PART 7B: EFFECTS OTHER THAN CANCER

7.10. INTRODUCTION

Human exposure to 2,3,7,8-TCDD has been associated with noncancer effects in most systems. The majority of effects have been reported among occupationally exposed groups, such as chemical production workers, pesticide users, and individuals who handled or were exposed to materials treated with 2,3,7,8-TCDD-contaminated pesticides, and among residents of communities contaminated with tainted waste oil (Missouri, USA) and industrial effluent (Seveso, Italy).

These effects represent a complex network of responses ranging from changes in hepatic enzyme levels which, based on current evidence, do not appear to be related to clinical disease, to observable alterations in the character and physiology of the sebaceous gland, as in chloracne (Calvert et al., 1992; Taylor, 1979). This section of Chapter 7 describes, by system , the noncancer effects associated with exposure to 2,3,7,8-TCDD. The characterization of the effects by system provides a context within which to compare the results of the various studies. However, it is important to recognize that the observed effects are not independent events but rather may be one outcome in a series of interrelated outcomes, some of which we may be incapable of measuring with the present technology or which we currently do not recognize as an outcome of exposure to 2,3,7,8-TCDD.

The information describing human effects attributed to exposure to 2,3,7,8-TCDD-contaminated materials is derived from a wide variety of sources, including clinical assessments (case reports) of exposed individuals and analytic epidemiologic studies using case-control, cross-sectional, and cohort designs. The case reports describe the acute outcomes of exposure to 2,3,7,8-TCDD and provide the basis for hypothesis generation for controlled epidemiologic studies; however, they are not suitable for testing causal relationships between exposure and related effects (Ashe and Suskind, 1950; Suskind et al., 1953; Bauer et al., 1961; Goldman, 1972).

As described in the previous section, cohort and case-control studies have been used to investigate hypothesized increases in malignancies among the various 2,3,7,8-TCDD-exposed populations (Fingerhut et al., 1991a, b; Steenland et al., 1999; Manz et al., 1991; Eriksson et al., 1990). Cross-sectional studies have been conducted to evaluate the prevalence or extent of disease in living 2,3,7,8-TCDD-exposed groups (Suskind and Hertzberg, 1984; Moses et al., 1984; Lathrop et al., 1984, 1987; Roegner et al., 1991; Grubbs et al. 1995; Sweeney et al., 1989; Centers for Disease Control Vietnam Experience Study, 1988a; Webb et al., 1989; Ott and Zober, 1994). Many of the earliest studies were unable to define exposure-outcome relationships owing to a variety of shortcomings, including small sample size, poor participation, short latency periods, selection of inappropriate controls, and the inability to quantify exposure to

2,3,7,8-TCDD or to identify confounding exposures. In more recent cross-sectional studies of U.S. chemical workers (Sweeney et al., 1989), U.S. Air Force Ranch Hand personnel (Roegner et al., 1991; Grubbs et al., 1995), and Missouri residents (Webb et al., 1989), serum or adipose tissue levels of 2,3,7,8-TCDD were measured to evaluate 2,3,7,8-TCDD-associated effects in exposed populations. The ability to measure tissue or serum levels of 2,3,7,8-TCDD for all or a large sample of the subjects confirmed exposure to 2,3,7,8-TCDD and permitted the investigators to test hypothesized dose-response relationships.

7.11. CROSS-SECTIONAL STUDIES: USES AND LIMITATIONS

Most of the studies that describe nonmalignant effects were designed as cross-sectional medical studies. These types of studies are useful for assessing the current status of the surviving study population; however, they are inherently limited by a number of factors, including survivor and participation biases, exposure and disease misclassification, recall bias, and interobserver variability. Survivor and participation biases may have occurred because the studies included only those who were living at the time of the study, and did not or could not obtain similar information on those who died or were too ill to participate. Studies of groups exposed to agents that contribute to early deaths or cause severe illnesses may exclude the populations who were at highest risk. Exclusion of the sick and deceased whose condition was associated with 2,3,7,8-TCDD may erroneously cause the risk estimate to be closer to the null than the true risk.

Disease misclassification may be introduced in a variety of ways. Medical tests in many reviewed studies were most often performed once, without follow-up. For some disorders, multiple testing is preferred to obviate normal variations in some test parameters, e.g., immunologic tests or hormone levels. In other situations, self-reported medical histories were collected and, by design, were not or could not be confirmed by medical records. When the exposed group incorrectly reports more disease than the unexposed group, recall and reporting biases may falsely raise the risk estimate.

Exposure misclassification, particularly in the early studies, was a major limitation. In the earlier studies of production workers or community residents, exposure to 2,3,7,8-TCDD was determined only by an individual's presence (residing or working) in an area that was contaminated with 2,3,7,8-TCDD (Suskind and Hertzberg, 1984; Moses et al., 1984; Hoffman et al., 1986; Poland et al., 1971; May, 1973, 1982; Martin, 1984; Bond et al., 1983, 1989; Filippini et al., 1981; Ideo et al., 1985; Mocarelli et al., 1986). The lack of a measurement to quantify exposure hindered the ability to confirm exposure and to assess the magnitude of an exposure-response relationship. If the misclassification is nondifferential, it tends to bias the measure of effect toward the null.

As described above, in studies conducted a decade later (Roegner et al., 1991; Grubbs et al., 1995; Sweeney et al., 1989; Centers for Disease Control Veterans Health Studies, 1988; Webb et al., 1989) researchers were able to confirm and quantify exposure to 2,3,7,8-TCDD in serum or adipose tissue. This breakthrough helped establish that certain previously exposed populations had 2,3,7,8-TCDD levels well above the background level of less than 20 picograms per gram of lipid (pg/g) (Patterson et al., 1989) and that the nonexposed comparison group was truly not exposed to greater than background levels. Yet, because the occupational populations were exposed to 2,3,7,8-TCDD-contaminated substances for as many as 40 years before being tested, levels attained at the time of exposure can only be estimated. It appears that for some populations with higher exposures, such as workers, the estimate reflects continuous exposure over an extended period. For example, despite the intervening period between last exposure to 2,3,7,8-TCDD and the determination of serum 2,3,7,8-TCDD levels in workers employed in the production of 2,4,5-TCP and 2,4,5-T (15 to 37 years after last occupational exposure), the duration of occupational exposure was highly correlated to serum levels of 2,3,7,8-TCDD obtained at the time of the study (1987-1988) (Pearson product moment correlation coefficient [r] = 0.7) (Sweeney et al., 1989). These data suggest a strong relationship between length of occupational exposure and serum 2,3,7,8-TCDD regardless of the length of the intervening period.

The deposition, metabolism, and excretion of high doses of 2,3,7,8-TCDD in the human system have not been fully described. A study by Pirkle et al. (1989) suggests that 2,3,7,8-TCDD decays by one-half in approximately 7.1 years, based on a one-compartment model and using a standard half-life equation. If this is true, exposures to trichlorophenol production workers may have been as high as 30,000 pg/g (Fingerhut et al., 1991a) and in excess of 50,000 pg/g in some residents of Seveso (Mocarelli et al., 1991). The data may be limited by the lack of complete information on the manner in which human metabolism handles 2,3,7,8-TCDD exposure. In a separate analysis, Michalek et al. (1996) estimated the half-life among veteran Ranch Hands to be 8.7 years (95% CI = 8.0-9.5 years). This calcuation was based on a mean decay rate of 0.0797 per year. In this analysis half-life increased with increasing body fat, but not age.

This section of Chapter 7 is a selective review of studies that, to date, provide the most information on the relationship between nonmalignant outcomes and exposure to 2,3,7,8-TCDD-contaminated materials. Animal studies have been reviewed in other chapters of this document and will not be discussed in detail. Case reports will not be reviewed, but will be used to provide support for the analytic studies. In the assessment of mortality from nonmalignant causes of death, only cohort studies in which standardized mortality ratios (SMR) or equivalent population-based risk ratios were calculated will be discussed.

7.12. DESCRIPTION OF PRINCIPAL STUDIES

In the following section, we have provided a summary of the population description and methods of the studies that reported results for two or more systems or effects. Studies that are referenced only once will be described when cited. Results of these studies will be described in subsequent sections.

7.12.1. Occupational Studies

7.12.1.1. *U.S. Chemical Workers: West Virginia*

In March 1949, an explosion of a TCP reaction kettle in a chemical plant in Nitro, West Virginia, and the subsequent cleanup exposed approximately 450 workers to 2,3,7,8-TCDD-contaminated substances. Examination of the workers in 1949 revealed a number of acute symptoms "characterized by skin, eye and respiratory tract irritation, headache, dizziness and nausea" (Suskind and Hertzberg, 1984). The acute symptoms subsided but were followed within a week or two by "acneform eruption, severe muscle pain affecting the extremities, thorax and shoulders, fatigue, nervousness and irritability, dyspnea, complaint of decreased libido and intolerance to cold" (Suskind and Hertzberg, 1984). Thirty years later, two independent, cross-sectional medical studies were conducted to evaluate the long-term consequences of exposure to 2,3,7,8-TCDD-contaminated substances among the surviving workers (Suskind and Hertzberg, 1984; Moses et al., 1984).

In a study by Suskind and Hertzberg (1984), a group of 204 (of a total of 419) active and retired white male workers exposed between 1948 and 1969 to the 2,4,5-T production process and to the reactor release were included in a clinical examination program. The control group consisted of 163 (46% participation) current or former employees of the same plant who had no self-reported exposure to 2,4,5-T production or maintenance of the facility. The study collected demographic and medical histories and performed clinical chemistries, urinalysis, pulmonary function tests, dermatologic examinations, and conduction velocities of the sural sensory and peroneal motor. Multiple linear regression analysis was used to compare the exposed and nonexposed groups.

For participation in a separate study of workers at the Nitro plant, Moses et al. (1984) invited all workers documented in union records to have worked in 2,4,5-T production and a systematic random sample of workers with no known 2,4,5-T production exposure. Fifty-five percent (N = 226) of the persons invited participated in the study. Lifetime occupational and medical histories were collected and clinical chemistries, urinalysis, and dermatologic examinations were conducted. Exposure to 2,3,7,8-TCDD could not be discerned because of irreconcilable inconsistencies in self-reported work histories and the lack of good company

records to estimate and confirm the likelihood of exposure. Although the authors recognized that absence of chloracne did not preclude exposure, they compared the results of the group with chloracne with those without chloracne. Thus, the study design was revised to explore the differences in health status in individuals with and without chloracne. Because exposed workers may have been included in the group diagnosed without chloracne, the usefulness of the data to quantify exposure-disease relationships is limited.

Two studies of this cohort also examined cancer and noncancer mortality of subsets of workers from this plant (Zack and Suskind, 1980; Collins et al., 1993).

7.12.1.2. *U.S. Chemical Workers: The NIOSH Study*

The study conducted by the National Institute for Occupational Safety and Health is a cross-sectional medical study of living workers who were previously employed for at least 1 day in one of two plants located in Newark, New Jersey, and Verona, Missouri. From 1951 to 1969, 490 workers employed at the New Jersey plant produced sodium 2,4,5-trichlorophenate (NaTCP), 2,4,5-trichlorophenoxy acetic acid (2,4,5-T), and 2,4-dichlorophenoxy acetic acid (2,4-D). A high proportion of chloracne and other dermatologic abnormalities and cases of porphyria and hypomania were reported among the workers at the New Jersey facility (Poland et al., 1971; Bleiberg et al., 1964), which produced some of the most heavily 2,3,7,8-TCDD-contaminated NaTCP and 2,4,5-T among production facilities whose products were surveyed (Fee et al., 1975). At the Missouri plant, NaTCP and 2,4,5-T were produced intermittently for 4 months in 1968, and NaTCP and hexachlorophene were produced continuously for 22 months between April 1970 and January 1972.

For comparison, unexposed neighborhood referents were recruited using a random sampling procedure described by Sweeney et al. (1989). Referents were selected if they reported no prior history of occupational exposure to 2,3,7,8-TCDD and matched the worker by age (within 5 years), race, and gender. A total of 586 workers were eligible for inclusion in the study, of which 400 (68.3%) were living, 142 (24.2%) were deceased, and 44 (7.5%) could not be located. All 400 living workers were invited to participate in the study; 281 (70%) were examined. A description of the study population is included in the results.

Worker and referent health and exposure status were assessed in 1987-1988 through an interviewer-administered medical and occupational history and comprehensive physical and psychological examinations (Sweeney et al., 1989). A lifetime medical history was obtained from each participant by interviewers who were blind to the exposure status of the respondent. Results of the pulmonary, hepatic, gastrointestinal, porphyria, mood dysfunction, and neurologic

examinations have been published or accepted for publication (Calvert et al., 1991, 1992, 1993, 1994, 1996, 1998, 1999; Sweeney et al., 1993; Egeland et al., 1994; Halperin et al., 1995, 1998).

As a surrogate for cumulative exposure, serum 2,3,7,8-TCDD levels were measured in 237 workers and a random sample of 79 referents. Procedures for sample collection, preparation, adjustment for lipids, and statistical analysis were described in earlier reports (Fingerhut et al., 1989; Patterson et al., 1986a; Sweeney et al., 1990). The mean lipid-adjusted serum 2,3,7,8-TCDD level for workers was 220 pg/g, median 80 pg/g, ranging to 3,400 pg/g. The mean level was statistically significantly greater than that for referents (7 pg/g) (p<0.001). Analyses of other congeners of dioxins and dibenzofurans were also conducted; only the 2,3,7,8-TCDD levels were different in the two exposure groups (Piacitelli et al., 1992).

7.12.1.3. *BASF Accident Cohort*

"On 17 November 1953, an uncontrolled decomposition reaction occurred during the production of 2,4,5-trichlorophenol at a BASF AG facility in Ludwigshafen, Germany" (Ott et al., 1994). The reactor contents, which contained 2,3,7,8-TCDD, contaminated the building in which the TCP autoclave was housed. A series of studies have documented the effects and mortality experience of the workers present at the time of the decomposition reaction and exposed during the initial cleanup and equipment maintenance (May 1954 medical department list) (Cohort C1, N = 69), individuals present during subsequent clean-demolition activities between 1954 and 1969 (Cohort C2, N = 84), and a mixed group of workers identified as of December 1987 through interviews that include individuals who worked in the laboratory as safety inspectors and others who participated in the 1968-1969 demolition activities (Cohort C3, N = 101) (Zober et al., 1990; Ott et al., 1993). Two hundred forty-seven study subjects were included in a mortality study that found a significantly elevated SMR for all malignant neoplasms among workers with chloracne and 20 or more years since first exposure to 2,3,7,8-TCDD-contaminated chemicals (Zober et al., 1990).

Among 79% of the living subjects, lipid-adjusted serum 2,3,7,8-TCDD levels were measured in 138 (54%) of 254 study subjects during the period 1988-1992 (Ott et al., 1993). The geometric mean of the 2,3,7,8-TCDD levels in the entire group was 15.4 ppt (ranging from <1 to 553.0 ppt) (Ott et al., 1993) or 43 pg/g of lipid (M. G. Ott, personal communication, 1993). Geometric means for the cohorts are as follows: C1 = 1,009.5 pg/g lipid; C2 = 48.8 pg/g of lipid; and C3 = 83.7 pg/g of lipid. Background levels were determined in separate analyses of 102 unexposed individuals from Germany (Päpke et al., 1992). The geometric mean for 2,3,7,8-TCDD of the external referent group was 3.0, ranging from 0.6 to 9.1 pg/g of lipid. On the basis of regression analyses, serum 2,3,7,8-TCDD levels were highly correlated (R2=0.65) to duration

of exposure and location of exposure. Chloracne severity was positively and significantly related to 2,3,7,8-TCDD concentrations.

Comprehensive batteries of clinical chemistry measurements were also measured for the 138 subjects between 1988 and 1993 (Ott et al., 1994). Referents were selected from among BASF employees between the ages of 50 and 69 who participated in routine occupational medical examinations from 1989 to 1991. For some tests, there were as many as 6,000 referent values. For the immunologic parameters, the referent values were obtained from a group of 42 unexposed BASF employees who participated in a separate study that examined the immunologic function of 21 extruder personnel exposed to 2,3,7,8-tetrabrominated dibenzo-p-dioxin (2,3,7,8-TBDD) and -furan (2,3,7,8-TBDF) (Ott and Zober, 1996b). Cause-specific mortality and cancer incidence was also evaluated in the 243 male study subjects who were followed through 1992 (Ott and Zober, 1996a).

7.12.2. Studies of Community Residents

7.12.2.1. *The Missouri Experience*

During 1971, 2,3,7,8-TCDD-contaminated stillbottoms were removed from a hexachlorophene production facility and mixed with waste oil. This mixture was deposited on 45 residential, recreational, and industrial sites in southeastern Missouri in 1971 and 1972 (Daryl Roberts, personal communication). Waste oil mists were commonly used in the summer for dust control on roadways, horse arenas, truck depots, and other unpaved surfaces. Estimated contamination of the areas ranges from 1 to 2,200 ppb (Hoffman and Stehr-Green, 1989). A listing of potentially exposed persons was created (volunteers), and a survey was conducted to obtain baseline information to identify persons at high risk of exposure. Beginning in 1984, a series of studies were conducted to evaluate potential effects (Hoffman et al., 1986; Evans et al., 1988; Webb et al., 1989), including reproductive events, of residential exposure to 2,3,7,8-TCDD (Stockbauer et al., 1988).

The study by Hoffman et al. (1986) was conducted on 154 individuals (74% of total eligible) who were residents of the Quail Run Mobile Home Park between 1971 and 1983, because soil concentrations around the site were 2,200 ppb 2,3,7,8-TCDD. The comparison group of 155 (77% of total eligible) individuals was recruited from residents of a nearby mobile home park. The examination included tests for delayed hypersensitivity (the multitest Cornell Medical Index [CMI]; Merieux Institute, Miami, Florida) and neurobehavioral effects, blood chemistries, urinalysis, height, weight, vital signs, and examination of the skin, peripheral pulses, lymph nodes, abdomen, and peripheral nervous system. The results of this study were plagued by the exclusion of skin test results of 150 of 294 participants because of high reader error. Furthermore, information on subject exposure to 2,3,7,8-TCDD was limited because the study was based on a

minimum residence of 6 months in areas with contaminated soil. Actual contact with contaminated soil was not assessed.

In the follow-up study by Evans et al. (1988), 50 persons from the initial study who did not respond to the delayed hypersensitivity skin tests were retested. These subjects were thought to have impaired immune function. The multitest CMI was reapplied to all test subjects.

Webb and colleagues (1989) examined 41 of 51 persons with various histories of exposure to 2,3,7,8-TCDD (residential, recreational, and occupational exposure) and for whom adipose tissue levels of 2,3,7,8-TCDD were measured. Of the 41 participants, 16 had adipose 2,3,7,8-TCDD levels less than 20 pg/g (within background range), 13 had levels between $\geq$20 and 60 pg/g, and 12 subjects had levels above 60 pg/g. Standard medical examinations were conducted, and complete blood count with differential, a panel of automated chemistry tests, serum immunoglobulins, tests for porphyrins, and the multitest CMI were performed.

7.12.2.2. *Seveso, Italy*

In 1976, an explosion of a trichlorophenol reactor in a 2,4,5-T production facility in Medina, Italy, caused the contamination by 2,3,7,8-TCDD of the neighboring city of Seveso, Italy. Several reports (Biscanti et al., 1978; Homberger et al., 1979; Pocchiari, 1980a; Pocchiari et al., 1980b; Reggiani, 1978, 1980; Rehder et al., 1978; Tuchmann-Duplessis, 1977) compared four potentially affected communities (Seveso, Meda, Cesano, and Desio) to nearby unexposed communities. The contaminated area was subdivided into three zones (A, B, and R) of decreasing mean soil levels of 2,3,7,8-TCDD (Mocarelli et al., 1988). The mean 2,3,7,8-TCDD concentration in zone A was 230 $\mu g/m^2$; in Zone B, 3.0 $\mu g/m^2$; and in zone R, 0.9 $\mu g/m^2$.

Mean serum 2,3,7,8-TCDD concentrations among a sample of residents who were 13 years and older at the time of the explosion were: zone A, 443 pg/g lipid (N = 177); zone B, 87 pg/g lipid (N = 54); zone R, 15 pg/g (N = 17) (IARC, 1997). Geometric mean serum 2,3,7,8-TCDD concentrations measured were: zone A, 53.2 pg/g lipid (N = 7); zone B, 11 pg/g lipid (N = 51); zone R, 4.9 pg/g (N = 55) (Landi et al., 1997).

In 1979, Pocchiari et al. (1979) reported on initial efforts to screen residents in zones A, B, and R for 2,3,7,8-TCDD-related effects. Since then, a series of cross-sectional medical studies have reported the final results of the screening (Caramaschi et al., 1981; Ideo et al., 1985; Mocarelli et al., 1986; Assennato et al., 1989). Within 1 year of the reactor release, 193 cases of chloracne were identified among residents of zones A, B, and R, most of which resolved with time (Assennato et al., 1989). For Seveso residents, a standard diagnosis of chloracne was developed, in which all cases were stratified by severity: 0, no lesions; 1, a few comedones (up to

10, minimum stage); 2, numerous comedones and cysts (light stage); 3, comedones and cysts in specific regions (medium stage); and 4, comedones and cysts spreading from the face to other regions of the body (serious stage) (Caramaschi et al., 1981). Four studies investigated possible biochemical changes, particularly liver enzyme induction and lipid levels, among the 170 children diagnosed with chloracne and control groups (Caramaschi et al., 1981; Ideo et al., 1985; Mocarelli et al., 1986; Assennato et al., 1989).

In addition to chloracne, several other studies also evaluated peripheral neuropathy. In an early report by Pocchiari et al. (1979), tests for presence of peripheral nerve dysfunction were conducted for Seveso residents and for workers at the Icmesa production facility. Assennato et al. (1989) and Filippini et al. (1981) assessed the prevalence of peripheral neuropathy, comparing residents with and without chloracne (Assennato et al., 1989) or comparing residents having chloracne or abnormal serum hepatic enzyme levels with residents with no manifestations of 2,3,7,8-TCDD exposure (Filippini et al., 1981).

Two mortality studies, one of children ages 1-19 years and another of adults 20 years and older, examined death rates in residents of zones A, B, and R 10 years after the explosion (1976-1986) (Bertazzi et al., 1989, 1992). The comparison population was composed of approximately 100,000 inhabitants of uncontaminated areas surrounding Seveso. Follow-up for both the young and older cohorts was 99%. A third study examined the mortality of the three cohorts from July 1976 through June 1991 (Pesatori et al., 1998).

To date, although there are a few individual measurements of exposure among residents of the exposure zones, the general limitation of the studies conducted in Seveso residents is the classification of exposure of subjects by residence in zones A, B, or R, which is based on mean soil concentration of 2,3,7,8-TCDD. The weaknesses of using soil 2,3,7,8-TCDD levels to classify extent of exposure to 2,3,7,8-TCDD were aptly described by Bertazzi et al. (1989): "This (use of soil contamination) is a rather poor surrogate of exposure, and by no means an indicator of intake, since it does not take into consideration all the possible sources and ignores interindividual variability." This is the same problem encountered by some researchers investigating 2,3,7,8-TCDD-related effects among Missouri residents. One might also expand this limitation to each of the studies where environmental rather than personal levels of 2,3,7,8-TCDD contamination were used for exposure classification.

7.12.2.3. *Developmental Studies From The Netherlands*

In the early 1990s, scientists from several Dutch communities collected data on postnatal developmental outcomes and related them to total PCB-dioxin-furan TEQ from breast milk, and PCBs in cord blood and maternal blood. The results from these studies will be addressed by

system in Section 7.13. These studies are similar in design; a number come from the same study group including women and infants from Rotterdam, and from both Rotterdam and Grongenin. The outcomes studied in both communities included a neurological examination (Prechtl/Touwen) at 10 days and 18 months of age and Obstetrical Optimality score at 10 days of age. In Rotterdam only, mental and psychomotor development (Bayley Scales of Infant Development) was measured at 3, 7, and 18 months of age, and visual recognition memory (Fagan Infant test) at 3 and 7 months of age. At 42 months, these children were assessed for cognitive abilities (Kaufman Assessment Battery) and a subgroup was assessed for verbal comprehension (Reynell Language Developmental Scales). Another group in Amsterdam has also examined postnatal developmental outcomes and breast milk level; this study will be discussed at the end of this section.

The reports that covered Rotterdam, or Rotterdam and Groningen together, include the same infants. Data were collected beginning in June 1990 and continued until February 1992 or June 1992, depending on the outcome examined. Women were introduced to the project by their obstetricians or midwives, and details were explained to the women at home. First contact took place in the third trimester (32-34 weeks), and women were screened for their intentions to breast feed for at least 6 weeks ("exposed") or for their intention not to breast feed at all (comparisons). Only women with full-term deliveries to first- or second-born infants, among other characteristics, were selected for inclusion in the study. Originally 489 women who were willing to participate were identified; 71 of these were lost to the final study when they were not able to breast feed for the required 6 weeks.

Formula was supplied to those women in the study who did not intend to breast feed. In each community, the goal was to get approximately 100 in each group. The investigators did not describe the response rate, or the comparability of respondents to nonrespondents. All the reports included blood samples from the mother during the last month of pregnancy, cord blood, and breast milk collected in the second week after delivery. PCB levels (congeners 118, 138, 153, 180) were measured in the blood samples and used to estimate the prenatal exposures to the children, and PCB and dioxin-furan levels were measured in the breast milk (seventeen 2,3,7,8-substituted PCDDs and PCDFs, 3 coplanar PCBs, and 23 nonplanar PCB congeners) (Table 7-21b). These data were then used to rank exposure in the women and infants into categories to examine exposure outcome. The total breast milk values were calculated using the levels measured and multiplying this by the number of weeks of breast feeding. This approach assumes only small changes in the levels of these exposures over the duration of breast feeding when actual numbers are used, or that the relative magnitude of exposure remains consistent. As long as all the women are breast feeding, this is a useful approach to determine relative magnitude. Breast milk samples collected on the second week and about the sixth week after delivery were

compared for the following exposure groupings: dioxins-furans: IUPAC 48, 54, 66, 67, 70, 73, 75, 83, 94, 114, 118, 121, 124, 130, 131, 134, 135), coplanar PCBs (IUPAC 77, 126, 169), mono-ortho PCBs (IUPAC 105, 118, 156), di-ortho PCBs (IUPAC 170, 180), and total PCB-dioxin-furans, which includes all of the above. This terminology will be used in the descriptions of individual reports. With continued breast feeding, a drop in the levels of these would be expected as the body burden decreases. Decreases in levels were observed for a number of these; not all were statistically significant, in part because of the small number of women evaluated: decrease in dioxin-furans (no. women = 27, $p = 0.07$), coplanar PCBs (no. women = 44, $p = 0.91$), mono-ortho PCBs (no. women = 180, $p = 0.002$), di-ortho PCBs (no. women = 180, $p = 0.001$), and total PCB-dioxin-furans (no. women = 19, $p = 0.10$) (Koopman-Esseboom et al., 1994b). It seems likely that the measured levels in breast milk would continue to drop with an extended period of breast feeding. Thus, the studies' assumption of a steady-state level of dioxins, furans and PCBs in the breast milk, with the women's different lengths of breast feeding, could overestimate the actual exposure levels to varying degrees. Therefore, any effects observed might occur at a lower level of exposure than reported. Effects of exposure in these studies were assessed by analyses of the dioxin-furan TEQ or total PCB-dioxin-furan TEQ (for dioxins, furans and dioxin-like PCBs) based on levels observed in breastmilk, and $\sum PCB_{\text{cord blood}}$ or $\sum PCB_{\text{maternal blood}}$ (for IUPAC 118, 138, 153, 180), controlling for such items as sociodemographic indicators and personal habits. Because data from other sources have shown breast milk levels to be well correlated with adipose tissues, the breast milk level during the second week after delivery is a reasonable estimate for prenatal exposures (at least the relative magnitude) to these agents (Jensen, 1987).

About half the 418 mothers and infants were in Rotterdam and half in Gronginen. A comparison of a variety of demographic factors, personal habits, and health characteristics for the two communities showed no differences for a number of them (e.g. maternal age, weight, smoking status), and significantly higher parental education, maternal alcohol consumption, and birth weight of the child in Groningen; the length of gestation was one-half week longer in Groningen. A number of factors, socioeconomic status (SES) indicators, health, and exposure were different in the breast-fed versus formula-fed group (Huisman, 1995b): for example, more than 60% of both parents in the breast-fed group had higher education versus 31% or less in the formula-fed group. In addition, breast-feeding mothers were more likely to have consumed alcohol during pregnancy (37% versus 18%) and less likely to have smoked (16% versus 35%). The breast-fed pregnancies were also more highly exposed prior to breast–feeding: $\sum PCB_{\text{cord blood}}$ and $\sum PCB_{\text{maternal blood}}$ were significantly higher in this group. Analysis of breast milk levels in the Rotterdam area and in Groningen (Koopman-Esseboom et al, 1994a) showed that the dioxin-furan TEQ and some individual dioxin and congener levels (D66, D67, F83, F118, F121, F130, PCB66,

PCB118, PCB137, and PCB187) were significantly higher in the more urbanized Rotterdam area. For some of the analyses, the infants were placed into a "low" or "high" group based on the median value for the dioxin-furan TEQ (at 30.8 pg TEQ/g fat, for the total PCB-dioxin-furan TEQ (at 72.4 pg TEQ/g fat) or into "low," "medium," or "high" based on the PCB-dioxin-furan TEQ times the number of weeks breast fed. In addition, in one report different congeners were analyzed separately. These different analysis strategies make integrating the data from the various reports difficult.

Two individuals performed some of the tests (e.g., Neurologic Optimality Score in Huisman et al., 1995a and Huisman et al., 1995b; Fluency Cluster Score in Huisman et al., 1995b), one each in Rotterdam versus Grongenin. The authors reported that their comparison of the data from the two cities suggested a systematic difference between the two for some outcomes. They controlled for this potential discrepancy in the logistic regressions. As there was no direct comparison of the scoring on the same children, the above is a possible explanation, as is the possibility that there are community differences (for some unknown reason) for these specific outcomes.

A smaller series of women and their infants were studied by another research team in Amsterdam; between June 1990 and May 1991 these women were identified while still pregnant. Only women who intended to breast feed for 12 weeks were included in this study group. The reports from this group (Pluim et al., 1993; Pluim et al., 1994; Pluim et al., 1996) had a different final number of subjects (38 versus 35), but from the general description, they do appear to be from the same general group. Maternal blood was collected around the time of delivery, cord blood was collected, infant blood samples were collected at 1 and 11 weeks of age, and three weeks after delivery, two breast milk samples were collected. The authors assumed that the breast milk levels also reflected *in utero* exposures to the infants. They did not include bottle-fed children as comparisons for the following reasons: first, the SES of women who choose to breast feed tends to be different from those who choose to bottle-feed; and second, comparison of breast milk levels is not possible for the mothers of bottle-fed infants, either directly or using these levels as a surrogate for the *in utero* exposures. The 17 most toxic congeners, 7 dioxins and 10 dibenzofurans, were used to develop a dioxin-furan TEQ (see Table 7-21b for the listing of these congeners and the mean levels for the study group). The final score for consumption for each child used the amount of milk consumed (assumed to be 700 g/day while the child received only breast milk, and half that amount later), the amount of milk fat in the milk (assumed to be an average of 2.5%), and the levels of the 17 congeners. The levels determined were split at the median into "low" and "high" groups (28.0 pg dioxin-furan TEQ g/fat; high = 29.2-62.7 pg TEQ/g and low was less than 28.0 pg TEQ/g). These reports compared differences in the high

and low groups (1) with thyroid hormone concentrations (Pluim et al., 1993) (see Section 7.13.4.2); (2) with alanine aminotransferase and aspartate aminotransferase activities, and platelet count in the infants' plasma (Pluim et al., 1994) (see Section 7.13.2.5.2); and (3) against several growth measures (neonatal weight and length, liver size and quetelet index) over the first six months of life (Pluim et al., 1997) (see Section 7.13.12.8).

