kg	0.083 mg/kg
Bathing—15 min	–	0.24 mg/kg	0.154 mg/kg
Bathing—5 min	0.08 mg/kg	0.08 mg/kg	0.051 mg/kg

<sup>a</sup>Adapted from Fan ([1988](#)).

#### 4.10.1.1.2. Early lifestage-specific toxicokinetics

Chapter 3 describes the toxicokinetics of TCE. However, toxicokinetics in developmental lifestages are distinct from toxicokinetics in adults ([Benedetti et al., 2007](#); [Ginsberg et al., 2004a](#); [Ginsberg et al., 2004b](#); [Hattis et al., 2003](#); [Ginsberg et al., 2002](#)) due to, for example, altered ventilation rates, percentage of adipose tissue, and metabolic enzyme expression. Early lifestage-specific information is described below for absorption, distribution, metabolism, and excretion, followed by available early lifestage-specific PBPK models.

#### 4.10.1.1.2.1. Absorption

As discussed in Section 3.1, exposure to TCE may occur via inhalation, ingestion, and dermal absorption. In addition, prenatal exposure may result in absorption via the transplacental route. Exposure via inhalation is proportional to the ventilation rate, duration of exposure, and concentration of expired air, and children have increased ventilation rates per kg body weight compared to adults, with an increased alveolar surface area per kg body weight for the first 2 years ([U.S. EPA, 2008c](#)). It is not clear to what extent dermal absorption may be different for children compared to adults; however, infants have a twofold increase in surface area compared to adults, although similar permeability (except for premature babies) compared to adults ([U.S. EPA, 2008c](#)).

#### 4.10.1.1.2.2. Distribution

Both human and animal studies provide clear evidence that TCE distributes widely to all tissues of the body (see Section 3.2). For lipophilic compounds such as TCE, percentage adipose tissue, which varies with age, will affect absorption and retention of the absorbed dose. Infants have a lower percentage of adipose tissue per body weight than adults, resulting in a higher concentration of the lipophilic compound in the fat of the child ([NRC, 1993](#)).

During pregnancy of humans and experimental animals, TCE is distributed to the placenta ([Ghantous et al., 1986](#); [Withey and Karpinski, 1985](#); [Laham, 1970](#); [Beppu, 1968](#); [Helliwell and Hutton, 1950](#)). In humans, TCE has been found in newborn blood after exposure to TCE during childbirth with ratios of concentrations in fetal:maternal blood ranging from approximately 0.5 to approximately 2 ([Laham, 1970](#)). In childhood, blood level concentrations of TCE were found to range from 0.01 to 0.02 ng/mL ([Sexton et al., 2005](#)). Pregnant rats exposed to TCE vapors on GD 17 resulted in concentrations of TCE in fetal blood approximately one-third the concentration in corresponding maternal blood, and was altered based upon the position along the uterine horn ([Withey and Karpinski, 1985](#)). TCE has also been found in the organs of prenatal rabbits including the brain, liver, kidneys, and heart ([Beppu, 1968](#)). Rats prenatally exposed to TCE had increased levels measured in the brain at PND 10, compared to rats exposed as adults ([Rodriguez et al., 2007](#)). TCE can cross the blood:brain barrier during both prenatal and postnatal development, and may occur to a greater extent in younger children. It is also important to note that it has been observed in mice that TCE can cycle from the fetus into the amniotic fluid and back to the fetus ([Ghantous et al., 1986](#)).

Studies have examined the differential distribution by age to a mixture of six VOCs including TCE to children aged 3–10 years and adults aged 20–82 years ([Mahle et al., 2007](#)) and in rats at PND 10, 2 months (adult), and 2 years (aged) ([Mahle et al., 2007](#); [Rodriguez et al., 2007](#)). In humans, the blood:air partition coefficient for male or female children was

significantly lower compared to adult males ([Mahle et al., 2007](#)). In rats, the difference in tissue:air partition coefficients increased with age ([Mahle et al., 2007](#)). Higher peak concentrations of TCE in the blood were observed in the PND 10 rat compared to the adult rat after inhalation exposure, likely due to the lower metabolic capacity of the young rats ([Rodriguez et al., 2007](#)).

#### **4.10.1.1.2.3. Metabolism**

Section 3.3 describes the enzymes involved in the metabolism of TCE, including CYP and GST. Expression of these enzymes changes during various stages of fetal development ([Shao et al., 2007](#); [Dorne et al., 2005](#); [Hines and McCarver, 2002](#); [Hakkola et al., 1998a](#); [1998b](#); [van Lieshout et al., 1998](#); [Hakkola et al., 1996a](#); [Hakkola et al., 1996b](#)) and during postnatal development ([Blake et al., 2005](#); [Dorne et al., 2005](#); [Tateishi et al., 1997](#)), and may result in altered susceptibility.

Expression of CYP enzymes have been shown to play a role in decreasing the metabolism of TCE during pregnancy in rats, although metabolism increased in young (3-week-old) rats compared to adult (18-week-old) rats ([Nakajima et al., 1992b](#)). For TCE, CYP2E1 is the main metabolic CYP enzyme, and expression of this enzyme has been observed in humans in prenatal brain tissue at low levels beginning at 8 weeks of gestation and increasing throughout gestation ([Brzezinski et al., 1999](#)). Very low levels of CYP2E1 have been detected in some samples of fetal liver during the second trimester (37% of samples) and third trimester (80% of samples) ([Johnsrud et al., 2003](#); [Carpenter et al., 1996](#)), although hepatic expression surges immediately after birth in most cases ([Johnsrud et al., 2003](#); [Vieira et al., 1996](#)) and in most infants, reaches adult values by 3 months of age ([Johnsrud et al., 2003](#); [Vieira et al., 1996](#)).

Although there is some uncertainty as to which GST isoforms mediate TCE conjugation, it should be noted that their expression changes with fetal development ([McCarver and Hines, 2002](#); [Raijmakers et al., 2001](#); [van Lieshout et al., 1998](#)).

#### **4.10.1.1.2.4. Excretion**

The major processes of excretion of TCE and its metabolites are discussed in Section 3.4, yet little is known about whether there are age-related differences in excretion of TCE. The major pathway for elimination of TCE is via exhalation, and its metabolites via urine and feces, and it is known that renal processes are not mature until about 6 months of age ([NRC, 1993](#)). Only one case study was identified that measured TCE or its metabolites in exhaled breath and urine in a 17-year-old who ingested a large quantity of TCE ([Brüning et al., 1998](#)). TCE has also been measured in the breast milk in lactating women ([Fisher et al., 1997](#); [Pellizzari et al., 1982](#)), goats ([Hamada and Tanaka, 1995](#)), and rats ([Fisher et al., 1990](#)).

#### **4.10.1.1.2.5. PBPK models**

Early lifestage-specific information regarding absorption, distribution, metabolism, and excretion needs to be considered for a child-specific and chemical-specific PBPK model. To adequately address the risk to infants and children, age-specific parameters for these values should be used in PBPK models that can approximate the internal dose an infant or child receives based on a specific exposure level (see Section 3.5).

Fisher and colleagues developed PBPK models to describe the toxicokinetics of TCE in the pregnant rat ([Fisher et al., 1989](#)), lactating rat and nursing pup ([Fisher et al., 1990](#)). The prenatal study demonstrates that approximately two-thirds of maternal exposure to both TCE and TCA reached the fetus after maternal inhalation, gavage, or drinking water exposure ([Fisher et al., 1989](#)). After birth, only 2% of maternal exposure to TCE reaches the pup; however, 15 and 30% of maternal TCA reaches the pup after maternal inhalation and drinking water exposure, respectively ([Fisher et al., 1990](#)). One analysis of PBPK models examined the variability in response to VOCs including TCE between adults and children, and concluded that the intraspecies uncertainty factor (UF) for pharmacokinetics is sufficient to capture variability between adults and children ([Pelekis et al., 2001](#)).

#### **4.10.1.1.3. Early lifestage-specific effects**

Although limited data exist on TCE toxicity as it relates to early lifestages, there is enough information to discuss the qualitative differences. In addition to the evidence described below, Section 4.8 contains information on reproductive and developmental toxicity. In addition, Sections 4.3 on neurotoxicity and Section 4.6 on immunotoxicity characterize a wide array of postnatal developmental effects.

##### **4.10.1.1.3.1. Differential noncancer outcomes in early lifestages**

Some adverse health outcomes, in particular birth defects, are observed only after early lifestage exposure to TCE. A summary of structural developmental outcomes that have been associated with TCE exposures is presented in Sections 4.8.3.3.

Cardiac birth defects have been observed after exposure to TCE in humans (ATSDR, 2006a; [Yauck et al., 2004](#); [Goldberg et al., 1990](#); [Lagakos et al., 1986](#)), rodents ([Johnson et al., 2005, 2003](#); [Johnson et al., 1998b](#); [Johnson et al., 1998a](#); [Dawson et al., 1993](#); [Smith et al., 1992](#); [Dawson et al., 1990](#); [Smith et al., 1989](#)), and chicks ([Rufer et al., 2008](#); [Drake et al., 2006a](#); [Drake et al., 2006b](#); [Mishima et al., 2006](#); [Boyer et al., 2000](#); [Loeber et al., 1988](#); [Bross et al., 1983](#)). However, it is notable that cardiac malformations were not observed in a number of other studies in humans ([Taskinen et al., 1989](#); [Lagakos et al., 1986](#); [Tola et al., 1980](#)), rodents ([Carney et al., 2006](#); [Fisher et al., 2001](#); [Narotsky and Kavlock, 1995](#); [Narotsky et al., 1995](#); [Coberly et al., 1992](#); [Cosby and Dukelow, 1992](#); [Healy et al., 1982](#); [Hardin et al., 1981](#);

[Dorfmueller et al., 1979](#); [Schwetz et al., 1975](#)), and rabbits ([Hardin et al., 1981](#)). See Section 4.8.3.3.2.3 for further discussion on cardiac malformations.

Structural CNS birth defects were observed in humans ([ATSDR, 2001](#); [Bove, 1996](#); [Bove et al., 1995](#); [Lagakos et al., 1986](#)). In addition, a number of postnatal nonstructural adverse effects on the CNS system have been observed in humans and experimental animals following prenatal exposure to TCE. See Sections 4.3.10 and 4.8.3.3.4 for further discussion on developmental neurotoxicity.

A variety of other birth defects have been observed—including eye/ear birth anomalies in humans and rats ([Narotsky and Kavlock, 1995](#); [Narotsky et al., 1995](#); [Lagakos et al., 1986](#)); lung/respiratory tract disorders in humans and mice ([Das and Scott, 1994](#); [Lagakos et al., 1986](#)); and oral cleft defects ([Bove, 1996](#); [Bove et al., 1995](#); [Lagakos et al., 1986](#)), kidney/urinary tract disorders, musculoskeletal birth anomalies ([Lagakos et al., 1986](#)), and anemia/blood disorders ([Burg and Gist, 1999](#)) in humans. See Section 4.8.3.3.3 for further discussion on other structural developmental outcomes. A current follow-up study of the Camp Lejeune cohort will examine birth defects and may provide additional insight ([ATSDR, 2009](#); [U.S. GAO, 2007b, a](#); [ATSDR, 2003a](#)).

#### **4.10.1.1.3.2. Susceptibility to noncancer outcomes in early lifestages**

There are a number of adverse health outcomes observed after exposure to TCE that are observed in both children and adults. Below is a discussion of differential exposure, incidence, and/or severity in early lifestages compared to adulthood.

Occupational TCE poisonings via inhalation exposure resulted in an elevated percentage of cases in the adolescents aged 15–19 years old compared those  $\geq 20$  years old ([McCarthy and Jones, 1983](#)). In addition, there is concern for intentional exposure to TCE during adolescence, including a series of deaths involving inhaling typewriter correction fluid ([King et al., 1985](#)) a case of glue sniffing likely associated with cerebral infarction in a 12-year-old boy with a 2-year history of exposure ([Parker et al., 1984](#)), and a case of attempted suicide by ingestion of 70 mg TCE in a 17-year-old boy ([Brüning et al., 1998](#)).

##### **4.10.1.1.3.2.1. Neurotoxicity**

Adverse CNS effects observed after early lifestage exposure to TCE in humans include delayed newborn reflexes ([Beppu, 1968](#)); impaired learning or memory ([White et al., 1997](#); [Bernad et al., 1987](#)); aggressive behavior ([Blossom et al., 2008](#); [Bernad et al., 1987](#)); hearing impairment ([Burg and Gist, 1999](#); [Burg et al., 1995](#)); speech impairment ([Burg and Gist, 1999](#); [White et al., 1997](#); [Burg et al., 1995](#)); encephalopathy ([White et al., 1997](#)); impaired executive and motor function ([White et al., 1997](#)); attention deficit ([Bernad et al., 1987](#)) ([White et al., 1997](#)); and ASD ([Windham et al., 2006](#)). One analysis observed a trend for increased adversity during development, with those exposed during childhood demonstrating more deficits than

those exposed during adulthood ([White et al., 1997](#)). In experimental animals, observations include decreased specific gravity of newborn brains until weaning ([Westergren et al., 1984](#)), reductions in myelination in the brains at weaning, significantly decreased uptake of 2-deoxyglucose in the neonatal rat brain, significant increase in exploratory behavior ([Isaacson and Taylor, 1989](#); [Noland-Gerbec et al., 1986](#); [Taylor et al., 1985](#)), decreased rearing activity ([Fredriksson et al., 1993](#)), and increased time to cross the first grid in open field testing ([George et al., 1986](#)).

Few studies addressed whether or not children are more susceptible to CNS effects compared to adults ([Burg and Gist, 1999](#); [White et al., 1997](#); [Burg et al., 1995](#)). An analysis of three residential exposures of TCE observed speech impairments in younger children and not at any other lifestage ([White et al., 1997](#)). A national TCE exposure registry also observed statistically significant speech impairment and hearing impairment in 0–9 year olds and no other age group ([Burg and Gist, 1999](#); [Burg et al., 1995](#)). However, a follow-up study did not find a continued association with speech and hearing impairment in these children, although the absence of acoustic reflexes remained significant ([ATSDR, 2002](#)). See Section 4.3 for further information on CNS toxicity, and Section 4.8.3.3.4 for further information on developmental neurotoxicity.

#### **4.10.1.1.3.2.2. Liver toxicity**

No early lifestage-specific effects were observed after TCE exposure. See Section 4.5 for further information on liver toxicity.

#### **4.10.1.1.3.2.3. Kidney toxicity**

Residents of Woburn, Massachusetts including 4,978 children were surveyed on residential and medical history to examine an association between observed adverse health outcomes and wells contaminated with TCE and other chemicals; among these children, an association was observed for higher cumulative exposure measure and history of kidney and urinary tract disorders (primarily kidney or urinary tract infections) and with lung and respiratory disorders (asthma, chronic bronchitis, or pneumonia) ([Lagakos et al., 1986](#)). Comparisons were not made for the adults living in this community. See Section 4.4 for further information on kidney toxicity.

#### **4.10.1.1.3.2.4. Immunotoxicity**

Several studies in exposure to TCE in early lifestages of humans ([Lehmann et al., 2002](#); [Lehmann et al., 2001](#)) and experimental animals ([Blossom et al., 2008](#); [Peden-Adams et al., 2008](#); [Blossom and Doss, 2007](#); [Peden-Adams et al., 2006](#); [Adams et al., 2003](#)) were identified that assessed the potential for developmental immunotoxicity. While some noted evidence of immune system perturbation ([Blossom et al., 2008](#); [Blossom and Doss, 2007](#); [Peden-Adams et](#)

[al., 2006](#); [Adams et al., 2003](#); [Lehmann et al., 2002](#)), others did not ([Peden-Adams et al., 2008](#); [Lehmann et al., 2001](#)). However, none of these studies assessed whether exposure during early life resulted in evidence of increased susceptibility as compared to exposure during adulthood; this is an area for future research. See Section 4.6 for further information on immunotoxicity, and Section 4.8.3.3.5 for further discussion on developmental immunotoxicity.

#### **4.10.1.1.3.2.5. Respiratory toxicity**

Residents of Woburn, Massachusetts including 4,978 children were surveyed on residential and medical history to examine an association between observed adverse health outcomes and wells contaminated with TCE and other chemicals; among these children, an association was observed for lung and respiratory disorders (asthma, chronic bronchitis, or pneumonia) ([Lagakos et al., 1986](#)). Comparisons were not made for the adults living in this community. See Section 4.7 for further information on respiratory tract toxicity.

#### **4.10.1.1.3.3. Susceptibility to cancer outcomes in early lifestages**

The epidemiologic and experimental animal evidence is limited regarding susceptibility to cancer from exposure to TCE during early lifestages. The human epidemiological evidence is summarized above for cancer diagnosed during childhood (see Sections 4.8.2.1 and 4.8.3.3.6), including a discussion of childhood cancers of the nervous system including neuroblastoma and the immune system including leukemia (see Section 4.6.1.2). A current follow-up study of the Camp Lejeune cohort will examine childhood cancers and may provide additional insight ([ATSDR, 2009](#); [U.S. GAO, 2007b, a](#); [ATSDR, 2003a](#)). No studies of cancers in experimental animals in early lifestages have been observed.

##### **4.10.1.1.3.3.1. Total childhood cancer**

Total childhood cancers have been examined in relationship to TCE exposure ([ATSDR, 2006a](#); [Morgan and Cassady, 2002](#)). Two studies examining total childhood cancer in relation to TCE in drinking water did not observe an association. A study in Endicott, New York contaminated by a number of VOCs, including “thousands of gallons” of TCE observed fewer than six cases of cancer diagnosed between 1980 and 2001 in children aged 0–19 years, and did not exceed expected cases or types ([ATSDR, 2006a](#)). A California community exposed to TCE in drinking water from contaminated wells was examined for cancer, with a specific emphasis on childhood cancer (<15 years old); however, the incidence did not exceed those expected for the community ([Morgan and Cassady, 2002](#)). A third study of childhood cancer in relation to TCE in drinking water in Camp Lejeune, North Carolina is currently underway ([U.S. GAO, 2007b, a](#)).

#### 4.10.1.1.3.3.2. Childhood leukemia

Childhood leukemia has been examined in relationship to TCE exposure ([Costas et al., 2002](#); [Shu et al., 1999](#); [Cohn et al., 1994b](#); [McKinney et al., 1991](#); [Lowengart et al., 1987](#); [Lagakos et al., 1986](#)). In a study examining drinking water exposure to TCE in 75 New Jersey towns, childhood leukemia (including ALL) was significantly increased for girls (n = 6) diagnosed before age 20 years, but this was not observed for boys ([Cohn et al., 1994b](#)). A community in Woburn, Massachusetts with contaminated well water including TCE experienced 20 cases of childhood leukemia, significantly more than expected ([Lagakos et al., 1986](#)); however, the incidence of leukemia among children was not compared to the incidence rate among adults living in this community. Further analysis by Costas et al. ([2002](#)) also observed a greater than twofold increase over expected cases of childhood leukemia. Cases were more likely to be male (76%), <9 years old at diagnosis (62%), breast-fed (OR: 10.17, 95% CI: 1.22–84.50), and exposed during pregnancy (OR<sub>adj</sub>: 8.33, 95% CI: 0.73–94.67). The highest risk was observed for exposure during pregnancy compared to preconception or postnatal exposure, and a dose-response was seen for exposure during pregnancy ([Costas et al., 2002](#)). In addition, family members of those diagnosed with childhood leukemia, including 13 siblings under age 19 at the time of exposure, had altered immune response, but an analysis looking at only these children was not done ([Byers et al., 1988](#)).

Case-control studies examined children diagnosed with ALL for parental occupational exposures and found a nonsignificant two- to fourfold increase of childhood leukemia risk for exposure to TCE during preconception, pregnancy, postnatally, or all developmental periods combined ([Shu et al., 1999](#); [McKinney et al., 1991](#); [Lowengart et al., 1987](#)). Some studies showed an elevated risk for maternal ([Shu et al., 1999](#)) or paternal exposure ([McKinney et al., 1991](#); [Lowengart et al., 1987](#)), while others did not show an elevated risk for maternal ([McKinney et al., 1991](#)) or paternal exposure ([Shu et al., 1999](#)), possibly due to the small number of cases. No variability was observed in the developmental stages in Shu et al. ([1999](#)), although Lowengart et al. ([1987](#)) observed the highest risk to be paternal exposure to TCE after birth.

#### 4.10.1.1.3.3.3. CNS tumors

In a case-control study of parental occupational exposures, paternal self-reported exposure to TCE was not significantly associated with neuroblastoma in the offspring (OR = 1.4, 95% CI: 0.7–2.9) ([De Roos et al., 2001](#)). Brain tumors have also been observed in the offspring of fathers exposed to TCE, but the OR could not be determined ([Peters et al., 1985](#); [Peters et al., 1981](#)).

#### 4.10.1.1.3.3.4. ADAFs

According to EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* ([U.S. EPA, 2005e](#)), there may be increased susceptibility to early-life

exposures for carcinogens with a mutagenic mode of action. Therefore, because the weight of evidence supports a mutagenic mode of action for TCE carcinogenicity in the kidney (see Section 4.4.7), the lack of data suggesting an absence of GSTT1 expression in neonates, and in the absence of chemical-specific data to evaluate differences in susceptibility, early-life susceptibility should be assumed and the ADAFs should be applied, in accordance with the *Supplemental Guidance*.

#### 4.10.1.2. Later Lifestages

Few studies examine the differential effects of TCE exposure for elderly adults (>65 years old). These limited studies suggest that older adults may experience increased adverse effects than younger adults. However, there is no further evidence for elderly individuals exposed to TCE beyond these studies.

Toxicokinetics in later lifestages can be distinct from toxicokinetics in younger adults ([Benedetti et al., 2007](#); [Ginsberg et al., 2005](#)), although there is limited evidence showing a possible age-related difference in CYP expression ([Dorne et al., 2005](#); [Parkinson et al., 2004](#); [George et al., 1995b](#)). GST expression has been observed to decrease with age in human lymphocytes, with the lowest expression in those aged 60–80 years old ([van Lieshout and Peters, 1998](#)).

Studies have examined the age differences in TK after exposure to a mixture of six VOCs including TCE for humans ([Mahle et al., 2007](#)) and rats ([Mahle et al., 2007](#); [Rodriguez et al., 2007](#)). In humans, the blood:air partition coefficient for adult males (20–82 years) was significantly ( $p \leq 0.05$ ) higher ( $11.7 \pm 1.9$ ) compared to male ( $11.2 \pm 1.8$ ) or female ( $11.0 \pm 1.6$ ) children (3–10 years) ([Mahle et al., 2007](#)); when the data was stratified for adults above and below 55 years of age, there was no significant difference observed between adults (20–55 years) and aged (56–82) (data not reported). In rats, the difference in tissue:air partition coefficients also increased from PND 10 to adult (2 months) to aged (2 years) rat ([Mahle et al., 2007](#)). TCE has also been measured in the brain of rats, with an increased level observed in older (2-year-old) rats compared to adult (2-month-old) rats ([Rodriguez et al., 2007](#)). It was also observed that aged rats reached steady state slower with higher concentrations compared to the adult rat; the authors suggest that the almost twofold greater percentage of body fat in the elderly is responsible for this response ([Rodriguez et al., 2007](#)).

One cohort of TCE exposed metal degreasers found an increase in psychoorganic syndrome and increased vibration threshold related to increasing age ([Rasmussen et al., 1993b](#); [Rasmussen et al., 1993c, d](#)), although the age groups were  $\leq 29$ , 30–39, and 40+ years, but the age ranged only from 18 to 68 years and did not examine >65 years as a separate category.

#### **4.10.2. Other Susceptibility Factors**

Aside from age, many other factors may affect susceptibility to TCE toxicity. A partial list of these factors includes gender, genetic polymorphisms, preexisting disease status, nutritional status, diet, and previous or concurrent exposures to other chemicals. The toxicity that results due to changes in multiple factors may be quite variable, depending on the exposed population and the type of exposure. Qualitatively, the presence of multiple susceptibility factors will increase the variability that is seen in a population response to TCE toxicity.

##### **4.10.2.1. Gender**

Individuals of different genders are physiologically, anatomically, and biochemically different. Males and females can differ greatly in many physiological parameters such as body composition, organ function, and ventilation rate, which can influence the toxicokinetics of chemicals and their metabolites in the body ([Gochfeld, 2007](#); [Gandhi et al., 2004](#)).

##### **4.10.2.1.1. Gender-specific toxicokinetics**

Chapter 3 describes the toxicokinetics of TCE. Gender-specific information is described below for absorption, distribution, metabolism, and excretion, followed by available gender-specific PBPK models.

##### **4.10.2.1.1.1. Absorption**

As discussed in Section 3.1, exposure to TCE may occur via inhalation, ingestion, and skin absorption. Exposure via inhalation is proportional to the ventilation rate, duration of exposure, and concentration of expired air, and women have increased ventilation rates during exercise compared to men ([Gochfeld, 2007](#)). Percentage of body fat varies with gender ([Gochfeld, 2007](#)), which for lipophilic compounds such as TCE will affect absorption and retention of the absorbed dose. After experimental exposure to TCE, women were found to absorb a lower dose due to lower alveolar intake rates compared to men ([Sato, 1993](#); [Sato et al., 1991b](#)).

##### **4.10.2.1.1.2. Distribution**

Both human and animal studies provide clear evidence that TCE distributes widely to all tissues of the body (see Section 3.2). The distribution of TCE to specific organs will depend on organ blood flow and the lipid and water content of the organ, which may vary between genders ([Gochfeld, 2007](#)). After experimental exposure to humans, higher distribution of TCE into fat tissue was observed in women leading to a greater blood concentration 16 hours after exposure compared to men ([Sato, 1993](#); [Sato et al., 1991b](#)). In experimental animals, male rats generally have higher levels of TCE in tissues compared to female rats, likely due to gender differences in metabolism ([Lash et al., 2006](#)). In addition, TCE has been observed in the male reproductive organs (epididymis, vas deferens, testis, prostate, and seminal vesicle) ([Zenick et al., 1984](#)).

#### 4.10.2.1.1.3. Metabolism

Section 3.3 describes the metabolic processes involved in the metabolism of TCE, including CYP and GST enzymes. In addition, the role of metabolism in male reproductive toxicity is discussed in Section 4.8.1.3.2.1. In general, there is some indication that TCE metabolism is different between males and females, with females more rapidly metabolizing TCE after oral exposure to rats ([Lash et al., 2006](#)), i.p. injections in rats ([Verma and Rana, 2003](#)), and in mouse, rat, and human liver microsomes ([Elfarra et al., 1998](#)).

In general, CYP expression may differ between genders ([Gochfeld, 2007](#); [Gandhi et al., 2004](#); [Parkinson et al., 2004](#)), although no gender-related difference in CYP2E1 activity is observed in the human liver microsomes ([Parkinson et al., 2004](#); [George et al., 1995a](#)). After exposure to TCE, CYP2E1 was detected in the epididymis and testes of mice ([Forkert et al., 2002](#)), and CYP2E1 and GST-alpha has been detected in the ovaries of rats ([Wu and Berger, 2008](#)), indicating that metabolism of TCE can occur in both the male and female reproductive tracts. One study of TCE exposure in mice observed induced CYP2E1 expression in the liver of males only ([Nakajima et al., 2000](#)). Male rats have been shown to have higher levels of TCE metabolites in the liver ([Lash et al., 2006](#)), and lower levels of TCE metabolites in the kidney ([Lash et al., 2006](#)) compared to female rats. However, another study did not observe any sex-related differences in the metabolism of TCE in rats ([Nakajima et al., 1992b](#)).

Unlike CYP-mediated oxidation, quantitative differences in the polymorphic distribution or activity levels of GST isoforms in humans are not presently known. However, the available data ([Lash et al., 1999a](#); [Lash et al., 1999b](#)) do suggest that significant variation in GST-mediated conjugation of TCE exists in humans. One study observed that GSH conjugation is higher in male rats compared to female rats ([Lash et al., 2000b](#)); however, it has also been speculated that any gender difference may be due to a polymorphism in GSH conjugation of TCE rather than a true gender difference ([Lash et al., 1999b](#)). Also, induction of PPAR $\alpha$  expression in male mice after TCE exposure was greater than that in females ([Nakajima et al., 2000](#)).

#### 4.10.2.1.1.4. Excretion

The major processes of excretion of TCE and its metabolites are discussed in Section 3.4. Two human voluntary inhalation exposure studies observed the levels of TCE and its metabolites in exhaled breath and urine ([Kimmerle and Eben, 1973a](#); [Nomiyama and Nomiyama, 1971](#)). Increased levels of TCE in exhaled breath in males were observed in one human voluntary inhalation exposure study of 250–380 ppm for 160 minutes ([Nomiyama and Nomiyama, 1971](#)), but no difference was observed in another study of 40 ppm for 4 hours or 50 ppm for 4 hours for 5 days ([Kimmerle and Eben, 1973a](#)).

After experimental exposure to TCE, women were generally found to excrete higher levels of TCE and TCA compared to men ([Kimmerle and Eben, 1973a](#); [Nomiya and Nomiya, 1971](#)). However, other studies observed an increase in TCE in the urine of males ([Inoue et al., 1989](#)), an increase in TCA in the urine of males ([Sato et al., 1991b](#)), or no statistically significant ( $p > 0.10$ ) gender difference for TCA in the urine ([Inoue et al., 1989](#)). Others found that the urinary elimination half-life of TCE metabolites is longer in women compared to men ([Ikeda, 1977](#); [Ikeda and Imamura, 1973](#)).

In addition to excretion pathways that occur in both genders, excretion occurs uniquely in men and women. In both humans and experimental animals, it has been observed that females can excrete TCE and metabolites in breast milk ([Fisher et al., 1997](#); [Hamada and Tanaka, 1995](#); [Fisher et al., 1990](#); [Pellizzari et al., 1982](#)), while males can excrete TCE and metabolites in seminal fluid ([Forkert et al., 2003](#); [Zenick et al., 1984](#)).

#### **4.10.2.1.1.5. PBPK models**

Gender-specific differences in uptake and metabolism of TCE were incorporated into a PBPK model using human exposure data ([Fisher et al., 1998](#)). The chemical-specific parameters included cardiac output at rest, ventilation rates, tissue volumes, blood flow, and fat volume. This model found that gender differences for the toxicokinetics of TCE are minor.

#### **4.10.2.1.2. Gender-specific effects**

##### **4.10.2.1.2.1. Gender susceptibility to noncancer outcomes**

###### **4.10.2.1.2.1.1. Liver toxicity**

No gender susceptibility to noncancerous outcomes in the liver was observed. A detailed discussion of the studies examining the effects of TCE on the liver can be found in Section 4.5.

###### **4.10.2.1.2.1.2. Kidney toxicity**

A detailed discussion of the studies examining the noncancer effects of TCE on the kidney can be found in Section 4.4. A residential study found that females aged 55–64 years old had an elevated risk of kidney disease (RR = 4.57, 99% CI: 2.10–9.93) compared to males, although an elevated risk of urinary tract disorders was reported for both males and females ([Burg et al., 1995](#)). Additionally, a higher rate of diabetes in females compared to males exposed to TCE was reported in two studies ([Davis et al., 2005](#); [Burg et al., 1995](#)). In rodents, however, kidney weights were increased more in male mice than in females ([Kjellstrand et al., 1983a](#); [Kjellstrand et al., 1983b](#)), and male rats have exhibited increased renal toxicity to TCE compared to females ([Lash et al., 2001b](#); [Lash et al., 1998a](#)).

#### **4.10.2.1.2.1.3. Immunotoxicity**

A detailed discussion of the studies examining the immunotoxic effects of TCE can be found in Section 4.6. Most of the immunotoxicity studies present data stratified by sex. The prevalence of exposure to TCE is generally lower in women compared with men. In men, the studies generally reported ORs between 2.0 and 8.0, and in women, the ORs were between 1.0 and 2.0 ([Cooper et al., 2009](#)). Based on small numbers of cases, an occupational study of TCE exposure found an increased risk for systemic sclerosis for men (OR: 4.75, 95% CI: 0.99–21.89) compared to women (OR: 2.10; 95% CI: 0.65–6.75) ([Diot et al., 2002](#)). Another study found similar results, with an elevated risk for men with a maximum intensity, cumulative intensity, and maximum probability of exposure to TCE compared to women ([Nietert et al., 1998](#)). These two studies, along with one focused exclusively on the risk of scleroderma to women ([Garabrant et al., 2003](#)), were included in a meta-analysis conducted by the EPA resulting in a combined estimate for “any” exposure, was OR = 2.5 (95% CI: 1.1, 5.4) for men and OR = 1.2 (95% CI: 0.58, 2.6) in women.

#### **4.10.2.1.2.1.4. Respiratory toxicity**

No gender susceptibility to noncancerous outcomes in the respiratory tract after TCE exposure was observed. A detailed discussion of the studies examining the respiratory effects of TCE can be found in Section 4.7.

#### **4.10.2.1.2.1.5. Reproductive toxicity**

A detailed discussion of the studies examining the gender-specific noncancer reproductive effects of TCE can be found in Section 4.8.1.

Studies examining males after exposure to TCE observed altered sperm morphology and hyperzoospermia ([Chia et al., 1996](#)), altered endocrine function ([Goh et al., 1998](#); [Chia et al., 1997](#)), decreased sexual drive and function ([Saihan et al., 1978](#); [El Ghawabi et al., 1973](#); [Bardodej and Vyskocil, 1956](#)), and altered fertility to TCE exposure. Infertility was not associated with TCE exposure in other studies ([Forkert et al., 2003](#); [Sallmen et al., 1998](#)), and sperm abnormalities were not observed in another study ([Rasmussen et al., 1988](#)).

There is more limited evidence for reproductive toxicity in females. There are epidemiological indicators of a possible effect of TCE exposure on female fertility ([Sallmen et al., 1998](#)), increased rate of miscarriage ([ATSDR, 2001](#)), and menstrual cycle disturbance ([ATSDR, 2001](#); [Zielinski, 1973](#); [Bardodej and Vyskocil, 1956](#)). In experimental animals, the effects on female reproduction include evidence of reduced in vitro oocyte fertilizability in rats ([Wu and Berger, 2008, 2007](#); [Berger and Horner, 2003](#)). However, in other studies that assessed reproductive outcome in female rodents ([Cosby and Dukelow, 1992](#); [George et al., 1986](#); [George et al., 1985](#); [Manson et al., 1984](#)), there was no evidence of adverse effects of TCE exposure on female reproductive function.

#### **4.10.2.1.2.1.6. Developmental toxicity**

A detailed discussion of the studies examining the gender-specific noncancer developmental effects of TCE can be found in Section 4.8.3. Only one study of contaminated drinking water exposure in Camp Lejeune, North Carolina observed a higher risk of SGA in males compared to females ([Sonnenfeld et al., 2001](#); [ATSDR, 1998a](#)).

#### **4.10.2.1.2.2. Gender susceptibility to cancer outcomes**

A detailed discussion of the studies examining the carcinogenic effects of TCE can be found on the liver in Section 4.5, on the kidney in Section 4.4, in the immune system in Section 4.6, in the respiratory system in Section 4.7, and on the reproductive system in Section 4.8.2.

##### **4.10.2.1.2.2.1. Liver cancer**

An elevated risk of liver cancer was observed for females compared to males in both human ([Raaschou-Nielsen et al., 2003](#)) and rodent ([Elfarra et al., 1998](#)) studies. In addition, gallbladder cancer was significantly elevated for women compared to men ([Raaschou-Nielsen et al., 2003](#)). A detailed discussion of the studies examining the gender-specific liver cancer effects of TCE can be found in Section 4.5.

##### **4.10.2.1.2.2.2. Kidney cancer**

One study of occupational exposure to TCE observed an increase in RCC for women compared to men ([Dosemeci et al., 1999](#)), but no gender difference was observed in other studies ([Raaschou-Nielsen et al., 2003](#); [Pesch et al., 2000b](#)). Blair et al. ([1998](#)) and Hansen et al. ([2001](#)) also present some results by sex, but both of these studies have too few cases to be informative about a sex difference for kidney cancer. Exposure differences between males and females in Dosemeci et al. ([1999](#)) may explain their finding. These studies, however, provide little information to evaluate susceptibility between sexes because of their lack of quantitative exposure assessment and lower statistical power. A detailed discussion of the studies examining the gender-specific kidney cancer effects of TCE can be found in Section 4.4.

##### **4.10.2.1.2.2.3. Cancers of the immune system**

Two drinking water studies suggest that there may be an increase of leukemia ([Cohn et al., 1994b](#); [Fagliano et al., 1990](#)) and NHL ([Cohn et al., 1994b](#)) among females compared to males. An occupational study also observed an elevated risk of leukemia in females compared to males ([Raaschou-Nielsen et al., 2003](#)), although a study of contaminated drinking water in Woburn, Massachusetts observed an increased risk of childhood leukemia in males compared to

females ([Costas et al., 2002](#)). A detailed discussion of the studies examining the gender-specific cancers of the immune system following TCE exposure can be found in Section 4.6.

#### **4.10.2.1.2.2.4. Respiratory cancers**

One study observed significantly elevated risk of lung cancer following occupational TCE exposure for both men and women, although the risk was found to be higher for women compared to men ([Raaschou-Nielsen et al., 2003](#)). This same study observed a nonsignificant elevated risk in both men and women for laryngeal cancer, again with an increased risk for women compared to men ([Raaschou-Nielsen et al., 2003](#)). Conversely, a study of Iowa residents with TCE-contaminated drinking water observed a sevenfold increased annual age-adjusted incidence for males compared to females ([Isacson et al., 1985](#)). However, other studies did not observe a gender-related difference ([ATSDR, 2002](#); [Hansen et al., 2001](#); [Blair et al., 1998](#)). A detailed discussion of the studies examining the gender-specific respiratory cancers following TCE exposure can be found in Sections 4.7.

#### **4.10.2.1.2.2.5. Reproductive cancers**

Breast cancer in females and prostate cancer in males were reported after exposure to TCE in drinking water ([Isacson et al., 1985](#)). A statistically elevated risk for cervical cancer, but not breast, ovarian, or uterine cancer, was observed in women in another study ([Raaschou-Nielsen et al., 2003](#)). This study also did not observe elevated prostate or testicular cancer ([Raaschou-Nielsen et al., 2003](#)). A detailed discussion of the studies examining the gender-specific reproductive cancers following TCE exposure can be found in Section 4.8.2.