The Amsterdam reports are based on small groups of infants with undescribed selection procedures. Thus it is not possible to evaluate volunteer bias. These authors did attempt to more closely estimate the dioxin-furan TEQ, using changes in feeding patterns as well as the measured levels in the early sample, but did not evaluate the potential for decreasing levels with increasing length of breast feeding.

Both sets of studies split the group of breast-fed infants at the median of the TEQ into high and low exposure groups, and used these for logistic regression and other analyses. This approach for quantification of exposure is a reasonable estimation of the general magnitude of exposures. Mean levels of exposure over the total period may be somewhat lower than reported, since the breast milk evaluations occurred early in lactation (1-2 weeks after delivery), while levels were likely to decrease with longer periods of breast feeding (see Part I., Vol. II Chapter 6, of "Estimating Exposure to Dioxin-Like Compounds"). For both of these Dutch study groups, it is important to note that the levels of dioxins, furans, and PCBs were within common/background environmental ranges.

7.12.3. Studies of Vietnam Veterans

7.12.3.1. *The Vietnam Experience Study*

The Vietnam Experience Study is described by its authors as a "multidimensional assessment of the health of Vietnam veterans" (Centers for Disease Control Vietnam Experience Study, 1988a-d). This study was designed to examine effects among men who served in Vietnam.

The study population was composed of a random sample of men who enlisted in the U.S. Army from 1965 through 1971, whose military occupational status was other than "duty soldier," who enlisted for a single term with a minimum of 16 weeks active duty and who were discharged at pay grades of E-1 to E-5. The controls were selected from among veterans enlisting during the same period but whose duty station was the United States, Germany, or Korea. Participation involved completion of a telephone survey of current and past health status by 7,924 veterans who served in Vietnam and 7,364 veterans who served outside of Vietnam. A random subsample of 2,940 Vietnam and 1,972 non-Vietnam veterans participated in the health evaluation component.

In a separate study, serum 2,3,7,8-TCDD levels were measured in a subset of the examined population: 646 Vietnam veterans who served in Vietnam during 1967 and 1968 and

97 non-Vietnam veterans (Centers for Disease Control Veterans Health Studies, 1988). The mean serum 2,3,7,8-TCDD level was not different between Vietnam (mean = 4.1 pg/g lipid [standard deviation (SD) ± 2.3]) and non-Vietnam (4.2 pg/g, SD ± 2.6) veterans. Two Vietnam veterans had levels above the background level of 20 pg/g: 25 pg/g and 45 pg/g.

The overall strengths of this study are that it is a large study, with good power to detect many common disorders; participation in the questionnaire part of the study was good (87% for Vietnam veterans; 84% for non-Vietnam veterans); there was good comparability between the two cohorts in demographic characteristics, although there were differential participation rates in the examination; and validation of selected self-reported effects was conducted. This study is limited primarily by the differential participation rates in the examination (75% for Vietnam veterans; 63% for non-Vietnam veterans). The low level of 2,3,7,8-TCDD in the sample of veterans made it impossible to conduct dose-response analyses.

7.12.3.2. *U.S. Air Force Ranch Hand Study*

One of the largest epidemiologic studies of U.S. military personnel stationed in Vietnam is being conducted by the U.S. Air Force. The study population consists of Air Force personnel who served in Operation Ranch Hand units in Vietnam from 1962 to 1971 and who were employed in the dissemination of Agent Orange through aerial spraying. Comparisons included Air Force personnel who flew or maintained C-130 aircraft in Southeast Asia during the same time period.

The study design includes a series of cross-sectional medical studies conducted at 5-year intervals beginning with the baseline study in 1982 (N = 1,045 exposed, 1,224 unexposed). Two follow-up evaluations were conducted in 1985 (N = 1,016 exposed, 1,293 unexposed) and 1987 (N = 995 exposed, 1,299 unexposed). Each cross-sectional study included comprehensive physical and psychological evaluations. In the 1982 baseline and 1985 and 1987 follow-up studies, exposure was based on the comparison of the Ranch Hand group versus the comparison group. An additional analysis approximated exposure (low, medium, high) for the Ranch Hand group by using historical military data and herbicide procurement and usage records. The results of these analyses were prepared by Lathrop and colleagues (1984 and 1987). In 1988, serum 2,3,7,8-TCDD levels were measured for a sample of the 1987 Ranch Hand group (N = 866) and the 1987 comparison group (N = 804). The 1987 examination data were then reanalyzed using lipid-adjusted serum 2,3,7,8-TCDD levels as the relative measure of exposure. The median serum 2,3,7,8-TCDD level adjusted for lipids for the Ranch Hand group was 12.8 pg/g, ranging to 618 pg/g. For the comparison group, the median level was 4.2, ranging to 54.8 pg/g (Roegner et al., 1991). For later studies, veterans who refused to give serum samples in 1987 or received a nonquantifiable result were resampled in 1992.

The overall strengths of this study are that it is a large study, with good power to detect many common disorders; follow-up was very good, as is continued participation of Ranch Hand and comparison populations. The physical and psychological examinations are extensive, planned to evaluate most, if not all, outcomes hypothetically associated with 2,3,7,8-TCDD. Continued reevaluation of the subjects (every 5 years) permits investigators to monitor the development of chronic diseases and to test for additional outcomes as new biochemical and toxicological data become available. Finally, the determination of serum 2,3,7,8-TCDD levels permitted validation of the exposure matrix based on historical records and the subsequent development of disease-specific dose-response models. Repeated measures of serum 2,3,7,8-TCDD over time will also provide valuable information on its half-life in humans.

Noteworthy caveats in the study include the fact that the majority of the population had serum levels under the background level of 20 pg/g (median = 12.8 pg/g, range to 600 pg/g in 1987). These data suggest that, although there are some Ranch Hands who were exposed to very high levels of 2,3,7,8-TCDD, most of the study group had lower exposures, if any at all. In addition, serum 2,3,7,8-TCDD levels indicated that the exposure matrix used in the analysis of the baseline and 1984 studies did not appropriately describe the potential for exposure. Therefore, data described in this chapter will refer only to the baseline and 1984 results where Ranch Hands as a group were compared to the comparisons and, most often, to the reanalysis of the 1987 data (plus relevant 1992 data) using serum levels. The adjusted odds ratios for the three categories of serum 2,3,7,8-TCDD selected by Roegner and colleagues (1991) are presented and discussed. The categories are: ≤10 pg/g 2,3,7,8-TCDD; 15-≤33.3 pg/g 2,3,7,8-TCDD; and >33.3 pg/g 2,3,7,8-TCDD.

In the categorical analysis of the 1992 followup examination results (Grubbs et al, 1995), results were presented in relation to the current 2,3,7,8-TCDD concentration (current dioxin level) and to the concentration estimated since time in duty in southeast Asia (initial level) in the following categories: comparison, current dioxin level ≤10 pg/g of lipid; background (Ranch Hand), current dioxin level≤10 pg/g of lipid; low (Ranch Hand), current dioxin > 10 pg/g of lipid, 10 pg/g<initial dioxin ≤143 pg/g; high (Ranch Hand), current dioxin > 10 pg/g of lipid, 10 pg/g<initial dioxin >143 pg/g. In subsequent analyses, for the low and high categories the definitions were changed to the following: low (Ranch Hand), 10 <current and initial <94 pg/g; high (Ranch Hand), 10>current and initial >94 pg/g (Michalek et al., 1997).

A consequence of conducting a comprehensive study in which a large number of statistical tests are performed is an increased possibility of spurious findings. The reader should be cognizant of this limitation, looking for consistencies with the results of other studies and in the toxicological literature rather than statistical significance alone.

7.12.3.3. *Studies of the Effect of Ingestion of Rice Oil Contaminated With Polychlorinated Dibenzofurans, Quaterphenyls, and Biphenyls in Japan (YUSHO) and Taiwan (YU-CHENG)*

This section also briefly reviews the noncancer effects observed in Yusho (Japan) and Yu-Cheng (Taiwan) victims, individuals exposed by ingestion to large concentrations of compounds structurally related to dioxins, namely polychlorinated dibenzofurans, quaterphenyls, and biphenyls. In addition, other reviews have summarized the numerous papers dedicated to Yusho and Yu-Cheng (Lü and Wong, 1984; Kuratsune, 1989; Rogan, 1989).

Reports describing effects among individuals who ingested the contaminated rice oil both in Taiwan and Japan were generally limited descriptions of acute rather than general effects. However, because more than 25 years have passed since Yusho and 15 years since Yu-Cheng, more studies are evaluating potential chronic effects.

Recent epidemiologic studies have concentrated on the development of offspring of Yu-Cheng mothers. These children were exposed *in utero* at the time the contaminants were ingested or were conceived after the poisoning and were exposed to residual contaminants transplacentally or through breast milk (Chen et al., 1992; Lai et al., 1993, 1994; Hsu et al., 1993; Guo et al., 1992, 1993,1994a,b, 1995a,b,1996; Chao et al., 1997; Yu et al., 1994, 1998, 2000).

7.12.3.3.1. *Yusho.* The initial recognition of the effects of the ingestion of contaminated rice oil by the Yusho population occurred in 1968. As of 1983, a total of 2,060 individuals were identified as part of the affected Yusho population (Masuda et al., 1985). Five years after exposure ended, the mean concentrations of PCBs in the adipose tissue, liver, and blood of Yusho cases were 1.9 ppm, 0.08 ppm, and 6.7 ppb (Masuda et al., 1985), respectively, which were about twice the levels in the control group. Adipose tissue levels of PCDFs ranged from 6 to 13 ppb (Masuda et al., 1985). Sixteen years after exposure, mean PCQ level in adipose tissue of Yusho cases was 207 ppb, approximately 100 times the level in Japanese controls (Kashimoto et al., 1985).

7.12.3.3.2. *Yu-Cheng*. The initial recognition of Yu-Cheng occurred in 1979. As of 1983, approximately 2,000 individuals were found to have been exposed to the contaminated rice oil. Within the first year of exposure, mean serum PCB, PCDF, and PCQ levels in blood for 15 cases were 60 ppm (range 4-188 ppm), 0.14 ppb (range <0.005-0.27 ppb), and 19.3 ppb (range 0.9-63.8), respectively (Kashimoto et al., 1985). Analysis of PCB levels in blood in 1980-1981 in 165 cases (mean 38 ppb, range 10-720) (Rogan, 1989) and in 1985 in 32 cases (mean 15.4 ppb, range

0.6-86.8) (Lundgren et al., 1988) suggested that some PCBs were being eliminated. It is not clear from the reports if the samples were drawn from distinctly different individuals or included some of the same individuals.

7.13. REVIEW OF EFFECTS ASSOCIATED WITH EXPOSURE TO 2,3,7,8-TCDD

The following section contains a review of the case reports and epidemiologic studies that describe effects associated with exposure to materials contaminated with 2,3,7,8-TCDD.

7.13.1. Dermal Effects

7.13.1.1. *Chloracne*

The most widely recognized dermal effect of exposure to 2,3,7,8-TCDD-contaminated substances is chloracne. Chloracne is a persistent acneiform condition characterized by comedones, keratin cysts, and inflamed papules with hyperpigmentation and a unique anatomic distribution, occurring subsequent to acute and chronic exposure to a variety of chlorinated aromatic compounds (Crow et al., 1978; Moses and Prioleau, 1985). This acne-like condition is reported to have occurred with and without other TCDD-related health effects in at least a few workers after all reported accidents at TCP production facilities (Ashe and Suskind, 1950; Suskind et al., 1953; Goldman, 1972; May, 1973; Zober at al., 1990), among individuals involved in daily production of 2,3,7,8-TCDD-contaminated products (Bleiberg et al., 1964; Poland et al., 1971; Pazderova-Vejlupkova et al., 1981; Moses et al., 1984; Moses and Prioleau, 1985; Suskind and Hertzberg, 1984; Bond et al., 1989), among three laboratory workers exposed to pure 2,3,7,8-TCDD (Oliver, 1975), and among at least 193 (0.6%) Seveso residents, mostly children (Reggiani, 1978; Caramaschi et al., 1981; Ideo et al., 1985; Mocarelli et al., 1986; Assennato et al., 1989). Chloracne was not found among Missouri residents (Hoffman et al., 1986; Webb et al., 1989) examined 10 years after exposure or among Ranch Hand personnel (Burton et al., 1998; Roegner et al., 1991). In U.S. Army Vietnam veterans, chloracne-like skin lesions were rarely observed on examination (0.9% in Vietnam veterans versus 0.8% in non-Vietnam veterans, OR = 1.4, 95% CI = 0.7-2.9) (Centers for Disease Control Vietnam Experience Study, 1988a).

Based on reports from Seveso and studies of chemical workers, chloracne appeared shortly after exposure to 2,3,7,8-TCDD-contaminated chemicals (Caramaschi et al., 1981; Zober et al., 1990). The eruption of blackheads, usually accompanied by cysts, was observed between 2 weeks and 2 months after the reactor release (Reggiani, 1980). In Seveso, within 6 months of the explosion, 34 cases of chloracne were identified among children, whereupon a more intensive search was undertaken among schoolchildren (Caramaschi et al., 1981). In chemical workers involved in the TCP reactor release at BASF Ludwigshafen, Germany, most cases of chloracne (that were also diagnosed with cancer) developed within 2 days after first exposure (Ott et al.,

1994; Zober et al., 1990). One case of chloracne did not develop for 2 years, but the authors suggest that the etiology of this case is unclear.

For many affected individuals, the condition disappeared after discontinuation of exposure (Assennato et al., 1989) despite high serum 2,3,7,8-TCDD levels (Mocarelli et al., 1991). But for a few, the chloracne remained for many years (Suskind and Hertzberg, 1984; Moses and Prioleau, 1985). Of the 204 exposed workers in the study by Suskind and Hertzberg (1984), 52% had persistent chloracne for at least 10 years after the TCP and 2,4,5-T processes ceased, 34% reported a history of chloracne, and 14% reported no history of chloracne. Moses et al. (1984) reported that the mean duration for persistent chloracne was 26.1±5.9 years.

There are very few human data from which to determine definitively the threshold level of 2,3,7,8-TCDD at which chloracne occurs or who is at greatest risk to develop chloracne. Data from analyses of chloracne cases among chemical workers and chloracne's relationship to serum and adipose tissue levels of 2,3,7,8-TCDD and hexachlorinated (HxCDD) dioxins provide some basic yet useful information on the characteristics of chloracne cases, particularly the inter-individual susceptibility to chloracne (Bond et al., 1989; Ott et al., 1987; Beck et al., 1989; Mocarelli et al., 1991). Bond et al. (1989) described 325 cases (15%) of chloracne among 2,192 workers exposed to 2,3,7,8-TCDD and HxCDDs or octachlorinated (OCDD) dioxins between 1938 and 1982 during chemical production activities (not as a result of a reactor accident). Cases were identified through company-maintained medical records; age- and calendar year-specific incidence rates were estimated based on age- and calendar year-specific person-years of employment contributed by the cohort; and risk factors were adjusted for by logistic regression. The analysis found that risk of chloracne was highest among workers who were exposed at younger ages, among those who had the longest length of exposure to 2,4,5-trichlorophenol or pentachlorophenol production operations, and among jobs rated at the highest intensity of exposure (Ott et al., 1987). These characteristics of chloracne cases in Michigan workers are consistent with those observed in chloracne cases from the BASF accident cohort (Ott et al., 1993). Although this study may underestimate the incidence rate of chloracne owing to possible underreporting or misdiagnosis of cases, and misclassification of exposure may have occurred, this study was the first of its kind to explore analytically the risk factors associated with occupationally acquired chloracne. It is unfortunate that these exposure estimates have not been validated by serum or adipose tissue 2,3,7,8-TCDD, HxCDD, and OCDD levels.

Serum levels of 2,3,7,8-TCDD and HxCDD have been measured in chloracne cases of Seveso residents (Mocarelli et al., 1991) and German chemical workers (Beck et al., 1989; Ott et al., 1993). Mocarelli et al. (1991) described chloracne in persons from zone A who had very high serum 2,3,7,8-TCDD levels ranging from 820 to 56,000 pg/g measured within 1 year of the

reactor release (Table 7-22). The study also included other individuals from Zone A, but without chloracne, who had serum 2,3,7,8-TCDD levels that ranged from 1,770 to 10,400 pg/g. With the exception of one person with chloracne who was 16 years old at the time of the accident, all of the cases were in children under age 11. Those without chloracne, for the most part, were over age 30. It is not clear whether the children were more susceptible to the chloranegenic effects or whether they had greater exposure to 2,3,7,8-TCDD-contaminated soil or airborne effluent.

As documented by others, adult TCP production workers also developed chloracne (Beck et al., 1989; Suskind and Hertzberg, 1984; Bond et al., 1989). Adipose tissue levels of 2,3,7,8-TCDD and HxCDD measured in adult chloracne cases of German chemical production workers suggest that these cases may have been a function of the combined exposure, making it difficult to isolate the contribution of the different chlorinated compounds. All cases had estimated adipose levels of greater than 200 pg/g 2,3,7,8-TCDD and in excess of 2,000 pg/g lipid HxCDD at the time of diagnosis. Estimated levels were based on the half-life extrapolation of the adipose tissue level measured in 1986 to the date of last occupational exposure, which may have occurred between 1949 and 1984. Similarly, Ott (1993) found that 80% of the severe chloracne cases had estimated (back-calculated) lipid-adjusted serum 2,3,7,8-TCDD levels of 250 pg/g. Yet 26% of nonchloracne cases had estimated 2,3,7,8-TCDD concentrations of 250 pg/g.

Data from studies of Seveso residents conducted from 1982 to 1985 indicate that, despite high serum 2,3,7,8-TCDD levels, the chloracne resolved in all but one person by 1983 (Assennato et al., 1989). The fact that the cases of chloracne in Seveso residents resolved within 10 years may explain why no chloracne was observed in the Ranch Hand group, although some serum 2,3,7,8-TCDD levels exceeded 600 pg/g in 1988 (Roegner et al., 1991) and may have been as high as 2,400 pg/g at the time of last occupational exposure, assuming 21 years since last exposure and a 7-year half-life. Nevertheless, residual chloracne was observed 30 years after first exposure among workers from Nitro, West Virginia, which may suggest that chronic high exposure to 2,3,7,8-TCDD or exposures higher than experienced by the Ranch Hands may account for long-term persistence of chloracne.

7.13.1.2. *Dermatologic Disorders Other Than Chloracne*

Dermal effects other than chloracne attributed to 2,3,7,8-TCDD exposure include a variety of symptoms and conditions that occurred less frequently than chloracne but appeared in several groups subsequent to acute and continuous exposure to 2,3,7,8-TCDD-contaminated TCP and 2,4,5-T. Two reports indicated that after acute episodes of exposure, e.g., accidents, individuals complained of red and irritated eyes, conjunctivitis, and blepharitis (inflammation of the eyelids) (Ashe and Suskind, 1950; Baader and Bauer, 1951). Other investigators also found cases of

eyelid cysts several months after acute exposure (Suskind et al., 1953; Kimmig and Schulz, 1957a,b; Poland et al., 1971; Reggiani, 1980) and up to 25 years after exposure (Moses et al., 1984; Suskind and Hertzberg, 1984).

Hyperpigmentation and hirsutism (also known as hypertrichosis or abnormal distribution of hair) were diagnosed among chemical workers in the United States (West Virginia and New Jersey) (Ashe and Suskind, 1950; Suskind et al., 1953; Bleiberg et al., 1964; Poland et al., 1971), Germany (Bauer et al., 1961; Goldman, 1972), and Czechoslovakia (Jirasek et al., 1974) who were exposed to 2,3,7,8-TCDD-contaminated TCP during manufacturing processes or industrial accidents and among laboratory workers in England exposed while synthesizing pure 2,3,7,8-TCDD (Oliver, 1975). Upon reexamination 25 years later, hypertrichosis was observed in exposed workers (5.4% exposed vs. 1.8% unexposed) from the West Virginia plant, particularly among workers with persistent chloracne on clinical examination (10.3% with persistent chloracne vs. 0% with history of chloracne only) ($p<0.001$) in one of two independent studies (Suskind and Hertzberg, 1984). A second study by Moses et al. (1984) found no evidence of hypertrichosis, although 31% of the exposed workers had evidence of residual chloracne (Moses et al., 1984). Studies of Vietnam veterans have reported no significant increase in the prevalence of either hyperpigmentation or hypertrichosis (Roegner et al., 1991; Centers for Disease Control Vietnam Experience Study, 1988a). Three cases of hypertrichosis but not hyperpigmentation were observed among Missouri residents, one with serum levels less than 20 pg/g and two with levels between 20 and 60 pg/g (Webb et al., 1989). Neither disorder was noted on examination among residents of the Quail Run Mobile Home Park (Hoffman et al., 1986).

Actinic or solar elastosis was found to be more prevalent among West Virginia workers diagnosed with active chloracne at the time of their examinations in 1979 (Suskind and Hertzberg, 1984) (exposed = 59.1% vs. unexposed = 30.1%, $p<0.01$). No significant difference was observed in the age-adjusted prevalence of actinic elastosis in workers with or without chloracne in the study by Moses et al. (1984). Actinic elastosis is known to be directly related to sun exposure; however, the amount of sun exposure, skin type, or other factors contributing to the sensitivity of the skin to sunlight were not assessed in the report. No other studies of TCP production workers, the Ranch Hands, or U.S. Army Vietnam veterans have found an increase in the prevalence of actinic elastosis.

Among the group of workers studied by Suskind and Hertzberg (1984), three cases of Peyronie's disease were noted. Peyronie's disease is a rare condition characterized by progressive scarring of the penile membrane. No explanation for this finding was expressed, nor has the condition been noted (or perhaps looked for) in other studies (Bond et al., 1989; Moses et al., 1984; Roegner et al., 1991).

In 1984, a statistically significant excess of nonmelanotic skin cancer was reported among Ranch Hand personnel involved in the aerial spraying of herbicides over Vietnam compared with a matched comparison group (Lathrop et al., 1984). The comparison group was composed of Air Force personnel assigned to cargo missions outside the sprayed areas of Vietnam. A follow-up study of the same cohorts in 1987 confirmed the excess of basal cell carcinoma and attributed the increase to sunlight exposure (Lathrop et al., 1987). However, in the reanalysis of the 1987 examination data, skin neoplasms of any kind were not related to serum 2,3,7,8-TCDD level (Roegner et al., 1991).

7.13.1.3. *Comment*

From an epidemiologic perspective, chloracne is a common consequence of exposure to chemicals contaminated with 2,3,7,8-TCDD and some other polyhalogenated hydrocarbons. Available data on serum or adipose tissue levels of 2,3,7,8-TCDD have not determined the threshold at which a case of chloracne occurs. Evidence from Bond et al. (1989) suggests that for chemical production workers the risk of chloracne may be related to the part of the process in which the workers were engaged, the amount of time spent in the contaminated region, and the intensity of the exposure while in the area. Chloracne is also related to short-term high-intensity exposures, as observed in Seveso residents (Reggiani, 1980) and occupational cohorts (Ott et al., 1993). Few studies have been successful in evaluating the relationship between history of chloracne and long-term nonmalignant effects. However, Zober et al. (1990) noted that the SMR for all malignant neoplasms for workers with chloracne and who had at least 20 years of latency (time since first exposure) was statistically significantly elevated (SMR 201; 90% CI = 122, 135). Future studies that are able to evaluate the association between history of chloracne and effects would provide useful information in this regard.

Other conditions, such as hyperpigmentation and hypertrichosis, may be more acute effects of 2,3,7,8-TCDD exposure that resolve over time, because they were not observed in studies where the cohorts were examined years after the cessation of exposure. Actinic keratosis, Peyronie's disease, and basal cell carcinoma may not be due to 2,3,7,8-TCDD because actinic keratosis and Peyronie's disease have been observed in a single cohort. Likewise, the excess basal cell carcinoma was noted only in one study group, and the results could not be replicated when a better indicator of personal exposure to 2,3,7,8-TCDD, serum 2,3,7,8-TCDD, was used as a surrogate for exposure in the statistical models.

7.13.2. Gastrointestinal Effects

7.13.2.1. *Hepatic Effects*

Changes in liver function and structure after exposure to 2,3,7,8-TCDD are commonly observed in experimental animals (Greig et al., 1973; Vos et al., 1974; Jones and Greig, 1975; McConnell et al., 1978a,b; Jones et al., 1981; Zinkl et al., 1973; Kociba et al., 1976, 1978; Gasiewicz et al., 1980; DeCaprio et al., 1986). The changes are not always consistent from one species to another, but they have prompted examination of hepatic effects among exposed human populations. As with animals, there is wide variation in the type and degree of hepatic effects reported in humans after exposure to 2,3,7,8-TCDD-contaminated materials. This section describes selected hepatic effects associated with 2,3,7,8-TCDD exposure observed in humans, including hepatomegaly and hepatic enzyme changes.

7.13.2.2. *Liver Size*

Increased liver size is consistently reported in treated animals after exposure to 2,3,7,8-TCDD (Vos et al., 1974; Allen et al., 1977; McConnell et al., 1978a,b; Kociba et al., 1978; Gasiewicz et al., 1980). Among exposed human populations, four case reports in three populations, but not controlled epidemiologic studies, described evidence of enlarged livers or hepatomegaly. Liver size was reportedly increased among two TCP production workers in West Virginia within a few months after a TCP reactor explosion (Ashe and Suskind, 1950; Suskind et al., 1953) and among "several" production workers in Czechoslovakia exposed to TCP, the butyl ester of 2,4,5-T, and sodium pentachlorophenol (Jirasek et al., 1974). Temporary liver enlargement was observed in 5 of 22 Seveso residents who had severe chloracne (Reggiani, 1980). The hepatomegaly lasted "several" months without concomitant elevation in hepatic enzymes. Fortunately, the effect appeared to be transient. Cross-sectional medical studies of TCP production workers (Bond et al., 1983; Suskind and Hertzberg, 1984; Moses et al., 1984; Calvert et al., 1992), Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991), and Missouri residents (Webb, 1989; Hoffman et al., 1986) have found little evidence of excess hepatomegaly in the exposed populations. Additionally, no dose-response relationship was observed between serum levels of 2,3,7,8-TCDD and physical findings of an enlarged liver for the 1987 or 1992 examinations of Ranch Hands (≤10 pg/g 2,3,7,8-TCDD, RR = 0.39, 95% CI = 0.11-1.33; 15-≤33.3 pg/g 2,3,7,8-TCDD, RR = 1.47, 95% CI = 0.57-3.79; >33.3 pg/g 2,3,7,8-TCDD, RR = 1.69, 95% CI = 0.60-4.75) (Roegner et al., 1991) (Background: RR = 0.51 95% CI = 0.19, 1.38; Low: RR = 0.26, 95% CI = 0.06, 1.09; High: RR = 1.02, 95% CI = 0.43, 2.44) (Grubbs et al., 1995) or the NIOSH study of TCP production workers (two workers; four referents; OR = 0.46, 95% CI = 0.09, 2.43) (Calvert et al., 1992).

One Missouri resident was found to have hepatomegaly, but he also was suffering from diabetes mellitus. His adipose tissue 2,3,7,8-TCDD level was 430 pg/g (Webb et al., 1989). The differences in findings between the case reports and the controlled epidemiologic studies suggest that hepatomegaly may be a resolvable, acute effect as a result of exposure to high levels of 2,3,7,8-TCDD.

7.13.2.3. *Enzyme Levels*

Laboratory studies have demonstrated changes in hepatic enzyme levels after 2,3,7,8-TCDD exposure, although there is considerable interspecies variation in the observed effect (Zinkl et al., 1973; Kociba et al., 1976, 1978; Gasiewicz et al., 1980; Olson et al., 1980). Epidemiologic studies and case reports describe elevated liver enzymes among exposed TCP production workers, Ranch Hand veterans (Roegner et al., 1991), and among Seveso residents (Mocarelli et al., 1986; May, 1982; Martin, 1984; Moses et al., 1984; Calvert et al., 1992; Ott et al., 1994).

7.13.2.4. *GGT*

Increased levels of gamma glutamyl transferase (GGT) may suggest activity such as cholestasis, liver regeneration, or drug or xenobiotic metabolism (Table 7-23). The studies of Seveso children demonstrate an increase in GGT levels occurring shortly after the explosion and then a gradual decline to near normal levels within 5 years. In one of the earliest studies of Seveso children with (N = 141) and without chloracne (N = 138), 2.8% of the children with chloracne had out-of-range GGT levels, but none of the children without chloracne had an out-of-range level ($p<0.001$) (Caramaschi et al., 1981). These results were echoed in a study of children from zones A, B, and R of Seveso, in which enzyme levels were measured yearly between June 1977 and June 1982 (Mocarelli et al., 1986). GGT levels were elevated in children of zone A, particularly in boys, during the first 2 years after the explosion (1977: exposed = 9.73 U/L; unexposed = 7.28 U/L; $p<0.01$; 1978: exposed = 9.88 U/L; unexposed = 8.26 U/L; $p<0.05$) (Mocarelli et al., 1986). Levels in girls during the same years were elevated but did not achieve statistical significance. For the next 4 years of the study, GGT levels remained elevated in boys and girls from zone A compared to unexposed children, but the values declined to normal levels with time.

GGT was found to be elevated among TCP production workers from one plant in Great Britain and in workers from West Virginia, Missouri, and New Jersey up to 30 years after last occupational exposure to 2,3,7,8-TCDD-contaminated chemicals (May, 1982; Martin, 1984; Moses et al., 1984; Calvert et al., 1992). The findings of two studies of British workers were

similar. Mean GGT levels were increased, but not statistically elevated, in workers with chloracne compared to unexposed controls tested 10 years after exposure to 2,3,7,8-TCDD-contaminated chemicals as a result of a TCP reactor explosion (chloracne, GGT = 39 U/L; controls, 27.7 U/L [May 1982]) (chloracne, GGT = 32 U/L; controls, 32 U/L [Martin, 1984]). Similarly, a statistically significant excess in the proportion of individuals with abnormally high GGT levels was found among West Virginia workers with chloracne who were examined as many as 30 years after exposure (Moses et al., 1984) (chloracne, mean GGT = 26.3 U/L, 23% abnormal; no chloracne, mean GGT = 17.4 U/L, 9% abnormal; $p<0.003$). Yet, compared to controls, GGT was not elevated in another study of West Virginia workers (Suskind and Hertzberg, 1984).