#### **4.10.2.1.2.2.6. Other Cancers**

Bladder and rectal cancer was increased in men compared to women after exposure to TCE in drinking water, but no gender difference was observed for colon cancer ([Isacson et al., 1985](#)). After occupational TCE exposure, bladder, stomach, colon, and esophageal cancer was nonsignificantly elevated in women compared to men ([Raaschou-Nielsen et al., 2003](#)).

#### **4.10.2.2. Genetic Variability**

Section 3.3 describes the metabolic processes involved in the metabolism of TCE. Human variation in response to TCE exposure may be associated with genetic variation. TCE is metabolized by both CYP and GST; therefore, it is likely that polymorphisms will alter the response to exposure ([Garte et al., 2001](#); [Nakajima and Aoyama, 2000](#)), as well as exposure to other chemicals that may alter the metabolism of TCE ([Lash et al., 2007](#)) (see Section 4.10.2.6). It is important to note that even with a given genetic polymorphism, metabolic expression is not static, and depends on lifestage (see Section 4.10.1), obesity (see Section 4.10.2.4), and alcohol intake (see Section 4.10.2.5).

#### 4.10.2.2.1. CYP genotypes

In general, variability in CYP expression occurs within humans ([Dorne et al., 2005](#)), and variability in CYP expression has been observed in experimental animals exposed to TCE ([Nakajima et al., 1993](#)). In particular, increased CYP2E1 activity may lead to increased susceptibility to TCE ([Lipscomb et al., 1997](#)). The CYP2E1\*3 allele and the CYP2E1\*4 allele were more common among those who developed scleroderma who were exposed to solvents including TCE ([Povey et al., 2001](#)). A PBPK model of CYP2E1 expression after TCE exposure has been developed for rats and humans ([Yoon et al., 2007](#)).

In experimental animals, toxicokinetics of TCE differed among CYP2E1 knockout and wild-type mice ([Kim and Ghanayem, 2006](#)). This study found that exhalation was more prevalent among the knockout mice, whereas urinary excretion was more prevalent among the wild-type mice. In addition, the dose was found to be retained to a greater degree by the knockout mice compared to the wild-type mice.

#### 4.10.2.2.2. GST genotype

There is a possibility that GST polymorphisms could play a role in variability in toxic response to TCE ([Caldwell and Keshava, 2006](#)), but this has not been sufficiently tested ([NRC, 2006](#)). One study of renal cell cancer in workers exposed to TCE demonstrated a significant increased risk for those with GSTM1+ and GSTT1+ polymorphisms, compared to a negative risk for those with GSTM1- and GSTT1- polymorphisms ([Brüning et al., 1997a](#)). Another study of occupational TCE exposure found that RCC was significantly associated with the GSTT1+ polymorphism but not with GSTT1- ([Moore et al., 2010](#)). However, another study did not confirm this hypothesis, observing no clear relationship between GSTM1 and GSTT1 polymorphisms and RCC among TCE-exposed individuals, although they did see a possible association with the homozygous wild-type allele GSTP1\*A ([Wiesenhütter et al., 2007](#)). Unrelated to TCE exposure, Sweeney et al. ([2000](#)) found GSTT1- to be associated with an increased risk of RCC, but no difference was seen for GSTM1 and GSTP1 alleles. The role of GST polymorphisms in the development of RCC is an area in need of future research.

#### 4.10.2.2.3. Other genotypes

Other genetic polymorphisms could play a role in variability in toxic response, in particular TCE-related skin disorders. Studies have found that many TCE-exposed patients diagnosed with skin conditions exhibited the slow-acetylator NAT2 genotype ([Nakajima et al., 2003](#); [Huang et al., 2002](#)), whereas there was no difference in NAT2 status for those diagnosed with RCC ([Wiesenhütter et al., 2007](#)). Other studies have found that many TCE-exposed patients diagnosed with skin conditions expressed variant HLA alleles ([Li et al., 2007](#); [Yue et al., 2007](#)), in particular HLA-B\*1301, which is more common in Asians compared to whites ([Cao et](#)

[al., 2001](#); [Williams et al., 2001](#)), or TNF  $\alpha$ -308 allele ([Dai et al., 2004](#)). Also, an in vitro study of human lung adenocarcinoma cells exposed to TCE varied in response based on their p53 status, with p53-wild-type cells resulting in severe cellular damage, but not the p53-null cells ([Chen et al., 2002a](#)).

#### **4.10.2.3. Race/Ethnicity**

Different racial or ethnic groups may express metabolic enzymes in different ratios and proportions due to genetic variability ([Garte et al., 2001](#)). In particular, ethnic variability in CYP ([Dorne et al., 2005](#); [Parkinson et al., 2004](#); [McCarver et al., 1998](#); [Shimada et al., 1994](#); [Stephens et al., 1994](#)) and GST ([Nelson et al., 1995](#)) expression has been reported.

It has been observed that the metabolic rate for TCE may differ between the Japanese and Chinese ([Inoue et al., 1989](#)). Also, body size varies among ethnic groups, and increased body size was related to increased absorption of TCE and urinary excretion of TCE metabolites ([Sato et al., 1991b](#)).

#### **4.10.2.4. Preexisting Health Status**

It is known that kidney and liver diseases can affect the clearance of chemicals from the body, and therefore, poor health may lead to increased half-lives for TCE and its metabolites. There are some data indicating that obesity/metabolic syndrome, diabetes, and hypertension may increase susceptibility to TCE exposure through altered toxicokinetics. In addition, some of these conditions lead to increased risk for adverse effects that have also been associated with TCE exposure, though the possible interaction between TCE and known risk factors for these effects is not understood.

##### **4.10.2.4.1. Obesity**

TCE is lipophilic and stored in adipose tissue; therefore, obese individuals may experience altered toxicokinetics of TCE compared to thin individuals. The absorption of TCE is increased in obese individuals compared to thin individuals ([Clewell et al., 2000](#)), as observed by lower blood concentrations immediately after exposure in obese men compared to thin men ([Sato, 1993](#); [Sato et al., 1991b](#)). Once absorbed, obese individuals have increased storage of TCE in the adipose tissue compared to thin men ([Clewell et al., 2000](#)), which prolongs internal exposures ([Lash et al., 2000b](#); [Davidson and Beliles, 1991](#)). Obesity also likely alters TCE metabolism, since increased CYP2E1 expression has been observed in obese individuals compared to thin individuals ([McCarver et al., 1998](#)). Finally, delayed excretion has been observed in obese individuals compared to thin individuals in both exhaled air ([Monster, 1979](#)) and urine ([Sato, 1993](#); [Sato et al., 1991b](#)). In sum, obese individuals have altered toxicokinetics of TCE compared to thin individuals due to increased storage of TCE, increased CYP2E1 metabolism, and a slower rate of elimination.

In addition, individuals with high BMI are at increased risk of some of the same health effects associated with TCE exposure. For example, RCC, liver cancer, and prostate cancer may be positively associated with BMI or obesity ([Wigle et al., 2008](#); [El-Serag and Rudolph, 2007](#); [Benichou et al., 1998](#); [Asal et al., 1988a](#); [Asal et al., 1988b](#)). However, whether and how TCE interacts with known risk factors for such diseases is unknown, as existing epidemiologic studies have only examined these factors as possible confounders for effects associated with TCE, or vice versa ([Krishnadasan et al., 2008](#); [Charbotel et al., 2006](#)).

#### **4.10.2.4.2. Diabetes**

A higher rate of diabetes in females compared to males exposed to TCE was reported in two studies ([Davis et al., 2005](#); [Burg et al., 1995](#)). Whether the TCE may have caused the diabetes or the diabetes may have increased susceptibility to TCE is not clear. However, it has been observed that CYP2E1 expression is increased in obese Type II diabetics ([Wang et al., 2003](#)), and in poorly controlled Type I diabetics ([Song et al., 1990](#)), which may consequently alter the metabolism of TCE.

#### **4.10.2.4.3. Hypertension**

One study found no difference in risk for RCC among those diagnosed with hypertension among those living in an area with high TCE exposure; however, a slightly elevated risk was seen for those being treated for hypertension (OR: 1.57, 95% CI: 0.90–2.72) ([Charbotel et al., 2006](#)). Unrelated to TCE exposure, hypertension has been associated with increased risk of RCC in women compared to men ([Benichou et al., 1998](#)).

#### **4.10.2.5. Lifestyle Factors and Nutrition Status**

##### **4.10.2.5.1. Alcohol intake**

A number of studies have examined the interaction between TCE and ethanol exposure in both humans ([McCarver et al., 1998](#); [Sato, 1993](#); [Sato et al., 1991a](#); [Barret et al., 1984](#); [Sato et al., 1981](#); [1975](#); [Stewart et al., 1974b](#); [Bardodej and Vyskocil, 1956](#)) and experimental animals ([Kaneko et al., 1994](#); [Nakajima et al., 1992a](#); [Okino et al., 1991](#); [Nakajima et al., 1990](#); [Larson and Bull, 1989](#); [Nakajima et al., 1988](#); [Sato and Nakajima, 1985](#); [Sato et al., 1983](#); [White and Carlson, 1981b](#); [Sato et al., 1980](#)).

The co-exposure causes metabolic inhibition of TCE in humans ([Windemuller and Ettema, 1978](#); [Muller et al., 1975](#)), male rats ([Kaneko et al., 1994](#); [Okino et al., 1991](#); [Nakajima et al., 1990](#); [Larson and Bull, 1989](#); [Nakajima et al., 1988](#); [Sato and Nakajima, 1985](#); [Sato et al., 1981](#); [Nakanishi et al., 1978](#)), and rabbits ([White and Carlson, 1981b](#)). Similarly, individuals exposed to TCE reported an increase in alcohol intolerance ([Rasmussen and Sabroe, 1986](#); [Bardodej and Vyskocil, 1956](#); [Grandjean et al., 1955](#)). Disulfiram, used to treat alcoholism, has also been found to decrease the elimination of TCE and TCA ([Bartonicek and Teisinger, 1962](#)).

A “degreasers flush” has been described, reflecting a reddening of the face of those working with TCE after drinking alcohol, and measured an elevated level of TCE in exhaled breath compared to nondrinkers exposed to TCE ([Stewart et al., 1974a](#)). This may be due to increased CYP2E1 expression in those that consume alcohol compared to nondrinkers, unrelated to TCE exposure ([Caldwell et al., 2008b](#); [Liangpunsakul et al., 2005](#); [Lieber, 2004](#); [Parkinson et al., 2004](#); [McCarver et al., 1998](#); [Perrot et al., 1989](#)).

In experimental animals, male rats pretreated with ethanol experienced an induction of TCE metabolism ([Nakajima et al., 1992a](#)), although another study of male rats observed that pretreatment with ethanol did not decrease CYP activity ([Okino et al., 1991](#)). It is important to note that a further increased response of TCE and ethanol has been reported when also combined with low-fat or low-carbohydrate diets in male rats ([Sato et al., 1983](#)).

Since the liver is a target organ for both TCE and alcohol, decreased metabolism of TCE could be related to cirrhosis of the liver as a result of alcohol abuse ([McCarver et al., 1998](#)), and an increase in clinical liver impairment along with degreasers flush has been observed ([Barret et al., 1984](#)).

The CNS may also be impacted by the co-exposure. Individuals exposed to TCE and ethanol reported an increase in altered mood states ([Reif et al., 2003](#)), decreased mental capacity as described as small increases in functional load ([Windemuller and Ettema, 1978](#)), and those exposed to TCE and tetrachloroethylene who consumed alcohol had an elevated color confusion index ([Valic et al., 1997](#)).

#### **4.10.2.5.2. Tobacco smoking**

Individuals who smoke tobacco may be at increased risk of the health effects from TCE exposure. One study examining those living in an area with high TCE exposure found an increasing trend of risk ( $p = 0.008$ ) for RCC among smokers, with the highest OR among those with  $\geq 40$  pack-years (OR = 3.27, 95% CI: 1.48–7.19) ([Charbotel et al., 2006](#)). It has been shown that RCC is independently associated with smoking in a dose-response manner ([Yuan et al., 1998](#)), particularly in men ([Benichou et al., 1998](#)). While Charbotel et al. (2006) adjusted for smoking effects in analyses examining TCE exposure and RCC, this study provides no information on potential effect modification of TCE exposure by smoking.

A number of factors correlated to smoking (e.g., SES status, diet, alcohol consumption) may positively confound results if greater smoking rates were over-represented, as observed in an occupational cohort exposed to TCE ([Raaschou-Nielsen et al., 2003](#)). Absence of smoking information, on the other hand, could introduce a negative bias. In a drinking water study with exposures to TCE and perchlorate, Morgan and Cassidy (2002) noted that the relatively high education and high income levels as well as high access to health care of subjects in this study compared to the averages for the county as a whole likely leads to a lower smoking rate.

#### 4.10.2.5.3. Nutritional status

Malnutrition may also increase susceptibility to TCE. Bioavailability of TCE after oral and i.v. exposure increased with fasting from approximately 63% in nonfasted rats to >90% in fasted rats, with blood levels in fasted rats were elevated two- to threefold, and increased half-life in the blood of fasted rats ([D'Souza et al., 1985](#)). Food deprivation ([Sato and Nakajima, 1985](#)) and carbohydrate restriction ([Sato and Nakajima, 1985](#); [Nakajima et al., 1982](#)) enhanced metabolism of TCE in male rats, but this was not observed for dietary changes in protein or fat levels ([Nakajima et al., 1982](#)).

Vitamin intake may also alter susceptibility to TCE. An in vitro study of cultured normal human epidermal keratinocyte demonstrated an increased lipid peroxidation in a dose-dependent manner after exposure to TCE, which were then attenuated by exposure to Vitamin E ([Ding et al., 2006](#)).

#### 4.10.2.5.4. Physical activity

Increased inhalation during physical activity increases TCE concentrations in the alveoli when compared to inhalation in a resting state ([Astrand, 1975](#)). Studies have examined the time course of inhaled TCE and metabolites in blood and urine in individuals with different workloads ([Jakubowski and Wieczorek, 1988](#); [Astrand and Ovrum, 1976](#); [Monster et al., 1976](#); [Vesterberg and Astrand, 1976](#); [Vesterberg et al., 1976](#)). These studies demonstrate that an increase in pulmonary ventilation increases the amount of TCE taken up during exposure ([Sato, 1993](#); [Jakubowski and Wieczorek, 1988](#); [Astrand and Ovrum, 1976](#); [Monster et al., 1976](#)).

The Rocketdyne aerospace cohort exposed to TCE (and other chemicals) found a protective effect with high physical activity, but only after controlling for TCE exposure and SES status (OR = 0.55, 95% CI: 0.32–0.95, *p* trend = 0.04) ([Krishnadasan et al., 2008](#)). In general, physical activity may provide a protective effect for prostate cancer ([Wigle et al., 2008](#)) (see Section 4.8.2.1.1).

#### 4.10.2.5.5. SES

SES can be an indicator for a number of co-exposures, such as increased tobacco smoking, poor diet, education, income, and health care access, which may play a role in the results observed in the health effects of TCE exposure ([Morgan and Cassady, 2002](#)).

Children's exposure to TCE was measured in a low SES community, as characterized by income, educational level, and receipt of free or reduced cost school meals ([Sexton et al., 2005](#)); however, this study did not compare data to a higher SES community, nor examine health effects.

An elevated risk of NHL and esophagus/adenocarcinoma after exposure to TCE was observed for blue-collar workers compared to white collar workers and workers with unknown

SES ([Raaschou-Nielsen et al., 2003](#)). Authors speculate that these results could be confounded due to other related factors than SES such as smoking.

#### 4.10.2.6. Mixtures

TCE exposure often occurs concurrently with other chemical substances. In general, the effects of exposures to multiple chemicals is considered by EPA in the *Framework for Cumulative Risk Assessment* ([U.S. EPA, 2003a](#)). A summary of the interactive effects of TCE and other chemical co-exposures is addressed in Caldwell et al. ([2008b](#)) and in Chapter 10 of the NRC's report *Assessing the Human Health Risks of Trichloroethylene: Key Scientific Issues* ([NRC, 2006](#)).

Chapter 2 discusses that other parent compounds produce similar metabolites to TCE (see Table 2-1) or have similar properties or industrial uses (see Tables 2-3 and 2-14). The metabolic pathway of TCE is discussed in Section 3.3; due to its metabolism into multiple compounds, exposure to TCE itself can be considered as exposure to a mixture ([NRC, 2006](#)). Many of the studies discussed above in Chapter 4 demonstrate that exposure to TCE and other chemical substances often occur together in both occupational and nonoccupational settings.

Co-exposures to other solvents may induce or saturate toxicokinetic pathways, altering the way in which TCE is metabolized and cleared from the body. The limited data summarized by the ATSDR in its interaction profile on TCE, 1,1,1-trichloroethane, 1,1-dichloroethane, and tetrachloroethylene suggest that additive joint action is plausible ([ATSDR, 2004b](#); [Pohl et al., 2003](#)). Joint exposure to TCE and the fungicide fenarimol has been shown to alter TCE metabolism and genetic expression in mice ([Hrelia et al., 1994](#)). Joint exposure to TCE, benzene, and methyl mercury has been shown to induce genetic expression in the liver and the kidney of rats ([Hendriksen et al., 2007](#)). Metabolic competition was also observed for TCE and various agents in another study by Jakobson et al. ([1986](#)).

PBPK models have been developed demonstrating the interaction between 1,1-DCE and TCE ([Andersen et al., 1987b](#)) and the interaction between TCE, tetrachloroethylene, and 1,1,1-trichloroethane in rats ([Dobrev et al., 2001](#)) and humans ([Dobrev et al., 2002](#)). Other PBPK models also showed metabolic inhibition at higher doses for TCE and toluene ([Thrall and Poet, 2000](#)), and for TCE and chloroform ([Isaacs et al., 2004](#)). Another PBPK model of TCE and multiple VOCs showed metabolic inhibition and induction when exposure occurs concurrently ([Haddad et al., 2000](#)).

#### 4.10.3. Uncertainty of Database and Research Needs for Susceptible Populations

There is some evidence that certain populations may be more susceptible to exposure to TCE. These populations include early and later lifestages, gender, genetic polymorphisms, race/ethnicity, preexisting health status, and lifestyle factors and nutrition status. In general, this

database would be improved by future epidemiologic and toxicological studies of TCE exposure that provide data on effect modification, including the factors discussed here.

Although the toxicokinetic variability has been characterized by population PBPK modeling (see Section 3.5), the available data are limited due to the relative small numbers of individuals ( $n < 100$ ), their all being adults, and the fact that subjects were selected nonrandomly (healthy volunteers).

Although there is more information on early life exposure to TCE than on other potentially susceptible populations, there remain a number of uncertainties and data gaps regarding children's susceptibility. Improved PBPK modeling for using childhood parameters early lifestages as recommended by the NRC (2006), and validation of these models, will aid in determining how variations in metabolic enzymes affect TCE metabolism. In particular, the NRC states that it is prudent to assume children need greater protection than adults—unless sufficient data are available to justify otherwise (NRC, 2006).

More studies specifically designed to evaluate effects in early and later lifestages are needed in order to more fully characterize potential lifestage-related TCE toxicity. Because the neurological effects of TCE constitute the most sensitive endpoints of concern for noncancer effects, it is quite likely that the early lifestages may be more susceptible to these outcomes than are adults. Lifestage-specific neurotoxic effects, particularly in the developing fetus, need further evaluation. It is important to consider the use of age-appropriate testing for assessment of these and other outcomes, both for cancer and noncancer outcomes. Data specific to the carcinogenic effects of TCE exposure during the critical periods of development of experimental animals and humans also are sparse.

There is a need to better characterize the implications of TCE exposures to susceptible populations. There is suggestive evidence that there may be greater susceptibility for exposures to the elderly. Gender and race/ethnic differences in susceptibility are likely due to variation in physiology and exposure, and genetic variation likely has an effect on the toxicokinetics of TCE. In particular, the relationship between genetic variation and generalized hypersensitivity skin diseases is relevant for future study (see Sections 4.6.1.1.2 and 4.10.2.2). Diminished health status (e.g., impaired kidney liver or kidney), alcohol consumption, tobacco smoking, and nutritional status will likely affect an individual's ability to metabolize TCE. In addition, further evaluation of the effects due to co-exposures to other compounds with similar or different modes of action need to be evaluated. Future research should better characterize possible susceptibility for certain lifestages or populations.

## 4.11. HAZARD CHARACTERIZATION

### 4.11.1. Characterization of Noncancer Effects

#### 4.11.1.1. Neurotoxicity

Both human and animal studies have associated TCE exposure with effects on several neurological domains. The strongest neurological evidence of hazard in humans is for changes in trigeminal nerve function or morphology and impairment of vestibular function. Fewer and more limited evidence exists in humans on delayed motor function, and changes in auditory, visual, and cognitive function or performance. Acute and subchronic animal studies show morphological changes in the trigeminal nerve, disruption of the peripheral auditory system leading to permanent function impairments and histopathology, changes in visual evoked responses to patterns or flash stimulus, and neurochemical and molecular changes. Additional acute studies reported structural or functional changes in hippocampus, such as decreased myelination or decreased excitability of hippocampal CA1 neurons, although the relationship of these effects to overall cognitive function is not established. Some evidence exists for motor-related changes in rats/mice exposed acutely/subchronically to TCE, but these effects have not been reported consistently across all studies.

Epidemiologic evidence supports a relationship between TCE exposure and trigeminal nerve function changes, with multiple studies in different populations reporting abnormalities in trigeminal nerve function in association with TCE exposure ([Mhiri et al., 2004](#); [Kilburn, 2002a](#); [Kilburn and Warshaw, 1993a](#); [Feldman et al., 1992](#); [Ruijten et al., 1991](#); [Feldman et al., 1988](#); [Barret et al., 1987](#); [Barret et al., 1984](#); [Barret et al., 1982](#)). Of these, two well-conducted occupational cohort studies, each including >100 TCE-exposed workers without apparent confounding from multiple solvent exposures, additionally reported statistically significant dose-response trends based on ambient TCE concentrations, duration of exposure, and/or urinary concentrations of the TCE metabolite TCA ([1987](#); [Barret et al., 1984](#)). Limited additional support is provided by a positive relationship between prevalence of abnormal trigeminal nerve or sensory function and cumulative exposure to TCE (most subjects) or CFC113 (<25% of subjects) ([Rasmussen et al., 1993a](#)). Test for linear trend in this study was not statistically significant and may reflect exposure misclassification since some subjects included in this study did not have TCE exposure. The lack of association between TCE exposure and overall nerve function in three small studies (trigeminal ([El Ghawabi et al., 1973](#)); ulnar and medial ([Triebig et al., 1983](#); [1982](#))) does not provide substantial evidence against a causal relationship between TCE exposure and trigeminal nerve impairment because of limitations in statistical power, the possibility of exposure misclassification, and differences in measurement methods. Laboratory animal studies have also shown TCE-induced changes in the trigeminal nerve. Although one study reported no significant changes in TSEP in rats exposed to TCE for 13 weeks ([Albee et al., 2006](#)), there is evidence of morphological changes in the trigeminal nerve following short-term exposures in rats ([Barret et al., 1992](#); [1991](#)).

Human chamber, occupational, geographic-based/drinking water, and laboratory animal studies clearly established TCE exposure causes transient impairment of vestibular function. Subjective symptoms such as headaches, dizziness, and nausea resulting from occupational ([Liu et al., 1988](#); [Rasmussen and Sabroe, 1986](#); [Smith, 1970](#); [Grandjean et al., 1955](#)), environmental ([Hirsch et al., 1996](#)), or chamber exposures ([Smith, 1970](#); [Stewart et al., 1970](#)) have been reported extensively. A few laboratory animal studies have investigated vestibular function, either by promoting nystagmus or by evaluating balance ([Umezumi et al., 1997](#); [Niklasson et al., 1993](#); [Tham et al., 1984](#); [1979](#)).

In addition, mood disturbances have been reported in a number of studies, although these effects also tend to be subjective and difficult to quantify ([Gash et al., 2008](#); [Kilburn, 2002b, a](#); [Kilburn and Warshaw, 1993a](#); [Tröster and Ruff, 1990](#); [McCunney, 1988](#); [Rasmussen and Sabroe, 1986](#); [Mitchell and Parsons-Smith, 1969](#)), and a few studies have reported no effects from TCE on mood ([Reif et al., 2003](#); [Triebig et al., 1977a](#); [Triebig et al., 1976](#)). Few comparable mood studies are available in laboratory animals, although both Moser et al. ([2003](#)) and Albee et al. ([2006](#)) reported increases in handling reactivity among rats exposed to TCE. Finally, significantly increased number of sleep hours was reported by Arito et al. ([1994](#)) in rats exposed via inhalation to 50–300-ppm TCE for 8 hours/day for 6 weeks.

Four epidemiologic studies of chronic exposure to TCE observed disruption of auditory function. One large occupational cohort study showed a statistically significant difference in auditory function with cumulative exposure to TCE or CFC113 as compared to control groups after adjustment for possible confounders, as well as a positive relationship between auditory function and increasing cumulative exposure ([Rasmussen et al., 1993c](#)). Of the three studies based on populations from ATSDR's TCE Subregistry from the National Exposure Registry, more limited than Rasmussen et al. ([1993c](#)) due to inferior exposure assessment, Burg et al. ([1995](#)) and Burg and Gist ([1999](#)) reported a higher prevalence of self-reported hearing impairments. The third study reported that auditory screening revealed abnormal middle ear function in children <10 years of age, although a dose-response relationship could not be established and other tests did not reveal differences in auditory function ([ATSDR, 2002](#)). Further evidence for these effects is provided by numerous laboratory animal studies demonstrating that high dose subacute and subchronic TCE exposure in rats disrupts the auditory system leading to permanent functional impairments and histopathology.

Studies in humans exposed under a variety of conditions, both acutely and chronically, report impaired visual functions such as color discrimination, visuospatial learning tasks, and visual depth perception in subjects with TCE exposure. Abnormalities in visual depth perception were observed with a high acute exposure to TCE under controlled conditions ([Vernon and Ferguson, 1969](#)). Studies of lower TCE exposure concentrations also observed visuofunction effects. One occupational study ([Rasmussen et al., 1993c](#)) reported a statistically significant positive relationship between cumulative exposure to TCE or CFC113 and visual gestalts

learning and retention among Danish degreasers. Two studies of populations living in a community with drinking water containing TCE and other solvents furthermore suggested changes in visual function ([Reif et al., 2003](#); [Kilburn, 2002b, a](#)). These studies used more direct measures of visual function as compared to Rasmussen et al. ([1993c](#)), but their exposure assessment is more limited because TCE exposure is not assigned to individual subjects (Kilburn 2002a), or because there are questions regarding control selection and exposure to several solvents ([Reif et al., 2003](#); [Kilburn, 2002b, a](#)).

Additional evidence of effects of TCE exposure on visual function is provided by a number of laboratory animal studies demonstrating that acute or subchronic TCE exposure causes changes in visual evoked responses to patterns or flash stimulus ([Boyes et al., 2005a](#); [Boyes et al., 2003](#); [Blain et al., 1994](#)). Animal studies have also reported that the degree of some effects is correlated with simultaneous brain TCE concentrations ([Boyes et al., 2005a](#); [Boyes et al., 2003](#)) and that, after a recovery period, visual effects return to control levels ([Blain et al., 1994](#); [Rebert et al., 1991](#)). Overall, the human and laboratory animal data together suggest that TCE exposure can cause impairment of visual function, and some animal studies suggest that some of these effects may be reversible with termination of exposure.

Studies of human subjects exposed to TCE either acutely in chamber studies or chronically in occupational settings have observed deficits in cognition. Five chamber studies reported statistically significant deficits in cognitive performance measures or outcome measures suggestive of cognitive effects ([Triebig et al., 1977a](#); [Gamberale et al., 1976](#); [Triebig et al., 1976](#); [Stewart et al., 1970](#)). Danish degreasers with high cumulative exposure to TCE or CFC113 had a high risk (OR = 13.7, 95% CI: 2.0–92.0) for psychoorganic syndrome characterized by cognitive impairment, personality changes, and reduced motivation, vigilance, and initiative compared to workers with low cumulative exposure. Studies of populations living in a community with contaminated groundwater also reported cognitive impairments ([Kilburn, 2002b, a](#); [Kilburn and Warshaw, 1993a](#)), although these studies carry less weight in the analysis because TCE exposure is not assigned to individual subjects and their methodological design is weaker.

Laboratory studies provide some additional evidence for the potential for TCE to affect cognition, although the predominant effect reported has been changes in the time needed to complete a task, rather than impairment of actual learning and memory function ([Umezu et al., 1997](#); [Kishi et al., 1993](#); [Kulig, 1987](#)). In addition, in laboratory animals, it can be difficult to distinguish cognitive changes from motor-related changes. However, several studies have reported structural or functional changes in the hippocampus, such as decreased myelination ([Isaacson et al., 1990](#); [Isaacson and Taylor, 1989](#)) or decreased excitability of hippocampal CA1 neurons ([Ohta et al., 2001](#)), although the relationship of these effects to overall cognitive function is not established.

Two studies of TCE exposure, one chamber study of acute exposure duration and one occupational study of chronic duration, reported changes in psychomotor responses. The chamber study of Gamberale et al. (1976) reported a dose-related decrease in performance in a CRT test in healthy volunteers exposed to 100 and 200 ppm TCE for 70 minutes as compared to the same subjects without exposure. Rasmussen et al. (1993a) reported a statistically significant association with cumulative exposure to TCE or CFC113 and dyscoordination trend among Danish degreasers. Observations in a third study (Gun et al., 1978) are difficult to judge given the author's lack of statistical treatment of data. In addition, Gash et al. (2008) reported that 14 out of 30 TCE-exposed workers exhibited significantly slower fine motor hand movements as measured through a movement analysis panel test. Studies of populations living in communities with TCE and other solvents detected in groundwater supplies reported significant delays in SRTs and CRTs in individuals exposed to TCE in contaminated groundwater as compared to referent groups (Kilburn, 2002b, a; Kilburn and Thornton, 1996; Kilburn and Warshaw, 1993a). Observations in these studies are more uncertain given questions of the representativeness of the referent population, lack of exposure assessment to individual study subjects, and inability to control for possible confounders including alcohol consumption and motivation. Finally, in a presentation of two case reports, decrements in motor skills as measured by the grooved pegboard and finger tapping tests were observed (Tröster and Ruff, 1990).

Laboratory animal studies of acute or subchronic exposure to TCE observed psychomotor effects, such as loss of righting reflex (Shih et al., 2001; Umezu et al., 1997) and decrements in activity, sensory-motor function, and neuromuscular function (2003; Moser et al., 1995; Kishi et al., 1993). However, two studies also noted an absence of significant changes in some measures of psychomotor function (Albee et al., 2006; Kulig, 1987). In addition, less consistent results have been reported with respect to locomotor activity in rodents. Some studies have reported increased locomotor activity after an acute i.p. dosage (Wolff and Siegmund, 1978) or decreased activity after acute or short-term gavage dosing (2003; Moser et al., 1995). No change in activity was observed following exposure through drinking water (Waseem et al., 2001), inhalation (Kulig, 1987), or orally during the neurodevelopment period (Fredriksson et al., 1993).

Several neurochemical and molecular changes have been reported in laboratory investigations of TCE toxicity. Kjellstrand et al. (1987) reported inhibition of sciatic nerve regeneration in mice and rats exposed continuously to 150 ppm TCE via inhalation for 24 days. Two studies have reported changes in GABAergic and glutamatergic neurons in terms of GABA or glutamate uptake (Briving et al., 1986) or response to GABAergic antagonistic drugs (Shih et al., 2001) as a result of TCE exposure, with the Briving et al. (1986) conducted at 50 ppm for 12 months. Although the functional consequences of these changes is unclear, Tham et al. (1984; 1979) described central vestibular system impairments as a result of TCE exposure that may be related to altered GABAergic function. In addition, several in vitro studies have demonstrated that TCE exposure alters the function of inhibitory ion channels such as receptors

for GABA<sub>A</sub> glycine, and serotonin ([Lopreato et al., 2003](#); [Beckstead et al., 2000](#); [Krasowski and Harrison, 2000](#)) or of voltage-sensitive calcium channels ([Shafer et al., 2005](#)).

#### 4.11.1.2. Kidney Toxicity

There are few human data pertaining to TCE-related noncancer kidney toxicity. Observation of elevated excretion of urinary proteins in the available studies ([Bolt et al., 2004](#); [Green et al., 2004](#); [Brüning et al., 1999a](#); [Brüning et al., 1999b](#); [Rasmussen et al., 1993b](#)) indicates the occurrence of a toxic insult among TCE-exposed subjects compared to unexposed controls. Two studies are of subjects with previously diagnosed kidney cancer ([Bolt et al., 2004](#); [Brüning et al., 1999a](#)), while subjects in the other studies are disease free. Urinary proteins are considered nonspecific markers of nephrotoxicity and include  $\alpha$ 1-microglobulin, albumin, and NAG ([Lybarger et al., 1999](#); [1999](#); [Price et al., 1996](#)). Four studies measure  $\alpha$ 1-microglobulin with elevated excretion observed in the German studies ([Bolt et al., 2004](#); [Brüning et al., 1999a](#); [Brüning et al., 1999b](#)) but not Green et al. (2004). However, Rasmussen et al. (1993b) reported a positive relationship between increasing urinary NAG, another nonspecific marker of tubular toxicity, and increasing exposure duration; and Green et al. (2004) found statistically significant group mean differences in NAG. Observations in Green et al. (2004) provide evidence of tubular damage among workers exposed to TCE at current occupational levels. Elevated excretion of NAG has also been observed with acute TCE poisoning ([Carriero et al., 2007](#)). Some support for TCE nephrotoxicity in humans is provided by a study of ESRD in a cohort of workers at Hill Air Force Base ([Radican et al., 2006](#)), although subjects in this study were exposed to hydrocarbons, JP-4 gasoline, and solvents in addition to TCE, including 1,1,1-trichloroethane, and a second reporting a twofold elevated risk for progression of glomerulonephritis to ESRD with TCE exposure ([Jacob et al., 2007](#)).

Laboratory animal and in vitro data provide additional support for TCE nephrotoxicity. Multiple studies with both gavage and inhalation exposure show that TCE causes renal toxicity in the form of cytomegaly and karyomegaly of the renal tubules in male and female rats and mice (summarized in Section 4.4.4). Further studies with TCE metabolites have demonstrated a potential role for DCVC, TCOH, and TCA in TCE-induced nephrotoxicity. Of these, available data suggest that DCVC-induced renal effects most like those of TCE and is formed in sufficient amounts following TCE exposure to account for these effects. TCE or DCVC have also been shown to be cytotoxic to primary cultures of rat and human renal tubular cells ([Cummings and Lash, 2000](#); [Cummings et al., 2000a](#); [Cummings et al., 2000c](#)).

Overall, multiple lines of evidence support the conclusion that TCE causes nephrotoxicity in the form of tubular toxicity, mediated predominantly through the TCE GSH conjugation product DCVC.

#### 4.11.1.3. Liver Toxicity

Few studies on liver toxicity and TCE exposure are found in humans. Of these, three studies reported significant changes in serum liver function tests, widely used in clinical settings in part to identify patients with liver disease, in metal degreasers whose TCE exposure was assessed using urinary trichloro-compounds as a biomarker ([Xu et al., 2009](#); [Nagaya et al., 1993](#); [Rasmussen et al., 1993b](#)). Two additional studies reported plasma or serum bile acid changes ([Neghab et al., 1997](#); [Driscoll et al., 1992](#)). One study of subjects from the TCE subregistry of ATSDR's National Exposure Registry is suggestive of liver disorders but limitations preclude inferences whether TCE caused these conditions is not possible given the study's limitations ([Davis et al., 2005](#)). Furthermore, a number of case reports exist of liver toxicity including hepatitis accompanying immune-related generalized skin diseases described as a variation of erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis patients, and hypersensitivity syndrome ([Kamijima et al., 2007](#)) in addition to jaundice, hepatomegaly, hepatosplenomegaly, and liver failure in TCE-exposed workers ([Huang et al., 2002](#); [Thiele et al., 1982](#)). Cohort studies have examined cirrhosis mortality and either TCE exposure ([Radican et al., 2008](#); [Boice et al., 2006b](#); [ATSDR, 2004a](#); [Boice et al., 1999](#); [Ritz, 1999a](#); [1998](#); [Morgan et al., 1998](#); [Blair et al., 1989](#); [Garabrant et al., 1988](#)) or solvent exposure ([Leigh and Jiang, 1993](#)), but are greatly limited by their use of death certificates where there is a high degree (up to 50%) of underreporting ([Blake et al., 1988](#)), so these null findings do not rule out an effect of TCE on cirrhosis. Overall, while some evidence exists of liver toxicity as assessed from liver function tests, the data are inadequate for making conclusions regarding causality.