In a study by Calvert et al. (1992), the mean GGT level and the proportion of workers with out-of-range levels were statistically significantly elevated among TCP workers from New Jersey and Missouri (workers, mean GGT = 58.5 U/L; unexposed referents, mean GGT = 47.4 U/L, $p<0.03$; workers, 11% abnormal; referents, 5% abnormal, OR = 2.27, 95% CI = 1.17-4.39). Based on the logistic regression model in Table 7-24, the increases in GGT were limited to workers with high serum 2,3,7,8-TCDD levels (>100 pg/g) and high lifetime alcohol consumption (>30 alcohol years) (alcohol year = 1 alcoholic beverage/day for 1 year). The contribution of other potentially confounding exposures that may have affected GGT levels was not explored in this study. Other studies of TCP production workers in Michigan and West Virginia or the BASF accident cohort did not report elevations in GGT levels (Bond et al., 1983; Suskind and Hertzberg, 1984; Ott et al., 1994).

Both the Vietnam Experience Study and the U.S. Air Force Ranch Hand Study found statistically significant elevations in GGT levels (Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991). In Army Vietnam veterans, mean GGT levels were 43.2 U/L compared with 41.1 U/L in non-Vietnam veterans (OR for out-of-range value = 1.3, 95% CI = 1.0-1.8) (Centers for Disease Control Vietnam Experience Study, 1988a). In the 1987 follow-up study, the comparison of the adjusted mean GGT level in the comparison group and in each of the three Ranch Hand groups defined by 2,3,7,8-TCDD level found statistically significant increases in the Ranch Hand population (≤10 pg/g 2,3,7,8-TCDD, $p<0.017$; 15-≤33.3 pg/g 2,3,7,8-TCDD, $p<0.043$; >33.3 pg/g 2,3,7,8-TCDD, $p<0.001$) (Roegner et al., 1991). In the 1992 followup, significant increseases were noted in Ranch Hands classified as either low or high exposure (Grubbs et al., 1995).

7.13.2.5. *AST and ALT*

7.13.2.5.1. ***Adult measures*****.** Abnormal levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) may indicate liver cell damage from a number of causes, including

hepatic necrosis, metastatic carcinoma, or obstructive jaundice (AST and ALT) or infectious or toxic hepatitis and cirrhosis (AST). Elevated levels of these enzymes may also be due to nonhepatic origins, such as myocardial infarction, acute pancreatitis (AST and ALT), or skeletal, cerebral, or renal necrosis (AST).

With respect to exposure to 2,3,7,8-TCDD, elevations in serum ALT and AST appear to be transient effects of acute exposure (Table 7-25). Case reports note that some populations have increased serum ALT levels shortly after exposure (Seveso children, British and Czechoslovakian TCP production workers) (May, 1973; Jirasek et al., 1974; Caramaschi et al., 1981; Mocarelli et al., 1986). Whereas epidemiologic studies conducted 10 to 30 years after last exposure reported no effects in exposed workers, Vietnam veterans, and Missouri residents compared to unexposed control groups (Table 7-25) (Suskind and Hertzberg, 1984; May, 1982; Martin, 1984; Bond et al., 1983; Calvert et al., 1992; Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991; Grubbs et al., 1995; Hoffman et al., 1986; Webb et al., 1989; Ott et al., 1994), or workers with and without chloracne (Moses et al., 1984). Furthermore, it appears from a single study that current exposure to 2,3,7,8-TCDD-contaminated substances must exceed some threshold to produce detectable enzyme elevations (Poland et al., 1971). Normal levels of AST (AST = 10.6 U/L) were found in workers who volunteered to participate in a medical study conducted concurrently with their employment in a New Jersey chemical facility producing TCP and 2,4,5,-T (Poland et al., 1971). This was the same plant included in a later cross-sectional medical study of workers that found, in 1988, a high mean serum 2,3,7,8-TCDD level for the group (220 pg/g) but no elevations in AST or ALT (Calvert et al., 1992; Fingerhut et al., 1991a). Similarly, no increases in AST were noted in the BASF accident cohort (Ott et al., 1994).

Several reports illustrate the probable transiency of ALT and AST elevations after heavy 2,3,7,8-TCDD exposure. Both enzymes were reported to be elevated among TCP production workers employed in Czechoslovakia who were described as exhibiting symptoms of “chemical intoxication” (Jirasek et al., 1974). The authors reported that AST and ALT levels were “different” in 11 (20%) of the 55 examined workers. These workers were exposed to 2,3,7,8-TCDD-contaminated chemicals, pentachlorophenol, and its production by-products, which include hexa-, hepta-, and octachlorinated dioxins and dibenzofurans. The exposures occurred between 1965 and 1968, with the ensuing effects beginning shortly thereafter. The observation period lasted from 1967 to 1973. In a follow-up report of the same population conducted in approximately 1977, Pazderova-Vejlupkova et al. (1981) did not report liver enzyme levels but suggested that the levels were not abnormal.

Similarly, ALT was increased in 5 of 14 TCP workers from Great Britain who were in the manufacturing building at the time of a TCP reactor explosion in 1968 (May, 1973). Levels for

AST were not reported. In 1977, workers from the same facility were reevaluated. No elevations in AST or ALT were found in production and laboratory workers with chloracne (May, 1982).

Finally, during the first year after the TCP reactor explosion, Caramaschi et al. (1981) evaluated AST and ALT among Seveso children with and without chloracne. Only ALT was statistically significantly elevated in children with chloracne. In a larger study, Mocarelli et al. (1986) tested liver enzyme levels yearly from 1977 to 1982 in male and female children from Seveso and from the unexposed surrounding area. In the 1977, 1979, 1980, and 1981 test series ALT, but not AST, was statistically significantly ($p<0.05$) elevated among male children in Seveso compared with unexposed comparisons. Female children had normal levels compared with controls for all years. In 1982, ALT levels in the exposed boys returned to normal.

None of the studies reporting elevations in ALT or AST identified clinical evidence of liver disease in the study populations. Therefore, in the absence of reports of hepatic or nonhepatic diseases related to changes in ALT or AST levels among exposed individuals, it is possible that the increases in ALT and AST are related to high-level, acute exposure to 2,3,7,8-TCDD-contaminated chemicals and that, barring additional exposure, the enzyme levels decrease with time.

7.13.2.5.2. ***Developmental measures*****.** One report (Pluim et al., 1994) has examined blood measures in 35 babies (the study and exposure groupings are described in detail in Section 7.12.2.3.). Four blood samples were taken: maternal blood around delivery, cord blood, and the infant's blood at 1 and 11 weeks of age. These samples were used to measure leucocytes (WBC), platelets, and differential, along with plasma, activity of gamma glutamyltransferase (GGT), AST, and ALT, and levels of cholesterol and bilirubin. Dioxin and furan levels were measured in breast milk collected about 3 weeks post-delivery. None of the maternal blood measurements were outside the normal range. A statistically significant inverse correlation was observed in an uncorrected comparison of the number of polynuclear neutrophils and dioxin-furan levels in breast milk $r = -0.53$, $p = 0.022$); this disappeared when regression analysis compared these and controlled for gestational age. None of the other factors compared in the cord blood or at 1 week or 11 weeks of age were statistically significant. The next set of analyses used the estimated cumulative dioxin-furan intake from breast feeding at 11 weeks of age; the PCDD/PCDF TEQs ranged from 5.7 to 123.7 pg TEQ/g fat, with a mean of 44.7 pg. The statistically significant correlations are presented in Table 7-26. These results are unadjusted, but remain significant after adjusting for maternal age, gestational age, and birth weight (regression coefficients were not presented). The author proposed that the changes in ALT and AST suggest an effect on the liver, associated with the cumulative exposure to dioxin-furans, and notes that all but three of the

children had ALT and AST within "normal" ranges, but the distribution of some of these findings (e.g., an increase in platelets) did vary. From this study, it is not possible to determine the reversibility of these changes.

7.13.2.6. *D-Glucaric Acid*

In five studies of Seveso residents, TCP production workers, and Vietnam veterans, urinary excretion of D-glucaric acid was measured to determine if exposure to 2,3,7,8-TCDD induced hepatic microsomal activity (Table 7-27). D-glucaric acid excretion is an indirect but valid indicator of enzyme induction.

Ideo and colleagues (1985) measured urinary D-glucaric acid in adults and children from all zones of Seveso and from nearby uncontaminated towns. Of adults tested in 1978, D-glucaric acid excretion was significantly elevated in adults residing in Seveso, Italy, at the time of the reactor explosion compared with residents of unexposed communities (Seveso = 27.1 μmol/g of creatinine vs. unexposed = 19.8 μmol/g of creatinine, $p<0.05$). No further studies of adults have been published. A series of studies evaluated D-glucaric acid excretion in Seveso children (Ideo et al., 1985) (Table 7-27). In 1976, the levels in children from zone A with chloracne (39 μmol/g of creatinine) were significantly greater than in children without chloracne (20.5 μmol/g of creatinine). Additional studies, conducted until 1981, found significant yearly decreases in urinary D-glucaric acid excretion. By 1981, levels were within normal range.

These early analyses of D-glucaric acid levels were conducted without the benefit of individual exposure measurements. In 1989-1994, serum 2,3,7,8-TCDD concentrations were analyzed in blood samples collected in 1976-77 from the original study group. In a comparison of D-glucaric acid levels and serum 2,3,7,8-TCDD concentrations, Cassaniga et al. (1999) found the median D-glucaric acid levels of the 26 controls and of the 11 exposed children with 2,3,7,8-TCDD concentrations below 1,000 pg/g lipid were similar (Controls = 22.8 μmol/g creatinine; <1,000 pg/g lipid TCDD = 21.6 μmol/g creatinine); the median D-glucaric acid levels of the five children with 2,3,7,8-TCDD concentrations above 1,000 pg/g were twice as high (59.2 μmol/g creatinine). These data suggest that D-glucaric acid levels may be a useful marker of recent, very high exposure to 2,3,7,8-TCDD because within 5 years after exposure, D-glucaric acid levels returned to normal.

D-glucaric acid-creatinine ratios were used to assess urinary excretion in TCP production workers tested within a year of suspension of TCP production and 10 years after a TCP reactor explosion. For exposed workers with or without chloracne at the time of the study, the D-glucaric acid-creatinine ratio was significantly higher than that of the unexposed controls (exposed = 2.09; unexposed controls = 1.59; $p<0.05$) (Martin, 1984).

Other studies of Air Force Ranch Hands or TCP production workers that examined D-glucaric acid excretion did not find increases in exposed populations 10 to 37 years after last exposure to 2,3,7,8-TCDD-contaminated chemicals (Roegner et al., 1991; Calvert et al., 1992).

7.13.2.6.1. ***Comment.*** The evidence presented by the large number of case reports and epidemiologic studies of groups exposed to 2,3,7,8-TCDD-contaminated chemicals suggests that hepatic enzyme induction occurred in some populations within a short time after high-level 2,3,7,8-TCDD exposure. In most cases, enzyme levels decreased as the time from exposure increased. However, even after more than 15 years since last exposure, levels of GGT continue to be significantly elevated in relation to serum 2,3,7,8-TCDD in TCP production workers with above-average alcohol consumption (Calvert et al., 1992) and in Air Force Ranch Hands (Roegner et al., 1991; Grubbs et al., 1995). Other Vietnam veterans (U.S. Army ground troops) also have significantly increased GGT levels compared with non-Vietnam veterans, but this increase in the Army veterans is probably not due to exposure to high levels of 2,3,7,8-TCDD. In the population of Army Vietnam veterans studied, the mean serum 2,3,7,8-TCDD was approximately 4 pg/g (Centers for Disease Control Veterans Health Studies, 1988), compared to a mean of 220 pg/g (range to 3,400 pg/g) in the production workers and a median of 12 pg/g (range to 600 pg/g) in the Ranch Hands.

The finding of continued elevation of GGT in the NIOSH and Army veterans may be a spurious result or it may reflect activity related to the continued presence of above-background levels of 2,3,7,8-TCDD in exposed individuals.

7.13.2.7. ***Porphyrin Metabolism***

In rats and mice, exposure to 2,3,7,8-TCDD has been clearly shown to produce alterations in porphyrin metabolism (Goldstein et al., 1973, 1982; Smith et al., 1982; Jones et al., 1981; DeVerneuil et al., 1983; Cantoni et al., 1981). Whether 2,3,7,8-TCDD is associated with porphyrin changes in humans, particularly porphyria cutanea tarda (PCT), is a subject of some debate. PCT is a form of acquired or inherited porphyria caused by a deficiency of the enzyme uroporphyrinogen decarboxylase and the resulting overproduction and excretion of uroporphyrin (Sweeney, 1986). The predominant characteristics of PCT include skin fragility, blistering upon sun exposure, dark pigmentation, excess hair growth, hepatomegaly, reddish-colored urine, and urinary excretion of uro- and heptacarboxylicoporphyrins (Strik, 1979). PCT has been associated with excessive alcohol intake, oral estrogens, iron overload, hepatomas, and exposure to polyhalogenated hydrocarbons (Strik, 1979). A particularly large outbreak of PCT occurred after consumption of grain treated with hexachlorobenzene (HCB) (Cam and Nigogosyan, 1963).

Cases of PCT were described in two populations of TCP production workers (Bleiberg et al., 1964; Jirasek et al., 1974) and among members of a family with inherited uroporphyrin decarboxylase deficiency who were living in Seveso at the time of the reactor explosion (Strik, 1979).

In 1964, Bleiberg reported that based on the Watson-Schwartz test, 11 of 29 New Jersey TCP production workers with chloracne had PCT as a result of increased urinary uroporphyrins, coproporphyrins, and urobilinogen. In a later study of 73 workers from the same plant in New Jersey, including four of the individuals that Bleiberg et al. (1964) found to have elevated urinary porphyrins, Poland et al. (1971) identified one individual with uroporphyrinuria. The report did not explain if this individual was one of the four described by Bleiberg et al. (1964). In the NIOSH study that examined workers from the same plant in New Jersey, the pattern of urinary porphyrin excretion for each participant was assessed to determine the presence of PCT (Calvert et al., 1994). No difference in the prevalence of PCT was found between workers and an unexposed control group (OR = 0.93, 95% CI = 0.19, 4.54). Furthermore, there were no differences in the risk between workers and the control group for an out-of-range uroporphyrin concentration or an out-of-range coproporphyrin concentration. Because this study was conducted at least 15 years after last occupational exposure to TCDD, it was not possible to determine whether porphyrinuria occurred during the years more proximal to occupational 2,3,7,8-TCDD exposure.

Jirasek et al. (1974) found 11 of 55 Czechoslovakian TCP production workers to have elevated urinary uroporphyrins that decreased during the observation period; the authors did not describe the test used to measure urinary uroporphyrins or coproporphyrins. Ten years later in a follow-up study, Pazderova-Vejlupkova et al. (1981) found no evidence of increased excretion of uroporphyrins or dermatological indications of PCT in the same group of workers.

There is some question that the porphyria noted in the New Jersey (Bleiberg et al., 1964) and Czechoslovakian workers was due to 2,3,7,8-TCDD exposure. Jones and Chelsky suggest that the observed cases of PCT in both plants may be due to exposure to HCB or a combination of both 2,3,7,8-TCDD and HCB (Jones and Chelsky, 1986). HCB was manufactured at the New Jersey plant from 1951 until 1960 and was produced at the facility in Czechoslovakia during the production of pentachlorophenol and TCP (Jirasek et al., 1973). In addition, there is some question as to the appropriateness of the clinical test Bleiberg et al. (1964) used to measure porphyrin levels. The Watson-Schwartz test was capable of measuring only the presence of porphobilinogen. The test was rarely positive in cases of exposure to hepatotoxins. Bleiberg's findings suggest that either other unspecified tests were used to measure uro- and coproporphyrin levels or the authors misinterpreted the function of the Watson-Schwartz test.

Evidence of porphyria in other studies of individuals exposed to 2,3,7,8-TCDD-contaminated substances is minimal. Although Suskind and Hertzberg (1984) sampled urine for porphyrins in the examination of West Virginia TCP workers, the authors report that the data are not valid. However, there was no dermatologic evidence of porphyria identified among the exposed workers. Moses et al. (1984) found no difference in porphyrin levels when comparing TCP workers with and without chloracne. Finally, no PCT was reported among three laboratory workers exposed to pure 2,3,7,8-TCDD (Oliver, 1975), the only humans known to have documented unintentional exposure to uncontaminated 2,3,7,8-TCDD.

In 1977, 60 Seveso residents were tested for elevated porphyrins, exclusive of the family with inherited deficiency of uroporphyrinogen decarboxylase. None of the 60 residents developed PCT; however, 13 (22%) exhibited secondary coproporphyrinuria, 5 of whom showed a slight increase of urocarboxyporphyrins, heptacarboxyporphyrins, and coproporphyrins classified as a "transition constellation to CHP type A" (Doss et al., 1984). Porphyrin levels were retested in 1980. Porphyrin levels returned to normal in 12 individuals. In three of those with transition CHP, porphyrin levels were higher than those in 1977 and were attributed to liver damage and alcohol consumption. Doss et al. (1984) suggested that in the Seveso family with uroporphyrinogen dicarboxylase deficiency, the exposure to 2,3,7,8-TCDD-contaminated effluent caused an exacerbation of a preexisting enzyme deficiency.

7.13.2.7.1. ***Comment.*** It is possible that the PCT and elevated urinary porphyrins observed in the New Jersey and Czechoslovakian workers were a direct result of exposure to hexachlorobenzene. In the follow-up studies, urinary porphyrin levels in workers were not elevated (Pazderova-Vejlupkova et al., 1981; Poland et al., 1971) or did not differ from levels in the control group (Calvert et al., 1992). The transient elevations in coproporphyrins among 22 Seveso residents described by Doss et al. (1984) may be a direct result of acute exposure to 2,3,7,8-TCDD.

The association is not clear. However, 2,3,7,8-TCDD is a potent porphyrigen in rats and mice and, therefore, high acute exposures may have contributed to the observed changes in porphyrin levels in these populations.

7.13.2.8. *Lipid Levels*

Animal studies provide conflicting evidence on the relationship between exposure to 2,3,7,8-TCDD and serum lipid levels. Some studies suggest that short-term high exposure to 2,3,7,8-TCDD increases serum cholesterol (Greig et al., 1973; Zinkl et al., 1973; Poli et al., 1980; Gasiewicz et al., 1980; Schiller et al., 1986; Gasiewicz and Neal, 1979; Olson et al., 1980) and triglyceride fractions (Schiller et al., 1986; McConnell et al., 1978a,b; Gasiewicz and Neal, 1979),

whereas other studies suggest a decrease (Gasiewicz et al., 1980; Olson et al., 1980) or no change in triglyceride levels (Poli et al., 1980). The human data appear to be similarly confusing. A number of case reports and epidemiologic studies have described increases in the levels of serum lipid fractions, particularly total cholesterol and triglycerides, in TCP production workers, laboratory workers, Seveso and Missouri residents, and Vietnam veterans. Others report no differences between subject and reference levels. A summary of the reported levels is included in Tables 7-28 and 7-29.

7.13.2.9. *Total Cholesterol*

Two case reports of workers with presumably high exposures to 2,3,7,8-TCDD-contaminated chemicals described elevations in total cholesterol. In 50% of 55 Czechoslovakian TCP production workers examined between 1968 and 1969 who exhibited signs of "chemical intoxication," total cholesterol was noted as elevated (Jirasek et al., 1974). In a follow-up study 10 years later, lipid levels among workers removed from exposure were not significantly different from referent levels, but total cholesterol levels remained significantly increased (Pazderova-Vejlupkova et al., 1981). In a separate report, three laboratory workers who were exposed during the synthesis of pure 2,3,7,8-TCDD developed serum cholesterol levels in excess of 7.7 mmol/L (Oliver, 1975). No information on pre-exposure cholesterol levels was provided in either report.

The results of epidemiologic studies conflict. Among British TCP production workers whose last exposure to 2,3,7,8-TCDD-contaminated chemicals was less than 1 year at the time of the study, total cholesterol levels in exposed workers with (6.02 mmol/L) and without (6.14 mmol/L) chloracne were significantly elevated compared to unexposed controls (5.6 mmol/L) (Martin, 1984) (Table 7-28), whereas May (1982) found unexposed workers (6.6 mmol/L) to have cholesterol levels higher than those of exposed workers with chloracne (5.97 mmol/L). Martin (1984) also found reduced, but not significantly, HDL cholesterol among exposed workers with chloracne (1.19 mmol/L) compared to unexposed controls (1.25 mmol/L). The differences in the results may be due to differences in the control groups and inclusion of different workers in the exposed groups.

Cholesterol levels in West Virginia TCP production workers were compared with unexposed workers from the same plant (Suskind and Hertzberg, 1984). No difference was identified in mean cholesterol levels between workers and controls. However, when lipid fractions were examined, there was a larger but nonsignificant percentage of exposed workers with elevated LDL cholesterol (7.7%) compared to unexposed controls (6.3%). A comparison of workers with persistent chloracne, no chloracne, or a history of chloracne found a significant

association (p<0.05) between the proportion of out-of-range LDL cholesterol values and persistent chloracne. An out-of-range LDL was defined as above the 90th percentile of the total range of values. In a second study that compared West Virginia workers with and without chloracne, no difference was found in mean cholesterol levels (Moses et al., 1984). In the NIOSH study, there was little difference between the adjusted mean total cholesterol levels for workers (5.7 mmol/L) and referents (5.6 mmol/L) and no relation to increasing serum 2,3,7,8-TCDD levels (Calvert et al., 1996). The mean levels were adjusted for age, body mass index, age, and gender.

Mean cholesterol levels were no different between workers in the BASF accident cohort (6.14 mmol/L) and the referent population (6.37 mmol/L) and were not related to current or log TCDD back-calculated levels (Ott et al., 1994). In addition, no significant differences were noted between the exposed and unexposed populations for HDL and LDL levels.

In general, cholesterol levels among exposed community residents were not increased. Despite their high exposure to 2,3,7,8-TCDD-contaminated TCP, neither children nor adults from Seveso were found to have elevated serum cholesterol levels compared to controls (Mocarelli et al., 1986; Assennato et al., 1989). Evaluated from 1976 through 1985, cholesterol levels in this population remained constant throughout the study period (Table 7-28). Similarly, among Missouri residents, serum cholesterol was not related to residence in the Quail Run Mobile Home Park (Hoffman et al., 1986) or to adipose tissue 2,3,7,8-TCDD levels (Webb et al., 1989).

Among U.S. Army veterans, there was no difference in total cholesterol levels between groups serving in Vietnam or other arenas (Centers for Disease Control Vietnam Experience Study, 1988a). In contrast, there was a statistically significant positive relationship between Ranch Hands with serum 2,3,7,8-TCDD levels above 33.3 pg/g and total cholesterol in Air Force Ranch Hands (Roegner et al., 1991). The total cholesterol-HDL ratio was also highest in this serum 2,3,7,8-TCDD category. In the 1992 analysis, although cholesterol concentrations remained higher in the High Ranch Hand category compared to the Low, Background and Comparison group, the difference was not great enough to achieve statistical significance (Grubbs et al., 1995).

7.13.2.10. *Triglycerides*

Elevated triglyceride levels were reported in only three of the studied populations. Among British TCP workers, triglycerides were significantly higher in exposed workers with (1.97 mmol/L) and without (1.90 mmol/L) chloracne compared to unexposed controls (1.41 mmol/L) (Martin, 1984). TCP production workers from West Virginia who had chloracne had a statistically nonsignificant increase in mean triglyceride levels (chloracne = 1.69 mmol/L; without chloracne = 1.46 mmol/L) (Moses et al., 1984). In addition, compared to the unexposed

Comparison population, triglyceride levels in Air Force Ranch Hands were significantly elevated for all serum 2,3,7,8-TCDD categories (Table 7-29). Among workers in the NIOSH study there appeared to be a small rise in triglyceride levels with increasing serum 2,3,7,8-TCDD (Calvert et al., 1996). The mean adjusted triglyceride levels and the percent of abnormal triglyceride values increase with increasing serum 2,3,7,8-TCDD level (<158 femtograms/liter [fg/L], mean = 1.04 mmol/L, % abnormal = 5.7; 158-520 fg/L, mean = 1.26 mmol/L, % abnormal = 6.1; 521-1,515 fg/L, mean = 1.23 mmol/L, % abnormal = 6.1; 1,516-19,717 fg/L, 1.35 mmol/L, % abnormal = 1.7, $p<0.05$ compared to referents [1.15 mmol/L]). Odds ratios and 95% confidence intervals for the quartiles are OR = 0.7 (95% CI = 0.2, 1.9), OR = 1.1 (95% CI = 0.4, 3.2), OR = 0.9 (95% CI = 0.3, 2.9), and OR = 1.7 (95% CI = 0.6, 4.6), respectively. The authors suggest that despite this small rise with 2,3,7,8-TCDD level, the influence of factors such as gender, body mass index, use of beta-blocker medication, and smoking had far greater effects on lipid concentration than did 2,3,7,8-TCDD level. Likewise, triglyceride levels in the BASF accident cohort were similar to those in the referent cohort and not related to 2,3,7,8-TCDD level (Ott et al., 1994).

Triglyceride levels were not elevated in Missouri (Hoffman et al., 1986; Webb et al., 1989) or Seveso residents (Mocarelli et al., 1986; Assennato et al., 1989) or in U.S. Army Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988a). However, in the 1987 examination, mean adjusted triglyceride concentrations were statistically significantly higher among Ranch Hands whose serum 2,3,7,8-TCDD levels were above 15 pg/g of lipid (Roegner et al., 1991). In the 1991 examination, mean adjusted triglyceride concentrations only in the high group were significantly different from the comparison group (Grubbs et al., 1995).

7.13.2.10.1. ***Comment.*** The effect of exposure to 2,3,7,8-TCDD-contaminated chemicals on cholesterol or triglyceride levels is not consistently well defined in the available studies. It is possible the transient elevations in total cholesterol and triglyceride levels may have occurred after high 2,3,7,8-TCDD exposure, as in the experience of the British and Czechoslovakian TCP workers and British laboratory workers. However, this scenario does not concur with the evidence from Seveso or among Ranch Hands. Despite their very high exposure to 2,3,7,8-TCDD-contaminated chemicals, neither adults nor children from Seveso had lipid levels above the referent level. In contrast, Ranch Hands continue to have elevated lipid levels despite the extended length of time between exposure and testing. Other factors, such as dietary fat intake, familial hypercholesterolemia, alcohol consumption, and exercise, which also affect cholesterol and other lipid levels, may be factors that were not considered in many of these studies.

7.13.3. Other Gastrointestinal Disorders

A variety of gastrointestinal disorders other than liver conditions were reported among TCDD-exposed groups. After heavy, acute, or chronic exposure, chemical workers in West Virginia (Ashe and Suskind, 1950), West Germany (Baader and Bauer, 1951; Bauer et al., 1961), and Czechoslovakia (Jirasek et al., 1974) consistently reported transient episodes of right upper quadrant pain, loss of appetite, and nausea. None of the reports suggest an etiology for these symptoms, nor were the symptoms reported in later follow-up studies of any cohorts (Suskind and Hertzberg, 1984; Moses et al., 1984; Pazderova-Vejlupkova et al., 1981).

Three investigations of TCP production workers reported an increased prevalence of a history of upper gastrointestinal tract ulcer across all age strata of West Virginia workers (exposed = 20.7% vs. unexposed = 5.5%) (Suskind and Hertzberg, 1984) and all digestive system diseases (type not specified) among workers employed in a plant in Midland, Michigan (prevalence: exposed = 1.5% vs. unexposed = 0.5%) (Bond et al., 1983). The factors contributing to these conditions have not been examined fully. Neither the Ranch Hand study (Roegner et al., 1991; Grubbs et al., 1995) or the NIOSH study (Calvert et al., 1992) found increased risk of upper gastrointestinal tract ulcers with increasing serum TCDD level.

7.13.4. Thyroid Function

7.13.4.1. *Adult Effects*

The thyroid plays an essential role in the maintenance of metabolic rate, food intake, and differentiation and maturation of various cell types. Because many of the toxic effects of 2,3,7,8-TCDD noted in animals resemble the signs of thyroid dysfunction, researchers considered the role of the thyroid in 2,3,7,8-TCDD toxicity (Neal et al., 1979). Some studies found a single high dose of 2,3,7,8-TCDD resulted in decreased levels of serum thyroxine (T4), indicating hypothyroidism, but no consistent findings were reported for alterations in 3,5,3′-triiodothyronine (T3); researchers report decreases, no change, and increases in levels of T3 (Bastomsky, 1977; Potter et al., 1983; Pazdernik and Kozman, 1985; Potter et al., 1986; Henry and Gasiewicz, 1987; Roth et al., 1988; Muzi et al., 1989). Furthermore, hypothyroidism induced in rats was protective against 2,3,7,8-TCDD-induced weight loss, immunotoxicity, and mortality (Rozman et al., 1984; Pazdernik and Kozman, 1985). This protective effect was reversed when T4 supplements were given to these animals (Rozman et al., 1985). Henry and Gasiewicz (1987), however, found that in hamsters serum T3 and T4 levels increased after 2,3,7,8-TCDD administration, putting in question the role of the thyroid in 2,3,7,8-TCDD-induced toxicity.

These animal findings suggest that if the thyroid plays a role in human toxicity, hypothyroidism would be manifested as a reduction in serum T4; in extreme cases of 2,3,7,8-

TCDD toxicity, however, one may experience hyperthyroidism. Only three studies of production workers examined this issue (Suskind and Hertzberg, 1984; Ott et al., 1994; Calvert et al., 1999). Suskind and Hertzberg (1984) performed T4 radioimmunoassay and thyroxine-binding globulin (TBG) tests and found no significant differences between exposed and unexposed workers. Quantitative results were not presented. Similarly, thyroid stimulating hormone (TSH), T4, and TBG levels were within normal range in the BASF accident workers and the means were not statistically different (Table 7-30). However, TGB and T4 levels were positively related to 2,3,7,8-TCDD levels in regression analyses (Ott et al., 1994). In the NIOSH study, TSH, total T4, and thyroid binding resin were measured. Overall, workers had a significantly higher adjusted mean free T4 index; however, there was no trend with serum 2,3,7,8-TCDD concentrations (Calvert et al., 1999). TSH was not different between workers and referents.

The 1987 Ranch Hand study indicated a nonsignificant reduction of T3% uptake but this was not measured in the 1991 study (Table 7-31). A slight increase in the mean level of TSH (Table 7-32) with increasing serum 2,3,7,8-TCDD level was noted in both 1987 and 1991; these results, however, did not reach statistical significance (Roegner et al., 1991; Grubbs et al., 1995). Among Army Vietnam veterans, mean TSH levels, but not mean free thyroxine index (FTI) levels, were statistically significantly higher than among non-Vietnam veterans, after adjustment for the six entry characteristics of age and year of enlistment, race, enlistment status, general technical test score, and primary military occupation (Table 7-31) (Centers for Disease Control Vietnam Experience Study, 1988a). However, the percent of values that were out of reference range did not differ significantly for TSH (Vietnam veterans, 1.0%; non-Vietnam veterans, 0.6%, OR = 2.0, 95% CI = 0.9-4.3) and FTI (Vietnam veterans, 5.4%; non-Vietnam veterans, 4.6%, OR = 1.2, 95% CI = 0.9-1.5). The exposure levels were low, based on the sample for which 2,3,7,8-TCDD was measured.

7.13.4.1.1. ***Comment.*** Few human studies examined the relationship between 2,3,7,8-TCDD exposure and thyroid function, and the results of present research are equivocal. These studies examined individuals with lower exposure to 2,3,7,8-TCDD than the animals, and exposure was chronic. The data from the Ranch Hand and the NIOSH studies, which measured serum 2,3,7,8-TCDD concentrations, suggest that in adults there are few long-term effects on adult thyroid function.