In laboratory animals, TCE exposure is associated with a wide array of hepatotoxic endpoints. Like humans, laboratory animals exposed to TCE have been observed to have increased serum bile acids ([Neghab et al., 1997](#); [Bai et al., 1992b](#)), although the toxicological importance of this effect is unclear. Most other effects in laboratory animals have not been studied in humans, but nonetheless provide evidence that TCE exposure leads to hepatotoxicity. These effects include increased liver weight, small transient increases in DNA synthesis, cytomegaly in the form of "swollen" or enlarged hepatocytes, increased nuclear size probably reflecting polyploidization, and proliferation of peroxisomes. Liver weight increases proportional to TCE dose are consistently reported across numerous studies and appear to be accompanied by periportal hepatocellular hypertrophy ([Laughter et al., 2004](#); [Nunes et al., 2001](#); [Nakajima et al., 2000](#); [Tao et al., 2000](#); [Berman et al., 1995](#); [Dees and Travis, 1993](#); [Goel et al., 1992](#); [Merrick et al., 1989](#); [Goldsworthy and Popp, 1987](#); [Melnick et al., 1987](#); [Buben and O'Flaherty, 1985](#); [Elcombe et al., 1985](#); [Kjellstrand et al., 1983a](#); [Kjellstrand et al., 1983b](#); [Tucker et al., 1982](#); [Kjellstrand et al., 1981b](#)). There is also evidence of increased DNA synthesis in a small portion of hepatocytes at around 10 days in vivo exposure ([Channel et al., 1998](#); [Dees and Travis, 1993](#); [Mirsalis et al., 1989](#); [Elcombe et al., 1985](#)). The lack of correlation of hepatocellular mitotic figures with whole-liver DNA synthesis or DNA synthesis

observed in individual hepatocytes ([Dees and Travis, 1993](#); [Elcombe et al., 1985](#)) supports the conclusions that cellular proliferation is not the predominant cause of increased DNA synthesis and that nonparenchymal cells may also contribute to such synthesis. Indeed, nonparenchymal cell activation or proliferation has been noted in several studies ([Goel et al., 1992](#); [Kjellstrand et al., 1983a](#)). Moreover, the histological descriptions of TCE-exposed livers are consistent with and, in some cases, specifically note increased polyploidy ([Buben and O'Flaherty, 1985](#)). Interestingly, changes in TCE-induced hepatocellular ploidy, as indicated by histological changes in nuclei, have been noted to remain after the cessation of exposure (Kjellstrand et al., 1983a). In regard to apoptosis, TCE has been reported either to have no effect or to cause a slight increase at high doses ([Channel et al., 1998](#); [Dees and Travis, 1993](#)). Some studies have also noted effects from dosing vehicle alone (such as corn oil, in particular) not only on liver pathology, but also on DNA synthesis ([Channel et al., 1998](#); [Merrick et al., 1989](#)). Available data also suggest that TCE does not induce substantial cytotoxicity, necrosis, or regenerative hyperplasia, as only isolated, focal necroses and mild to moderate changes in serum and liver enzyme toxicity markers have been reported ([Channel et al., 1998](#); [Dees and Travis, 1993](#); [Elcombe et al., 1985](#)). Data on peroxisome proliferation, along with increases in a number of associated biochemical markers, show effects in both mice and rats ([Channel et al., 1998](#); [Goldsworthy and Popp, 1987](#); [Elcombe et al., 1985](#)). These effects are consistently observed across rodent species and strains, although the degree of response at a given mg/kg/day dose appears to be highly variability across strains, with mice on average appearing to be more sensitive.

While it is likely that oxidative metabolism is necessary for TCE-induced effects in the liver, the specific metabolite or metabolites responsible is less clear. TCE, TCA, and DCA exposures have all been associated with induction of changes in liver weight, DNA synthesis, and peroxisomal enzymes. The available data strongly support TCA *not* being the sole or predominant active moiety for TCE-induced liver effects, particularly with respect to hepatomegaly. In particular, TCE and TCA dose-response relationships are quantitatively inconsistent, for TCE leads to greater increases in liver/body weight ratios than expected from predicted rates of TCA production (see analysis in Section 4.5.6.2.1). In fact, above a certain dose of TCE, liver/body weight ratios are greater than that observed under any conditions studied so far for TCA. Histological changes and effects on DNA synthesis are generally consistent with contributions from either TCA or DCA, with a degree of polyploidization, rather than cell proliferation, likely to be significant for TCE, TCA, and DCA.

Overall, TCE, likely through its oxidative metabolites, clearly leads to liver toxicity in laboratory animals, with mice appearing to be more sensitive than other laboratory animal species, but there is only limited epidemiologic evidence of hepatotoxicity being associated with TCE exposure.

#### 4.11.1.4. Immunotoxicity

Studies in humans provide evidence of associations between TCE exposure and a number of immunotoxicological endpoints. The relation between systemic autoimmune diseases, such as scleroderma, and occupational exposure to TCE has been reported in several recent studies. A meta-analysis of scleroderma studies ([Garabrant et al., 2003](#); [Diot et al., 2002](#); [Nietert et al., 1998](#)) conducted by the EPA resulted in a statistically significant combined OR for any exposure in men (OR: 2.5, 95% CI: 1.1, 5.4), with a lower RR seen in women (OR: 1.2, 95% CI: 0.58, 2.6). The incidence of systemic sclerosis among men is very low (approximately 1 per 100,000 per year), and is approximately 10 times lower than the rate seen in women ([Cooper and Stroehla, 2003](#)). Thus, the human data at this time do not allow determination of whether the difference in effect estimates between men and women reflects the relatively low background risk of scleroderma in men, gender-related differences in exposure prevalence or in the reliability of exposure assessment ([Messing et al., 2003](#)), a gender-related difference in susceptibility to the effects of TCE, or chance. Changes in levels of inflammatory cytokines were reported in an occupational study of degreasers exposed to TCE ([Iavicoli et al., 2005](#)) and a study of infants exposed to TCE via indoor air ([2002](#); [Lehmann et al., 2001](#)).

Experimental studies provide additional support for these effects. Numerous studies have demonstrated accelerated autoimmune responses in autoimmune-prone mice ([Cai et al., 2008](#); [Blossom et al., 2007](#); [Blossom et al., 2004](#); [Griffin et al., 2000a](#); [Griffin et al., 2000b](#)). With shorter exposure periods, effects include changes in cytokine levels similar to those reported in human studies. More severe effects, including autoimmune hepatitis, inflammatory skin lesions, and alopecia, were manifest at longer exposure periods, and interestingly, these effects differ somewhat from the “normal” expression in these mice. Immunotoxic effects, including increases in anti-dsDNA antibodies in adult animals, decreased thymus weights, and decreased PFC response with prenatal and neonatal exposure, have been also reported in B6C3F<sub>1</sub> mice, which do not have a known particular susceptibility to autoimmune disease ([Keil et al., 2009](#); [Peden-Adams et al., 2006](#); [Gilkeson et al., 2004](#)). Recent mechanistic studies have focused on the roles of various measures of oxidative stress in the induction of these effects by TCE ([Wang et al., 2008](#); [Wang et al., 2007b](#)).

There have been a large number of case reports of a severe hypersensitivity skin disorder, distinct from contact dermatitis and often accompanied by hepatitis, associated with occupational exposure to TCE, with prevalences as high as 13% of workers in the same location ([2008](#); [Kamijima et al., 2007](#)). Evidence of a treatment-related increase in delayed hypersensitivity response accompanied by hepatic damage has been observed in guinea pigs following intradermal injection ([Tang et al., 2008](#); [Tang et al., 2002](#)), and hypersensitivity response was also seen in mice exposed via drinking water pre- and postnatally (GD 0 through to 8 weeks of age) ([Peden-Adams et al., 2006](#)).

Human data pertaining to TCE-related immunosuppression resulting in an increased risk of infectious diseases is limited to the report of an association between reported history of bacteria or viral infections in Woburn, Massachusetts ([Lagakos et al., 1986](#)). Evidence of localized immunosuppression, as measured by pulmonary response to bacterial challenge (i.e., risk of Streptococcal pneumonia-related mortality and clearance of *Klebsiella* bacteria) was seen in an acute exposure study in CD-1 mice ([Aranyi et al., 1986](#)). A 4-week inhalation exposure in Sprague-Dawley rats reported a decrease in PFC response at exposures of 1,000 ppm ([Woolhiser et al., 2006](#)).

Overall, the human and animal studies of TCE and immune-related effects provide strong evidence for a role of TCE in autoimmune disease and in a specific type of generalized hypersensitivity syndrome, while there are less data pertaining to immunosuppressive effects.

#### **4.11.1.5. Respiratory Tract Toxicity**

There are very limited human data on pulmonary toxicity and TCE exposure. Two recent reports of a study of gun manufacturing workers reported asthma-related symptoms and lung function decrements associated with solvent exposure ([Saygun et al., 2007](#); [Cakmak et al., 2004](#)), but these studies are limited by multiple solvent exposures and the significant effect of smoking on pulmonary function. Laboratory studies in mice and rats have shown toxicity in the bronchial epithelium, primarily in Clara cells, following acute exposures to TCE by inhalation (see Section 4.7.2.1.1). A few studies of longer duration have reported more generalized toxicity, such as pulmonary fibrosis 90 days after a single 2,000 mg/kg i.p. dose in mice and pulmonary vasculitis after 13-week gavage exposures to 2,000 mg/kg-day in rats ([Forkert and Forkert, 1994](#); [NTP, 1990](#)). However, respiratory tract effects were not reported in other longer-term studies. Acute pulmonary toxicity appears to be dependent on oxidative metabolism, although the particular active moiety is not known. While earlier studies implicated chloral produced in situ by CYP enzymes in respiratory tract tissue was responsible for toxicity ([reviewed in Green, 2000](#)), the evidence is inconsistent, and several other possibilities are viable. First, substantial “accumulation” of chloral is unlikely, as it is likely either to be rapidly converted to TCOH in respiratory tract tissue or to diffuse rapidly into blood and be converted to TCOH in erythrocytes or the liver. Conversely, a role for systemically produced oxidative metabolites cannot be discounted, as CH and TCOH in blood have both been reported following inhalation dosing in mice. In addition, a recent study reported DCAC protein adducts in the lungs of mice to which TCE was administered by i.p. injection, suggesting DCAC, which is not believed to be derived from chloral, may also contribute to TCE respiratory toxicity. Although humans appear to have lower overall capacity for enzymatic oxidation in the lung relative to mice, CYP enzymes do reside in human respiratory tract tissue, suggesting that, qualitatively, the respiratory tract toxicity observed in rodents is biologically plausible in humans. However, quantitative estimates of differential sensitivity across species due to respiratory metabolism are highly uncertain due to

limited data. Therefore, overall, data are suggestive of TCE causing respiratory tract toxicity, based primarily on short-term studies in mice and rats, and no data suggest that such hazards would be biologically precluded in humans.

#### 4.11.1.6. Reproductive Toxicity

Reproductive toxicity related to TCE exposure has been evaluated in human and experimental animal studies for effects in males and females. Only a limited number of studies have examined whether TCE causes female reproductive toxicity. Epidemiologic studies have identified possible associations of TCE exposure with effects on female fertility ([ATSDR, 2001](#); [Sallmén et al., 1995](#)) and with menstrual cycle disturbances ([ATSDR, 2001](#); [Sagawa et al., 1973](#); [Zielinski, 1973](#); [Bardodej and Vyskocil, 1956](#)). Reduced in vitro oocyte fertilizability has been reported as a result of TCE exposure in rats ([Wu and Berger, 2007](#); [Berger and Horner, 2003](#)), but a number of other laboratory animal studies did not report adverse effects on female reproductive function ([Cosby and Dukelow, 1992](#); [George et al., 1986](#); [George et al., 1985](#); [Manson et al., 1984](#)). Overall, there are inadequate data to conclude whether adverse effects on human female reproduction are caused by TCE.

By contrast, a number of human and laboratory animal studies suggest that TCE exposure has the potential for male reproductive toxicity. In particular, human studies have reported TCE exposure to be associated, in several cases statistically-significantly, with increased sperm density and decreased sperm quality ([Chia et al., 1996](#); [Rasmussen et al., 1988](#)), altered sexual drive or function ([Saihan et al., 1978](#); [El Ghawabi et al., 1973](#); [Bardodej and Vyskocil, 1956](#)), or altered serum endocrine levels ([Goh et al., 1998](#); [Chia et al., 1997](#)). In addition, three studies that reported measures of fertility did not or could not report changes associated with TCE exposure ([Forkert et al., 2003](#); [ATSDR, 2001](#); [Sallmen et al., 1998](#)), although the statistical power of these studies is quite limited. Further evidence of similar effects is provided by several laboratory animal studies that reported effects on sperm ([Kumar et al., 2001b](#); [Veeramachaneni et al., 2001](#); [Kumar et al., 2000a](#); [Kumar et al., 2000b](#); [George et al., 1985](#); [Land et al., 1981](#)), libido/copulatory behavior ([Veeramachaneni et al., 2001](#); [George et al., 1986](#); [Zenick et al., 1984](#)), and serum hormone levels ([Veeramachaneni et al., 2001](#); [Kumar et al., 2000a](#)). As with the human database, some studies that assessed sperm measures did not report treatment-related alterations ([Xu et al., 2004](#); [Cosby and Dukelow, 1992](#); [George et al., 1986](#); [Zenick et al., 1984](#)). Additional adverse effects on male reproduction have also been reported, including histopathological lesions in the testes or epididymides ([Kan et al., 2007](#); [Forkert et al., 2002](#); [Kumar et al., 2001b](#); [Kumar et al., 2000b](#); [George et al., 1986](#)) and altered in vitro sperm-oocyte binding or in vivo fertilization due to TCE or metabolites ([DuTeaux et al., 2004a](#); [Xu et al., 2004](#)). While reduced fertility in rodents was only observed in one study ([George et al., 1986](#)), this is not surprising given the redundancy and efficiency of rodent reproductive capabilities. Furthermore, while George et al. ([1986](#)) proposed that the adverse male reproductive outcomes

observed in rats were due to systemic toxicity, the database as a whole suggests that TCE does induce reproductive toxicity independent of systemic effects. Therefore, overall, the human and laboratory animal data together support the conclusion that TCE exposure poses a potential hazard to the male reproductive system.

#### **4.11.1.7. Developmental Toxicity**

The relationship between TCE exposure (direct or parental) and adverse developmental outcomes has been investigated in a number of epidemiologic and laboratory animal studies. Prenatal effects examined include death (spontaneous abortion, perinatal death, pre- or postimplantation loss, resorptions), decreased growth (low birth weight, SGA, IUGR, decreased postnatal growth), and congenital malformations, in particular eye and cardiac defects. Postnatal developmental outcomes examined include growth and survival, developmental neurotoxicity, developmental immunotoxicity, and childhood cancers.

A few epidemiological studies have reported associations between parental exposure to TCE and spontaneous abortion or perinatal death ([ATSDR, 2001](#); [Taskinen et al., 1994](#); [Windham et al., 1991](#)), although other studies reported mixed or null findings ([ATSDR, 2008b, 2006a](#); [Bove, 1996](#); [Bove et al., 1995](#); [Goldberg et al., 1990](#); [Lindbohm et al., 1990](#); [Taskinen et al., 1989](#); [Lagakos et al., 1986](#)). Studies examining associations between TCE exposure and decreased birth weight or SGA have reported small, often nonstatistically significant, increases in risk for these effects ([ATSDR, 2008b, 2006a](#); [Windham et al., 1991](#)). However, other studies observed mixed or no association ([Rodenbeck et al., 2000](#); [Bove, 1996](#); [Bove et al., 1995](#); [Lagakos et al., 1986](#)). While comprising both occupational and environmental exposures, these studies are overall not highly informative due to their small numbers of cases and limited exposure characterization or to the fact that exposures to mixed solvents were involved. However, a number of laboratory animal studies show analogous effects of TCE exposure in rodents. In particular, pre- or postimplantation losses, increased resorptions, perinatal death, and decreased birth weight have been reported in multiple well-conducted studies in rats and mice ([Kumar et al., 2000b](#); [Narotsky and Kavlock, 1995](#); [Narotsky et al., 1995](#); [George et al., 1986](#); [George et al., 1985](#); [Healy et al., 1982](#)). Interestingly, the rat studies reporting these effects used F344 or Wistar rats, while several other studies, all of which used Sprague-Dawley rats, reported no increased risk in these developmental measures ([Carney et al., 2006](#); [Hardin et al., 1981](#); [Schwetz et al., 1975](#)). Overall, based on weakly suggestive epidemiologic data and fairly consistent laboratory animal data, it can be concluded that TCE exposure poses a potential hazard for prenatal losses and decreased growth or birth weight of offspring.

Epidemiologic data provide some support for the possible relationship between maternal TCE exposure and birth defects in offspring, in particular cardiac defects. Other developmental outcomes observed in epidemiology and experimental animal studies include an increase in total birth defects ([ATSDR, 2001](#); [Flood, 1988](#)), CNS defects ([ATSDR, 2001](#); [Bove, 1996](#); [Bove et](#)

al., 1995; [Lagakos et al., 1986](#)), oral cleft defects ([Lorente et al., 2000](#); [Bove, 1996](#); [Bove et al., 1995](#); [Lagakos et al., 1986](#)), eye/ear defects ([Narotsky and Kavlock, 1995](#); [Narotsky et al., 1995](#); [Lagakos et al., 1986](#)), kidney/urinary tract disorders ([Lagakos et al., 1986](#)), musculoskeletal birth anomalies ([Lagakos et al., 1986](#)), lung/respiratory tract disorders ([Das and Scott, 1994](#); [Lagakos et al., 1986](#)), and skeletal defects ([Healy et al., 1982](#)). Occupational cohort studies, while not consistently reporting positive results, are generally limited by the small number of observed or expected cases of birth defects ([Lorente et al., 2000](#); [Taskinen et al., 1989](#); [Tola et al., 1980](#)).

While only one of the epidemiological studies specifically reported observations of eye anomalies ([Lagakos et al., 1986](#)), studies in rats have identified increases in the incidence of fetal eye defects following oral exposures during the period of organogenesis with TCE ([Narotsky and Kavlock, 1995](#); [Narotsky et al., 1995](#)) or its oxidative metabolites, DCA and TCA ([Warren et al., 2006](#); [Smith et al., 1992](#); [Smith et al., 1989](#)). No other developmental or reproductive toxicity studies identified abnormalities of eye development following TCE exposures, which may have been related to the administered dose or other aspects of study design (e.g., level of detail applied to fetal ocular evaluation). Overall, the study evidence suggests a potential for the disruption of ocular development by exposure to TCE and its oxidative metabolites.

The epidemiological studies, while individually limited, as a whole show relatively consistent elevations, some of which were statistically significant, in the incidence of cardiac effects in TCE-exposed populations compared to reference groups ([ATSDR, 2008b, 2006a](#); [Yauck et al., 2004](#); [ATSDR, 2001](#); [Bove, 1996](#); [Bove et al., 1995](#); [Goldberg et al., 1990](#)). Interestingly, Goldberg et al. (1990) noted that the OR for congenital heart disease in offspring declined from threefold to no difference as compared to controls after TCE-contaminated drinking water wells were closed, suggestive of a causal relationship. However, this study reported no significant differences in cardiac lesions between exposed and nonexposed groups ([Goldberg et al., 1990](#)). One additional community study reported that, among the five cases of cardiovascular anomalies, there was no significant association with TCE ([Lagakos et al., 1986](#)), but due to the small number of cases, this does not support an absence of effect. In laboratory animal models, avian studies were the first to identify adverse effects of TCE exposure on cardiac development, and the initial findings have been confirmed multiple times ([Rufer et al., 2008](#); [Drake et al., 2006a](#); [Drake et al., 2006b](#); [Mishima et al., 2006](#); [Boyer et al., 2000](#); [Loeber et al., 1988](#); [Bross et al., 1983](#)). Additionally, administration of TCE and TCE metabolites TCA and DCA in maternal drinking water during gestation has been reported to induce cardiac malformations in rat fetuses ([Johnson et al., 2005, 2003](#); [Johnson et al., 1998b](#); [Johnson et al., 1998a](#); [Dawson et al., 1993](#); [Epstein et al., 1992](#); [Smith et al., 1992](#); [Dawson et al., 1990](#); [Smith et al., 1989](#)). However, it is notable that a number of other studies, several of which were well conducted, did not report induction of cardiac defects in rats or rabbits from TCE administered by inhalation ([Carney et al., 2006](#); [Healy et al., 1982](#); [Hardin et al., 1981](#); [Dorfmueller et al.,](#)

[1979](#); [Schwetz et al., 1975](#)) or in rats and mice by gavage ([Fisher et al., 2001](#); [Narotsky and Kavlock, 1995](#); [Narotsky et al., 1995](#); [Cosby and Dukelow, 1992](#)).