7.13.4.2. *Developmental Effects*

Two series of studies, both in The Netherlands but conducted in different communities, examined thyroid function in infants and related this to dioxin, furans and/or PCB levels in breast milk, cord blood, or third-trimester materal serum samples.

The first study, among infants in Amsterdam (Pluim et al., 1992; Pluim et al, 1993), examined thyroid function among 38 full-term breast-fed infants in relation to the total toxic equivalents per kg of breast milk fat (TEQ/kg) of dioxins and furans (listed in Table 7-21b and described in detail in Section 7.12.2.3) . The authors measured total T4, thyroxine-binding globulin (TBG), and thyroid stimulating hormone (TSH) levels sequentially in cord blood, infants at 1 week of age, and infants at 11 weeks of age (Tables 7-30 and 7-33). Total T3 was measured in cord blood and at 11 weeks, and free T4 was measured in cord blood. Infants were classified into "high" and "low" groups at the median of the range. At 1 week and 11 weeks postnatally, total T4 and total T4/TBG ratios were significantly higher among infants in the high group. At 11 weeks, TSH was also significantly higher for the high group. The authors suggest that exposure to high levels of dioxins and furans, either *in utero* or through breast milk, modulates the hypothalamic-pituitary-thyroid regulatory system of the infant (Pluim et al., 1992; Pluim et al., 1993).

A study conducted over approximately the same time period in Rotterdam, The Netherlands, examined thryoid function in 105 mother-infant pairs (Koopman-Esseboom et al., 1994c). Exposure was estimated using breast milk collected in the 1-2 weeks after delivery. As in the other study, the mother-infant pairs were split into two groups at the median dioxin-furan TEQ based on the congeners listed in Table 7-21b (see details in Section 7.12.2.3.). The authors measured total T4, total T3, free T4, and TSH levels in the mother during the last month of pregancy and 9-14 days post delivery, in cord blood, and in infants at 9-14 days and three months after birth (Tables 7-30 and 7-33). Of those enrolled in the study, 78 mother-infant pairs met all criteria and were included in the final analyses. All the thyroid measures were within normal ranges, with the exception of TSH for one woman. All of the TEQs (dioxin-furan TEQ, coplanar PCB TEQ, nonplanar PCB TEQ, and total PCB-dioxin-furan TEQ) were significantly correlated with infant plasma levels of TSH at the second week and third month, and inversely correlated with total T3 pre-delivery and with total T3 and total T4 post-delivery for the mothers. The only exception is that the nonplanar PCB TEQ was not significantly correlated with the mothers total T4 after delivery and the infants' third month TSH. Measures from the infants during their second week of life showed a significant increase in TSH (Table 7-33) and a significant decrease for total T4 and free T4 (Table 7-30) for infants in the "high" group.

7.13.4.2.1. ***Comment.*** Two studies of nursing infants suggest that ingestion of breast milk with a higher dioxin-furan TEQ may alter thyroid function (Pluim et al., 1993; Koopman-Esseboom et al., 1994c). Both studies had similar exposure groupings and some findings in common: both had significant increases in TSH at about 3 months of age with higher TEQs, and in one report, significant increases at about 2 weeks of age and in the cord blood. Significant changes were found with high TEQ for total T4, but they were in opposite directions, increased in neonates at 1 and 11 weeks of age (Pluim et al., 1993) and decreased for infants during the second postnatal week (Koopman-Esseboom et al., 1994c). Koopman-Esseboom and her colleagues also noted a significant increase in T4/TBG and a significant decrease in free T4. Both studies covered a short observation period, which limits the examination of persistent or long-term changes in thyroid status, and analyses did not control for other factors that might affect thyroid status. These findings suggest a possible shift in the distribution of thyroid hormones, and point out the need for collection of longitudinal data to assess the potential for long-term effects associated with developmental exposures.

These two developmental studies investigated relatively small numbers of individuals with thyroid parameters in the normal range. However, the high group, at about 3 months of age, had increased TSH levels in comparison to the low group. Total T4 levels and total T4 to thyroid binding globulin (TBG) ratio were generally elevated in the high infants.

The exact processes accounting for these observations in humans are unknown, but when put in perspective of animal responses, the following might apply: dioxin-furan increases the metabolism and excretion of thyroid hormone, mainly T4, in the liver. Reduced T4 levels stimulate the pituitary to secrete more TSH, which enhances thyroid hormone production. Early in the disruption process, the body can overcompensate for the loss of T4, which may result in a small excess of circulating T4 to the increased TSH. In animals, given higher doses of dioxin, the body is unable to maintain homeostasis, and TSH levels remain elevated and T4 levels decrease.

7.13.5. Adult Reproductive System

7.13.5.1. *Hormones*

In laboratory rats, high doses of 2,3,7,8-TCDD have been related to decreased testosterone levels, with evidence that dioxin decreases testosterone synthesis (Kleeman et al., 1990; Mebus et al., 1987; Moore and Peterson, 1988; Moore et al., 1985).

A reported symptom of men who were exposed to 2,3,7,8-TCDD-contaminated materials as a result of daily exposure and industrial accidents is reduced libido (Baader and Bauer, 1951; Bauer et al., 1961; Suskind et al., 1953). Two independently conducted studies of West Virginia TCP workers noted that exposed study subjects also reported this condition approximately 50%

more often than either the unexposed controls or individuals without chloracne (Moses et al., 1984; Suskind and Hertzberg, 1984). Endocrine studies or evaluations of conditions or situations that may lead to a reduction in libido were not conducted.

In the NIOSH study of TCP production workers, questions regarding libido were not asked; however, reproductive hormone levels were measured and related to serum 2,3,7,8-TCDD levels. In linear regression analyses, serum 2,3,7,8-TCDD was positively and significantly related to serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and inversely related to total testosterone after adjustment for potential confounders ($p<0.05$) (Egeland et al., 1994). The prevalence of abnormally low testosterone was two to four times higher among workers with serum 2,3,7,8-TCDD levels of 20-75 pg/g (OR = 3.9, 95% CI = 1.3, 11.3), 76-243 pg/g (OR = 2.7, 95% CI = 0.9, 8.2), or ≥244 pg/g (OR = 2.1, 95% CI = 0.8, 5.8) than among unexposed referents (4.8%) (mean serum 2,3,7,8-TCDD = 7 pg/g). Workers in these same serum 2,3,7,8-TCDD quartiles had a higher prevalence of abnormally high LH than workers with serum 2,3,7,8-TCDD levels of 244 pg/g to 3,400 pg/g, but the differences between each serum 2,3,7,8-TCDD category and referents were not significant.

The Ranch Hand veterans study provides the only other human data available that evaluate the relationship between serum 2,3,7,8-TCDD and testosterone (Roegner et al., 1991). Ranch Hand veterans with current serum dioxin levels exceeding 33.3 pg/g were reported to have a lower mean total serum testosterone level (515.0 ng/dL) than the nonexposed comparison group (525.2 ng/dL), but the difference was statistically nonsignificant. No association was observed with FSH and LH. In additional analysis by Henriksen et al. (1997) no association was found between abnormally high or abnormally low testosterone level and dioxin exposure category. Testicular abnormality was assessed by physician palpation at the 1987 physical examination (Roegner et al., 1991). Because of this finding, testicular volume was measured by ultrasound at the 1992 physical examination (Grubbs et al., 1995). No association was found between testicular volume measured by ultrasound and dioxin exposure category in 1992 (Grubbs et al., 1995).

Testosterone, FSH, and LH were also measured in U.S. Army veterans and non-Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988a). No significant differences in hormone means were noted between the two groups. Additionally, the proportions of values outside the reference range were also similar.

7.13.5.1.1. ***Comment.*** The human data offer some evidence of alterations in male reproductive hormone levels associated with substantial occupational exposure to 2,3,7,8-TCDD. The results support the animal literature, in which dioxin-related effects have been observed on the hypothalamic-pituitary-Leydig-cell axis and on testosterone synthesis.

7.13.5.2. ***Endometriosis***

After noting the prevalence and severity of endometriosis in rhesus monkeys chronically exposed to 2,3,7,8-TCDD (Rier et al. 1993), investigators started looking at endometriosis in humans. The first reported effort was a case-control study (Mayani et al., 1997) comparing 79 women, all treated in an infertility clinic during 1991-1995, some with endometriosis (N = 44), and the comparisons with tubal infertility (N = 35). All women underwent laparoscopic examination for diagnosis and scoring of endometriosis. Altogether, 9 women had 2,3,7,8-TCDD above the limits of detection: 2.9% of the controls (N = 1 of 35), 12.5% of those women with Stage I-II endometriosis (N = 3 of 24), and 25% of those with Stage III-IV endometriosis (N = 5 of 20). Logistic regression was used to control for potential effects of the different racial/ethic compositions of the cases and controls. The results of this analysis, compared to other unadjusted analyses, are not explicitly identified, but are probably OR = 7.6 (95% CI = 0.87-169.7). The authors did not present sufficient information on their data analyses to evaluate them (for example, whether actual levels of 2,3,7,8-TCDD were entered, or whether detectable levels were observed), but did note the limited power of this effort. An exposure-severity relationship was not observed. However, the frequency of exposure increased with increasing severity (not statistically tested).

A recent report (Pauwels et al., 1999) examined 101 infertile women treated at a collaborating Center for Reproductive Medicine in Belgium between 1995 and 1998. The couple were defined as infertile after attempting pregnancy for at least 1 year without success. Using laproscopic examination, 42 women were diagnosed with endometriosis; 25 women had mechanical infertility (e.g., tubal disease), 8 husbands of 20 without diagnosis were found to be infertile. Fourteen women were excluded from analysis because of ovulatory dysfunction. CALUX TEQs were generated using serum (N = 101), adipose tissue (N = 46) and follicular fluid (N = 8) based on major PCB congeners and chlorinated pesticides. In preliminary analyses and using a cut point of 100 pg TEQ/g serum lipid weight, the investigators observed proportionately more women with endometriosis with high TEQs (17%) compared to the controls (4%) (OR = 4.0, NS).

Finally, a study of endometriosis looked at each woman's history of breast milk consumption (Ikezuki et al., 1999). The authors hypothesized that women who were breast fed as infants would have higher dioxin levels and subsequently higher rates of endometriosis than would those who had been formula fed. A total of 2,848 women were queried: 2,281 from 8 companies participating in the project, and 567 women in the Japanese Endometriosis Association or who had surgery at Tokyo University Hospital. The proportions observed were the reverse of those hypothesized: more controls had been breast fed (68%) than had women with endometriosis

(51%). These data are of questionable use because of limited ascertainment of exposure, lack of knowledge about potential cases missed by the recruitment methods, and lack of detail about the comparability of the case and comparison groups.

7.13.5.2.1. ***Comments.*** The first two reports of infertility patients (Mayani et al., 1997; Pauwels et al., 1999) raised the potential for an association between endometriosis and 2,3,7,8-TCDD or TEQ exposure. These studies are small and of limited power. Studies currently underway will greatly add to the database on this outcome. The study comparing breast-fed women to those who were bottle-fed contained little concrete data on exposure and, most likely, incomplete and potentially biased selection of cases, and thus are of limited use for examining the relationship of dioxin to endometriosis.

7.13.6. Diabetes

Diabetes and fasting serum glucose levels were evaluated in cross-sectional medical studies because of the apparently high prevalence of diabetes and abnormal glucose tolerance tests in one case report of 55 TCP workers (Pazderova-Vejlupkova et al., 1981). In this group, evaluated 10 years after exposure ended, approximately 50% of the subjects had either confirmed cases of diabetes or abnormal glucose tolerance tests.

The results of later medical studies are mixed. Cross-sectional studies of workers from Nitro, West Virginia (Suskind and Hertzberg, 1984; Moses et al., 1984), found no difference in glucose levels between the exposed and control populations, although no quantitative values were presented in either study. Similarly, the adjusted odds ratio for out-of-range fasting glucose levels comparing Vietnam veterans to non-Vietnam veterans was not statistically significant (OR = 1.0, 95% CI = 0.4-2.2) (Centers for Disease Control Vietnam Experience Study, 1988a). But a comparison of the adjusted mean fasting glucose levels between the two groups was marginally significant (Vietnam veterans, 5.2 mmol/L; non-Vietnam veterans, 5.1 mmol/L, $p<0.05$). Mean fasting glucose levels in the BASF accident cohort were marginally elevated compared to the referent population and were associated with current levels of 2,3,7,8-TCDD ($p = 0.062$), but not the back-extrapolated level (Ott et al., 1994). In the NIOSH study, 9.3% of workers and 7.0% of age-matched comparisons met the case definition for diabetes. Although 60% of workers with serum 2,3,7,8-TCDD concentrations above 1,500 pg/g lipid met the case definition for diabetes, prevalence of diabetes mellitus was not related to serum 2,3,7,8-TCDD concentrations (Calvert et al., 1999). However, adjusted mean serum glucose levels (5.45 mmol/l, $p<0.03$) were significantly elevated for workers with half-life extrapolated 2,3,7,8-TCDD concentrations between 1,860 and 30,000 pg/g lipid.

Results from the Ranch Hand study (Henriksen et al., 1997) suggest that serum 2,3,7,8-TCDD levels may be positively and significantly related to diabetes and 2-hour postprandial glucose levels. Every participant who underwent at least one examination (1982-1992) was considered for inclusion in this analysis. Diabetic status was assessed by measuring 2-hour postprandial glucose and by using a case definition of diabetes. Diabetes was defined as having a verified history of diabetes mellitus by diagnosis or an oral glucose tolerance test of $\geq$11.1 mmol/L (200 mg/dL). In this analyses, the cohort was categorized by current and half-life extrapolated serum 2,3,7,8-TCDD concentrations (in pg/g lipid): Comparisons current < 10; Ranch Hands—background current < 10, low 10 < current and half-life extrapolated < 94, high 10 < current and half-life extrapolated > 94. Median serum 2,3,7,8-concentrations in pg/g lipid for the Ranch Hand groups are: low: 15.0 (range 10.0 - 26.6); high: 46.2 (range 18.0 - 617.8). Risk of diabetes mellitus was moderately increased for both low (RR = 1.3, 95% CI = 1.0-1.7) and high (RR = 1.5, 95% CI = 1.2-2.0) groups but not for the background group. For Ranch Hands participating in the 1992 examination, the risk for being classified as having an impaired fasting glucose level (140 mg/dl-200 mg/dl based on the 2-hour postprandial glucose test) was also moderately increased for the high group (RR = 1.6, 95% CI = 1.2-2.2). The data also suggest that Ranch Hands in the high group have a shorter time to diabetes onset and are more likely to use some kind of control for their diabetes, with a greater percentage using oral medication.

The outcome measures used in the Ranch Hand study, presence of diabetes, 2-hour postprandial, and fasting, did not permit a determination of the type of diabetes involved. However, it shows that nondiabetic Ranch Hands with higher current (>10 pg/g lipid) or past (>94 pg/g lipid half-life extrapolated) serum 2,3,7,8-TCDD concentration may be at a slightly greater risk for developing diabetes than individuals with background levels of 2,3,7,8-TCDD. These individuals also appear to have a greater prevalence of elevated fasting glucose levels, which may be a precursor to conversion to a diabetic state.

Mortality from diabetes was assessed in the NIOSH and IARC occupational cohorts (Steenland et al., 1999; Vena et al., 1998) and among adult residents of zones A, B and R in Seveso, Italy (Pesatori et al., 1998). In the NIOSH cohort, mortality due to diabetes was slightly but nonsignificantly elevated when considering diabetes only as an underlying cause of death (SMR = 118, 95% CI = 77-173) or if there was any mention of diabetes on the death certificate (multiple causes of death analysis) (SMR=89, 95% CI = 87-133). The results were the same in a cohort of 608 workers with chloracne (SMR=106, 95% CI = 29-271). When compared to an internal control group, there was a inverse dose-response relationship between mortality from diabetes and exposure score.

In the subset of workers in the IARC cohort exposed to 2,3,7,8-TCDD or chlorophenols N=13,831), there were modest, nonsignificant elevations in the risk of death from diabetes controlling for any workplace exposure to TCDD/HCDD (RR = 2.25, 95% CI = 0.53-9.50 N = 33) for duration of exposure (RR = 2.52, 95% CI = 0.89-7.11, N = 10 for 10-19 years), time since first exposure (latency) (RR = 2.34, 95% CI = 0.56-9.83, N = 14 for 10-19 years) and year of first exposure (1955-1964, 1965+) (RR = 1.76, 95% CI = 0.58-5.31, N = 10 for 1965+) using Poisson regression analysis.

In the Seveso cohort, very modest, statistically significant elevations in mortality from diabetes were observed only in females of zones B (RR = 1.9, 95% CI = 1.1-3.2, N = 13) and R (RR = 1.2, 95% CI = 1.0-1.6, N = 74) but not in females of zone A or males from any zone (Pesatori et al., 1998). Among males, statistically nonsignificant increased mortality was seen in zone B (RR = 1.3, 95% CI = 0.6-2.9) and in zone R (RR = 1.1, 95% CI = 0.8-1.6). No deaths from diabetes occurred in males of zone A. Given the small number of individuals in zone A, there is little power to detect an effect of TCDD exposure on mortality from diabetes.

Analysis of the risk of alteration in glucose metabolism among individuals exposed only to environmental levels of 2,3,7,8-TCDD was conducted using subjects who participated as comparisons in the 1992 examination phase of the Ranch Hand study (Longnecker and Michalek, 2000). Diabetes prevalence and serum insulin and glucose levels were compared to serum 2,3,7,8-TCDD concentration. Median serum 2,3,7,8-TCDD concentration was 4.0 ng/kg (or 4 pg/g) lipid, which included 108 subjects with levels below the limit of detection and who were assigned a level of 0.625 mg/kg. Multivariate regression analysis (adjusted for age, race, body mass index, waist size, family history of diabetes, body mass index at the time the dioxin was measured, military occupation, and triglycerides) suggests a slight increase in prevalence of diabetes with increasing 2,3,7,8-TCDD serum concentration; however, the risk did not rise monotonically with increasing serum concentration. All odds ratios calculated were compared to subjects in Quartile 1 who had < 2.8 ng/kg [Quartile 2, 2.8 - <4.0 ng/kg, OR 0.91, 95% CI = 0.50-1.68; Quartile 3, 4.0 - <5.2 ng/kg, OR 1.77 95% CI = 1.04-3.02; Quartile 4, < 5.2 ng/kg OR 1.56, 95% CI = 0.91-2.6]. Similar results were found when comparing serum 2,3,7,8-TCDD level and the estimated change in level of insulin: [Quartile 2, 2.8 - <4.0 ng/kg, 0.13, 95% CI = 0.00-0.25; Quartile 3, 4.0 - <5.2 ng/kg, 0.11 95% CI = -0.02 - 0.24; Quartile 4, < 5.2 ng/kg 0.14, 95% CI = 0.01-0.27].

7.13.6.1. *Comment*

Results of the available epidemiologic studies provide a patchwork of evidence that limits a conclusion of a strong causal relationship between occupational or environmental exposure to

2,3,7,8-TCDD and alterations in glucose metabolism. Diabetes is a particularly difficult outcome to study using the designs described above. Although the cross-sectional studies attempted to limit methodologic biases by using standard case definitions for diabetes and eliminating cases that occurred prior to exposure, cases that did not participate in the study or died prior to the study were lost to the analysis. This may have the effect of depressing the outcome measure, which may have been the case in the NIOSH study (Calvert et al., 1999). Mortality studies commonly miss diabetes as a cause of death because the many potentially fatal conditions manifested as a result of the diabetes are recorded as the primary cause of death. This should not introduce differential bias into the study because it is assumed that the death certificates of both the worker and comparison populations are treated in the same way. Steenland et al. (1999) attempted to remedy this problem by including in the analysis all recorded causes of death, yet they did not find excess mortality from diabetes among workers, even the group thought to be more highly exposed. This approach, called "multiple cause of death analysis" was not used in the analysis of the IARC or Seveso cohorts. In addition, it appears that in none of the above mentioned mortality studies was onset of diabetes in relationship to exposure considered in the analysis. Therefore, workers whose onset of diabetes occurred prior to exposure were included in the analysis, potentially overestimating the risk of exposure.

Furthermore, the patchwork of findings may also be attributed, in part, to the heterogeneous etiology of diabetes and, perhaps, the interrelationship between the intrinsic factors and exposure to 2,3,7,8-TCDD .

In addition, the National Academy of Sciences' Institute of Medicine (IOM) conducted a review of the scientific evidence on the relationship between exposure to 2,3,7,8-TCDD and Type 2 diabetes (IOM, 2000). In this review, the committee examined the results of four mortality studies of chemical workers (Steenland et al, 1999; Vena et al, 1998) and Seveso residents (Pesatori et al., 1998) and also morbidity studies of chemical workers (Calvert et al., 1999), Ranch Hands (Longnecker and Michalek, 2000; Air Force Health Study, 2000) and Vietnam veterans from Australia (Commonwealth Department of Veterans' Affairs, 1998a,b). The committee concluded that based on the collective evidence of the data and not on a single study that ". . . there is limited/suggestive evidence of an association between exposure to the herbicides used in Vietnam or the contaminant dioxin and Type 2 diabetes." They also noted that the observed increased risk of Type 2 diabetes in Vietnam veterans related to herbicide exposure was small and that, in this population, known risk factors, including family history, obesity and physical inactivity were more strongly related to the prevalence of diabetes.

7.13.7. Immunologic Effects

7.13.7.1. *Adult Effects*

Animal toxicologicalal studies have demonstrated numerous immunologic effects after exposure to 2,3,7,8-TCDD (see Chapter 4 for a more comprehensive review). In humans, the information with which to assess the immunologic consequences of exposure is sparse. A number of epidemiologic studies and one case report have described the immunologic function of populations exposed to 2,3,7,8-TCDD (Evans et al., 1988; Hoffman et al., 1986; Webb et al., 1989; Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991; Jennings et al., 1988; Reggiani, 1978; Ott et al., 1994; Tonn et al., 1996; Ernst et al., 1998; Halperin et al., 1998; Jung et al., 1998; Michalek et al., 1999). One study of extruder personnel exposed to brominated dioxins and furans was also reported (Zober et al., 1992).

Evaluation of the immunologic status in exposed residential populations has not found a relationship between exposure and impaired status. Immunocompetence was tested twice in 44 children who were residents of the region of Seveso with the highest 2,3,7,8-TCDD contamination and in 43 age-matched children who did not reside in the contaminated area (Reggiani, 1978). Twenty of the exposed children had chloracne and 24 had no skin lesions. The tests included serum immunoglobulins, complement levels, lymphocyte subpopulations, and lymphocyte activity analysis. Although no data were presented, the authors reported that the various measures were within normal range and that there was no difference between the two groups.

Initial studies of Missouri residents who had the potential for exposure to 2,3,7,8-TCDD-contaminated soil suggested that 2,3,7,8-TCDD caused depression in cell-mediated immunity (delayed hypersensitivity), as demonstrated by a statistically significant increase in anergy (exposed vs. nonexposed: 11.8% vs. 1.1%) (Hoffman et al., 1986). This study, however, was limited by the exclusion from test results of 61% and 32% of the exposed and unexposed groups, respectively. A follow-up study confirmed the presence of substantial bias in the first study. Evans et al. (1988) retested 28 of the 50 exposed residents and 15 of the 27 unexposed residents who did not respond (anergic) or responded weakly (relatively weakly) to an antigen challenge in the first study. A follow-up study could not confirm the presence of anergy. Both studies found in the exposed residents increased frequencies in CD4/CD8 ratio of less than 1.0 (Table 7-34). No other abnormalities were noted by Hoffman et al. (1986) (Tables 7-35 to 7-40).

In a later study of Missouri residents, Webb et al. (1989) found no clinical evidence of immunosuppression in 40 individuals whose adipose 2,3,7,8-TCDD levels ranged from under 20 pg/g to over 430 pg/g (top of range not given). Tests included serum immunoglobulins, T-cell surface markers OKT3, OKT4, OKT8, OKT11, Leu11c, CD4/CD8 ratio, (CD4 + CD8)/CD3, and B1 and B2 cells. In logistic regression, significant (p<0.05) relationships were noted for IgG,

%CD3, %T11, %CD8, and %CD4 + LEU8 POS, controlling for age and sex (Tables 7-35 to 7-40).

The effect of past occupational exposure on immunologic function was examined in 18 British workers who were evaluated 17 years after accidental industrial exposure to chemicals contaminated with 2,3,7,8-TCDD (Jennings et al., 1988). It is not clear from the article when occupational exposure to 2,3,7,8-TCDD ended for these workers. Exposed workers and unexposed controls were matched for age, race, sex, smoking habits, alcohol consumption, and percent of ideal body weight. There were no significant differences in the levels of immunoglobulins, T and B lymphocytes, responsiveness to phytohemagglutinin A, and in the number of CD4 and CD8 counts. Three measures were found to be statistically significantly ($p<0.05$) higher in workers than in controls: antinuclear antibodies (ANA) (8 workers vs. 0 controls, $p<0.01$) (when Hep2 cells were used as substrate but not when rat liver cells were used), immune complexes (workers = 11 vs. 3 controls, $p<0.05$), and natural killer cells (NK) (workers = 0.21×10^6/L vs. controls = 0.59×10^6/L, $p<0.002$) identified by the monoclonal antibody Leu-7 (Table 7-41). No evidence of a consistent relationship between dioxin exposure and immune system alteration, as indicated by antinuclear antibodies, were found in the Ranch Hands Study (Michalek at al., 1999). In the discussion, the authors could not explain the physiologic basis of their findings and suggested that further research was needed.

Among participants in the BASF accident study cohort, with the exception of natural killer cells and helper-inducer cells, the proportions of some lymphocyte populations (B cells, T-cells, T helper cells, T suppressor cells) were lower among workers, but the distribution of cells in referents and workers was equivalent (Ott et al., 1994). Levels for IgA, IgG, IgM, and complement C4 and C3 were slightly higher in workers than in the unexposed referent population. There also appeared to be slight dose-related increases in IgA, IgG, and complement C4 with 2,3,7,8-TCDD levels measured in October 1988 and February 1992. IgA and IgG were related to the half-life extrapolated 2,3,7,8-TCDD levels. It must be noted, however, that the statistically significant relationship between IgA and 2,3,7,8-TCDD is most likely due to a case of liver cirrhosis and the association with IgG due to a liver carcinoma.

Eleven German workers, exposed to chemicals contaminated with 2,3,7,8-TCDD and other polychlorinated dioxins between 1966 and 1976 during production of 2,4,5-T or maintenance activities were studied by Tonn et al. (1996). Between 1989-1992, serum 2,3,7,8-TCDD concentrations were measured. The levels ranged from 43 to 874 pg/g lipid. No differences were noted between the exposed and control groups for the lymphocyte subsets tested or response to mitogen stimulation. However, in TCDD-exposed workers, the response of T-cells to irradiated stimulator cells or IL-2 was statistically significantly decreased.

In the NIOSH study, measures of immunologic status and function were evaluated (Halperin et al., 1998). Of all the parameters examined, only a slight decrease in the number and

proportion of activated T-cells appeared to be related to 2,3,7,8-TCDD serum concentration: CD3/Ta1 k/mm^3 (number of activated cells) (TCDD 52-125 pg/g, OR = 2.7, 95% CI = 1.4-1.5; TCDD 126-297 pg/g, OR = 2.6, 95% CI = 1.4-4.9; TCDD 298-3389 pg/g, OR = 2.4, 95% CI = 1.3-4.6), and CD3/Ta1 (%) (TCDD 52-125 pg/g, OR = 2.5, 95% CI = 1.3-4.8; TCDD 126-297 pg/g, OR = 2.3, 95% CI = 1.2-4.3; TCDD 298-3389 pg/g, OR=2.4, 95% CI = 1.3-4.5).

Jung et al. (1998) and Ernst et al. (1998) evaluated the immunologic status and function of workers exposed to PCDDs and PCDFs during production of 2,4,5-T and other chlorophenols. The cohort of exposed workers was previously described by Flesch-Janys et al. (1992). Jung et al. (1998) included all 192 workers in their analysis. The median serum TCDD concentration of this group was 36.1 pg/g lipid, with a range from 1.2 to 893.2 pg/g lipid. With the exception of a significant negative correlation between $CD3^+/CD8^+$ cells and logTEQ, no other parameters were found to be exposure-related.

Workers included in the analysis by Ernst et al. (1998) had a median serum TCDD level of 217 pg/g lipid, ranging from 33.6 to 2,732 pg/g lipid. The serum TCDD concentrations in the 28 age-matched control approximated normal at 4 pg/g lipid. Exposed subjects worked a minimum of 7 years in the tricholorophenol and 2,4,5-T departments. Compared to controls, the exposed group had significantly increased proportions of $CD8^+CA45R0^+$ (cytotoxic memory T-cells) and activated $CD8^{dim}CA57^+$, and a significantly lower number of lymphocytes with naive phenotype $CD45R0^+$. The authors also report that interferon release in diluted whole-blood cultures, but not in isolated peripheral blood mononuclear cells, was statistically significantly decreased in TCDD-exposed workers.

Among the 21 extruder personnel exposed to both 2,3,7,8-TBDD and 2,3,7,8-TBDF, of the 16 parameters tested, only complement C4 was statistically significantly ($p<0.01$) associated with concentrations of 2,3,7,8-TBDD and 2,3,7,8-TBDF (Zober et al., 1992). Borderline associations were noted between 2,3,7,8-TBDF and decreases in total lymphocyte count ($p = 0.056$), T-cell count ($p = 0.045$), T-helper cell count (CD4) ($p = 0.045$) and an increase in complement C3 (0.054), and between 2,3,7,8-TBDD and a decreased percent lymphocyte count ($p = 0.054$). However, with the exception of complement C3, the associations appear to be driven by a single individual with the highest 2,3,7,8-TBDD levels (478 pg/g of lipid) who, at the time of the study, exhibited no evidence of clinical immunodeficiency.

Two studies extensively evaluated immunologic function in Vietnam veterans. No significant differences were noted among U.S. Army ground troops and the comparison population in lymphocyte subset populations, T-cell populations, or serum immunoglobulins (Tables 7-34 to 7-40) (Centers for Disease Control Vietnam Experience Study, 1988a). Comprehensive immunologic profiles were developed for each participant of the U.S. Air Force Ranch Hand Study (Tables 7-34 to 7-40) (Roegner et al., 1991). Significant positive associations

were found between IgA and serum 2,3,7,8-TCDD. The authors suggest that the rise in IgA is consistent with a subclinical inflammatory response, but the authors could not explain the source of the inflammatory response. In analysis of the 1992 examination round, Michalek et al. (1999) found no relationship between category of serum TCDD concentration and immunologic changes. The study population was classified by median serum TCDD concentration: Comparison: 4.0 pg/g lipid, range 1-10 (current level); Ranch Hand background: 5.7 pg/g lipid, range 1-10 (current level); Ranch Hand low: 52.8 pg/g lipid, range 28-94 (half-life extrapolated); Ranch Hand high: 194.7 pg/g lipid, range 94-3,290 (half-life extrapolated). Of all the tests performed, only prevalence of an abnormal skin test, represented as a composite score and the proportion of CD20 cells in the background group, and the absolute count for $CD16^{+}CD56^{+}CD3^{-}$ were significantly different than the referent population.