The potential importance of these effects warrants a more detailed discussion of possible explanations for the apparent inconsistencies in the laboratory animal studies. Many of the studies that did not identify cardiac anomalies used a traditional free-hand section technique on fixed fetal specimens ([Healy et al., 1982](#); [Hardin et al., 1981](#); [Dorfmueller et al., 1979](#); [Schwetz et al., 1975](#)). Detection of such anomalies can be enhanced through the use of a fresh dissection technique as described by Staples ([1974](#)) and Stuckhardt and Poppe ([1984](#)) and this was the technique used in the study by Dawson et al. ([1990](#)) with further refinement of the technique used in the positive studies by Dawson et al. ([1993](#)) and Johnson et al. ([2005, 2003](#)). However, two studies that used the same or similar fresh dissection technique did not report cardiac anomalies ([Carney et al., 2006](#); [Fisher et al., 2001](#)), although it has been suggested that differences in experimental design (e.g., inhalation versus gavage versus drinking water route of administration, exposure during organogenesis versus the entire gestational period, or varied dissection or evaluation procedures) may have been contributing factors to the differences in observed response. A number of other limitations in the studies by Dawson et al. ([1993](#)) and Johnson et al. ([2005, 2003](#)) have been suggested ([Watson et al., 2006](#); [Hardin et al., 2005](#)). One concern is the lack of clear dose-response relationship for the incidence of any specific cardiac anomaly or combination of anomalies, a disparity for which no reasonable explanation has been put forth. In addition, analyses on a fetal- rather than litter-basis and the pooling of data collected over an extended period, including nonconcurrent controls, have been criticized. With respect to the first issue, the study authors provided individual litter incidence data to EPA for analysis (see Chapter 5, Dose-Response Assessment), and, in response to the second issue, the study authors provided further explanation as to their experimental procedures ([Johnson et al., 2004](#)). In sum, while the studies by Dawson et al. ([1993](#)) and Johnson et al. ([2005, 2003](#)) have significant limitations, there is insufficient reason to dismiss their findings.

Finally, mechanistic studies, particularly based on the avian studies mentioned above, provide additional support for TCE-induced fetal cardiac malformation, particularly with respect to defects involving septal and valvular morphogenesis. As summarized by NRC ([2006](#)), there is substantial concordance in the stages and events of cardiac valve formation between mammals and birds. While quantitative extrapolation of findings from avian studies to humans is not possible without appropriate kinetic data for these experimental systems, the treatment-related alterations in endothelial cushion development observed in avian in ovo and in vitro studies ([Mishima et al., 2006](#); [Ou et al., 2003](#); [Boyer et al., 2000](#)) provide a plausible mechanistic basis for defects in septal and valvular morphogenesis observed in rodents, and consequently support the plausibility of cardiac defects induced by TCE in humans.

Postnatal developmental outcomes examined after TCE prenatal and/or postnatal exposure in both humans and experimental animals include developmental neurotoxicity,

developmental immunotoxicity, and childhood cancer. Effects on the developing nervous system included a broad array of structural and behavioral alterations in humans ([Windham et al., 2006](#); [Laslo-Baker et al., 2004](#); [ATSDR, 2002](#); [Till et al., 2001a](#); [Burg and Gist, 1997](#); [White et al., 1997, abstract](#); [Burg et al., 1995](#); [Bernad et al., 1987](#); [Beppu, 1968](#)) and animals ([Blossom et al., 2008](#); [Narotsky and Kavlock, 1995](#); [Fredriksson et al., 1993](#); [Isaacson and Taylor, 1989](#); [George et al., 1986](#); [Noland-Gerbec et al., 1986](#); [Taylor et al., 1985](#); [Westergren et al., 1984](#)). Adverse immunological findings in humans following developmental exposures to TCE were reported by Lehmann et al. ([2002](#)) and Byers et al. ([1988](#)). In mice, alterations in T-cell subpopulations, spleen and/or thymic cellularity, cytokine production, autoantibody levels (in an autoimmune-prone mouse strain), and/or hypersensitivity response were observed after exposures during development ([Blossom et al., 2008](#); [Peden-Adams et al., 2008](#); [Blossom and Doss, 2007](#); [Peden-Adams et al., 2006](#)). Childhood cancers included leukemia and NHL ([Costas et al., 2002](#); [Morgan and Cassady, 2002](#); [Shu et al., 1999](#); [Flood, 1997a](#); [MDPH, 1997a](#); [Cohn et al., 1994b](#); [McKinney et al., 1991](#); [ADHS, 1990](#); [Kioski et al., 1990a](#); [Kioski et al., 1990b](#); [Flood, 1988](#); [Lowengart et al., 1987](#); [Cutler et al., 1986](#); [Lagakos et al., 1986](#)), CNS tumors ([Morgan and Cassady, 2002](#); [De Roos et al., 2001](#); [Flood, 1997a](#); [ADHS, 1990](#); [Kioski et al., 1990a](#); [Flood, 1988](#); [Peters et al., 1985](#); [Peters et al., 1981](#)), and total cancers ([ATSDR, 2006a](#); [Flood, 1997a](#); [Porter, 1993](#); [ADHS, 1990](#); [Flood, 1988](#)). These outcomes are discussed in the other relevant sections for neurotoxicity, immunotoxicity, and carcinogenesis.

#### **4.11.2. Characterization of Carcinogenicity**

Following EPA ([2005b](#)) *Guidelines for Carcinogen Risk Assessment*, TCE is characterized as “carcinogenic to humans” by all routes of exposure. This conclusion is based on convincing evidence of a causal association between TCE exposure in humans and kidney cancer. The kidney cancer association cannot be reasonably attributed to chance, bias, or confounding. The human evidence of carcinogenicity from epidemiologic studies of TCE exposure is strong for NHL but less convincing than for kidney cancer, and more limited for liver and biliary tract cancer. In addition to the body of evidence pertaining to kidney cancer, NHL, and liver cancer, the available epidemiologic studies also provide more limited evidence of an association between TCE exposure and other types of cancer, including bladder, esophageal, prostate, cervical, breast, and childhood leukemia. Differences between these sets of data and the data for kidney cancer, NHL, and liver cancer are observations from fewer numbers of studies, a mixed pattern of observed risk estimates, and the general absence of exposure-response data from the studies using a quantitative TCE-specific exposure measure.

There are several lines of supporting evidence for TCE carcinogenicity in humans. First, TCE induces multiple types of cancer in rodents given TCE by gavage and inhalation, including cancers in the same target tissues identified in the epidemiologic studies – kidney, liver, and lymphoid tissues. Second, toxicokinetic data indicate that TCE absorption, distribution,

metabolism, and excretion are qualitatively similar in humans and rodents. Finally, there is sufficient weight of evidence to conclude that a mutagenic mode of action is operative for TCE-induced kidney tumors, and this mode of action is clearly relevant to humans. Modes of action have not been established for other TCE-induced cancers in rodents, and no mechanistic data indicate that any hypothesized key events are biologically precluded in humans.

#### **4.11.2.1. Summary Evaluation of Epidemiologic Evidence of TCE and Cancer**

The available epidemiologic studies provide convincing evidence of a causal association between TCE exposure and cancer. The strongest epidemiologic evidence consists of reported increased risks of kidney cancer, with more limited evidence for NHL and liver cancer, in several well-designed cohort and case-control studies (discussed below). The summary evaluation below of the evidence for causality is based on guidelines adapted from Hill (1965) by EPA (2005b), and focuses on evidence related to kidney cancer, NHL, and liver cancer.

##### **4.11.2.1.1. (a) Consistency of observed association**

Elevated risks for kidney cancer have been observed across many independent studies. Twenty-four studies in which there was a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) and which were judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis were identified in a systematic review of the epidemiologic literature. Of the 15 of these 24 studies reporting risks of kidney cancer (Moore et al., 2010; Radican et al., 2008; Charbotel et al., 2006; Zhao et al., 2005; Brüning et al., 2003; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Pesch et al., 2000b; Boice et al., 1999; Dosemeci et al., 1999; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994; Siemiatycki, 1991), most estimated RRs between 1.1 and 1.9 for overall exposure to TCE. Six of these 15 studies reported statistically significant increased risks either for overall exposure to TCE (Moore et al., 2010; Brüning et al., 2003; Raaschou-Nielsen et al., 2003; Dosemeci et al., 1999) or for one of the highest TCE exposure groups (Moore et al., 2010; Charbotel et al., 2006; Zhao et al., 2005; Raaschou-Nielsen et al., 2003). Thirteen other cohort, case-control, and geographic-based studies were given less weight because of their lesser likelihood of TCE exposure and other study design limitations that would decrease statistical power and study sensitivity (see Sections 4.1 and 4.4.2).

The consistency of the association between TCE exposure and kidney cancer is further supported by the results of the meta-analyses of the 15 cohort and case-control studies of sufficient quality and with high probability of TCE exposure to individual subjects. These analyses observed a statistically significant increased RRm estimate for kidney cancer of 1.27 (95% CI: 1.13, 1.43) for overall TCE. The RRms were robust and did not change appreciably with the removal of any individual study or with the use of alternate RR estimates from individual studies. In addition, there was no evidence for heterogeneity or publication bias.

The consistency of increased kidney cancer RR estimates across a large number of independent studies of different designs and populations from different countries and industries argues against chance, bias or confounding as the basis for observed associations. This consistency thus provides substantial support for a causal effect between kidney cancer and TCE exposure.

Some evidence of consistency is found between TCE exposure and NHL and liver cancer. In a weight-of-evidence review of the NHL studies, 17 studies in which there was a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) and which met, to a sufficient degree, the standards of epidemiologic design and analysis were identified. These studies generally reported excess RR estimates for NHL between 0.8 and 3.1 for overall TCE exposure. Statistically significant elevated RR estimates for overall exposure were observed in two cohort studies ([Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#)) and one case-control study ([Hardell et al., 1994](#)). The other 14 identified studies reported elevated RR estimates with overall TCE exposure that were not statistically significant ([Purdue et al., 2011](#); [Cocco et al., 2010](#); [Wang et al., 2009](#); [Radican et al., 2008](#); [Miligi et al., 2006](#); [Zhao et al., 2005](#); [Boice et al., 1999](#); [Persson and Fredrikson, 1999](#); [Morgan et al., 1998](#); [Nordström et al., 1998](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#); [Greenland et al., 1994](#); [Siemiatycki, 1991](#)). Fifteen additional studies were given less weight because of their lesser likelihood of TCE exposure and other design limitations that would decrease study power and sensitivity ([Sinks et al., 1992](#)) (see Sections 4.1 and 4.6.1.2). The observed lack of association with NHL in these studies likely reflects study design and exposure assessment limitations and is not considered inconsistent with the overall evidence on TCE and NHL.

Consistency of the association between TCE exposure and NHL is further supported by the results of meta-analyses. These meta-analyses found a statistically significant increased RR estimate for NHL of 1.23 (95% CI: 1.07, 1.42) for overall TCE exposure. This result and its statistical significance were not overly influenced by most individual studies. Some heterogeneity was observed across the 17 studies of overall exposure, although it was not statistically significant ( $p = 0.16$ ). Analyzing the cohort and case-control studies separately resolved most of the heterogeneity, but the result for the summary case-control studies was only about a 7% increased RR estimate and was not statistically significant. The sources of heterogeneity are uncertain but may be the result of some bias associated with exposure assessment and/or disease classification, or from differences between cohort and case-control studies in average TCE exposure. In addition, there is some evidence of potential publication bias in this data set; however, it is uncertain that this is actually publication bias rather than an association between SE and effect size resulting for some other reason (e.g., a difference in study populations or protocols in the smaller studies). Furthermore, if there is publication bias in this data set, it does not appear to account completely for the finding of an increased NHL risk.

There are fewer studies on liver cancer than for kidney cancer and NHL. Of nine studies, all of them cohort studies, in which there was a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review ([Radican et al., 2008](#); [Boice et al., 2006b](#); [Raaschou-Nielsen et al., 2003](#); [Hansen et al., 2001](#); [Boice et al., 1999](#); [Morgan et al., 1998](#); [Anttila et al., 1995](#); [Axelson et al., 1994](#); [Greenland et al., 1994](#)), most reported RR estimates for liver and gallbladder cancer between 0.5 and 2.0 for overall exposure to TCE. RR estimates were generally based on small numbers of cases or deaths, with the result of wide CIs on the estimates, except for one study ([Raaschou-Nielsen et al., 2003](#)). This study reported almost 6 times more cancer cases than the next largest study and observed a statistically significant elevated liver and gallbladder cancer risk with overall TCE exposure (RR = 1.35 [95% CI: 1.03, 1.77]). Ten additional studies were given less weight because of their lesser likelihood of TCE exposure and other design limitations that would decrease statistical power and study sensitivity (see Sections 4.1 and 4.5.2).

Consistency of the association between TCE exposure and liver cancer is further supported by the results of meta-analyses. These meta-analyses found a statistically significant increased RR<sub>m</sub> estimate for liver and biliary tract cancer of 1.29 (95% CI: 1.07, 1.56) with overall TCE exposure. Although there was no evidence of heterogeneity or publication bias and the summary estimate was fairly insensitive to the use of alternative RR estimates, the statistical significance of the summary estimate depends heavily on the one large study by Raaschou-Nielsen et al. ([2003](#)). However, there were fewer adequate studies available for meta-analysis of liver cancer (9 vs. 17 for NHL and 15 for kidney), leading to lower statistical power, even with pooling. Moreover, liver cancer is comparatively rarer, with age-adjusted incidences roughly half or less those for kidney cancer or NHL; thus, fewer liver cancer cases are generally observed in individual cohort studies.

#### **4.11.2.1.2. (b) Strength of the observed association**

In general, the observed associations between TCE exposure and cancer are modest, with RRs or ORs for overall TCE exposure generally <2.0 and higher RRs or ORs for high exposure categories. Among the highest statistically significant RRs were those reported for kidney cancer in the studies by Henschler et al. ([1995](#)) (7.97 [95% CI: 2.59, 8.59]) and Vamvakas et al. ([1998](#)) (10.80 [95% CI: 3.36, 34.75]). As discussed in Section 4.4.2.2.1, risk magnitude in both studies is highly uncertain due, in part, to possible selection biases, and neither was included in the meta-analyses. However, the findings of these studies were corroborated, though with lower reported RRs, by later studies, which overcame many of their deficiencies, such as Brüning et al. ([2003](#)) (2.47 [95% CI: 1.36, 4.49]), Charbotel et al. ([2006](#)) (2.16 [95% CI: 1.02, 4.60] for the high cumulative exposure group), and Moore et al. ([2010](#)) (2.05 [95% CI: 1.13, 3.73] for high confidence assessment of TCE). In addition, the very high apparent exposure in the subjects of

Henschler et al. (1995) and Vamvakas et al. (1998) may have contributed to their reported RRs being higher than those in other studies. Exposures in most population case-control studies are of lower overall TCE intensity compared to exposures in Brüning et al. (2003) and Charbotel et al. (2006), and, as would be expected, observed RR estimates are lower (1.24 [95% CI: 1.03, 1.49]), Pesch et al. (2000b); 1.30 [95% CI: 0.9, 1.9], Dosemeci et al. (1999)). A few high-quality cohort and case-control studies reported statistically significant RRs of approximately 2.0 with highest exposure, including Zhao et al. (2005) (4.9 [95% CI: 1.23, 19.6] for high TCE score), Raaschou-Nielsen et al. (2003) (1.7 [95% CI: 1.1, 2.4] for  $\geq 5$  year exposure duration, subcohort with higher exposure), Charbotel et al. (2006) (2.16 [95% CI: 1.02, 4.60] for high cumulative exposure and 2.73 [95% CI: 1.06, 7.07] for high cumulative exposure plus peaks) and Moore et al. (2010) (2.23 [95% CI: 1.07, 4.64] for high cumulative exposure and 2.41 [95% CI: 1.05, 5.56] for high average intensity TCE exposure).

Among the highest statistically significant RRs reported for NHL were those of Hansen et al. (2001) (3.1 [95% CI: 1.3, 6.1]), Hardell et al. (1994) (7.2 [95% CI: 1.3, 42]), the latter a case-control study whose magnitude of risk is uncertain because of self-reported occupational TCE exposure. A similar magnitude of risk was reported in Purdue et al. (2011) for highest exposure (3.3 [95% CI: 1.1, 10.1], >234,000 ppm-hour, and 7.9 [95% CI: 1.8, 34.3], >360 ppm-hour/week). Observed RR estimates for liver cancer and overall TCE exposure are generally more modest.

The strength of association between TCE exposure and cancer is modest with overall TCE exposure. Large RR estimates are considered strong evidence of causality; however, a modest risk does not preclude a causal association and may reflect a lower level of exposure, an agent of lower potency, or a common disease with a high background level (U.S. EPA, 2005b). Modest RR estimates have been observed with several well-established human carcinogens such as benzene and secondhand smoke. Chance cannot explain the observed association between TCE and cancer; statistically significant associations were found in a number of the studies that contribute greater weight to the overall evidence, given their design and statistical analysis approaches. In addition, other known or suspected risk factors cannot fully explain the observed elevations in kidney cancer RRs. All kidney cancer case-control studies included adjustment for possible confounding effects of smoking, and some studies included BMI, hypertension, and co-exposure to other occupational agents such as cutting or petroleum oils. Cutting and petroleum oils, known as metalworking fluids, have not been associated with kidney cancer (Mirer, 2010; NIOSH, 1998), and potential confounding by this occupational co-exposure is unable to explain the observed association with TCE. Additionally, the associations between kidney cancer and TCE exposure remained in these studies after statistical adjustment for possible known and suspected confounders. Charbotel et al. (2006) observed a nonstatistically significantly kidney cancer risk with exposure to TCE adjusted for cutting or petroleum oil exposures (1.96 [95% CI:

0.71, 5.37] for the high-cumulative exposure group and 2.63 [95% CI: 0.79, 8.83] for high-exposure group with peaks).

All kidney cancer case-control studies adjusted for smoking except the Moore et al. (2010) study, which reported that smoking did not significantly change the overall association with TCE exposure. Although direct examination of smoking and other suspected kidney cancer risk factors is usually not possible in cohort studies, confounding is less likely in Zhao et al. (2005), given their use of an internal referent group and adjustment for SES status, an indirect surrogate for smoking, and other occupational exposures. In addition, the magnitude of the lung cancer risk in Raaschou-Nielsen et al. (2003) suggests that a high smoking rate is unlikely and cannot explain their finding on kidney cancer. Last, a meta-analysis of the nine cohort studies that reported kidney cancer risks found an RRm estimate for lung cancer of 0.96 (95% CI: 0.76, 1.21) for overall TCE exposure and 0.96 (95% CI: 0.72, 1.27) for the highest exposure group. These observations suggest that confounding by smoking is not an alternative explanation for the kidney cancer meta-analysis results.

Few risk factors are recognized for NHL, with the exception of viruses and suspected factors such as immunosuppression or smoking, which are associated with specific NHL subtypes. Associations between NHL and TCE exposure are based on groupings of several NHL subtypes. Three of the seven NHL case-control studies adjusted for age, sex, and smoking in statistical analyses (Wang et al., 2009; Miligi et al., 2006) two others adjusted for age, sex, and education (Purdue et al., 2011; Cocco et al., 2010), and the other three case-control studies adjusted for age only or age and sex (Persson and Fredrikson, 1999; Nordström et al., 1998; Hardell et al., 1994). Like for kidney cancer, direct examination of possible confounding in cohort studies is not possible. The use of internal controls in some of the cohort studies is intended to reduce possible confounding related to lifestyle differences, including smoking habits, between exposed and referent subjects.

Heavy alcohol use and viral hepatitis are established risk factors for liver cancer, with severe obesity and diabetes characterized as a metabolic syndrome associated with liver cancer. Only cohort studies for liver cancer are available, and they were not able to consider these possible risk factors.

#### **4.11.2.1.3. (c) Specificity of the observed association**

Specificity is generally not as relevant as other aspects for judging causality. As stated in the EPA *Guidelines for Carcinogen Risk Assessment* (2005b), based on our current understanding that many agents cause cancer at multiple sites and that cancers have multiple causes, the absence of specificity does not detract from evidence for a causal effect. Evidence for specificity could be provided by a biological marker in tumors that was specific to TCE exposure. There is some evidence suggesting that particular *VHL* mutations in kidney tumors may be caused by TCE, but uncertainties in these data preclude a definitive conclusion.

#### 4.11.2.1.4. (d) Temporal relationship of the observed association

Each cohort study was evaluated for the adequacy of the follow-up period to account for the latency of cancer development. The studies with the greatest weight based on study design characteristics (e.g., those used in the meta-analysis) all had adequate follow-up to assess associations between TCE exposure and cancer. Therefore, the findings of those studies are consistent with a temporal relationship.

#### 4.11.2.1.5. (e) Biological gradient (exposure-response relationship)

Exposure-response relationships are examined in the TCE epidemiologic studies only to a limited extent. Many studies examined only overall “exposed” vs. “unexposed” groups and did not provide exposure information by level of exposure. Others do not have adequate exposure assessments to confidently distinguish between levels of exposure. For example, many studies used duration of employment as an exposure surrogate; however, this is a poor exposure metric given subjects may have differing exposure intensity with similar exposure duration ([NRC, 2006](#)).

Three studies of kidney cancer reported a statistically significant trend of increasing risk with increasing TCE exposure, Zhao et al. ([2005](#)) ( $p = 0.023$  for trend with TCE score), Charbotel et al. ([2006](#)) ( $p = 0.04$  for trend with cumulative TCE exposure) and Moore et al. ([2010](#)) ( $p = 0.02$  for trend with cumulative TCE exposure). Charbotel et al. ([2006](#)) was specifically designed to examine TCE exposure and had a high-quality exposure assessment, and the Moore et al. ([2010](#)) exposure assessment considered detailed information on jobs using solvents. Zhao et al. ([2005](#)) also had a relatively well-designed exposure assessment. A positive trend was also observed in one other study (Raaschou-Nielsen et al. ([2003](#)), with employment duration).

Biological gradient is further supported by meta-analyses for kidney cancer using only the highest exposure groups and accounting for possible reporting bias, which yielded a higher RRM estimate (1.58 [95% CI: 1.28, 1.96]) than for overall TCE exposure (1.27 [95% CI: 1.13, 1.43]). Although this analysis uses a subset of studies in the overall TCE exposure analysis, the finding of higher risk in the highest exposure groups, where such groups were available, is consistent with a trend of increased risk with increased exposure.

The NHL case-control study of Purdue et al. ([2011](#)) reported a statistically significant trend with TCE exposure ( $p = 0.02$  for trend with average-weekly TCE exposure), and NHL risk in Boice et al. ([1999](#)) appeared to increase with increasing exposure duration ( $p = 0.20$  for routine-intermittent exposed subjects). The borderline trend with TCE intensity in the case-control studies of Wang et al. ([2009](#)) ( $p = 0.06$ ) and Purdue et al. ([2011](#)) ( $p = 0.08$  for trend with cumulative TCE exposure) is consistent with their findings for average weekly TCE exposure. As with kidney cancer, further support was provided by meta-analyses using only the highest exposure groups, which yielded a higher RRM estimate (1.43 [95% CI: 1.13, 1.82]) than for

overall TCE exposure (1.23 [95% CI: 1.07, 1.42]). For liver cancer, the meta-analyses using only the highest exposure groups yielded a lower, and nonstatistically significant, RRm estimate (1.28 [95% CI: 0.93, 1.77]) than for overall TCE exposure (1.29 [95% CI: 1.07, 1.56]). There were no case-control studies on liver cancer and TCE, and the cohort studies generally had few liver cancer cases, making it more difficult to assess exposure-response relationships. The one large study ([Raaschou-Nielsen et al., 2003](#)) used only duration of employment, which is an inferior exposure metric.

#### **4.11.2.1.6. (f) Biological plausibility**

TCE metabolism is similar in humans, rats, and mice and results in reactive metabolites. TCE is metabolized in multiple organs and metabolites are systemically distributed. Several oxidative metabolites produced primarily in the liver, including CH, TCA and DCA, are rodent hepatocarcinogens. Two other metabolites, DCVC and DCVG, which can be produced and cleared by the kidney, have shown genotoxic activity, suggesting the potential for carcinogenicity. Kidney cancer, NHL, and liver cancer have all been observed in rodent bioassays (see below). The laboratory animal data for liver and kidney cancer are the most robust, and are corroborated in multiple studies, sexes, and strains, although each has only been reported in a single species and the incidences of kidney cancer are quite low. Lymphomas were only reported to be statistically significantly elevated in a single study in mice, but one additional mouse study reported elevated lymphoma incidence and one rat study reported elevated leukemia incidence. In addition, there is some evidence both in humans and laboratory animals for kidney, liver and immune system noncancer toxicity from TCE exposure. Several hypothesized modes of action have been presented for the rodent tumor findings, and the available evidence does not preclude the relevance of the hypothesized modes of action to humans. Activation of macrophages, NK cells, and cytokine production (e.g., tumor necrosis factor) may also play an etiologic role in carcinogenesis, and thus, the immune-related effects of TCE should also be considered. In addition, the decreased in lymphocyte counts and subsets, including CD4+ T cells, and decreased lymphocyte activation seen in TCE-exposed workers ([Lan et al., 2010](#)) also support the biological plausibility of a role of TCE exposure in NHL.

#### **4.11.2.1.7. (g) Coherence**

Coherence is defined as consistency with the known biology. As discussed under biological plausibility, the observance of kidney and liver cancer and NHL in humans is consistent with the biological processing and toxicity of TCE.

#### **4.11.2.1.8. (h) Experimental evidence (from human populations)**

Few experimental data from human populations are available on the relationship between TCE exposure and cancer. The only study of a “natural experiment” (i.e., observations of a temporal change in cancer incidence in relation to a specific event) notes that childhood leukemia cases appeared to be more evenly distributed throughout Woburn, Massachusetts, after closure of the two wells contaminated with TCE and other organic solvents ([MDPH, 1997c](#)).

#### **4.11.2.1.9. (i) Analogy**

Exposure to structurally related chlorinated solvents such as tetrachloroethylene and dichloromethane have also been associated with kidney, lymphoid, and liver tumors in human, although the evidence for TCE is considered stronger.

#### **4.11.2.1.10. Conclusion**

In conclusion, based on the weight-of-evidence analysis for kidney cancer and in accordance with EPA guidelines, TCE is characterized as “carcinogenic to humans.” This hazard descriptor is used when there is convincing epidemiologic evidence of a causal association between human exposure and cancer. Convincing evidence is found in the consistency of the kidney cancer findings. The consistency of increased kidney cancer relative risk estimates across a large number of independent studies of different designs and populations from different countries and industries provides compelling evidence given the difficulty, a priori, in detecting effects in epidemiologic studies when the relative risks are modest, the cancers are relatively rare, and therefore, individual studies have limited statistical power. This strong consistency argues against chance, bias, and confounding as explanations for the elevated kidney cancer risks. In addition, statistically significant exposure-response trends are observed in high-quality studies. These studies were designed to examine kidney cancer in populations with high TCE exposure intensity. These studies addressed important potential confounders and biases, further supporting the observed associations with kidney cancer as causal. In a meta-analysis of the 15 studies that met the inclusion criteria, a statistically significant RR<sub>m</sub> estimate was observed for overall TCE exposure (RR<sub>m</sub>: 1.27 [95% CI: 1.13, 1.43]). The RR<sub>m</sub> estimate was greater for the highest TCE exposure groups (RR<sub>m</sub>: 1.58 [95% CI: 1.28, 1.96]; n = 13 studies). Meta-analyses investigating the influence of individual studies and the sensitivity of the results to alternate risk estimate selections found the RR<sub>m</sub> estimates to be highly robust. Furthermore, there was no indication of publication bias or significant heterogeneity. It would require a substantial amount of negative data from informative studies (i.e., studies having a high likelihood of TCE exposure in individual study subjects and which meet, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review) to contradict this observed association.

The evidence is less convincing for NHL and liver cancer. While the evidence is strong for NHL, issues of (nonstatistically significant) study heterogeneity, potential publication bias, and weaker exposure-response results contribute greater uncertainty. The evidence is more limited for liver cancer mainly because only cohort studies are available and most of these studies have small numbers of cases. In addition to the body of evidence described above pertaining to kidney cancer, NHL, and liver cancer, the available epidemiologic studies also provide suggestive evidence of an association between TCE exposure and other types of cancer, including bladder, esophageal, prostate, cervical, breast, and childhood leukemia, breast. Differences between these sets of data and the data for kidney cancer, NHL, and liver cancer are observations are from fewer numbers of studies, a mixed pattern of observed risk estimates and the general absence of exposure-response data from the studies using a quantitative TCE-specific cumulative exposure measure.

#### **4.11.2.2. Summary of Evidence for TCE Carcinogenicity in Rodents**

Additional evidence of TCE carcinogenicity consists of increased incidences of tumors reported in multiple chronic bioassays in rats and mice. In total, this database identifies some of the same target tissues of TCE carcinogenicity also seen in epidemiological studies, including the kidney, liver, and lymphoid tissues.

Of particular note is the site-concordant finding of TCE-induced kidney cancer in rats. In particular, low, but biologically and sometimes statistically significant, increases in the incidence of kidney tumors were observed in multiple strains of rats treated with TCE by either inhalation or corn oil gavage ([NTP, 1990](#); [Maltoni et al., 1988](#); [NTP, 1988](#); [Maltoni et al., 1986](#)). For instance, Maltoni et al. ([1986](#)) reported that although only 4/130 renal adenocarcinomas were noted in rats in the highest dose group, these tumors had never been observed in over 50,000 Sprague-Dawley rats (untreated, vehicle-treated, or treated with different chemicals) examined in previous experiments in the same laboratory. In addition, the gavage study by NCI ([1976](#)) and two inhalation studies by Henschler et al. ([1980](#)), and Fukuda et al. ([1983](#)) each observed one renal adenoma or adenocarcinoma in some dose groups and none in controls. The largest (but still small) incidences were observed in treated male rats, only in the highest dose groups. However, given the small numbers, an effect in females cannot be ruled out. Several studies in rats were limited by excessive toxicity, accidental deaths, or deficiencies in reporting ([NTP, 1990, 1988](#); [NCI, 1976](#)). Individually, therefore, these studies provide only suggestive evidence of renal carcinogenicity. Overall, given the rarity of these types of tumors in the rat strains tested and the repeated similar results across experiments and strains, these studies taken together support the conclusion that TCE is a kidney carcinogen in rats, with males being more sensitive than females. No other tested laboratory species (i.e., mice and hamsters) have exhibited increased kidney tumors, although high incidences of kidney toxicity have been reported in mice ([NTP, 1990](#); [Maltoni et al., 1988](#); [Maltoni et al., 1986](#); [NCI, 1976](#)). The GSH-conjugation-

derived metabolites suspected of mediating TCE-induced kidney carcinogenesis have not been tested in a standard 2-year bioassay, so their role cannot be confirmed definitively. However, it is clear that GSH conjugation of TCE occurs in humans and that the human kidney contains the appropriate enzymes for bioactivation of GSH conjugates. Therefore, the production of the active metabolites thought to be responsible for kidney tumor induction in rats likely occurs in humans.

Statistically significant increases in TCE-induced liver tumors have been reported in multiple inhalation and gavage studies with male Swiss mice and B6C3F<sub>1</sub> mice of both sexes ([Bull et al., 2002](#); [Anna et al., 1994](#); [NTP, 1990](#); [Maltoni et al., 1988](#); [Herren-Freund et al., 1987](#); [Maltoni et al., 1986](#); [NCI, 1976](#)). On the other hand, in female Swiss mice, Fukuda et al. ([1983](#)) (CD-1 [ICR, Swiss-derived] mice) and Maltoni et al. ([1988](#); [1986](#)) both reported small, nonsignificant increases at the highest dose by inhalation. Henschler et al. ([1984](#); [1980](#)) reported no increases in either sex of Han:NMRI (also Swiss-derived) mice exposed by inhalation and ICR/HA (Swiss) mice exposed by gavage. However, the inhalation study ([Henschler et al., 1980](#)) had only 30 mice per dose group and the gavage study ([Henschler et al., 1984](#)) had dosing interrupted due to toxicity. Studies in rats ([NTP, 1990](#); [Maltoni et al., 1988](#); [NTP, 1988](#); [Maltoni et al., 1986](#); [Henschler et al., 1980](#); [NCI, 1976](#)) and hamsters ([Henschler et al., 1980](#)) did not report statistically significant increases in liver tumor induction with TCE treatment. However, several studies in rats were limited by excessive toxicity or accidental deaths ([NTP, 1990, 1988](#); [NCI, 1976](#)), and the study in hamsters only had 30 animals per dose group. These data are inadequate for concluding that TCE lacks hepatocarcinogenicity in rats and hamsters, but are indicative of a lower potency in these species. Moreover, it is notable that a few studies in rats reported low incidences (too few for statistical significance) of very rare biliary- or endothelial-derived tumors in the livers of some treated animals ([Maltoni et al., 1988](#); [Maltoni et al., 1986](#); [Fukuda et al., 1983](#); [Henschler et al., 1980](#)). Further evidence for the hepatocarcinogenicity of TCE is derived from chronic bioassays of the TCE oxidative metabolites CH, TCA, and DCA in mice (e.g., [DeAngelo et al., 2008](#); [Leakey et al., 2003a](#); [Leakey et al., 2003b](#); [George et al., 2000](#); [DeAngelo et al., 1999](#); [DeAngelo et al., 1996](#); [Bull et al., 1990](#)), all of which reported hepatocarcinogenicity. Very limited testing of these TCE metabolites has been done in rats, with a single experiment reported in both Richmond et al. ([1995](#)) and DeAngelo et al. ([1996](#)) finding statistically significant DCA-induced hepatocarcinogenicity. With respect to TCA, DeAngelo et al. ([1997](#)), often cited as demonstrating lack of hepatocarcinogenicity in rats, actually reported elevated adenoma multiplicity and carcinoma incidence from TCA treatment. However, statistically, the role of chance could not be confidently excluded because of the low number of animals per dose group (20–24 per treatment group at final sacrifice). Overall, TCE and its oxidative metabolites are clearly carcinogenic in mice, with males more sensitive than females and the B6C3F<sub>1</sub> strain appearing to be more sensitive than the Swiss strain. Such strain and sex differences are not unexpected, as they appear to parallel, qualitatively, differences in

background tumor incidence. Data in other laboratory animal species are limited. Thus, except for DCA, which is carcinogenic in rats, inadequate evidence exists to evaluate the hepatocarcinogenicity of these compounds in rats or hamsters. However, to the extent that there is hepatocarcinogenic potential in rats, TCE is clearly less potent in the strains tested in this species than in B6C3F<sub>1</sub> and Swiss mice.

Additionally, there is more limited evidence for TCE-induced lymphohematopoietic cancers in rats and mice, lung tumors in mice, and testicular tumors in rats. With respect to lymphomas, Henschler et al. (1980) reported statistically significant increases in lymphomas in female Han:NMRI mice treated via inhalation. While Henschler et al. (1980) suggested that these lymphomas were of viral origin specific to this strain, subsequent studies reported increased lymphomas in female B6C3F<sub>1</sub> mice treated via corn oil gavage (NTP, 1990) and leukemias in male Sprague-Dawley and female August rats (Maltoni et al., 1988; NTP, 1988; Maltoni et al., 1986). However, these tumors had relatively modest increases in incidence with treatment, and were not reported to be increased in other studies. With respect to lung tumors, rodent bioassays have demonstrated a statistically significant increase in pulmonary tumors in mice following chronic inhalation exposure to TCE (Maltoni et al., 1988; Maltoni et al., 1986; Fukuda et al., 1983). Pulmonary tumors were not reported in other species tested (i.e., rats and hamsters; (Maltoni et al., 1988; Maltoni et al., 1986; Fukuda et al., 1983; Henschler et al., 1980)). Chronic oral exposure to TCE led to a nonstatistically significant increase in pulmonary tumors in mice but, again, not in rats or hamsters (NTP, 1990; Maltoni et al., 1988; NTP, 1988; Maltoni et al., 1986; Henschler et al., 1984; Van Duuren et al., 1979; NCI, 1976). A lower response via oral exposure would be consistent with a role of respiratory metabolism in pulmonary carcinogenicity. Finally, increased testicular (interstitial cell and Leydig cell) tumors have been observed in rats exposed by inhalation and gavage (NTP, 1990, 1988; Maltoni et al., 1986). Statistically significant increases were reported in Sprague-Dawley rats exposed via inhalation (Maltoni et al., 1988; Maltoni et al., 1986) and Marshall rats exposed via gavage (NTP, 1988). In three rat strains, ACI, August, and F344/N, a high (>75%) control rate of testicular tumors was observed, limiting the ability to detect a treatment effect (NTP, 1990, 1988).

In summary, there is clear evidence for TCE carcinogenicity in rats and mice, with multiple studies showing TCE to cause different kinds of cancers. The apparent lack of site concordance across laboratory animal species may be due to limitations in design or conduct in a number of rat bioassays and/or genuine interspecies differences in sensitivity. Nonetheless, these studies have shown carcinogenic effects across different strains, sexes, and routes of exposure, and site-concordance is not necessarily expected for carcinogens. Of greater import is the finding that there is site-concordance between the main cancers observed in TCE-exposed humans and those observed in rodent studies—in particular, cancers of the kidney, liver, and lymphoid tissues.