7.13.7.1.1. ***Comment***. The information on immunologic function in humans, children or adults, relative to exposure to 2,3,7,8-TCDD is scarce. All but one of the epidemiologic studies are restricted to adults and do not describe a consistent pattern of effects among the studies. Natural killer cells (NK) were increased among one population of 2,3,7,8-TCDD chemical workers examined 17 years after exposure ended (Jennings et al., 1988). These findings were not corroborated in Ranch Hands (Roegner et al., 1991; Michalek et al, 1999), the BASF accident cohort (Ott et al., 1994), the NIOSH cohort (Halperin et al., 1998), the Hamburg cohort (Jung et al., 1998; Ernst et al., 1998), or workers exposed to 2,3,7,8-TBDD and TBDF (Zober et al., 1992). Dose-related elevations in IgA were observed in Ranch Hands in relation to current levels and in the BASF accident cohort with respect to both current and half-life extrapolated 2,3,7,8-TCDD levels. Yet, IgA was not higher in adult Missouri residents with adipose 2,3,7,8-TCDD levels above background (Webb et al., 1989). IgG was also significantly related to 2,3,7,8-TCDD in the BASF accident cohort but not in Ranch Hands (Michalek et al., 1998). While complement C3 and C4 were elevated in both the BASF accident cohort and the extruder personnel, no other study examined these endpoints.

The effect of acute, high exposure to 2,3,7,8-TCDD among children from Seveso was reportedly negative within 2 years after exposure (Reggiani, 1978). Although no data have been published illustrating the values obtained from the tests of immunologic function in these children, the author indicates that the measured parameters were no different in the exposed and unexposed children after two series of tests.

More advanced functional analyses have been conducted relating to the ability of T-cells to respond to intercellular stimulators such as the interleukins and interferon (Tonn et al., 1996; Ernst et al., 1998). These studies are suggestive of a decreased ability of T-cells to respond in individuals more heavily exposed to PCDDs and PCDFs. More work needs to be done in

similarly exposed populations to confirm these findings and to determine the mechanism of action.

More comprehensive evaluations of immunologic function with respect to 2,3,7,8-TCDD exposure are necessary to assess more definitively the relationships observed in nonhuman species. Longitudinal studies of the maturing human immunologic system may provide the greatest insight, particularly because animal studies have found many of their significant results in immature animals and breast milk is a source of 2,3,7,8-TCDD and other related compounds. Additional studies of highly exposed adults may also shed light on the effects of long-term chronic exposures. Therefore, there appears to be too little information to suggest definitively that 2,3,7,8-TCDD, at the levels observed, is an immunotoxin in humans.

7.13.7.2. *Developmental Effects*

One report (Weisglas-Kuperus et al., 1995) has examined direct and surrogate measures of immune status in 207 babies in Rotterdam (study described in detail in Section 7.12.2.3.). The surrogate measures were derived from questionnaires given to the mothers, covering incidence of rhinitis, bronchitis, tonsillitis, and otitis in children up to 18 months of age. Almost all of the children (205) were immunized against measles, rubella, and mumps; the children's antibody levels to these were used to assess humoral antibody production. For the purposes of this report, cord bloods from 48 of these children were analyzed to assess prenatal TEQ levels; at age 3 months, 47/48 bloods were drawn from the original group, with another child, randomly selected, added to this group. At 18 months, 37 of the original children gave blood, and 6 other children, randomly selected, were added to this group. In these samples, the following were measured: monocytes, granulocytes, and lymphocytes in whole blood; lymphocyte subpopulations were determined using monoclonal antibodies. No relationship was found between pre- and postnatal total TEQ levels and respiratory tract symptoms (i.e., number of periods with rhinitis, bronchitis, tonsillitis, and otitis) or humoral antibody production at 18 months to vaccination against mumps, measles, and rubella at 14 months. A higher prenatal exposure, estimated by cord blood levels, was associated with alterations in T cell subsets, with an increased number of $TcR\gamma\delta^+$ T cells; increased total numbers of T cells, $CD8^+$ cells, and $TcR\gamma\delta^+$ T cells at 18 months of age were associated with higher TEQ levels (Table 7). Higher TEQ levels were also associated with a decreased number of monocytes (Total TEQ, Dioxin-furan TEQ, mono-ortho PCB TEQ, and Di-ortho PCB TEQ) and granulocytes (Total TEQ only) at 3 months. All values were found to be within clinically "normal" ranges. The authors suggested that the subtle changes in the number of blood leukocytes do not necessarily mirror alterations in the cell composition of lymphoid and nonlymphoid organs, nor do they necessarily reflect functional defects (Weisglas-Kuperus et al., 1995).

In an update to the report described above (Weisglas-Kuperus et al. 2000), 193 children were examined at 42 months of age. Questionnaires were completed for 175, including questions on infection and allergic disease in the children. Blood samples were collected on a subsample of 85 children, limiting data on concurrent PCB levels (PCBs 118, 138, 153, 180) in plasma and immunologic marker analyses in lymphocytes. Examination of questionnaire reports of infectious diseases and allergies, only reduced levels of "attacks of shortness of breath with wheeze was associated with prenatal PCB exposure (n=175; OR=0.44; 95% CI: 0.18-0.99); more associations were observed with current PCB levels (n=85): increases in recurrent middle ear infections (OR=3.05; 95% CI: 1.17-7.98); chicken pox (OR=7.63; 95%CI:1.21-48.54); and reduced allergic reactions (OR=0.01; 95%CI:0.01-0.37). Dioxin-furan TEQ at birth was associated with increased coughing, chest congestion, and phlegm (OR=1.06; 95%CI:1.00-1.11). Total PCB levels at 42 months were significantly lower for formula fed children (0.21 ug/l versus 0.75 ug/l, $p<0.05$); however, no significant differences were observed for recurrent middle ear infections, chicken pox or allergic reaction. Interestingly, when these were examined by duration of breast feeding, infections and chicken pox were lower and allergic reactions were higher with longer breast feeding (all were borderline non-significant at p=0.06 or 0.07), even though the total PCB levels at 42 months were over 70% higher (not significant). Positive associations were observed between the total PCB levels at birth (cord blood, maternal blood or both) and lymphocytes, CD3+, CD3+CD8+, CD4+CD45RO+, and CD3+HLA-DR+ T-cells; no associations were observed with the current PCB levels or with the dioxin-furan TEQ at birth. The authors concluded that effects of perinatal exposures persist, and are associated with a greater likelihood of infectious disease but less likelihood of allergic conditions. In addition, they concluded that the benefits of longer periods of breast feeding helped to counteract the effects of exposure.

7.13.7.2.1. ***Comment***. These data suggest some long term effects are occurring. Repeating these investigations in other groups would be informative.

7.13.8. Neurologic Effects

Although there are few studies reporting neurologic abnormalities related to 2,3,7,8-TCDD exposure in adult animal models, neurologic effects are reported to have occurred shortly after exposure in occupationally exposed individuals (Ashe and Suskind, 1950; Baader and Bauer, 1951; Bauer et al., 1961; Goldman, 1972; Jirasek et al., 1974; Oliver, 1975; Pocchiari et al., 1979) (Table 7-43) and in Seveso residents (Filippini et al., 1981) (Table 7-44). Previous case reports and studies found symptoms referable to the central (CNS) and peripheral (PNS) nervous systems among workers and community residents exposed to 2,3,7,8-TCDD-contaminated materials. Although human studies reveal a wide spectrum of effects due to 2,3,7,8-TCDD

(Sweeney et al., 1989), very few toxicological studies have focused on the nervous system. Singer et al. (1982) reviewed the following animal studies, which examined the relationship of CNS dysfunction and 2,3,7,8-TCDD exposure: Elovaara et al. (1977) found anomalous CNS function in some rats exposed to a single dose of 2,3,7,8-TCDD, and Creso et al. (1978) reported CNS symptoms of irritability, restlessness, and increased aggression in rats administered 2,3,7,8-TCDD.

7.13.8.1. *Adult Neurobehavioral Assessments*

Numerous case reports cite symptoms referable to the nervous system occurring after acute exposure among occupationally exposed individuals (Creso et al., 1978; Ashe and Suskind, 1950; Suskind et al., 1953; Goldman, 1972), as well as chronic exposure to 2,3,7,8-TCDD-contaminated materials (Oliver, 1975; Baader and Bauer, 1951; Kimmig and Schulz, 1957a,b; Bauer et al., 1961; Poland et al., 1971). Symptoms include headache (Ashe and Suskind, 1950; Bauer et al., 1961; Jirasek et al., 1974; Oliver, 1975; Kimmig and Schulz, 1957a,b; Poland et al., 1971), insomnia (Ashe and Suskind, 1950; Suskind et al., 1953; Oliver, 1975; Kimmig and Schulz, 1957a,b), nervousness or irritability (Ashe and Suskind, 1950; Suskind et al., 1953; Bauer et al., 1961; Oliver, 1975), depression and anxiety (Bauer et al., 1961; Jirasek et al., 1974), loss of libido, and encephalopathy (Jirasek et al., 1974; Kimmig and Schulz, 1957a) (Table 7-43).

Some reports indicate that symptoms referable to the CNS and PNS may persist in some exposed individuals for as long as 25 years (Suskind et al., 1953; Poland et al., 1971; Jirasek et al., 1973; Jirasek et al., 1974; Creso et al., 1978; Ashe and Suskind, 1950; Suskind et al., 1953). In 1953, Suskind et al. reported a variety of CNS-related symptoms in 36 workers from a plant in Nitro, West Virginia, who had developed chloracne and other symptoms after exposure to contaminants subsequent to a TCP reactor explosion in March 1949 (N = 11) or during normal production process of TCP and 2,4,5-T (N = 25) between 1948 and 1953 (Suskind et al., 1953). Such symptoms reported among this relatively young group (average age = 36 years, range = 22-63 years) included fatigue (N = 21), nervousness and irritability (N = 17), and decreased libido (N = 13). No attempt was made to determine whether these symptoms also occurred among exposed individuals without chloracne or among the nonexposed plant population.

Between 1968 and 1969, Jirasek et al. (1974) “observed very closely” (by clinical evaluation) a group of 55 workers exposed to 2,3,7,8-TCDD in the production of 2,4,5-T, hexachlorobenzene, and pentachlorophenol. As described in the report, intoxication with 2,3,7,8-TCDD “occurred gradually from 1965 to 1968,” although further quantification of exposure is not described. Psychiatric examination revealed the following: severe neurotic symptoms (64%), neurasthenia syndrome with depressive component (11%), depressive syndromes (8%), pseudoneurasthenia syndromes in patients with arteriosclerosis of the CNS

(14%), and normal psychiatric examination (3%). One patient died at age 57 years with rapidly progressive dementia secondary to an atypical arteriosclerosis involving brain and other organs.

Ten years after the initial examination, Pazderova-Vejlupkova et al. (1981) evaluated the health status of 44 of the original 55 workers. They found the following on psychiatric examination: 58% continued to have neurotic symptoms without depressive or anxiety components, 18% developed severe neurasthenia syndromes with signs of dementia, and 24% were normal.

Poland et al. (1971) administered the Minnesota Multiphasic Personality Inventory (MMPI) to 52 male production workers who were exposed at that time to TCP, 2,4,5-T, 2,4-D, and other chemicals. Severity of acne correlated significantly with a high score on the hypomania scale of the MMPI. When production workers were compared with 17 presumably unexposed administrative workers, the two groups differed on only one MMPI symptom scale; exposed production workers scored higher on the hypochondriasis scale.

Table 7-45 describes the results of neurologic and neurobehavioral assessments of TCP production workers, Ranch Hands, and U.S. Army Vietnam veterans.

Moses et al. (1984) found a significant excess among workers with chloracne compared to those without lesions for the following symptoms: insomnia, decreased libido, and difficulties with ejaculation or erection. There was no difference between the two groups for the symptoms of fatigue, irritability, nervousness, depression, or personality changes.

In the study by Suskind and Hertzberg (1984), psychological symptoms were evaluated by interview and peripheral nerve function by nerve conduction velocity of the peroneal motor and sural sensory nerve fibers. The complaint of loss of libido was more frequent among exposed than unexposed, even after stratification by age. The complaint of nervousness, depression, or anxiety was not significantly related to exposure (16.3% vs. 11.7%) in the crude analysis, even though the sample size was sufficient to detect a twofold increase. However, in the group over 50 years old there was a significant exposure effect (19% vs. 6.4%) for the complaint of nervousness, depression, or anxiety. The complaint of impotence was significantly related to exposure in the crude analysis, but after stratification by age this effect disappeared.

The effects of exposure to 2,3,7,8-TCDD on measures of current symptoms of depression were evaluated (Alderfer et al., 1992) as part of the NIOSH cross-sectional medical study (Sweeney et al., 1989). Symptoms of depressed mood were measured by the Beck Depression Inventory and the depression subscale of the Self-Report Symptom Checklist-90-Revised (SCL-90-R). Neither serum 2,3,7,8-TCDD levels nor status as a worker was associated with depressed mood as assessed by either the Beck Depression Inventory or the SCL-90-R depression subscale (Alderfer et al., 1992). This finding supports the conclusion that current serum levels of 2,3,7,8-TCDD are not associated with current depression among a population of workers that was highly

exposed to TCDD. However, because this cross-sectional study was conducted many years after 2,3,7,8-TCDD exposure, this analysis could not address the question of whether 2,3,7,8-TCDD is associated with past depression that resolved before the study was performed. These findings are consistent with those of the U.S. Air Force study (Roegner et al., 1991) of personnel who applied Agent Orange during the Vietnam War: serum 2,3,7,8-TCDD was not associated with the depression subscale score of the SCL-90-R after controlling for covariates.

The Air Force study conducted neurologic and psychological assessments of the participating Ranch Hands and comparisons (Lathrop et al., 1984). In the first examination series published in 1984 (baseline), the psychological assessment included a self-report of psychological or emotional illness, the Diagnostic Interview Schedule (DIS), Cornell Medical Index (inventory of psychophysiologic symptoms), MMPI, Halstead-Reitan Battery (HRB), and Wechsler Adult Intelligence Scale (WAIS). In the 1987 follow-up examination, the psychological assessment included an interviewer-administered questionnaire in which each participant was asked about the occurrence of mental or emotional disorders and sleep disorders. The presence of posttraumatic stress disorder was based on a subset of questions from the MMPI; the WAIS IQ assessment was deleted and the Millon Clinical Multiaxial Inventory (MCMI) and the Symptom Checklist-90-Revised (SCL-90-R) were added (Lathrop et al., 1987).

Results of the 1984 baseline study revealed no difference between Ranch Hands and the comparison group for self-reported psychological or emotional illness. The DIS revealed significantly more fatigue, anger, erosion, and anxiety for high school-educated but not college-educated Ranch Hands. These outcomes were highly related to education. The Cornell Medical Index found 4 of 10 parameters abnormal for Ranch Hands: startle, psychosomatic, gastrointestinal nervousness, and anxiety. These parameters were inversely related to education level. On the MMPI, high school-educated Ranch Hands showed significant differences (more deficits) on subscales for hypochondria, masculinity/femininity, and mania/hypomania, but comparisons scored higher on the subscale for denial, a finding that might undermine the deficits noted for Ranch Hands. Again, MMPI scores were influenced by education level ($p<0.01$) but not exposure level. For both the HRB and WAIS, there was no difference between Ranch Hands and comparisons, and the scores were related to education level.

In the 1987 reanalysis with serum 2,3,7,8-TCDD (Roegner et al., 1991), there was no significant difference between groups or relationship with serum 2,3,7,8-TCDD levels on reported (and verified) data on lifetime psychological illness or sleep disorders or any SCL-90-R. Some of the MCMI parameters appeared to be related to serum 2,3,7,8-TCDD levels (significantly higher mean schizoid and schizotypal scores and significantly lower mean histrionic score in the group above 33.3 pg/g than in comparisons). However, these findings were inconsistent with similar variables in the SCL-90-R and the self-reported histories.

Comprehensive neurologic and psychological assessments were conducted on participants of the Vietnam Experience Study (Centers for Disease Control Vietnam Experience Study, 1988a, b). The neurobehavioral tests evaluated aptitude, concept formation and problem-solving, memory, manual dexterity, verbal skills, visuomotor skills, attention, and mental control. Among Vietnam veterans there was a significantly greater prevalence of alcohol abuse or dependence (Vietnam veterans, 13.7%; non-Vietnam veterans, 9.2%; OR = 1.5, 95% CI = 1.2-1.8), depression (Vietnam veterans, 4.5%; non-Vietnam veterans, 3.2%; OR = 2.0, 95% CI = 1.4-2.9), and a higher prevalence of poor psychological status (Centers for Disease Control Vietnam Experience Study, 1988b). The poor psychological status tended to be most prevalent in Vietnam veterans who were not white, who enlisted before their 19th birthday, and whose enlistment test scores fell below the group median.

In the study of residents of the Quail Run Mobile Home Park, neurobehavioral tests evaluated reaction time, mood, memory, visuo-motor coordination, intelligence, and indicators of psychological stress (Hoffman et al., 1986). Differences between Quail Run residents and controls were observed in the vocabulary subtest of the WAIS (Quail Run = 34.7, controls = 41.1, raw scores; $p<0.01$), tension/anxiety raw score (Quail Run = 13.7, controls = 11.1; $p<0.01$), and anger/hostility scale (Quail Run = 11.6, controls = 8.9; $p<0.05$) of the POMS inventory, and for the depression/dejection and fatigue/inertia scales (no data provided).

7.13.8.2. *Adult Neurologic Status*

On neurologic examination, 11 of 60 West Virginia workers with chloracne exhibited decreased sensitivity to pinprick, whereas none of the 34 subjects without chloracne had decreased pinprick sensation ($p<0.01$) (Moses et al., 1984). There were no other differences in performance of the neurologic examination noted in the text. When examined by Suskind and Hertzberg (1984), no significant differences were noted in the conduction velocities of either nerve fiber (sural sensory: exposed workers, mean = 42.06±0.49; unexposed workers, mean 41.49±0.54; peroneal motor: exposed workers, mean = 41.77±0.47; unexposed workers, mean = 42.62±0.52).

Among New Jersey and Missouri TCP workers, the overall neurologic status and peripheral nerve function were assessed for all 281 workers and 260 referents by self-reported medical history; neurologic examination; electrophysiologic tests of nerve conduction velocity, amplitude, and latency; and vibratory and thermal threshold (Sweeney et al., 1993). No differences in neurologic status or nerve function between workers or referents were detected. Additionally, although the mean serum 2,3,7,8-TCDD level in the workers was 220 pg/g, there was no relationship between neurologic function and levels of serum 2,3,7,8-TCDD.

The neurologic examination in the 1987 follow-up study evaluated cranial, CNS, and PNS function in participating Ranch Hands (Lathrop et al., 1987). In general, there was no difference in the prevalence of neurologic abnormalities in Ranch Hands and comparisons. However, Ranch Hands with serum 2,3,7,8-TCDD levels above 33.3 pg/g tended to have a higher proportion of individuals with abnormal coordination than the comparisons (Ranch Hands, 2.7%; comparisons, 0.4%; adjusted RR = 18.3, $p<0.001$) (Roegner et al., 1991).

Overall neurologic status of Army Vietnam veterans did not differ from that of non-Vietnam veterans (Vietnam veterans, 1.0%; non-Vietnam veterans, 0.8%; OR = 1.2, 95% CI = 0.6-2.3) (Centers for Disease Control Vietnam Experience Study, 1988b). Only self-reported symptoms related to nerve disorders were significantly more prevalent among Vietnam veterans than non-Vietnam veterans (Vietnam veterans, 8.2%; non-Vietnam veterans, 6.5%; OR = 1.2, 95% CI = 1.0-1.6).

Table 7-44 describes the results of neurologic and neurobehavioral studies of Seveso and Missouri residents. While three studies evaluated the neurologic status of residents of Seveso (Pocchiari et al., 1979; Filippini et al., 1981; Assennato et al., 1989), no studies evaluated neurobehavioral changes. In an effort to quantify exposure-related neurologic disorders among Seveso residents and among workers from the Icmesa plant, two government-sponsored screenings were conducted in 1977 and 1978 on 308 residents of Seveso and 200 workers. Among these workers, approximately 4% (N = 8) were found to have damage to nerve fibers of multiple (unspecified) nerves, controlling for confounding factors such as alcohol abuse, diabetes, kidney disease, and neurotoxic medication use (Pocchiari et al., 1979). The report did not describe the extent of worker exposure to 2,3,7,8-TCDD. Other potential neurotoxic occupational exposures do not appear to have been considered. Three workers were described as having polyneuropathies of the lower limbs.

In 1981, prevalence risk ratios (PRR) for neuropathy were calculated separately for the 308 Seveso residents (Filippini et al., 1981). PRRs for neuropathy were determined for residents who exhibited clinical indication of 2,3,7,8-TCDD exposure, defined as the presence of elevated liver enzyme levels (GGT, ALT, AST) (which are also indicative of nonspecific insults to the liver) or chloracne, and for those who exhibited conditions that are risk factors for neuropathy, e.g., alcoholism, inflammatory disease, diabetes, or potential occupational exposure to neurotoxins. Seveso residents who had clinical indication of 2,3,7,8-TCDD exposure (chloracne or elevated liver enzymes GGT, AST, ALT) or who had risk factors for neuropathy were found to have significantly greater prevalence of neuropathy than residents without either manifestation (PRR exposure = 2.8, 95% CI = 1.2-6.5; PRR for possible 2,3,7,8-TCDD-predisposing factors = 2.6, 95% CI = 1.2-5.6) (Filippini et al., 1981). Additional analysis showed that individuals who

met the definition for chloracne or abnormal levels of hepatic enzymes were significantly more at risk than residents without either condition.

Residents of Seveso who developed chloracne after the reactor release (N = 193) were invited to a series of three follow-up screenings in 1982-1983, 1983-1984, and 1985 (Assennato et al., 1989). A control group from a nearby but uncontaminated area was also examined. Conduction velocities of the median motor, peroneal motor, and sural sensory fibers were conducted in 1982-1983 and 1985 (Assennato et al., 1989). No increases in the prevalence of abnormal electrophysiologic measures were observed in the chloracne group when compared with controls without chloracne. In addition, there was no change in the conduction velocities for each fiber from the 1982-1983 to 1985 studies.

Quail Run residents reported significantly more "numbness" or "pins and needles" in the hands or feet (28.6%) than the controls (18.1%) ($p<0.05$), but there were no differences in mean threshold scores for the more objective neurosensory tests. Residents also reported more persistent severe headaches (Quail Run, 26.0%; control, 14.2%; $p<0.05$).

Participants in the study by Webb et al. (1989) did not complete neurobehavioral tests, but they were examined by a neurologist. The results were unremarkable. Of the 38 participants, two with levels above background had abnormal pinprick sensitivity ($\leq$20 pg/g, N = 2; 20-60 pg/g, N = 1; $\geq$60 pg/g, N = 1), three had abnormal vibration thresholds ($\leq$20 pg/g, N = 2; 20-60 pg/g, N = 1; $\geq$60 pg/g, N = 2), and four had abnormal reflexes ($\leq$20 pg/g, N = 2; 20-60 pg/g, N = 2; $\geq$60 pg/g, N = 2). The results of other components of the neurologic examination were not reported and are assumed to be normal.

7.13.8.2.1. ***Comment on adult studies.*** The overall results of these case reports and epidemiologic studies demonstrate that exposure to 2,3,7,8-TCDD-contaminated materials is associated with symptoms referable to the central and peripheral nervous systems shortly following exposure and, in some cases, lasting many years (Tables 7-44 and 7-45). Symptoms include fatigue, nervousness, anxiety, and decreased libido (Ashe and Suskind, 1950; Suskind et al., 1953; Oliver, 1975; Kimmig and Schulz, 1957a,b; Bauer et al., 1961; Jirasek et al., 1974). One case report found mania/hypomania on the MMPI (Poland et al., 1971). These symptoms are consistent with mood disorder. Although one study reported neurasthenia with signs of dementia lasting 10 or more years following exposure (Jirasek et al., 1974), the U.S. Air Force study of Vietnam veterans used neurobehavioral testing but was unable to demonstrate cognitive or other functional CNS deficits. However, this negative study did not investigate the relationship between serum 2,3,7,8-TCDD levels and neurobehavioral deficits. NIOSH investigated measures of depressed mood many years after exposure to 2,3,7,8-TCDD among production workers and

found no relationship between depressive symptoms and serum 2,3,7,8-TCDD levels (Alderfer et al., 1992).

Overall neurologic status of workers, community residents, and Vietnam veterans exposed to 2,3,7,8-TCDD and evaluated from 5 to 37 years after last exposure appears to be normal. These data suggest that, although exposure to 2,3,7,8-TCDD may have been extensive, as in the case of the exposed workers, Ranch Hands, and Seveso residents, and case reports describe many related symptoms, the effects may have been transient. If so, studies conducted years after the last exposure would not detect such changes. These results suggest that, in adults, no long-term neurologic effects were caused by even high exposure to 2,3,7,8-TCDD-contaminated materials. However, there is very little information with which to examine the effects of exposure on the developing human neurologic system.

7.13.8.3. *Developmental Neurobehavioral Effects*

Five recent reports from The Netherlands have examined neurologic/behavioral outcomes. Outcomes are summarized in Table 7-46. The overall design of these studies and the classification of biological exposure data are described in detail in Section 7.12.2.3.

A pair of studies examined the same group of children from Rotterdam (Table 7-46). These infants were tested for (1) psychomotor and mental development indices (PDI and MDI) based on the Dutch standardized verion of Bayley Scales of Infant Development (Koopman-Esseboom et al., 1996) at ages 3, 7, and 18 months; (2) Visual Recognition Memory Scores based on the Fagan Test of Infant Intelligence (Koopman-Esseboom et al., 1995b) at ages 3 and 7 months; and (3) use of the Prechtl neonatal neurologic examination (Koopman-Esseboom et al., 1995a) to classify infants (about 2 weeks of age) as to neurologic normality.

The first study (Koopman-Esseboom et al., 1995b) demonstrated a significant increase in the visual recognition memory test (Fagan Test of Infant Intelligence) for breast feeding and length of breast feeding when examined with maternal serum PCB levels. Neither the prenatal PCBs nor the non-dioxin-like PCBs in breast milk were associated with the Fagan outcome at either time period. In this report and another (Koopman-Esseboom et al., 1996), reported below, a cumulative score was developed for “low,” “medium,” and “high” exposure which multiplied the pg total PCB-dioxin-furan TEQ/g fat times weeks breast feeding (see Table 7-46). In the final regression analyses, significant differences were not observed for total PCB-dioxin-furan TEQ with outcomes at 3 months of age. However, at 7 months, there was a dose-related increase in scores with “medium” and “high” total PCB-dioxin-furan TEQ (Table 7-46). The authors suggested that these benefits resulted from (1) increased breast-feeding and (2) the high total PCB-dioxin-furan TEQ being an artifact of its correlation with the higher level of lipids or

lipophilic factors (e.g. hormones, long-chain polyunsaturated fatty acids [LCPUFAs]) beneficial to this aspect of development.

The second study (Koopman-Esseboom et al., 1996) observed a beneficial effect on PDI at 7 months of age (PDI-7) for breast feeding versus formula when the total PCB-dioxin-furan TEQ was low (Table 7-46). There were statistically significant deficits observed for PDI-7 in the regression analysis of "medium" levels of total PCB-dioxin-furan TEQ, and for medium and high levels of total PCB-dioxin-furan TEQ combined. MDI-7 showed a significant increase with duration of breastfeeding, but the total PCB-dioxin-furan TEQ did not have a significant effect. The other endpoints (MDI-3, PDI-3, MDI-18 and PDI-18) were not significantly associated with either duration of breast feeding or total PCB-dioxin-furan TEQ. In the analysis of prenatal PCB (using maternal blood levels collected late in pregnancy), PDI scores were lower at 3 months with higher PCB levels. The authors also examined thyroid hormone levels because they are necessary for brain development, and found no significant effects of thyroid hormone levels on PDI or MDI.

The third study (Koopman-Esseboom et al., 1995a) used the Prechtl neonatal neurologic examination to classify infants (about two weeks of age) as to neurologic normality: "normal," "mildly abnormal" (e.g., mild hypotonia or tremor), or "definitely abnormal" (e.g., hyper-excitability, hypotonia, hypertonia, or a hemisyndrome). Two infants in each location were classified as "definitely abnormal," and 20 total were classified as "mildly abnormal" (11 in Groningen and 9 in Rotterdam). One "definitely abnormal" child was eliminated from further analyses because of a birth trauma. Because of the small numbers, the remaining 23 children were grouped together and termed "neurologically abnormal." These groups were examined for obstetric optimality scores and thyroid levels (these thyroid levels were discussed in Section 7.13.4.2) and no significant findings were observed. The categorization of neurologically normal or abnormal, as expected, was highly correlated with the neurologic optimality scores (postural tone cluster and reflex cluster) (see Huisman et al., 1995a,b). The levels of coplanar PCB TEQ and total PCB-dioxin-furan TEQ were different in the two groups (Table 7-46). Only free T4 was significantly different in the two groups (total T3, total T4, free T4, and TSH were tested). The authors concluded that there was no signficant relationship of dioxins, furans, and PCBs with these "clinically relevant" outcomes and recommended follow-up of these children as they aged.

Two more studies examined the Rotterdam children and the Groningen children together (Table 7-46). These studies covered (1) neonatal neurologic development at 10-21 days post-birth (Huisman et al. 1995a) and (2) neurological condition at 18 months (Huisman, 1995b).

In the first report (Huisman et al., 1995a), infants were examined 10-21 days after birth, and several evaluations were made: (1) neonatal neurological condition (394 infants were normal, 20 suspect, and 4 abnormal); (2) Prechtl's Neurologic Optimality Scores (NOS), based on 21

items; and (3) these 21 items grouped to develop postural tone cluster scores and reflex cluster scores. The NOS and the cluster scores were then dichotomized for use in the statistical analyses: the NOS was divided at the median (a score of 57), the postural tone cluster score (less than or equal to 9 (43% of the children) versus greater than 9, and the reflex cluster score (less than or equal to 10 (22% of the children) versus greater than 10. Prenatal PCB measures (maternal blood and cord blood) were not associated with NOS or the clusters. Many individual PCBs, dioxins and furans in breast milk were associated with NOS (Table 7-46), as were most of the summary measures based on breast milk (total PCB-dioxin-furan TEQ, dioxin-furan TEQ, $\sum PCB_{breast\ milk}$, mono-ortho PCB TEQ, and di-ortho PCB TEQ.) Coplanar PCB TEQ was associated with hypotonia (measured through the postural tone cluster score): OR:1.64 (1.03-2.63). Because the data suggested observer differences in the two communities (by a shift in the distribution between them), analyses controlled for community. However, the scoring of the two observers was not compared for some common subjects.

The second report (Huisman et al., 1995b) examined the same groups of infants at 18 months of age. The infants were assessed during an observation of motor functions using techniques described by Touwen, Hempel, and colleagues (1992-3). Of the 418 children scored, 408 were considered "normal" and the remainder were "mildly abnormal" (9) or "abnormal" (1). Only the prenatal PCB exposure (estimated by either $\sum PCB_{cord}$ or $\sum PCB_{maternal\ blood}$) was associated with it at 18 months (Table 7-46) The authors observed an interaction with paternal smoking, so that the adverse outcome with exposure was observed only in children with nonsmoking fathers. The authors noted that maternal smoking was only collected during pregnancy, and so the association of maternal postnatal smoking could not be evaluated. None of the measures of PCB, dioxin, or furans were associated with the fluency cluster score, but breast-fed children in general did have a higher score than did formula -fed children.

These children were again assessed at 42 months of age (Patandin et al., 1999). In this round, 395 children (94% of the original study group) were assessed for cognitive abilities using the Kaufman Assessment Battery for Children (K-ABC), and a subgroup of 193 (the Rotterdam children) were assessed for verbal comprehension using the Reynell Language Developmental Scales (RDLS). $\sum PCB$ was calculated using PCBs (IUPAC numbers 118, 138, 153 and 180) from the mother's blood, cord blood, and plasma from the 42-month-old children. PCB, dioxin, and furan levels were available for the breast milk samples collected 2 weeks after delivery from breast-feeding mothers. Exposure metrics included $\sum PCB$, total TEQ (dioxin, furan and PCB), and the sum of 20 non-dioxin-like PCBs. Statistically significant deficits were associated with the natural log of the $\sum PCB_{maternal\ blood}$ for K-ABC for the entire group, and for those children who were formula fed. Significant deficits for the RDLS were noted only in the formula-fed children.

Analyses of the current body burden in the children were not associated with any cognitive deficits. Statistically significant changes were not observed in the breast-fed children, possibly because of the higher SES status, parental education, and parental verbal IQs. Another possibility is the beneficial effects of breast feeding in general.

7.13.8.4. *Comment on Developmental Studies*

One factor demonstrated in this series of studies is the benefit derived from breast feeding. Even though the level of environmental toxicants reaching the child through early dietary exposure may be greater with breast feeding, formula-fed children did not do as well overall on many behavioral and neurological measures in these studies. This may not be true with environmental "accidents," which could result in much higher levels to the child. These differences could also be attributed to the association of breast feeding with socioeconomic status of the households, parental education levels, etc.

A large number of dioxins, furans, and PCBs were evaluated at different developmental stages. Given the smaller volume in the collection of third-trimester blood from the mother and cord blood at birth, only four PCBs were measured (IUPAC 118, 138, 153, 180). Thus, prenatal dioxin-furan levels can only be approximated in these data. The statistically significant correlations between the different agents and biological sources suggest that it would be difficult to sort out effects of any individual group or class of agents.

In some of these studies, total breast-feeding time and breast milk levels were used to estimate the total exposure via breast feeding. This model is a reasonable relative estimate of broad categories, but may be problematic for estimation for women with widely different lengths of breast feeding. The levels in breast milk are likely to decrease over time, and the consumption of breast milk is likely to drop gradually as other food sources are increased. Thus the general levels of the broad groupings are useful, but the individual estimates should be used with caution.

Several of these studies based their results on crude (unadjusted) analyses. Given that there were significant differences between the breast-feeding parents and the bottle-feeding parents as to socioeconomic status (e.g., education, profession) and other lifestyle factors (e.g., smoking and drinking patterns), these results could change with a more in-depth analysis. The observation of hypotonia and prenatal PCB exposures is consistent with another study from the 1980s (Rogan et al., 1986). This study found effects of prenatal exposure (but not postnatal through breast feeding) on hypotonia, as did one of the Dutch studies (Huisman et al., 1995a). These associations with prenatal exposure have persisted up to 42 months of age (Patandin et al., 1999). These findings are consistent with findings of cognitive deficits in 11-year old children

exposed prenatally to PCBs in Michigan; as with the Dutch studies, deficits were not associated with exposures through breast feeding (Jacobson and Jacobson, 1996).

7.13.9. Circulatory System

The relationship between human exposure to 2,3,7,8-TCDD-contaminated chemicals and disorders of the circulatory system has been explored. A number of early case reports have described effects on the cardiovascular system among individuals reportedly exposed to chemicals contaminated with 2,3,7,8-TCDD. Myocarditis (Goldman, 1972), myocardial infarctions (Walker and Martin, 1979; Bauer et al., 1961), ectasia of the coronary arteries (England, 1981), and rapidly progressive atherosclerosis (Jirasek et al., 1974; Pazderova-Vejlupkova et al., 1981) have been reported.

Some of the earliest studies described mortality from diseases affecting the circulatory system among worker populations exposed to 2,3,7,8-TCDD (Bond et al., 1987; Coggon et al., 1991; Fingerhut et al., 1991b; Zober et al., 1990; Bueno de Mesquita et al., 1993; Bertazzi et al., 1989, 1992; Collins et al., 1993) (Table 7-47). The circulatory system includes ICD-9 codes 390-459 (International Classification of Diseases 9). Most early studies were designed primarily to test hypotheses relating to cancer and, secondarily, to characterize mortality compared to the general population from causes other than cancer, and without detailed characterization of confounders related to the circulatory system.

In many of the earlier studies, mortality from all diseases of the circulatory system among TCP production workers was similar to or less than mortality in the general population, as described by an SMR of 100 (Table 7-47). Examples include The Netherlands (Plant A) (SMR = 98, 95% CI = 65-142) (Bueno de Mesquita et al., 1993); the United States (Nitro, West Virginia) (SMR = 90, 95% CI = 80-100) (Collins et al., 1993); and Great Britain (SMR = 116, 95% CI = 91-146) (Coggon et al., 1991). In studies of workers with chloracne, mortality from circulatory diseases was decreased in U.S. workers (SMR = 95, 95% CI = 79-113 (Bond et al., 1987), and moderately increased in German workers, SMR = 121, 90% CI = 83-170 (Zober et al., 1990). In the follow-up study of the same cohort of German workers with chloracne —data not presented— this excess disappeared (Ott and Zober, 1996a).

More recent studies, many of which are updates of earlier reports (Flesch-Janys et al., 1995, Ott and Zober, 1996a; Hooiveld et al., 1998; Vena et al., 1998; Steenland et al., 1999), more thoroughly examined mortality from noncancer endpoints, including diseases of the circulatory system. In these studies, researchers employed more sophisticated analytical methods, such as adjustment for confounders, use of internal control groups, and measurement or estimation of

exposure to 2,3,7,8-TCDD or related compounds to refine calculated risk estimates for mortality from diseases of the circulatory system and other outcomes.

In a cohort of German chemical workers (Hamburg) who manufactured 2,4,5-TCP, and 2,4,5-T and chemicals contaminated with higher chlorinated PCDDs and PCDFs (Flesch-Janys et al., 1995), mortality for all circulatory diseases was positively related to estimated 2,3,7,8-TCDD concentrations and significantly related to estimated total TEQ concentrations above 39 ng/kg, lipid adjusted. Lipid-adjusted 2,3,7,8-TCDD concentrations and total TEQ estimates for the cohort were based on PCDD and PCDF measurements of 190 male workers.

In contrast, Ott and Zober (1996a) found no dose-dependent relation between estimated 2,3,7,8 -TCDD dose and mortality from diseases of the circulatory system (Conditional risk ratio = 0.93, 95% CI = 0.70, 1.24) when adjusted for cigarette smoking, body mass index, and age in workers from Ludwigshafen, Germany. Similarly, Vena et al. (1998) found significant deficits in mortality from circulatory diseases (SMR = 94, 95% CI = 88, 99) among workers in the expanded IARC international cohort exposed only to phenoxy herbicides and chlorophenols when compared to country-, gender-, age-, calendar period-, and cause-specific national death rates using the World Health Organization Mortality Data Bank. However, when compared to an internal control group of workers not exposed to TCDD/HCDD, mortality from all circulatory diseases among workers exposed to TCDD or HCDD was statistically significantly elevated (RR = 1.51, 95% CI = 1.17, 1.96). This significant increase occurred for workers exposed for 5-9 years and 10-19 years, but not for periods over 20 years.

In an update of the Dutch cohort, when compared to the general population (SMR = 100, 95% CI = 80, 140) or to an internal comparison group (adjusted RR = 1.4, 95% CI = 0.8, 2.5), little risk for mortality from circulatory disease was observed among the 549 workers exposed between 1955 and 1991 to 2,4,5-T; 2,4,5-TCP; and contaminants, or among the 140 workers exposed in a 1963 reactor release (SMR =110, 95% CI = 60, 170) (Hooiveld et al., 1998).

Mortality from more specific endpoints, such as ischemic heart disease, all heart diseases, and cerebrovascular disease, was noted in a few studies. The SMR from ischemic heart disease was decreased (SMR= 96, 95% CI = 51-164) in U.S. workers (Midland, Michigan) (Bond et al., 1987) and in German workers from Ludwigshafen (SMR = 70, 95% CI = 40-110) (Ott and Zober, 1996a). Overall mortality from ischemic heart disease was not elevated in a cohort of chemical workers from Hamburg, but it was significantly increased in workers only in the highest 2,3,7,8-TCDD quintile (RR = 2.48, 95% CI = 1.32-4.66). In an update of the Dutch cohort study through 1991, mortality from ischemic heart disease was elevated but did not achieve statistical significance (SMR = 190, 95% CI = 90-360) (Hooiveld et al., 1998). For the 29 workers with a

history of chloracne who were also exposed as a result of the 2,4,5-TCP reactor release, mortality was significantly elevated (SMR = 3.7, 95% CI = 1.4-8.1, N = 6).

For workers included in the IARC cohort exposed to phenoxy herbicides or chlorophenols, mortality from ischemic heart disease was decreased relative to the WHO comparison population (SMR = 97, 95% CI = 90-104) but significantly increased relative to an internal control group using Poisson regression (RR = 167, 95% CI = 123-226) (Vena et al., 1998). Using estimated cumulative exposure scores (Piacitelli et al., 1997), Steenland et al. (1999) found mortality from ischemic heart disease moderately increased with increasing exposure score, with an SMR = 93 in the lowest septile to an SMR = 123 for workers in the highest septile ($P_{\text{test for trend}} = 0.14$). In an analysis using Cox regression and an internal control group, only the rate ratio for the category with the highest exposure score (7^{th} septile) was statistically significantly elevated, the test for trend overall exposure score categories was statistically significant ($p = 0.5$). In the subcohort with chloracne mortality from ischemic heart disease was marginally increased (SMR = 117, 95% CI = 94-144).

Cerebrovascular disease mortality was increased by more than twofold in Michigan TCP production workers with chloracne (SMR = 208, 95% CI = 57,539) (Bond et al., 1987).
In an update of the 1993 study of Dutch TCP production workers (Bueno de Mesquita et al., 1993), mortality from cerebrovascular disease remained slightly elevated when compared both to the national population (SMR = 140, 95% CI = 60-260) and to an internal control group (SMR = 140, 95% CI = 40-510) (Hooiveld et al., 1998). Steenland et al. (1999) found a deficit of mortality from cerebrovascular disease in an update of the NIOSH cohort (SMR = 96, 95% CI = 74-121), as did Vena et al. (1998) in his study of nonneoplastic mortality in the IARC expanded cohort (SMR = 84, 95% CI = 71-98, international comparison population). When compared to the internal reference population, mortality was significantly elevated in the IARC expanded cohort (RR = 1.55, 95% CI = 0.83-2.28).

Mortality from IHD was lower than expected (SMR = 82, 95% CI = 0.67-1.02) in a cohort of 1,909 herbicide sprayers followed from 1972 through 1989. Workers eligible for study applied a mixture of 2,4-D and 2,4,5-T for 2 weeks or longer anytime during the period 1951-1971 (Asp et al., 1994). Similar trends were observed for other diseases of the circulatory system.

In veteran populations, among 1,261 Ranch Hand personnel, mortality from circulatory disease was nonsignificantly elevated (SMR = 110, 95% CI = 60-150) compared with that of a comparison population of 19,101 other Air Force veterans who were not exposed to herbicides (Michalek et al., 1990). These results were repeated in an updated mortality analysis (SMR = 105, 95% CI = 96-142) (Wolfe et al., 1994). After 15 years of followup, overall mortality from circulatory disease was as expected (SMR = 100, 95% CI = 70-130); only the SMR for enlisted

ground personnel, the subgroup with the highest dioxin levels, was greater than expected (SMR = 100, 95% CI = 90-150) (Michalek et al., 1998). Of the 118 total deaths, 3 were due to cerebrovascular disease (SMR = 2.7, 95% CI = not calculated). Similar nonsignificant increases in the relative mortality ratio (RMR) were observed for circulatory diseases (RMR = 1.6, 95% CI = 0.8-3.2) in Australian Vietnam veterans (N = 19,205; 260 deaths) compared to 25,677 (263 deaths) non-Vietnam veterans who served only in Australia (Fett et al., 1987). In contrast, the unadjusted relative risk of 0.49 (95% CI = 0.25-0.99) for all circulatory diseases suggested a deficit of deaths from this cause among 9,325 Vietnam Army veterans (246 deaths) compared to 8,989 non-Vietnam veterans (200 deaths) (Centers for Disease Control Vietnam Experience Study, 1988c).

Bertazzi and colleagues examined the mortality experience of Seveso residents ages 1-19 years (Bertazzi et al., 1992) and ages 20-74 years (Bertazzi et al., 1989) 10 years after the TCP reactor release. In the younger population, two deaths from circulatory diseases occurred only in female residents (RR = 1.63, 95% CI = 0.3-8.1). In the older population, circulatory disease mortality of residents from zone A (the most highly contaminated region) was elevated in both males (RR = 1.75, 95% CI = 1.0-3.2) and females (RR = 1.89, 95% CI = 0.8-4.2). In males, the highest death rate occurred during the first 5 years after the release, 1976-1981 (RR = 2.04, 95% CI = 1.0-4.2), and in females, the highest death rate occurred during the second 5 years post release, 1982-1986. As the authors suggest, the study was limited by a small number of subjects, a crude measure of 2,3,7,8-TCDD exposure, and a short followup period. The authors could not attribute the increased mortality from circulatory disease to 2,3,7,8-TCDD exposure, but suggested that the "high stress and pollution" imposed on the residents of zone A may have been a contributing factor.

An update of the 15-year (1976-1981) mortality experience of the cohorts of zones A, B and R was conducted by Pesatori et al. (1998). In zone A males, moderate increases in mortality from all circulatory diseases combined was observed (RR = 1.6, 95% CI = 1.1-2.5, N = 21) due, in part, to the threefold increase in mortality from chronic ischemic heart disease (RR = 3.0, 95% CI = 1.2-7.3, N = 5). Among females in zone A, rheumatic heart disease (RR = 15.8, 95% CI = 4.9-50.4, N = 3) and hypertension (RR = 3.6, 95% CI = 1.2-11.4, N = 3) were in excess. No significantly increased risks were observed for circulatory disease among residents of zone B, however, in zone R males and females, small but statistically significant elevations occurred for all circulatory diseases (RR = 1.1, 95% CI = 1.0-1.2, N = 719 males; RR = 1.1, 95% CI = 1.0-1.2, N = 759 females). Among males, chronic ischemic heart disease was 40% higher than expected (RR = 1.4, 95% CI = 1.1-1.7, N = 126) and 30% higher in females (RR = 1.3, 95% CI = 1.0-1.5, N = 133). Only in zone R was the risk for mortality from cerebrovascular disease increased.

Many cross-sectional medical studies also examined the association between 2,3,7,8-TCDD exposure and effects on the cardiovascular system (Suskind and Hertzberg, 1984; Moses et al., 1984; Bond et al., 1983; Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991; Grubbs et al., 1995; Calvert et al., 1998). In the NIOSH morbidity study cohort, after controlling for important confounders, no associations were observed between serum 2,3,7,8-TCDD concentrations and increased risk for myocardial infarction, angina, arrhythmia, hypertension, systolic or diastolic hypertension at the time of the study, or abnormal peripheral arterial flow (Calvert et al., 1998).

Statistically significant associations relative to 2,3,7,8-TCDD exposure were found in the Ranch Hand study only for diastolic blood pressure, arrhythmias detected on the electrocardiogram (ECG), and peripheral pulse abnormalities (Roegner et al., 1991). However, there is some doubt that the significant findings are dose related because significant increases in the mean diastolic blood pressure were found in Ranch Hands with serum 2,3,7,8-TCDD levels from 15 to 33.3 pg/g but not in the Ranch Hands with higher serum levels. The adjusted odds ratios for ECG-diagnosed arrhythmias among Ranch Hands were not entirely consistent with a dose-response relationship. For each serum 2,3,7,8-TCDD category the odds ratios were: serum 2,3,7,8-TCDD levels $\leq$10 pg/g, OR = 1.33 (95% CI = 0.68-2.58, p = 0.40); 15-33.3 pg/g, OR = 0.83 (95% CI = 0.31, 2.23, p = 0.71); and for serum 2,3,7,8-TCDD levels above 33.3 pg/g the OR was 2.34 (95% CI = 1.00-5.51, p = 0.051). The proportion of individuals in this group with arrhythmias (5.2%) was not much higher than in Ranch Hands whose serum levels were $\leq$10 pg/g (4.7%). Finally, relative to the comparison group, the proportion of abnormal peripheral pulses in all Ranch Hands, regardless of serum level, was elevated.

In the 1995 followup analysis, the findings closely reflect those of the 1992 analysis, although some results did vary (Grubbs et al., 1995). The prevalence of hypertension of hypertension continued to be significantly related to serum 2,3,7,8-TCDD levels, although there was no evidence for a dose-response association for either diastolic or systolic blood pressure. Overall ECG abnormalities were not different between Ranch Hands and comparisons. However, dose-related increases in risk were noted for RBBB, nonspecific ST and T-waves, and arrhythmias. Finally, no consistent evidence was found for a dose-related increase in the prevalence of abnormal peripheral pulses; however, the prevalence was statistically significant when Ranch Hands were compared to the comparisons.

No excess abnormalities or disorders of the circulatory system or heart were found in several groups of TCP production workers, although their potential for exposure to 2,3,7,8-TCDD-contaminated chemicals was high (Suskind and Hertzberg, 1984; Moses et al., 1984; Bond et al., 1983). The prevalence of hypertension or coronary artery disease (both self-reported),

abnormal ECG findings, atherosclerotic changes (not specified) on chest X-ray, or blood pressure elevation was not elevated in West Virginia TCP production workers (Suskind and Hertzberg, 1984). Similarly, when Moses et al. (1984) examined TCP production workers with chloracne and compared them with workers not affected by chloracne, they found no increased risk for self-reported abnormal ECG, self-reported angina, or self-reported myocardial infarction and no difference in the physical examination of the cardiovascular system. Finally, Bond et al. (1983) found no increased risk for self-reported hypertension among workers involved in the production of trichlorophenol and 2,4,5-T.

7.13.9.1. *Comment*

The SMRs for circulatory system diseases reported in the studies occupational are close to 100, suggesting that the "healthy worker effect" is diminished in these cohorts. Because employed workers are healthier than the general population, the SMR for cardiovascular disease in employed populations tends to be less than 100 (McMichael, 1976; Fox and Collier, 1976). It is also possible that because most of the cohorts exposed to TCDD and related compounds are composed mainly of retired or deceased workers, the healthy worker effect is reduced.

The picture relating exposure to 2,3,7,8-TCDD and diseases of the circulatory system is mixed. Animal data indicate that high doses of 2,3,7,8-TCDD affect cardiac and vascular integrity (Allen and Carstens, 1967; Allen et al., 1977; Norback and Allen, 1973). Data from a few animal studies suggest that relatively high doses of 2,3,7,8-TCDD cause damage to the myocardium and heart valves in rats (Kociba et al., 1978; Buu-Hoï, 1972) and to the arterial walls in rabbits (Brewster et al., 1987). Other research found that 2,3,7,8-TCDD may alter cardiac function in rats and guinea pigs (Hermansky et al., 1988; Kelling et al., 1987; Canga et al., 1988), causing reduced spontaneous and isoproterenol-induced heart contractility; this suggests that 2,3,7,8-TCDD may increase the risk of arrhythmias among the dosed animals. The authors postulate that 2,3,7,8-TCDD alters cyclic-AMP concentrations, altering the responsiveness of cardiac cells to β-adrenergic stimuli (Brewster et al., 1987). In contrast, histopathologic changes were not observed in the cardiovascular system of hamsters, which appear to be resistant to the effects of 2,3,7,8-TCDD at levels of 3,000 μg/kg of (Olson et al., 1980). Other experimental studies suggest an association between 2,3,7,8-TCDD and alterations in lipid levels (Poli et al., 1980; Albro et al., 1978; Bombick et al., 1984; Swift et al., 1981).

Findings from mortality and morbidity studies of production workers are not definitive, and suggest the need for analyses more specifically examining possible effects on the circulatory system and heart. The outcomes examined in the animal and human studies are different. Animal studies describe morphologic and chemical changes in the vascular and cardiac cells caused by

2,3,7,8-TCDD. On the other hand, the diseases and causes of death in the human studies assessed the possible consequences of exposure on long-term pathologic changes to the tissues, which cause cell and, sometimes, organ and system failure. Using the animal data, it is possible to project the long-term consequences of exposure to the organ or system. However, the animal studies do not account for the possibility of intervening events, such as the repair of tissue after exposure ends, or other events that reduce the hypothesized endpoint to a much less drastic outcome.

In recent studies, circulatory diseases and diseases of the heart were investigated as hypothesized health outcomes of exposure to 2,3,7,8-TCDD. A few mortality studies considered the possible confounding effect of other variables, including smoking, lipid levels, and other conditions that influence circulatory diseases and disorders of the heart. However, a general limitation of mortality studies is that only those conditions that ultimately caused the death of the individual are enumerated. Events such as myocardial infarctions, which may debilitate the individual but not cause death, may be missed if death is caused by another circumstance. Therefore, the effect of 2,3,7,8-TCDD on the coronary arteries might be missed because it was not coded as the underlying cause of death on the death certificate.

With the exception of the studies of the Ranch Hand and NIOSH cohorts (Roegner et al., 1991; Michalek et al., 1998; Calvert et al., 1999), cross-sectional analyses of other more highly exposed groups were limited by a lack of good exposure data and an inability to examine the relationship between serum 2,3,7,8-TCDD levels and diseases of the circulatory system or heart. Such studies may also be limited by the fact that they include a survivor population, as described in the introduction.

Further research would be useful to define the relationship between the pathologic endpoints observed in animals after high single doses of 2,3,7,8-TCDD and the disease outcomes observed in humans after high long-term exposure. To identify whether 2,3,7,8-TCDD has an effect on the human vasculature, additional work is needed to determine whether certain doses of 2,3,7,8-TCDD cause changes in the human vascular system; to determine whether there are changes in the action of chemicals associated with human cardiac muscle contraction caused by 2,3,7,8-TCDD exposure; and to assess mortality and morbidity in individuals with potential for 2,3,7,8-TCDD exposure while carefully controlling for other risk factors and using more accurate measures of exposure.

7.13.10. Pulmonary Effects

Studies of long-term exposure to 2,3,7,8-TCDD in Sprague-Dawley rats (Kociba et al., 1979; van Miller et al., 1977), B6C3F1 mice (NTP, 1982a), Swiss-Webster mice (NTP, 1982b),

and rhesus monkeys (Allen et al., 1977) have reported changes in bronchiolar or alveolar tissue ranging from epithelial hyperplasia and metaplasia to squamous cell carcinomas. The hyperplastic and metaplastic changes observed in exposed animals are similar to the pathologic picture of chronic bronchitis in humans (American Thoracic Society, 1962).

Case reports have described temporary respiratory irritation (Zack and Suskind, 1980) and tracheobronchitis (Goldman, 1972) among chemical workers exposed to 2,3,7,8-TCDD-contaminated herbicides following industrial accidents. In addition, Baader and Bauer (1951) reported chronic bronchitis in seven workers involved in pentachlorophenol production, which resolved in all but two workers within 2 weeks after production was discontinued.

There is conflicting evidence from controlled epidemiologic studies regarding an association between chronic respiratory system effects and human exposure to substances contaminated with 2,3,7,8-TCDD. One study of workers involved in the production of TCP and 2,4,5-T suggested that 2,3,7,8-TCDD exposure increases the risk for abnormal ventilatory function (Suskind and Hertzberg, 1984). This study found a statistically significantly increased risk for an abnormal forced expiratory volume at 1 second (FEV_1) ($p<0.01$), an abnormal forced vital capacity (FVC) ($p<0.001$), and an abnormal FEV_1/FVC ratio ($p<0.05$) among workers who were smoking at the time of the study. For workers, the percent predicted spirometric parameters for FEV_1, FVC, and FEV_1/FVC were 99.4%, 92.7%, and 76.5%, and for referents, 104.4%, 97.6%, and 79.9%, respectively. The only other study of TCP and 2,4,5-T production workers that reported ventilatory function findings found no association between serum 2,3,7,8-TCDD levels and declines in ventilatory function (Calvert et al., 1991). The disparity in results between the two studies may be related to the age of the unexposed populations and potential exposures of the exposed. In the Suskind and Hertzberg study, the exposed workers were, on average, 10 years older than the unexposed workers. Although the authors indirectly adjusted for age by analyzing age-adjusted ventilatory measures, it is not clear if these adjustments can completely control for a 10-year difference in age. In the study by Calvert et al. (1991), the difference in mean age between the exposed and unexposed groups was 0.6 years. The second difference involves the potential for exposure to 2,4,5-T acid dust at the plants studied. The 2,4,5-T acid that was produced at the plant studied by Suskind and Hertzberg was finished as a powder. At the plants studied by Calvert et al. (1991), the 2,4,5-T acid was finished as a liquid. Therefore, the potential for exposure to 2,4,5-T acid dust was greater at the plant studied by Suskind and Hertzberg (1984). Although we are not aware of any published reports supporting an association between ventilatory function and 2,4,5-T acid exposure, a respiratory burden of particles, in the absence of a specific toxic agent, can be a probable cause of ventilatory function declines (Becklake, 1985).

The Ranch Hand study also examined the association between serum 2,3,7,8-TCDD level and respiratory system effects (Roegner et al., 1991). This study found significant declines in the mean FEV_1 and the mean forced expiratory volume (FVC) for Ranch Hands with serum 2,3,7,8-TCDD levels above 33.3 pg/g (adjusted mean FEV_1 = 91.3%; mean FVC = 87.4) compared to a nonexposed comparison group (adjusted mean FEV_1 = 93.5%; mean FVC = 91.7) (Roegner et al., 1991). The 2,3,7,8-TCDD-related declines were small and were interpreted by the authors to be "subtle" and "not clinically significant." As expected, smoking appeared to have the greater influence on lung function although this has not been considered by the Air Force in their interpretation. In the followup examination conducted in 1992, no consistent relationship was found between serum 2,3,7,8-TCDD concentrations and respiratory parameters (Grubbs et al., 1995).

Mortality from respiratory diseases among the various exposed populations as is mixed. In productions workers, no excess mortality was observed from all diseases of the respiratory system among subcorhort of workers in the IARC study exposed to phenoxy herbicides or chlorophenols (SMR = 86, 95% CI = 73-101, N = 151) (Vena et al., 1999) or among German accident workers (N = 1) (Ott and Zober, 1996a).

7.13.10.1. *Other Mortality Studies of Production Workers*

Overall mortality from respiratory diseases among the Seveso population was less than expected for all exposure zones except males in zone A (Pesatori et al., 1998). However, chronic obstructive lung disease was twofold higher than expected in females in zone B (N = 7) and threefold higher in males of zone A (N = 4).

7.13.10.2. *Comment*

In conclusion, case reports indicate that intense acute exposure to 2,3,7,8-TCDD can produce respiratory irritation. However, the findings from controlled epidemiologic studies do not support an association between 2,3,7,8-TCDD exposure and chronic noncancer effects on the respiratory system.

7.13.11. Renal Effects

There is little evidence in the animal or human data to suggest that exposure to 2,3,7,8-TCDD is related to renal or bladder dysfunction. In a single case report, a child exposed to 2,3,7,8-TCDD after contact with soil sprayed with contaminated waste oil was diagnosed with focal pyelonephritis (Kimbrough et al., 1977). After diagnosis and treatment, the condition resolved with no reported recurrence. No major renal or bladder dysfunctions were noted among

Air Force Ranch Hands (Lathrop et al., 1984, 1987; Roegner et al., 1991) or among TCP production workers from West Virginia (Suskind and Hertzberg, 1984) or New Jersey (Poland et al., 1971).

7.13.12. Developmental/Reproductive Effects

Animal studies of reproductive effects have focused primarily on maternal 2,3,7,8-TCDD exposure and developmental toxicity. Prenatal exposure to 2,3,7,8-TCDD has been associated with increased pre- and postnatal mortality, cleft palate and kidney abnormalities, altered sexual development, and reduced fertility in studies of maternal exposure in a number of species. Fewer studies have focused on the effects of dioxin on the male reproductive system or on the results of matings in which only the males were exposed to dioxin. Studies of male exposures have not provided evidence of paternally mediated effects on the offspring. (Chapter 5 contains a detailed account of the animal literature.)

Thus, experimental research has emphasized maternally mediated developmental effects of 2,3,7,8-TCDD, while in humans, studies of paternal exposures have predominated. Moreover, assessment of exposed male animals has most commonly examined the effects of 2,3,7,8-TCDD on spermatogenesis, fertility, and sex organ development (Theobold and Peterson, 1984), whereas studies of human males have mainly targeted studies of congenital malformations and recognized spontaneous abortions. Experimental research designed to corroborate human investigations may provide critically needed data to plug the gaps in our understanding of the mechanisms through which 2,3,7,8-TCDD exposure may operate to produce adverse reproductive events in humans.

The origin of concerns regarding a potential link between exposure to chlorinated dioxins and adverse developmental events can be traced to early animal studies reporting increased incidence of developmental abnormalities in rats and mice exposed early in gestation to 2,4,5-T (Courtney and Moore, 1971). This was of grave concern, as the U.S. military's most widely used herbicide during the Vietnam conflict, Agent Orange, was composed of approximately equal proportions by weight of the n-butyl esters of 2,4-D and 2,4,5-T. The latter is contaminated by 2,3,7,8-TCDD during manufacture.

One dilemma encountered when attempting to review the epidemiologic literature dealing with dioxins and reproductive effects is the categorization of studies of sufficient similarity to allow for comparative analysis. These studies vary greatly in the nature (occupational, environmental) and route (inhalation, ingestion, absorption) of exposure; in the reproductive and developmental outcomes examined (often multiple endpoints were considered, and case definition differed across studies); in the assessment of parental exposure (maternal, paternal, or both); and in the timing of exposure relative to the pregnancy.

The examination of reproductive and developmental disorders poses several challenges to the researcher compared to other health outcomes. First, to understand both normal and pathologic reproduction, evaluation should include paternal and maternal, and sometimes fetal, contributions. Increased interest in male-mediated reproductive toxicity emphasizes the need to consider the couple as the unit of analysis in many reproductive study settings (Olshan and Mattison, 1994).

The second challenge to researchers is the interrelatedness of the spectrum of reproductive endpoints available for study. Fecundity (the joint potential to conceive), fertility (the production of live children), and very early pregnancy loss (those conceptions that do not survive to be recognized by usual diagnostic methods) as related to 2,3,7,8-TCDD exposure have not been evaluated in the same population. Clearly, these endpoints affect the rates of reproductive outcomes occurring later in the reproductive spectrum.

Another feature of developmental effects is the changing vulnerability of the developing organism throughout gestation. Exposure to a single developmental toxicant throughout pregnancy may result in different effects at various stages of gestation. The window of susceptibility varies; therefore, knowledge of the timing of exposure is critical in these studies.

Finally, although not restricted to studies of developmental events, care must be given to the collection and analysis of data on potential confounders. These factors may need to be obtained for both mother and father, with attention to the timing of specific characteristics, such as changes in smoking or occupation during pregnancy.