#### **4.11.2.3. Summary of Additional Evidence on Biological Plausibility**

Additional evidence from toxicokinetic, toxicity, and mechanistic studies supports the biological plausibility of TCE carcinogenicity in humans.

##### **4.11.2.3.1. Toxicokinetics**

As described in Chapter 3, there is no evidence of major qualitative differences across species in TCE absorption, distribution, metabolism, and excretion. In particular, available evidence is consistent with TCE being readily absorbed via oral, dermal, and inhalation exposures, and rapidly distributed to tissues via systemic circulation. Extensive *in vivo* and *in vitro* data show that mice, rats, and humans all metabolize TCE via two primary pathways: oxidation by CYPs and conjugation with GSH via GSTs. Several metabolites and excretion products from both pathways, including TCA, DCA, TCOH, TCOG, NAcDCVC, and DCVG, have been detected in blood and urine from exposed humans as well as from at least one rodent species. In addition, the subsequent distribution, metabolism, and excretion of TCE metabolites are qualitatively similar among species. Therefore, humans possess the metabolic pathways that produce the TCE metabolites thought to be involved in the induction of rat kidney and mouse liver tumors, and internal target tissues of both humans and rodents experience a similar mix of TCE and metabolites.

As addressed in further detail elsewhere (see Chapters 3 and 5), examples of quantitative interspecies differences in toxicokinetics include differences in partition coefficients, metabolic capacity and affinity in various tissues, and plasma binding of the metabolite TCA. These and other differences are addressed through PBPK modeling, which also incorporates physiological differences among species (see Section 3.5), and are accounted for in the PBPK model-based dose-response analyses (see Chapter 5). Importantly, these quantitative differences affect only interspecies extrapolations of carcinogenic potency, and do not affect inferences as to the carcinogenic hazard for TCE. In addition, available data on toxicokinetic differences do not appear sufficient to explain interspecies differences in target sites of TCE carcinogenicity (discussed further in Chapter 5: Dose-Response Assessment).

##### **4.11.2.3.2. Toxicity and mode of action**

Many different modes of action have been proposed for TCE-induced carcinogenesis. With respect to genotoxicity, although it appears unlikely that TCE, as a pure compound, causes point mutations, there is evidence for TCE genotoxicity with respect to other genetic endpoints, such as micronucleus formation (see Section 4.2.1.4.4). In addition, as discussed further below, several TCE metabolites have tested positive in genotoxicity assays. The mode-of-action conclusions for specific target organs in laboratory animals are summarized below. Only in the case of the kidney is it concluded that the data are sufficient to support a particular mode of

action being operative. However, the available evidence do not indicate that qualitative differences between humans and test animals would preclude any of the hypothesized key events in rodents from occurring in humans.

For the kidney, the predominance of positive genotoxicity data in the database of available studies of TCE metabolites derived from GSH conjugation (in particular DCVC, see Section 4.2.5), together with toxicokinetic data consistent with their systemic delivery to and in situ formation in the kidney, supports the conclusion that a mutagenic mode of action is operative in TCE-induced kidney tumors (see Section 4.4.7.1). Relevant data include demonstration of genotoxicity in available in vitro assays of GSH conjugation metabolites and reported kidney-specific genotoxicity after in vivo administration of TCE or DCVC. Mutagenicity is a well-established cause of carcinogenicity. While supporting the biological plausibility of this hypothesized mode of action, available data on the *VHL* gene in humans or transgenic animals do not conclusively elucidate the role of *VHL* mutation in TCE-induced renal carcinogenesis. Cytotoxicity and compensatory cell proliferation, also presumed to be mediated through metabolites formed after GSH-conjugation of TCE, have also been suggested to play a role in the mode of action for renal carcinogenesis, as high incidences of nephrotoxicity have been observed in animals at doses that also induce kidney tumors. Human studies have reported markers for nephrotoxicity at current occupational exposures, although data are lacking at lower exposures. Toxicity is observed in both mice and rats, in some cases with nearly 100% incidence in all dose groups, but kidney tumors are only observed at low incidences in rats at the highest tested doses. Therefore, nephrotoxicity alone appears to be insufficient, or at least not rate-limiting, for rodent renal carcinogenesis, since maximal levels of toxicity are reached before the onset of tumors. In addition, nephrotoxicity has not been shown to be necessary for kidney tumor induction by TCE in rodents. In particular, there is a lack of experimental support for causal links, such as compensatory cellular proliferation or clonal expansion of initiated cells, between nephrotoxicity and kidney tumors induced by TCE. Furthermore, it is not clear if nephrotoxicity is one of several key events in a mode of action, if it is a marker for an “upstream” key event (such as oxidative stress) that may contribute independently to both nephrotoxicity and renal carcinogenesis, or if it is incidental to kidney tumor induction. Therefore, although the data are consistent with the hypothesis that cytotoxicity and regenerative proliferation contribute to TCE-induced kidney tumors, the weight of evidence is not as strong as the support for a mutagenic mode of action. Moreover, while toxicokinetic differences in the GSH conjugation pathway, along with their uncertainty, are addressed through PBPK modeling, no data suggest that any of the proposed key events for TCE-induced kidney tumors rats are precluded in humans. Therefore, TCE-induced rat kidney tumors provide additional support for the convincing human evidence of TCE-induced kidney cancer, with mechanistic data supportive of a mutagenic mode of action.

The strongest data supporting the hypothesis of a mutagenic mode of action in either the lung or the liver are those demonstrating the genotoxicity of CH (see Section 4.2.4), which is produced in these target organs as a result of oxidative metabolism of TCE. It has been suggested that CH mutagenicity is unlikely to be the cause of TCE hepatocarcinogenicity because the concentrations required to elicit these responses are several orders of magnitude higher than achieved in vivo ([Moore and Harrington-Brock, 2000](#)). However, it is not clear how much of a correspondence is to be expected from concentrations in genotoxicity assays in vitro and concentrations in vivo, as reported in vivo CH concentrations are in whole liver homogenate while in vitro concentrations are in culture media. The use of i.p. administration, which leads to an inflammatory response, in many other in vivo genotoxicity assays in the liver and lung complicates the comparison with carcinogenicity data. Also, it is difficult with the available data to assess the contributions from genotoxic effects of CH along with those from the genotoxic and nongenotoxic effects of other oxidative metabolites (e.g., DCA and TCA). Therefore, while data are insufficient to conclude that a mutagenic mode of action mediated by CH is operant, a mutagenic mode of action in the liver or lung, either mediated by CH or by some other oxidative metabolite of TCE, cannot be ruled out.

A second mode-of-action hypothesis for TCE-induced liver tumors involves activation of the PPAR $\alpha$  receptor. Clearly, in vivo administration of TCE leads to activation of PPAR $\alpha$  in rodents and likely does so in humans as well (based on in vitro data for TCE and its oxidative metabolites). However, the evidence as a whole does not support the view that PPAR $\alpha$  is the sole operant mode of action mediating TCE hepatocarcinogenesis. Although metabolites of TCE activate PPAR $\alpha$ , the data on the subsequent elements in the hypothesized mode of action (e.g., gene regulation, cell proliferation, apoptosis, and selective clonal expansion), while limited, indicate significant differences between PPAR $\alpha$  agonists such as Wy-14643 and TCE or its metabolites. For example, compared with other agonists, TCE induces transient as opposed to persistent increases in DNA synthesis; increases (or is without effect on), as opposed to decreases, apoptosis; and induces a different H-ras mutation frequency or spectrum. These data support the view that mechanisms other than PPAR $\alpha$  activation may contribute to these effects; besides PPAR $\alpha$  activation, the other hypothesized key events are nonspecific, and available data (e.g., using knockout mice) do not indicate that they are solely or predominantly dependent on PPAR $\alpha$ . A second consideration is whether certain TCE metabolites (e.g., TCA) that activate PPAR $\alpha$  are the sole contributors to its carcinogenicity. As summarized above (see Section 4.11.1.3), TCA is not the only metabolite contributing to the observed noncancer effects of TCE in the liver. Other data also suggest that multiple metabolites may also contribute to the hepatic carcinogenicity of TCE. Liver phenotype experiments, particularly those utilizing immunostaining for c-Jun, support a role for both DCA and TCA in TCE-induced tumors, with strong evidence that TCA cannot solely account for the characteristics of TCE-induced tumors ([e.g., Bull et al., 2002](#)). In addition, H-ras mutation frequency and spectrum of TCE-induced

tumors more closely resembles that of spontaneous tumors or of those induced by DCA, and were less similar in comparison to that of TCA-induced tumors. The heterogeneity of TCE-induced tumors is similar to that observed to be induced by a diversity of carcinogens including those that do not activate PPAR $\alpha$ , and to that observed in human liver cancer. Taken together, the available data indicate that, rather than being solely dependent on a single metabolite (TCA) and/or molecular target (PPAR $\alpha$ ), multiple TCE metabolites and multiple toxicity pathways contribute to TCE-induced liver tumors.

Other considerations as well as new data published since the NRC (2006) review are also pertinent to the liver tumor mode of action conclusions. It is generally acknowledged that, qualitatively, there are no data to support the conclusion that effects mediated by the PPAR $\alpha$  receptor that contribute to hepatocarcinogenesis would be biologically precluded in humans (NRC, 2006; Klaunig et al., 2003). It has, on the other hand, been argued that due to quantitative toxicokinetic and toxicodynamic differences, the hepatocarcinogenic effects of chemicals activating this receptor are “unlikely” to occur in humans (NRC, 2006; Klaunig et al., 2003); however, several lines of evidence strongly undermine the confidence in this assertion. With respect to toxicokinetics, as discussed above, quantitative differences in oxidative metabolism are accounted for in PBPK modeling of available in vivo data, and do not support interspecies differences of a magnitude that would preclude hepatocarcinogenic effects based on toxicokinetics alone. With respect to the mode of action proposed by Klaunig et al. (2003), recent experiments have demonstrated that PPAR $\alpha$  activation and the sequence of key events in the hypothesized mode of action are not sufficient to induce hepatocarcinogenesis (Yang et al., 2007). Moreover, the demonstration that the PPAR $\alpha$  agonist DEHP induces tumors in PPAR $\alpha$ -null mice supports the view that the events comprising the hypothesized mode of action are not necessary for liver tumor induction in mice by this PPAR $\alpha$  agonist (Ito et al., 2007). Therefore, several lines of evidence, including experiments published since the NRC (2006) review, call into question the scientific validity of using the PPAR $\alpha$  mode-of-action hypothesis as the basis for evaluating the relevance to human carcinogenesis of rodent liver tumors (Guyton et al., 2009).

In summary, available data support the conclusion that the mode of action for TCE-induced liver tumors in laboratory animals is not known. However, a number of qualitative similarities exist between observations in TCE-exposed mice and what is known about the etiology and induction of human HCCs. Polyploidization, changes in glycogen storage, inhibition of GST-zeta, and aberrant DNA methylation status, which have been observed in studies of mice exposed to TCE or its oxidative metabolites, are all either clearly related to human carcinogenesis or are areas of active research as to their potential roles (PPAR $\alpha$  activation is discussed below). The mechanisms by which TCE exposure may interact with known risk factors for human HCCs are not known. However, available data do not suggest that TCE exposure to mice results in liver tumors that are substantially different in terms of their

phenotypic characteristics either from human HCCs or from rodent liver tumors induced by other chemicals.

Comparing various other, albeit relatively nonspecific, tumor characteristics between rodent species and humans provides additional support to the biologic plausibility of TCE carcinogenicity. For example, in the kidney and the liver, the higher incidences of background and TCE-induced tumors in male rats and mice, respectively, as compared to females parallels the observed higher human incidences in males for these cancers ([Ries et al., 2008](#)). For the liver, while there is a lower background incidence of liver tumors in humans than in rodents, in the United States, there is an increasing occurrence of liver cancer associated with several factors, including viral hepatitis, higher survival rates for cirrhosis, and possibly diabetes ([reviewed in El-Serag, 2007](#)). In addition, Leakey et al. ([2003a](#)) reported that increased body weight in B6C3F<sub>1</sub> mice is strongly associated with increased background liver tumor incidences, although the mechanistic basis for this risk factor in mice has not been established. Nonetheless, it is interesting that recent epidemiologic studies have suggested obesity, in addition to associated disorders such as diabetes and metabolic syndrome, as a risk factor for human liver cancer ([El-Serag, 2007](#); [El-Serag and Rudolph, 2007](#)). Furthermore, the phenotypic and morphologic heterogeneity of tumors seen in the human liver is qualitatively similar to descriptions of mouse liver tumors induced by TCE exposure, as well as those observed from exposure to a variety of other chemical carcinogens. These parallels suggest similar pathways (e.g., for cell signaling) of carcinogenesis may be active in mice and humans and support the qualitative relevance of mouse models of liver to human liver cancer.

For mouse lung tumors, mode-of-action hypotheses have centered on TCE metabolites produced via oxidative metabolism in situ. As discussed above, the hypothesis that the mutagenicity of reactive intermediates or metabolites (e.g., CH) generated during CYP metabolism contributes to lung tumors cannot be ruled out, although available data are inadequate to conclusively support this mode of action. An alternative mode of action has been posited involving other effects of such oxidative metabolites, particularly CH, including cytotoxicity and regenerative cell proliferation. Experimental support for this alternative hypothesis remains limited, with no data on proposed key events in experiments  $\geq 2$  weeks in duration. While the data are inadequate to support this mode-of-action hypothesis, the data also do not suggest that any proposed key events would be biologically plausible in humans. Furthermore, the focus of the existing mode-of-action hypothesis involving cytotoxicity has been CH, and, as summarized above (see Section 4.11.1.5), other metabolites may contribute to respiratory tract noncancer toxicity or carcinogenicity. In sum, the mode of action for mouse lung tumors induced by TCE is not known.

A mode of action subsequent to in situ oxidative metabolism, whether involving mutagenicity, cytotoxicity, or other key events, may also be relevant to other tissues where TCE would undergo CYP metabolism. For instance, CYP2E1, oxidative metabolites, and protein

adducts have been reported in the testes of rats exposed to TCE, and, in some rat bioassays, TCE exposure increased the incidence of rat testicular tumors. However, inadequate data exist to adequately define a mode-of-action hypothesis for this tumor site.

#### 4.11.3. Characterization of Factors Impacting Susceptibility

As discussed in more detail in Section 4.10, there is some evidence that certain populations may be more susceptible to exposure to TCE. Factors affecting susceptibility examined include lifestage, gender, genetic polymorphisms, race/ethnicity, preexisting health status, and lifestyle factors and nutrition status.

Examination of early lifestages includes exposures such as transplacental transfer ([Ghantous et al., 1986](#); [Withey and Karpinski, 1985](#); [Laham, 1970](#); [Beppu, 1968](#); [Helliwell and Hutton, 1950](#)) and breast milk ingestion ([Fisher et al., 1997](#); [Hamada and Tanaka, 1995](#); [Fisher et al., 1990](#); [Pellizzari et al., 1982](#)), early lifestage-specific toxicokinetics, PBPK models ([Fisher et al., 1990, 1989](#)), and differential outcomes in early lifestages such as developmental cardiac defects. Although there is more information on susceptibility to TCE during early lifestages than on susceptibility during later lifestages or for other populations with potentially increased susceptibility, there remain a number of uncertainties and data gaps regarding children's susceptibility. Improved PBPK modeling for using childhood parameters for early lifestages as recommended by the NRC ([2006](#)), and validation of these models will aid in determining how variations in metabolic enzymes affect TCE metabolism. In particular, the NRC states that it is prudent to assume children need greater protection than adults, unless sufficient data are available to justify otherwise ([NRC, 2006](#)). Because the weight of evidence supports a mutagenic mode of action for TCE carcinogenicity in the kidney (see Section 4.4.7), and there is an absence of chemical-specific data to evaluate differences in carcinogenic susceptibility, early-life susceptibility should be assumed and the ADAFs should be applied, in accordance with the Supplemental Guidance (discussed further in Chapter 5).

Fewer data are available on later lifestages, although there is suggestive evidence to indicate that older adults may experience increased adverse effects than younger adults ([Mahle et al., 2007](#); [Rodriguez et al., 2007](#)). In general, more studies specifically designed to evaluate effects in early and later lifestages are needed in order to more fully characterize potential lifestage-related TCE toxicity.

Examination of gender-specific susceptibility includes toxicokinetics, PBPK models ([Fisher et al., 1998](#)), and differential outcomes. Gender differences observed are likely due to variation in physiology and exposure.

Genetic variation likely has an effect on the toxicokinetics of TCE. In particular, differences in CYP2E1 activity may affect susceptibility of TCE due to effects on production of toxic metabolites ([Yoon et al., 2007](#); [Kim and Ghanayem, 2006](#); [Povey et al., 2001](#); [Lipscomb et al., 1997](#)). GST polymorphisms could also play a role in variability in toxic response

([Wiesenhütter et al., 2007](#); [Brüning et al., 1997a](#)), as well as other genotypes, but these have not been sufficiently tested. Differences in genetic polymorphisms related to the metabolism of TCE have also been observed among various race/ethnic groups ([Sato et al., 1991b](#); [Inoue et al., 1989](#)).

Preexisting diminished health status may alter the response to TCE exposure. Individuals with increased body mass may have an altered toxicokinetic response ([Clewell et al., 2000](#); [Lash et al., 2000a](#); [McCarver et al., 1998](#); [Sato, 1993](#); [Davidson and Beliles, 1991](#); [Sato et al., 1991b](#); [Monster et al., 1979a](#)), resulting in changes the internal concentrations of TCE or in the production of toxic metabolites. Other conditions, including diabetes and hypertension, are risk factors for some of the same health effects that have been associated with TCE exposure, such as RCC. However, the interaction between TCE and known risk factors for human diseases is not known, and further evaluation of the effects due to these factors is needed.

Lifestyle and nutrition factors examined include alcohol consumption, tobacco smoking, nutritional status, physical activity, and SES status. In particular, alcohol intake has been associated with metabolic inhibition (altered CYP2E1 expression) of TCE in both humans and experimental animals ([McCarver et al., 1998](#); [Kaneko et al., 1994](#); [Sato, 1993](#); [Nakajima et al., 1992a](#); [Okino et al., 1991](#); [Sato et al., 1991a](#); [Nakajima et al., 1990](#); [Larson and Bull, 1989](#); [Nakajima et al., 1988](#); [Sato and Nakajima, 1985](#); [Barret et al., 1984](#); [Sato et al., 1983, 1981](#); [White and Carlson, 1981a](#); [Sato et al., 1980](#); [Muller et al., 1975](#); [Stewart et al., 1974a](#); [Bardodej and Vyskocil, 1956](#)). In addition, such factors have been associated with increased baseline risks for health effects associated with TCE, such as kidney cancer (e.g., smoking) and liver cancer (e.g., alcohol consumption). However, the interaction between TCE and known risk factors for human diseases is not known, and further evaluation of the effects due to these factors is needed.

In sum, there is some evidence that certain populations may be more susceptible to exposure to TCE. Factors affecting susceptibility examined include lifestage, gender, genetic polymorphisms, race/ethnicity, preexisting health status, and lifestyle factors and nutrition status. However, except in the case of toxicokinetic variability characterized using the PBPK model described in Section 3.5, there are inadequate chemical-specific data to quantify the degree of differential susceptibility due to such factors.