The reproductive effects of dioxins in humans were succinctly and elegantly reviewed by Hatch in 1984 (Hatch, 1984b). In her review, Hatch employed type of exposure, i.e., populations that were exposed occupationally, environmentally, or through industrial accident or military service, as the classification scheme for the research presented. The same approach has been followed in this review. The earlier investigations of 2,3,7,8-TCDD and reproductive effects, i.e., those conducted prior to 1984, are presented separately from the more recent studies. The development of assays in the mid-1980s to quantitate 2,3,7,8-TCDD in serum and adipose tissue, allowing individual measurements of exposure, warrants this dichotomy of the research.

Review of the Literature Prior to 1984

7.13.12.1. *Occupational Studies*

Epidemiologic studies of occupational dioxin exposure and reproductive effects have focused on paternally mediated effects, with exposures occurring at varying intervals relative to conception. Townsend et al. (1982) interviewed 370 wives of employees exposed to dioxins at the Dow Michigan Division in Midland, Michigan (63% of those eligible for the study), and 345

control wives of Dow employees who were not exposed to dioxin (62% of the eligible control pool). Exposure classification was determined by an industrial hygienist familiar with the processes performed at the plant. Employees were considered exposed to dioxin if they had been assigned for at least 1 month to specific jobs associated with chlorophenol processes between 1939 and 1975. All outcomes were reported by the employee's spouse.

There was no systematic attempt to ascertain the reason(s) for the high refusal rate in both cohorts. "Unsolicited reasons" for refusal included divorce, death of spouse, or no pregnancies; no breakdown by cohort was provided. The possibility of differential rates of infertility or early pregnancy loss, as reflected by the reported absence of pregnancy, was not addressed in this study.

For the multiple endpoints of spontaneous abortion, stillbirth, birth defects, infant mortality, and childhood morbidity, no significant association between dioxin exposure and any adverse event was identified (Table 7-48). This study had a very long interval during which the exposure and event could have occurred, and a minimum requirement for paternal exposure of 1 month at any time during that interval. This exposure definition essentially assumes "irreparable" damage that would persist long after the father's active occupational exposure had terminated or a very long half-life, resulting in an elevated biological level up to 35 years later. Thus, an effect of exposure, if one existed, would have been diluted by this approach. The authors do not discuss whether time since last exposure was considered in the analysis.

In another study of occupational exposure to 2,4,5-T, Smith et al. (1982) identified 616 male chemical applicators from a list maintained by New Zealand's Agricultural Chemicals Board and 531 comparison workers at small agricultural contracting companies. Of these, 548 sprayers and 441 agricultural contractors and their spouses participated, with impressive response rates of 89% and 83%, respectively. Mailed questionnaires ascertained spraying activities from the males and reproductive histories from their spouses. The investigators noted that wives of the chemical sprayers anecdotally reported assisting their husbands in spraying activities, some performing this task during their pregnancy.

The questionnaires were completed in 1980 and elicited information on spraying activities and reproductive events that occurred between 1969 and 1980. Reported pregnancy outcomes were categorized into three groups based on whether the fathers had sprayed any chemicals at any time during, or prior to, the calendar year in which the pregnancy occurred and whether 2,4,5-T had been used. There were 1,122 births reported among the 441 control spouses and 1,172 among the 548 spouses of the exposed sprayers. If all other factors were comparable in both cohorts (maternal age, socioeconomic status, and maternal smoking histories were shown to be similar), then there appear to be 220 fewer births observed in the exposed group than might be expected. No associations between herbicide exposure and spontaneous abortion (OR = 0.89, 95% CI = 0.6-

1.3) or congenital malformations (OR = 1.2, 95% CI = 0.6-2.4) were identified. Other risk factors did not appear to be controlled in the analysis of these data: for example, the analysis of miscarriages did not appear to control for maternal age or occurrence of prior fetal loss. Multiple pregnancies per family unit were studied; however, the authors did not address the problem of nonindependent events.

This study is limited by the high probability of exposure to chemicals other than 2,4,5-T. Although exposure levels were not quantitated in this study, a later study of a subset from this same population was conducted to estimate 2,3,7,8-TCDD exposure.

In 1988, Smith and colleagues selected nine pesticide applicators from the above group with the greatest number of years and months per year of pesticide application and evaluated their serum 2,3,7,8-TCDD levels (Table 7-49). The mean serum 2,3,7,8-TCDD level, adjusted for total lipids, among the cases (53.3 pg/g) was 10 times that of age-matched controls (5.6 pg/g). However, exposure in this study was based on self-reports of pesticide application, and research has demonstrated that self-reports do not correlate with documented serum 2,3,7,8-TCDD levels (Needham et al., 1991). Therefore, it would be helpful if serum levels could be obtained from a subset of those applicators with lower self-reported exposures.

A clinical epidemiology study conducted in 1979 examined workers involved with the manufacture of 2,4,5-T between 1948 and 1969 in Nitro, West Virginia (Suskind and Hertzberg, 1984). All active and retired plant employees exposed to the 2,4,5-T process during that 22-year interval comprised the eligible pool of "exposed" subjects. The control group consisted of current and former plant employees who were never associated with the 2,4,5-T process, according to company records. The response rates for these cohorts were 61% (N = 204) and 46% (N = 163), respectively.

A reproductive history was obtained from these male employees during an interview and clinical examination. No attempt was made to verify reports of live births, infant deaths, miscarriages, birth defects, and stillbirths with the spouses or through medical records. There were no significant differences in rates of any adverse outcome by exposure status. Given the poor response rates, crude measure of exposure, and lack of verification of paternally reported reproductive histories, this study was not likely to detect an association between 2,3,7,8-TCDD and reproductive events if one existed.

7.13.12.2. *Environmental Studies*

The problem of documentation of exposure is perhaps of even greater concern in studies of subjects environmentally exposed to dioxins but lacking individual 2,3,7,8-TCDD measures. The route of exposure (inhalation, ingestion, absorption), length and intensity of exposure, and the

timing of the exposure are more difficult to estimate in free-living populations compared with workers in an occupational setting.

Selection bias is also a critical concern in environmental studies because there is no environmental equivalent to company records for defining the exposure status of the study group. Proximity of residence to a source (e.g., a toxic waste site or an industrial source) was generally the best option available for identifying the population at risk of exposure. Issues such as length of time living at that residence, the amount of time spent in the home, other potential sources of exposure (e.g., occupational), and the timing of the contamination episode relative to the outcome of interest may have greatly affected the degree of exposure.

The potential for volunteer bias, which is likely in studies that rely on subjects recruited through publicized requests for participation, is an additional concern in studies of this nature. Moreover, it is extremely difficult to conduct epidemiologic investigations under crisis situations such as the industrial accident that occurred in Seveso, Italy. Given these limitations, the efforts of these investigators have provided valuable impetus to the refinement of study designs and the development of more sophisticated techniques to explore this issue.

7.13.12.2.1. ***The Seveso, Italy, dioxin accident of 1976***. In 1976, during the production of trichlorophenol at the ICMESA plant in Seveso, Italy, a runaway reaction resulted in an explosion that ultimately contaminated 700 acres in the surrounding community. Environmental levels of 2,3,7,8-TCDD were determined by using wipe tests, evaluating toxic effects in small animals, and analyzing grass samples. Approximately two weeks later, more than 200 families were evacuated from high-contamination areas. Exposures were sufficiently high for chloracne to be observed in this environmentally exposed population. Following the reactor release, an extensive surveillance system was initiated to monitor the health of the exposed population. Changes in fetal loss rates occurring during the last quarter of 1976 and first quarter of 1977 were found in both exposed and unexposed communities. An estimated 150 women were in the first trimester of pregnancy at the time of the accident (Rehder et al., 1978); of these, 125 women wished therapeutic abortions by October 1976. Therapeutic abortions were approved for 30. Another estimate (Tuchmann-Duplessis, 1980a,b) reports a total of 108 (50 in 1976 and 58 in 1977) therapeutic abortions in the four affected communities. Several reports (Biscanti et al., 1978; Pocchiari, 1980; Pocchiari et al., 1980) suggested that a large number of women obtained unapproved, and therefore not reported, therapeutic abortions. This supposition was supported by a steep decrease in birth rates in the first 6 months of 1977, that was primarily observed in the exposed communities. (All communities had had decreases over time; however, the decrease in exposed communities at this key time was much larger.)

The Seveso Congenital Malformations Registry enrolled all live births and stillbirths occurring January 1, 1977, through December 31, 1982, to women residents of zones A, B, R, and non-ABR (Mastroiacovo et al., 1988). A total of 15,291 births (live and stillbirths) and 742 birth defects were recorded: zone A, 26 births and no birth defects; zone B, 435 births and 25 birth defects (57.5/1,000 births); zone R, 2,439 births and 110 birth defects (45.1/1,000 births); zone non-ABR, 12,391 births and 605 birth defects (48.8/1,000 births). Birth defects were confirmed by medical records. Relative risk estimate for total defects comparing zones A+B with zone non-ABR is 1.2 (90% CI = 0.88-1.64) (Table 7-48). The rate of birth defects occurring early in the observation period (first quarter of 1977) did not differ from the rate of birth defects occurring later in the observation period (data not shown), and there were no discernible patterns of defects within or between the exposure groups.

To document an increase in the rate of an event, sound baseline information is required. No reliable information regarding rates of birth defects in this area was available until the establishment of the Seveso Congenital Malformations Registry 6 months after the incident. Thus the potential contribution of 2,3,7,8-TCDD to the congenital malformation rate cannot be separated from improved case ascertainment. Prior to this time, only certain birth defects were required to be reported to Italian health officers (Reggiani, 1980b). In addition to the limitations due to legal requirements, there were other reasons for the underreporting of malformations: "Traditionally, Italian physicians have under-reported congenital malformations because of their severe negative social implications." (Tuchmann-Duplessis, 1980a) Although physicians and midwives were encouraged to do more complete recording, Reggiani (1978) concluded that the malformation data are "missing" for 1976, and "incomplete" for the first trimester of 1977. The influence of spontaneous abortions on the birth defect rate is likewise unknown. As with congenital malformations, reliable background data for spontaneous abortion in the study area were not available. The small number of conceptions available for study limits the power of these studies to detect an association between 2,3,7,8-TCDD exposure and spontaneous abortion and birth defects.

Another adverse outcome that has not been described in most epidemiologic studies is the effect of 2,3,7,8-TCDD on the chromosome. Pocchiari and colleagues described one study in which 30 therapeutic and 4 spontaneous abortions from the Seveso area were examined (Pocchiari, 1979). There were no indications of mutagenic, teratogenic, or embryotoxic effects that could be attributed to 2,3,7,8-TCDD exposure. However, it was difficult to determine maternal exposure status for any of the cases. A later study examined the association between 2,3,7,8-TCDD and cytogenetic abnormalities in fetuses from abortions induced shortly after the Seveso accident (Tenchini et al., 1983). The frequency of aberrant cells and the mean number of

aberrations per damaged cell were significantly higher in exposed versus unexposed fetuses. However, the paper omits important data, such as the timing of conception relative to the accident, how long after exposure the abortion occurred, the zone in which the mother and father resided at the time of conception and pregnancy, and tissue 2,3,7,8-TCDD concentration in the fetuses. No other studies have examined fetal tissue to corroborate these data. This approach offers exciting possibilities for future research, as genetic techniques in the area of DNA-adduct analyses become more widely available.

7.13.12.3. *Studies of Exposure to Agent Orange by Military Veterans and Vietnamese Civilians*

The problem of exposure documentation has also been a highly controversial issue in studies of potential exposure to Agent Orange in Vietnam and adverse health effects among both the Vietnam veterans and residents of Vietnam, and their offspring. An early study among the Vietnamese population encompassed a 10-year period in Vietnam from 1960 to 1969 (Cutting et al., 1970). Exposure was dichotomized into pre- or light-spraying years from 1960 to 1965 and heavy-spraying years from 1966 to 1969. A total of 480,087 births, 16,166 stillbirths, 2,866 hydatidiform moles, and 2,355 congenital malformations of all types were examined in this study. Pregnancy outcome data were collected from 22 hospitals.

Increases in the rates of stillbirths, molar pregnancies, and congenital malformations were noted in the coastal plain and delta areas following heavy spraying, although the authors emphasized the slight downward trend observed for all outcomes in the countrywide rates. Several biases in this sampling approach severely limit the interpretation of the study's findings. The births examined were not representative of the births in the country during that period. In addition, the hospital records were incomplete, and transport of the mothers to the selected hospitals resulted in uncertainty regarding maternal residence during the pregnancy.

A second investigation conducted in Vietnam used the HERBS tape (military records detailing Agent Orange spraying missions) covering the period from 1965 to 1971 to determine maternal exposure status according to area of residence (Kunstadter, 1982). HERBS data were matched to hospital records with the date of birth to generate an “estimated date of conception” and to maternal residence at birth to assign “potential for maternal exposure.” Birth outcome data were collected from hospital records, which were subject to incomplete ascertainment, inaccuracies, and incompleteness.

No association between spraying of Agent Orange and any type of birth defect or perinatal mortality was noted. Although cleft lip defects increased in proportion to other malformations

during the heavy spraying period, the total number of birth defects declined and continued to decrease after spraying activities ceased.

Finally, Australian investigators examined the relationship between service in Vietnam during 1962-1972 and birth defects (Report to the Minister for Veterans' Affairs, 1983). Cases were infants born with any of a defined set of congenital malformations in any of 34 hospitals in New South Wales, Victoria, and the Australian Capital Territory from 1966 to 1979. Control infants were matched to cases on maternal age, hospital, and time of birth, yielding 8,500 matched pairs for analysis.

Fathers of case and control infants were matched against a list of members who had served in the Australian Army during the specified time period. No associations were detected for Vietnam service and total birth defects (OR = 1.02, 95% CI = 0.8-1.3) or for any of the approximately 100 birth defects examined. Length of service in Vietnam, time between deplanement and conception, and Vietnam service prior to and following conception were considered in the analysis.

A critical point that should be emphasized regarding the Australian study is the assessment of service in Vietnam as the exposure of interest. The author clearly stated that investigations had indicated that exposure to herbicides was "infrequent and probably very low in Australian troops in Vietnam; the study does not exclude possible effects of herbicides in situations of substantial exposure" (Report to the Ministry for Veterans' Affairs, 1983).

A series of unpublished studies conducted by Vietnamese investigators was reviewed by Hatch (1984a) and should be mentioned in this review. While these reports also have limitations, including incomplete background data for rates of reproductive events and sparsity of epidemiologic details provided in the studies, the assessment of Vietnamese populations offered the opportunity to evaluate pregnancies with various patterns of parental exposure.

Investigations conducted in northern Vietnam assessed pregnancies with no maternal exposure to spraying activities; paternal exposure was presumed to have occurred only when the father had performed military service in the south. Studies of couples in southern Vietnam compared reproductive outcomes observed in sprayed versus nonsprayed areas and represented potential associations between either maternal and/or paternal herbicide exposure and risk of adverse pregnancy outcome.

Three studies examined presumed paternal herbicide exposure and birth defects among pregnancies in northern Vietnam. Lang and van compared the frequency of birth defects during the period 1975-1978 as a function of the father having served in south Vietnam (Hatch, 1984a). Among 2,547 offspring whose fathers had never served in the south, the birth defect rate was 6 per 1,000 (N = 15). Among 511 offspring whose fathers had served in the south, the rate was 29

per 1,000 (N = 15). Similar results were obtained in a study by Lang and colleagues at unspecified agricultural and handicraft cooperatives in northern Vietnam (Hatch, 1984a). The congenital malformation rate among "exposed" pregnancies (paternal service in southern Vietnam) was 23-26 per 1,000 (71 or 82 of 3,147). In comparison, the rate among the unexposed pregnancies was 5 per 1,000 (10 of 2,172).

In what is perhaps the most stringent of the Vietnamese studies by Can et al. (reported in Hatch, 1984a), 40,064 women from three rice-growing districts in northern Vietnam were assessed (although few details are provided on the method of selection). All of the women were married and pregnant at least once during the war and had no history of tuberculosis, syphilis, or malaria, or of using antibiotics or hormones during pregnancy. "Detailed" interviews were conducted by physicians and midwives, and district health records were consulted in an attempt to validate reported pregnancies and outcomes. Only pregnancies conceived during the conflict were considered in this study. There were a total of 121,993 pregnancies among the 29,041 women whose spouses were "nonexposed" and 32,069 pregnancies among the 11,023 women whose spouses had served in the south. Service in south Vietnam was associated with an increased rate of spontaneous abortion ($p = 0.05$). The increased rate of congenital malformations among the exposed pregnancies (0.6%) compared with the unexposed (0.4%) did not achieve statistical significance ($p = 0.10$).

In attempt to further assess this difference, these investigators conducted a case-control study in which they examined a random sample of 61 families of children who had survived with a birth defect. A control group of 183 families of normal children (matched on maternal age, number of deliveries, living environment, and age) was selected. Forty-nine percent of children with a birth defect had a father who had served in south Vietnam, compared with 21% of the children without malformations, yielding an odds ratio of 3.6 (95% CI = 1.9-6.7).

Additional unpublished studies conducted in Vietnam by Vietnamese investigators were reviewed by Constable and Hatch (1985). The reader is referred to this source for details of the studies. It was concluded that studies of presumptive paternal "herbicide" exposure prior to or at conception were suggestive of a relationship with congenital malformations. The evidence for an association with spontaneous abortion was "less convincing," and for molar pregnancies no relationship appeared to exist. Those studies that examined both maternal and paternal "herbicide" exposure were also suggestive of a relation to birth defects as well as spontaneous abortion, stillbirths, and molar pregnancies. Follow-up studies by these Vietnamese investigators supported these earlier findings (Huong et al., 1989; Phuong et al., 1989b).

In the next section, the impact of assays to measure individual levels of 2,3,7,8-TCDD is discussed, and studies of 2,3,7,8-TCDD exposure and reproductive effects that have been published since 1984 are presented.

Review of the Literature From 1984 Through 1995

During the interval since 1984, assays that had been developed to measure TCDD in serum and adipose tissue were being tested and refined. Several investigators have since used these assays in (usually small) subsets of their study populations to estimate exposure to 2,3,7,8-TCDD in their total sample and also in attempts to validate their assumptions regarding magnitude of exposure for their study subjects. Subsets were generally selected to represent subjects designated as "high" versus "low" exposure by the study investigators, utilizing various information sources including self-reports, company or military records, etc. Table 7-49 presents the results of the exposure analyses (Mocarelli et al., 1991; Patterson et al., 1986b; Smith et al., 1992; Centers for Disease Control Veterans Health Studies, 1988; Fingerhut et al., 1991a; Schecter et al., 1989; Kahn et al., 1988; Kang et al., 1991; Roegner et al., 1991; Phuong et al., 1989b).

These data illustrate wide variability in groups presumed to have been exposed to 2,3,7,8-TCDD at levels above background ($\leq$20 pg/g). For example, the mean and median serum levels of Vietnam ground combat troops with service in areas heavily sprayed with Agent Orange did not exceed the levels found in the U.S. general population. There is evidence for higher exposure to 2,3,7,8-TCDD among certain subgroups of Vietnam veterans (et al., 1988; Roegner et al., 1991) as well as residents of Vietnam (Phuong et al., 1989b), Seveso (Mocarelli et al., 1991), and Missouri (Patterson et al., 1986b), and occupational groups (Fingerhut et al., 1991a; Beck et al., 1989).

With the exception of the Ranch Hand study (Roegner et al., 1991), these subsets were selected to describe 2,3,7,8-TCDD exposure in the total study sample and not for an examination of the relationship between 2,3,7,8-TCDD and reproductive events. In addition, the data from the Ranch Hand population indicated a serum 2,3,7,8-TCDD half-life of 7.1 years (Pirkle et al., 1989), but serum samples in this group were collected and analyzed at 11- and 15-year intervals following exposure. Questions regarding the impact of initial dose, age, gender, and pregnancy itself on half-life in humans remain unanswered at this time.

7.13.12.4. ***Environmental Studies***

7.13.12.4.1. ***The Times Beach, Missouri, 2,3,7,8-TCDD episode***. In 1971, a waste oil dealer in Missouri disposed of waste sludge containing approximately 29 kg 2,3,7,8-TCDD by mixing it with waste oils as a dust control spray, which was subsequently distributed throughout the State. General media announcements from health officials were made, urging persons potentially exposed to 2,3,7,8-TCDD to participate in a survey and health screening process. People were warned of their potential exposure by virtue of their residence, employment, or engagement in recreational activities in the contaminated sites. A pilot study was initiated in 1983, in which a subset of those persons who had responded to the media announcements was assessed (Stehr et al., 1986). Approximately 800 completed questionnaires were screened to select participants; it was not clear if this number represents all of the questionnaires that had been returned up to that time.

This phase of the study was intended to identify potential problems for future investigations. Persons determined to be at "high" versus "low" risk for dioxin exposure, based on their completed surveys, were selected. "High risk" was defined as either (1) reported residence or occupation in areas with TCDD levels between 20 and 100 ppb for at least 2 years or in areas with TCDD levels >100 ppb for at least 6 months, or (2) participation in activities requiring close contact with soil in areas with TCDD concentrations as described for similar periods of time. "Low-risk" persons were determined to have had no access to, or "regular high-soil-contact activities" in, any known contaminated area. Controls were frequency matched with the high-exposed group on type of exposure site, age, sex, race, and socioeconomic status. Sixty-eight "high-risk" persons (83% of those eligible) and 36 "low-risk" persons (90% response) were evaluated through physical, neurological, and dermatological examinations; laboratory tests; and interviews.

Information on reproductive outcomes was obtained during the interview administered to the subjects "or their nearest relative." None of the outcomes observed differed significantly by exposure status (Table 7-48), although high-risk women had a later mean age at menarche ($p = 0.06$). A total of 30 births were available for assessment in this sample.

The following year, a more intensive study was undertaken to test the results found in the pilot study (Hoffman et al., 1986). The exposed group consisted of residents of the Quail Run Mobile Home Park in Gray Summit, Missouri, where TCDD levels in the soil were measured at up to 2,200 ppb. Data on 95 of the approximately 207 households in the park were available; 154 persons (74%) who were "both eligible and interested" agreed to participate. The unexposed group consisted of 155 residents of a nonexposed trailer park, representing 77% of those "both eligible and interested" in participation.

The protocol was similar to that employed in the pilot study described above. The authors reported that "no differences were found between the exposed and unexposed groups in the frequency of reproductive disorders or adverse pregnancy outcomes, such as fetal deaths, spontaneous abortions, and children with congenital malformations." No sample sizes or statistical test results for any of the outcomes were reported. Clearly, these studies of Missouri residents were not designed to investigate the relationship between dioxin exposure and reproductive outcomes, and not very much can be learned about this association from these results.

In a retrospective cohort study by Stockbauer et al. (1988), the association between 2,3,7,8-TCDD and reproductive outcomes was examined among residents of contaminated areas in Missouri. All live births and stillbirths that occurred in the nine residential areas identified as contaminated with TCDD during the period 1972-1982 were identified through Missouri vital statistics records. A total of 402 births were examined from six residential areas. TCDD levels in the soil from these six areas ranged from 241 to 2,200 ppb. 2,3,7,8-TCDD levels were not reported for the three areas where no births occurred during this interval, which would have been of interest to note.

A reference group of 804 unexposed births, matched for maternal age and race, hospital and year of birth, and plurality, was selected from the vital statistics records. Medical records were abstracted to ascertain birth defects in the matched sets. In addition, the births were linked to a statewide birth defects register that had been recently initiated. Data on several potentially confounding variables were obtained from birth certificates.

The exposed mothers tended to be less educated, had more children, were more likely to be in the extremes of the prepregnant weight distribution, and were more likely to smoke cigarettes. Statistical testing for these differences was not reported. Moreover, statistical adjustment for these potential confounders was performed only in the birth weight analyses, although there was little change in the birth weight risk ratios after adjustment.

Increased risk ratios were reported in the exposed group for infant death (OR = 2.0), fetal death (OR = 1.6), perinatal death (OR = 1.3), low birth weight (AOR = 1.5), and several subcategories of malformations, although none of these findings achieved statistical significance (Table 7-48).

Two approaches were used to determine a dose-response relationship. In the first, the study data set was matched against the Missouri central listing of dioxin-exposed persons, which yielded 98 of the exposed mothers and none of the control mothers. These 98 women were then dichotomized into "high" (N = 20) or "low" (N = 78) exposure groups. High exposure was defined as residence for at least 6 months in areas with $\geq$100 ppb TCDD in the soil or $\geq$2 years in

areas with TCDD levels from 20 to 100 ppb. Low exposure was defined as residence in areas with similar TCDD levels as described above, but for less than the required time period or residence at sites with 1-19 ppb TCDD. No evidence for a dose-response relationship was observed with this analysis.

The second approach categorized the births into two different intervals relative to the spraying of the soil with the TCDD-contaminated sludge in 1971 to 1973. When births in the 1972-1974 period were compared with births from 1975 to 1982, the authors reported that "the only birth outcomes with higher risk ratios in the earlier time period were very low birth weight, birth defects, and major birth defects." None of these observations were statistically significant, but sample sizes and testing results were not provided in the paper.

The authors acknowledged the possibility of exposure misclassification and also the "modest" power of the study to detect associations due to the small sample size.

7.13.12.5. *Studies of Vietnam Experience in Ground Troops and Ranch Hands Published 1984-1995*

7.13.12.5.1. ***Evaluation of exposure.*** Evidence from earlier studies to determine if Agent Orange exposure increased the risk of adverse pregnancy outcomes among Vietnam veterans has been described as "sparse, sometimes off the point, sometimes conflicting..." (Hatch and Stein, 1986). The general dissatisfaction with these studies had a common factor: the lack of a valid measure of dioxin exposure. Once the assays to document individual 2,3,7,8-TCDD exposure became available and served as the gold standard for assessing the exposure assumptions made by study investigators, this concern regarding exposure misclassification was shown to be justified. Until 1992, when the first study to examine reproductive outcomes among Vietnam veterans based on individual exposure measurements of 2,3,7,8-TCDD was published, this remained the major criticism of the research. Even though 2,3,7,8-TCDD serum levels were available for reanalysis of the 1984 data of the Ranch Hand study, they were not used in that report. A later reanalysis (in 1995) used estimated 2,3,7,8-TCDD level at the time of conception. Exposure indices were developed for several studies to aid in analysis of exposure outcome relationships.

7.13.12.5.2. ***Exposure indices: The CDC case-control study's exposure opportunity index.*** In the early 1980s, the CDC conducted a large case-control study that examined the relationship between service in Vietnam and risk of congenital malformations (Erickson et al., 1984). Although service in Vietnam was the major exposure variable, two additional measures of exposure were assessed. Vietnam veterans were asked if they believed they had been exposed to Agent Orange. In addition, an exposure opportunity index (EOI), developed by the Army Agent

Orange Task Force, assigned a score estimating the likelihood of exposure based on places and times of Vietnam service. These scores ranged from minimal (1) to high (5) opportunity for Agent Orange exposure.

The CDC then reported on a study, using the serum assay as the standard, to evaluate the validity of both self-reported exposure to Agent Orange and use of military records to fomulate the EOI used in the case-control study of military service in Vietnam and birth defects. The results revealed a poor correlation (no correlation coefficient was provided) between both of these exposure estimates and serum TCDD levels (Centers for Disease Control Veterans Health Studies, 1988). In addition, the distributions of serum 2,3,7,8-TCDD levels were nearly identical (median = 3.8 ppt) among 646 ground combat troops who had served in heavily sprayed areas compared with 97 veterans who had never served in Vietnam.

In a separate study, Kahn et al. (1988) measured serum 2,3,7,8-TCDD levels in 10 Vietnam veterans who reported that they had handled Agent Orange "regularly" while in Vietnam, 10 Vietnam veterans with little or no exposure to Agent Orange, and 27 Vietnam-era veterans. The levels of serum 2,3,7,8-TCDD among those men who had handled Agent Orange were significantly elevated (median = 25.1 pg/g blood fat) compared with the Vietnam control veterans (median = 5.3 pg/g) and the Vietnam-era veterans (median = 3.9 pg/g) ($p<0.01$).

The Baseline Ranch Hand study's exposure index. In 1984, the U.S. Air Force released preliminary results of a 20-year study designed to examine the personnel responsible for conducting the aerial spraying of herbicides in Vietnam (Lathrop et al., 1984). The analyses in this baseline study were based on cohort status, i.e., Ranch Hands ("exposed") versus controls ("nonexposed"). In an attempt to determine a link between exposure and clinical endpoints, an exposure index was developed to estimate individual 2,3,7,8-TCDD exposure. The exposure index was later found to be uncorrelated with dioxin levels in Ranch Hand veterans (Michalek, 1989).

In 1988, a report describing a U.S. Air Force and CDC collaborative pilot study utilizing the serum 2,3,7,8-TCDD assay among 200 Air Force Ranch Hand personnel was published. It was noted that the Ranch Hand personnel had significantly higher serum 2,3,7,8-TCDD levels than did controls (Wolfe et al., 1988). These data also indicated that the Ranch Hands as a whole were not as highly exposed to 2,3,7,8-TCDD as was the NIOSH cohort (Piacitelli et al., 1992) and the Seveso population (Mocarelli et al., 1991).

In 1992, the report describing reproductive outcomes among Ranch Hand personnel became available (Wolfe et al., 1992b). In addition to outcome verification, serum 2,3,7,8-TCDD levels were measured in a sample of Ranch Hands (N = 791) and the comparison population

(N = 942). A comparison of the exposure index used in the baseline Ranch Hand study with individual 2,3,7,8-TCDD levels revealed "considerable misclassification" among the study subjects.

As a result of these investigations, it became clear that the likelihood of exposure misclassification in studies of the relationship between 2,3,7,8-TCDD and reproductive events, without direct measures of individual exposure, casts considerable doubt as to the validity of the early findings.

An equally important finding resulting from these investigations was the inability of the exposure indices to classify exposure status in individuals (Needham et al., 1991). 2,3,7,8-TCDD analyses in small subsets of the total study sample, selected on the basis of presumed exposure as defined by an "exposure index" (either military or government records, self-reports, or 2,3,7,8-TCDD levels in soil samples), have demonstrated that the exposure classification schemes employed in these studies were not valid. Therefore, studies that defined exposure as paternal military service in Vietnam should be evaluated without inference to 2,3,7,8-TCDD exposure.

7.13.12.5.3. ***Study results: Atlanta Congenital Defects Program study***. In 1984, the CDC released the findings from the large case-control study that examined the relationship between service in Vietnam and risk of congenital malformations (Erickson et al., 1984). Case-group babies were infants with serious structural malformations born between 1968 and 1980 and registered with the Metropolitan Atlanta Congenital Defects Program (MACDP). Of 7,133 eligible cases, maternal interviews were obtained for 4,929 (69%). Control babies were selected from Georgia vital statistics records and were frequency matched to cases on race and year and hospital of birth. Of 4,246 eligible controls, maternal interviews were obtained for 3,029 (71%). Paternal interview rates were 56% and 57%, respectively.

While response rates were similar by case status overall, among nonwhites significantly more cases were not interviewed. The major reason for nonparticipation was the inability to locate subjects rather than subjects refusing to enroll.

Among the children with congenital malformations, 428 (9%) were fathered by Vietnam veterans and 4,387 (91%) were fathered by non-Vietnam veterans; identical percentages were noted among the control infants. The odds ratio for service in Vietnam and birth defects of any type was 0.97 (95% CI = 0.83-1.14). Odds ratios were also calculated for 96 separate categories of birth defects, with no significant associations observed.

The EOI developed for this study (as described above) also was not associated with total birth defects. However, significant associations were observed for spina bifida, cleft lip with or

without cleft palate, neoplasms, and coloboma. With the exception of the last defect, all three also showed evidence of a dose-response relationship.

For the analysis that examined potential associations between self-reported Agent Orange exposure and birth defects, the frequency of exposure among fathers and each type of malformation was compared with the frequency among fathers and all other defects in an attempt to reduce recall bias. Twenty-five percent (N = 74) of the Vietnam veterans reported that they believed they were exposed to Agent Orange during their service in Vietnam. The analysis of self-reported exposure and birth defects was negative on all counts, in contrast to the EOI analysis, which found significant associations as described above. However, the small numbers of cases of many individual defects resulted in a virtual lack of power to detect any associations for these specific defects.

CDC Vietnam Experience study. As part of a separate, large, multifaceted study mandated by Congress, the CDC evaluated the risk of service in Vietnam and adverse reproductive outcomes (Centers for Disease Control Vietnam Experience Study, 1988d, 1989). The Vietnam Experience Study protocol involved two phases. In the first phase, a random sample of male veterans who met eligibility criteria related to military service was selected for a telephone interview.

Of the eligible "exposed" group, i.e., those veterans who had served in Vietnam, 84% (N = 7,924) agreed to participate, and 84% (N = 7,364) of the nonexposed (those who had not served in Vietnam) were enrolled. Of these 15,288 veterans who completed the telephone interview, a random sample was selected for the second phase, which consisted of a comprehensive medical examination. For this phase, the response rates were 75% (N = 2,490) for the Vietnam veterans and 63% (N = 1,972) for the non-Vietnam veterans group.

During the telephone interview, veterans were questioned about miscarriage, induced abortion, ectopic pregnancy, live births, stillbirths, birth defects, as well as leukemia and other childhood cancers, and major health problems or impairments during the first 5 years of life.

A preliminary analysis of the interview data revealed that the Vietnam veterans had reported 40%-50% more birth defects than the non-Vietnam veterans. In addition, a difference between the cohorts for certain subcategories of malformations, including spina bifida, cleft lip with or without cleft palate, and hydrocephalus, was noted. Therefore, a substudy was conducted to compare the rates of total birth defects in Vietnam and non-Vietnam veterans as these events were recorded on hospital records.

The eligible sample for this substudy consisted of 2,282 veterans who had not yet received their medical examinations, as it would be easier to collect the additional information required and permission to obtain hospital records for their offspring from this group. Hospital records were

obtained for 92% (N = 1,791) of the offspring of Vietnam veterans and 91% (N = 1,575) of the offspring of non-Vietnam veterans.

When birth defects were identified from hospital records, there was no association of Vietnam service with total, major, minor, or suspected birth defects. From the telephone interview data, the odds ratio for Vietnam service and total birth defects was 1.3 (95% CI = 1.2-1.4); in the hospital records substudy, the odds ratio was 1.1 (95% CI = 0.7-1.8). It was concluded that this finding supported the explanation of differential reporting in the telephone interviews.

The odds ratios for selected categories of birth defects calculated from both the telephone interview and hospital records study are presented in Table 7-50. The rate of birth defects increased for both cohorts when malformations were identified in the medical records.

The extent of differential reporting between the two cohorts has also been described (Centers for Disease Control Vietnam Experience Study, 1989). Overall, the authors concluded that agreement between veterans' reports and hospital records for the presence of a birth defect was “relatively poor” for both cohorts. Positive predictive value, sensitivity, and the kappa statistic were slightly lower among Vietnam veterans (Table 7-51). It was further stated that there was no evidence of selection bias or participation bias in this substudy because no differences were noted between cohorts in health histories and demographic or military covariates, and the participation of both groups of veterans was high. However, the subjects in this substudy were selected from the group of veterans who completed the examination. The response rate for this phase of the study was not high, as noted above, with 75% of Vietnam veterans and only 63% of the non-Vietnam veterans participating in the medical examination. Although characteristics of the subset who agreed to undergo physical examination did not differ from the telephone interview sample, no data on those who refused the exam and no reasons for refusing to participate were provided in this paper.

Adjusted odds ratios for two additional outcomes verified in the hospital records substudy, low birth weight (AOR = 1.1, 95% CI = 0.8-1.4) and perinatal mortality (AOR = 1.6, 95% CI = 0.8-3.1), did not differ by Vietnam service status. A race-specific analysis of total, major, and minor defects showed an increased risk for total and minor birth defects among black veterans. The adjusted odds ratio was 3.3 (95% CI = 1.5-7.5) for total defects and 2.9 (95% CI = 1.1-8.0) for minor malformations. This finding is based on very small numbers, however, and on multiple occurrences of two minor defects in two families.

From data obtained during the telephone interview, the adjusted odds ratio for Vietnam service and spontaneous abortion was 1.3 (95% CI = 1.2-1.4). Although an excess among Vietnam veterans was noted across all three trimesters, only the association in the first trimester was significant. There was no attempt to confirm this endpoint by using hospital records. No

significant differences were observed for the reproductive outcomes of induced abortion (AOR = 1.0, 95% CI = 0.9-1.2), stillbirths (AOR = 0.9, 95% CI = 0.7-1.1), ectopic pregnancies (AOR = 1.0, 95% CI = 0.7-1.2), or childhood cancers (OR = 1.5, 95% CI = 0.8-2.8).

In addition to the reported excess of total birth defects in the telephone interview, Vietnam veterans also reported more neural tube defects and hydrocephalus than the non-Vietnam veterans. A second substudy was undertaken to examine the increase in cerebrospinal malformations (CSMs). In this substudy, an attempt was made to obtain hospital records for all of the offspring identified in the telephone interview as meeting one of the following criteria: (1) offspring with a CSM as reported by a veteran, (2) those with a reported condition that suggested a CSM, or (3) all reported stillbirths.

Of the 403 offspring reported to have a CSM, 109 were ineligible, 14% (N = 58) because of conception prior to father's military service and 12.6% (N = 51) classified as miscarriages. Again, the issue of the impact of spontaneous abortion on rates of birth defects is introduced.

The CSM substudy was limited by a poor response rate among the non-Vietnam veterans. The sample thus consisted of 127 offspring of Vietnam veterans (82.5%) and 94 children of the non-Vietnam veterans (67%). Compared with fathers who participated in the CSM study, nonparticipants were more likely to be nonwhite, less educated, unmarried, younger at the time of their child's birth, and have lower general technical test scores. There were also differences in paternal covariates between the participating cohorts that could confound the findings. Compared with Vietnam veterans, non-Vietnam veterans were better educated, had higher general technical test scores, were more likely to be married, were older when their child was born, were less likely to consume alcohol, and were more likely to have had a nontactical primary military occupational specialty (MOS) in the Army and to have served in both the later and earlier time periods.

The number of CSMs reported by the veterans that were verified by hospital records was examined separately by stillbirth and live birth status. Among the reported stillbirths, five CSMs were documented among children of Vietnam veterans and six among the non-Vietnam veterans. Ten of these 11 CSMs had not been reported by the veterans. Positive predictive values derived from this analysis were 6.5% for Vietnam veterans and 8.1% for non-Vietnam veterans.

Among the live births, 21 of 49 reported CSM cases were noted on hospital records for the Vietnam veterans; 6 of the reported 20 cases were observed among the non-Vietnam veterans group, yielding positive predictive values of 42.9% and 30%, respectively.

Tables 7-35 and 7-36 in the unpublished technical report of the study list the fathers' descriptions of the birth defects in their offspring obtained through the telephone interview by cohort (Centers for Disease Control Vietnam Experience Study, 1989). It was intriguing to note that among Vietnam veterans, for 22 of the 55 reported cases of birth defects, the hospital record

finding was "none." In five additional cases, the hospital record finding was "none of the nervous system." Of these 27 nondocumented reports of birth defects, 3 cases were reported as having died within the first year of life. Death during the first year of life was also reported for one of the eight unverified CSMs in the non-Vietnam veterans cohort.

In summary, the CDC investigators concluded that for "most reproductive and child health outcomes studied, Vietnam veterans were more likely to report an adverse event than were non-Vietnam veterans." In the two substudies conducted to compare rates that were identified through hospital records, no significant differences in adverse outcomes between the two cohorts were determined. However, the ability of this study to address the issue of 2,3,7,8-TCDD exposure and reproductive outcome is severely limited. The question of bias still remains in the two substudies. In addition, exposure was defined as service in Vietnam, which does not provide much insight into the question of the reproductive toxicity of 2,3,7,8-TCDD.

American Legion study. The relation of self-reported exposure to Agent Orange and reproductive outcomes was part of a study conducted among 6,810 American Legionnaires who had served during the Vietnam War (Stellman et al., 1988). Information was obtained through a questionnaire mailed to 2,858 veterans (42%) who had served in Southeast Asia and 3,933 veterans (58%) who had served elsewhere. No association between Agent Orange exposure and difficulty with conception, time to conception of the first child, or infant birth weight was observed. However, the proportion of spontaneous abortion was significantly higher among the spouses of veterans who served in Vietnam (7.6%) compared with controls (5.5%) ($p<0.001$). These figures were below the background rate for recognized spontaneous abortion (10%-15%) in the general population.

The significance of these findings is limited by the lack of verification of self-reported exposure, the low response rate, the lack of outcome verification through medical records, and the selection of veterans from the American Legion organization, as they may not be representative of all veterans who served during the Vietnam conflict.

Boston Hospital study. A case-control study to investigate the relationship between paternal military service in Vietnam and risk of spontaneous abortion was conducted at Boston Hospital for Women (Aschengrau and Monson, 1989). Cases identified through hospital records were spontaneous abortions $\leq$27 weeks gestation that occurred between July 1976 and February 1978 (N = 201). Paternal identity information from the hospital birth records was linked with national and State military records to identify those fathers who had served in Vietnam. Frequency of service in Vietnam was compared for cases and all full-term live birth controls (N = 1,119) born at

the hospital during this time period. No association was detected (OR = 0.88, 95% CI = 0.42-1.86).

The same investigators conducted a subsequent study that expanded the outcomes examined to include adverse outcomes in late pregnancy (Aschengrau and Monson, 1990). Case infants identified through hospital records included 857 congenital malformations, 61 stillbirths, and 48 neonatal deaths that occurred at the hospital during August 1977 and March 1980. "Exposure" was defined by using the same method as in the previous study. Frequency of paternal service in Vietnam was compared for cases and 998 normal-term infant controls. Again, no associations with any of these later adverse outcomes were detected (Table 7-48).

The Ranch Hand study -- reproductive outcomes: Baseline study - 1984. This initial report of the health of Ranch Hand personnel used cohort status (Ranch Hand vs. comparisons) as the basis for evaluating effects and exposure. This group of exposed veterans included those who served in Vietnam during 1962-1965, when Herbicides Purple, Pink, and Green were sprayed. These herbicides had higher TCDD concentrations (33 ppm, 66 ppm, and 66 ppm, respectively) than Herbicide Orange with 2 ppm TCDD (Lathrop et al., 1984).

The protocol consisted of a comprehensive personal and family health questionnaire and a physical examination, including an in-depth laboratory analysis. The response rates for each phase of the protocol were quite different both within and between cohorts. Participation in the questionnaire phase was 97% (N = 1,174) for the Ranch Hands and 93% (N = 956) for controls. In the physical examination phase, participation dropped to 87% (N = 1,045) for the Ranch Hands and 76% (N = 773) for controls.

Nonresponders were "on the average" younger than participants. Ranch Hand enlisted personnel had higher participation rates than officers, and black Ranch Hand officers had lower participation rates than nonblack officers. The difference in the response rates for the physical examination phase of the protocol was ascribed partially to the active encouragement of the Ranch Hand Association for participation and the intense media coverage that the study received. The authors stated that the "majority" of reasons given for nonparticipation were "no time-no interest" and passive refusal.

The reproductive outcomes evaluated in this phase of the study were ascertained through questionnaires obtained from both the veterans and their spouses or partners. A total of 7,399 conceptions were analyzed in this report. There were 3,293 conceptions among 1,174 Ranch Hands and 4,106 among the 1,531 controls.

No significant differences were reported for four "measures of fertility": (1) the number of childless marriages, (2) the number of couples having achieved their desired family size, (3) the

number of childless marriages per total number of marriages, and (4) the number of conceptions per years spent together, which included nonmarital relationships. The fertility analysis was performed on the total number of conceptions reported and was not adjusted for any confounding variables. Moreover, exposure in this analysis was defined by a simple dichotomy of Ranch Hands versus controls.

To examine the relationship between Ranch Hand status and spontaneous and induced abortion, stillbirths, and live births, exposure was stratified by pre- and post-Southeast Asia (SEA) service. The unadjusted analysis indicated that Ranch Hands had increased spontaneous abortion rates in both pre-SEA duty ($p = 0.06$) and post-SEA duty ($p = 0.13$). The report qualified this by stating that these inferences were based on analyses that were not adjusted and that "key factors affecting pregnancy outcome are of questionable value," although no similar qualification was given for the fertility analysis. After adjustment for maternal age, smoking, and alcohol use and paternal age, no significant difference was observed for spontaneous abortion.

Among the live births with complete data obtained to allow for adjustment of cofactors, no difference in risk of prematurity was noted. However, the estimate of gestational age, and all other outcomes studied, were based on parental report, which may be subject to errors in recall, and it was not clear whether prematurity was analyzed as only a dichotomous variable (<37 weeks, ≥ 37 weeks) or as a continuous variable. No analyses for birth weight differences by exposure status were performed in this effort; more recent data on IUGR and other pregnancy outcomes (e.g., infant death) are presented in Section 7.13.12.8.

Unadjusted analyses were conducted to examine the relationship between exposure and neonatal death, infant death, physical handicaps, birth defects, and learning disabilities. These analyses were stratified by pre- and post-SEA service periods. The results indicated that Ranch Hands were borderline statistically significantly more likely to report physical handicaps ($p = 0.07$), birth defects ($p = 0.08$), and neonatal deaths ($p = 0.02$) in the post-SEA analysis. After adjustment for the maternal and paternal covariates described above, the relationship with birth defects achieved statistical significance ($p = 0.04$); the other relationships were not statistically significant.

Twelve of the 76 birth defects reported to have occurred among the Ranch Hands after post-SEA service were skin anomalies (ICD Code 757). When these anomalies are excluded, this relationship is no longer statistically significant ($p = 0.14$), although "still of interest."

Finally, semen samples from Ranch Hands (N = 560) and controls (N = 409) were analyzed for sperm count and morphology. The response rates for this parameter were 72.5% and 76.5%, respectively, although some of the samples submitted were ineligible for analysis because of prior vasectomies and orchiectomies. Linear regression techniques examined sperm count as a

continuous variable and percentage of sperm with abnormal morphology as a dependent variable. Independent variables were age and exposure to industrial chemicals. No differences in either parameter were identified.

This finding contrasts with the semen analysis results obtained among 324 Vietnam veterans and 247 non-Vietnam veterans in the Vietnam Experience Study (Centers for Disease Control Vietnam Experience Study, 1988a). That analysis indicated that Vietnam veterans had significantly lower sperm concentrations (OR = 2.3, 95% CI = 1.2-4.3), below the clinical reference value (20 million cells/mL), than the non-Vietnam veterans. In addition, Vietnam veterans had a significantly lower average proportion of "normal" sperm heads. These analyses were adjusted for six covariates, although industrial chemicals were not among them.

The Ranch Hand study - 1992. The significant association between Ranch Hand status and birth defects found in the previous study was of sufficient interest to launch a massive project to verify all reported conceptions and pregnancy outcomes through medical record abstraction. In addition, in 1987 serum 2,3,7,8-TCDD levels were obtained from a subset of Ranch Hands and controls. In 1992, the Air Force released the results of the first study that examined the relationship between direct measure of individual serum 2,3,7,8-TCDD levels and verified reproductive outcomes (Wolfe et al., 1992b). A total of 4,607 conceptions were examined in this study; 2,533 were contributed by 791 Ranch Hands, and 2,074 were contributed by 768 controls.

Ranch Hand personnel were shown to have significantly higher 2,3,7,8-TCDD levels compared with the controls. The median values were 12.8 pg/g and 4.2 pg/g, respectively. The 98th percentile for Ranch Hands was 166.4 pg/g; for controls, 10.4 pg/g. 2,3,7,8-TCDD levels were determined in 1987. These results were used to estimate initial doses received during the veterans' tour in Southeast Asia but not the 2,3,7,8-TCDD level at the time of conception.

The fertility analysis performed in the earlier study was not repeated according to level of serum 2,3,7,8-TCDD (an omission currently being addressed; personal communication, J. Michalek, 2000). There was a significant variation in the association between 2,3,7,8-TCDD and miscarriage with time since SEA tour ($\leq$18.6 years or >18.6 years) and time of conception (pre- or post-SEA tour) among Ranch Hands with current 2,3,7,8-TCDD levels >10 ppt ($p = 0.01$) (Table 7-52). This was attributed to the low miscarriage rate among the pre-SEA Ranch Hands with current 2,3,7,8-TCDD levels >33.3 pg/g lipids. In examining post-SEA conceptions only, a linear trend can be seen for spontaneous abortions and increasing 2,3,7,8-TCDD levels among Ranch Hands who had "late tours" in SEA, i.e., less than or equal to 18.6 years had elapsed between their tour of duty and current 2,3,7,8-TCDD levels. The opposite trend is noted in Ranch Hands with "early tours," i.e., more than 18.6 years had elapsed between the end of duty and the 1987 blood

draw. It was concluded that 2,3,7,8-TCDD did not affect the rates of miscarriage because it seemed "implausible that dioxin would act differently in the two groups."

An alternative explanation might be that there is a relationship, but it cannot be detected by this type of analysis. To evaluate the relationship between 2,3,7,8-TCDD level and spontaneous abortion, 2,3,7,8-TCDD level at the time of conception must be considered. Assuming a half-life of 7 years in humans (Pirkle et al., 1989), it would seem reasonable, for example, to assume that the two groups of Ranch Hands with 2,3,7,8-TCDD levels of 10-14.9 pg/g of lipids with post-SEA conceptions may have had very different 2,3,7,8-TCDD levels at the time their children were conceived. This is possible because the early-tour veterans had more time to decrease their body burden of 2,3,7,8-TCDD before their bloods were drawn in 1987 than did their late-tour counterparts. Paternal TCDD level at the time of conception was estimated in a subsequent analysis of these data (Wolfe et al, 1995).

Table 7-48 illustrates the risk estimates for reproductive outcomes in the Ranch Hand study. Interestingly, the only statistically significant associations between 2,3,7,8-TCDD and adverse events (total birth defects, genital anomalies, and urinary system anomalies) occurred among Ranch Hands with 2,3,7,8-TCDD levels of 15-33.3 pg/g of lipids and not among those in the >33.3 pg/g group.

The report stated that the "expected dose-pattern" for 2,3,7,8-TCDD and total adverse reproductive outcomes (miscarriage, tubal pregnancy, other noninduced abortive pregnancy, or stillbirth) is the "linear one in which the highest anomaly rate occurs at the highest levels of dioxin." This statement raises at least two questions. If a linear response is assumed, might this imply that very early pregnancy losses occur at the highest 2,3,7,8-TCDD levels, so that the conceptus would not survive long enough to be clinically recognized? Or are very early pregnancy losses and clinically recognized spontaneous abortions two separate entities with different thresholds? Such a scenario has been suggested to explain changes in spontaneous abortions observed after exposure to radiation in Hiroshima (Miller and Blot, 1972).

These questions are of interest because the rate of each of these endpoints may directly affect the rates of all subsequent reproductive outcomes that are available for examination. The miscarriages do not include the very early losses, as 99.6% of reported miscarriages were verified through medical records. Very early losses are unlikely to be identified by the woman or clinically.

An analysis in which the 1987 dioxin levels are used to estimate dioxin level at time of conception would be a worthwhile effort. If a relationship between paternal 2,3,7,8-TCDD level and adverse reproductive outcome does exist, this may help determine the dose-response pattern of the relationship.

No evidence was found to support an association between 2,3,7,8-TCDD and total adverse outcomes. These findings should be viewed with caution in view of the above comments concerning the unexplored area of events early in gestation.

Overall, there was little convincing evidence to support an association between birth weight, examined as both a continuous variable and dichotomized (<2,500 g or ≥2,500 g), and paternal 2,3,7,8-TCDD level. Analyses that adjusted for covariates included maternal and paternal age, maternal alcohol use and smoking, and race of the father. No assessment of 2,3,7,8-TCDD and prematurity was reported.

The potential association between cohort status and birth defects was examined for all defects combined and 12 additional categories of malformations. The only categories with sufficient numbers of verified post-SEA cases to detect a relative risk of 2 were total birth defects (229 cases among 1,045 Ranch Hands and 289 cases among 1,602 controls) and musculoskeletal deformities (132 cases among Ranch Hands and 180 among controls).

A significant variation was observed in the association between total birth defects ($p = 0.03$), defects of the respiratory system ($p = 0.03$), and urinary system abnormalities ($p = 0.04$) by Ranch Hand versus control status with time of conception (pre- or post-SEA). All of these findings were due to a lower rate among Ranch Hands in the pre-SEA conceptions and a higher rate among the post-SEA conceptions for the Ranch Hands.

Analyses of birth defects by 2,3,7,8-TCDD level did not find any "consistent patterns" to support an association. For example, among children of enlisted flying and enlisted ground personnel, children of Ranch Hands with 2,3,7,8-TCDD levels ≤10 pg/g lipids had higher rates (433 per 1,000 and 317 per 1,000) than children of controls with background 2,3,7,8-TCDD levels <10 pg/g lipids (229 per 1,000). However, rates of children of enlisted ground personnel with 2,3,7,8-TCDD levels ≥33.3 pg/g lipids were not significantly elevated. Again, these analyses were not based on 2,3,7,8-TCDD level at time of conception. Moreover, if the higher 2,3,7,8-TCDD levels were related to early pregnancy loss, these results would make more biological sense, as the abnormal conceptuses due to 2,3,7,8-TCDD exposure would have been lost before the pregnancy was recognized.

There was also a significant association between 2,3,7,8-TCDD levels and neonatal death (OR = 5.5, 95% CI = 1.5-20.7). Insufficient numbers (N = 13) precluded the calculation of an adjusted odds ratio for this finding.

Finally, no association was detected between 2,3,7,8-TCDD level and either sperm count or percentage of abnormal sperm. These analyses were based on semen samples that had been collected in 1982.

The Ranch Hand study - 1995. This study includes pregnancies to those men described above; but the group has been restricted to confirmed pregnancies occurring after the beginning of service in Vietnam in those men who participated in the 1987 physical, gave blood to evaluate serum dioxin levels, and had usable laboratory measurements (Wolfe et al., 1995). This group potentially included 872 Ranch Hand veterans and 1,036 comparison subjects. In fact, 454 Ranch Hand veterans (RH) yielded 1,006 recognized conceptions and 419 veterans yielded 792 live births, while 570 comparison subjects yielded 1,235 recognized conceptions and 531 controls yielded 981 live births. Paternal dioxin level at the time of conception was used to generate four exposure groupings: (1) comparison with current level ≤ 10 ppt, (2) RH with current level ≤ 10 ppt, (3) RH with current level > 10 ppt and estimated initial level≤ 110ppt, and (4) RH with current level > 10 ppt and estimated initial level > 110 ppt. Comparisons with > 10 ppt dioxin were eliminated from the group, as having higher than "background" levels without an understanding of the probable source of the exposure. The children in the second group (RH with < 10 ppt) were considered separately because the fathers' levels could not be used to estimate exposure levels at the time of pregnancy. Levels at conception were estimated using a fixed 7.1 year half-life with a first-order decay rate. As the time between measurement (1987) and conception varied from 15 to 26 years, the number of half lives ranges from about two to a little over three.

All analyses were adjusted for paternal race, age and military occupation and maternal age, and smoking and drinking during pregnancy. In addition to these, analyses of spontaneous abortion were adjusted for spontaneous abortions occurring prior to service. The proportion of men who fathered recognized pregnancies or live births were about the same in both groups, Ranch Hand or comparison.

Analyses show modest, borderline significant increases in spontaneous abortion (Table 7-48), defects of the circulatory system and heart (OR: 2.3; 95% CI = 1.0-5.1), all anomalies (OR: 1.3; 95% CI = 1.0-1.6), major birth defects (OR: 1.7; 95% CI = 1.1-2.7), and some developmental delays (OR: 1.5; 95% CI = 1.0-2.3), all of these for the low RH group only. Dose-response patterns were not observed, and more detailed analyses were not possible because of the small number of adverse outcomes in each grouping.

7.13.12.5.4. ***Comment.*** The data described above do not present strong evidence for an association of paternal dioxins with developmental effects, occurring pre-conceptionally, prenatally, or identified around the time of birth. These studies are limited by the ability to accurately define exposure at the critical windows for the events. The developmental studies are retrospective, typically using a surrogate measure of exposure (recall, records of work place or service in Vietnam, etc.). Overall, only a few of the developmental studies have collected

biological measurements of dioxin levels at the time of the study (e.g., the Ranch Hand studies, the CDC studies of Vietnam experience in ground troops). Several efforts have compared surrogate exposure measures with serum levels: (1) a comparison of serum levels with CDC's exposure opportunity index and with the subject's self-report of exposure (CDC, 1988) showed that the surrogates were not a good estimate of the measured levels; (2) a rather small comparison of Vietnam veterans (10 reported exposed, 10 reported little or no exposure) and 27 Vietnam-era veterans (Kahn et al., 1988) showed a significant elevation for the exposed men compared to the other two groups; and (3) in the Ranch Hand study, comparisons of serum levels from a sample of men to the exposure index revealed "considerable misclassification" (Wolfe, 1992b). Given that the second study was rather small, and confidence intervals included background exposure, those data are of limited use. In general, the results described above suggest that many of the studies reported have subjects misclassified by exposure. If so, effects of exposure could be masked.

Few of the results in the studies give an indication of an effect of dioxin on pregnancy outcomes; some did show increased birth defects, but many of these were based on small numbers. As mentioned above, the potential effects of fetal loss on the examination of malformations is difficult to address, but might affect these results, especially in the higher exposure groups.

7.13.12.6. ***New Issues - Developmental Outcomes (1996-1999)***

7.13.12.6.1. ***Dental effects.*** An investigation of dioxin exposure and tooth development was done in Finnish children as a result of studies of dental effects in dioxin-exposed rats, mice, and nonhuman primates (Chapter 5), and in PCB-exposed children (Rogan et al., 1988). The Finnish investigators examined enamel hypomineralization of permanent first molars in 6-7 year old children (Alaluusua et al., 1996; Alaluusua et al., 1999). These molars were mineralized during the postnatal period, when the children are exposed through breast feeding. The population was first identified in Helsinki and Kuopio as part of an international effort, coordinated by WHO/EURO, to evaluate possible health effects associated with levels of PCDDs and PCDFs in breast milk. Approximately 150 women were recruited from each area, with the commitment to provide a breast milk sample at 4 weeks postpartum, if still lactating. A total of 167 samples were obtained, with about a 50% response rate in Helsinki (77 women provided samples) and about 60% from Kuopio (90 women provided samples). Exposure to each child was estimated using the TEQ for PCDDs and PCDFs and their elimination constant, plus the length of time the child was breast fed. At age 6 or 7, 102 children's teeth were examined (61% of those with breast milk samples). Defects were scored as to severity and size, blind to exposure score. Duration of lactation ranged from 1 to 36 months (mean 10.5 +/-5.5 sd) and TEQs from 3.8 to 99.4 pg/g milk

fat (mean 19.8 +/-10.9 sd). The length of time breast feeding was not significantly associated with mineralization changes, nor was the TEQ alone. However, when the levels and length of breast feeding were combined in an overall score, a statistically significant association was observed (r = 0.3, p = 0.003, regression analysis). The beta from the analysis was not presented, so the slope of the relationship is unknown. The levels in breast milk might also be a surrogate for *in utero* exposure; these samples were collected after 4 weeks of breast feeding, and so might not be as good a surrogate as samples collected earlier in lactation.

7.13.12.6.2. ***Comments*****.** These data present interesting findings relating hypomineralization of permanent first molars and TEQ exposure through breast feeding. Unfortunately, the presentation of the results is incomplete: They present limited information on adjustment for other risk factors/ confounders, have a small number of subjects and consequently low power, and since the beta was not presented for the regression analyses, the potential biological significance cannot be examined. This would be an interesting outcome to examine in additional studies.

7.13.12.7. ***Sex Ratio at Birth***

Sex ratio has been reported to vary with a great number of factors, including race, timing of conception within the cycle, certain parental diseases, and gestational age (James, 1987; Kellokumpu-Lehtinen and Pelliniemi, 1984). The sex ratio is defined by demographers as (number of male births)/(number of female births)*100. However, many of the papers covered in this section present the proportion of male births of the total, rather than an actual ratio. In this discussion, while the endpoint will still be called "sex ratio," all data will be presented as the proportion for consistency with the original literature and ease of comparison among the studies.

In response to a report of hormonal variations in men occupationally exposed to dioxin (Egeland et al., 1994), James (1995, and repeated later [1997a,b]) proposed that with high gonadotropin and low testosterone levels, sex ratios could be lowered (fewer male births compared to female births). A 1996 letter (Mocarelli et al., 1996) reported an excess in female births conceived following the Seveso accident. This included births from April 1977 to December 1984, a time period approximating the half-life of dioxin. Seventy-four births occurred during this time within the A-zone; 26 (35.1%) were males and 48 females (65.9%), compared to the expected value (51.4% males [James, 1987]) used by the investigators. Since 1988, these investigators measured dioxin levels in archived serum samples. Of the 74 births, 17 occurred in families with both parents in the A-zone. "Elevated" dioxin level was defined in this report as > 100 ppt (lipid adjusted), and ranged from 104 to 2,340 pg/g lipid in fathers and 126-1,650 pg/g lipid in mothers; 100 ppt or less was considered within normal range. Of this group, 100% (N =

12) births were female. Eighty percent of those with "low" dioxin levels were male (N = 5). In an unadjusted analysis, the overall sex ratio (0.235) was significantly different from the "expected value" ($X^2 = 12.68$, $p<0.001$), in an analysis unadjusted for other factors related to variations in sex ratio. After this time period, from 1985 to 1994, the sex ratio increased to 0.484 (N = 124), a value not significantly different from the "expected value." The authors mentioned that the reduced sex ratio in this small series of births could have resulted from excess males in spontaneous abortions (a theory that cannot be assessed with existing data), or that changes in sex ratio could result from the changes in hormonal balance.

The investigators revisited the Seveso Cohort, identifying all births for those who lived in A, B, or R contaminated at the time of the 1976 explosion (Mocarelli et al., 2000). Parents with less than or equal to 15 ppt TCDD were compared to those above (all together, or split into categories: >15-80 ppt, >80 ppt). After comparing levels in mothers and fathers in 1976, for a total of 674 births in 452 families, a pattern of reduced birth ratios was noted for paternal exposure only, or where both parents were exposed, but not where only the mother was exposed. More detailed analyses focused on paternal exposure, with the observation that the reduction in sex ratio was greater for those fathers who were less 19 years old at exposure in 1976 (sex ratio: 0.382; 95% confidence intervals: 0.30-0.47) versus those who were older (sex ratio: 0.469; 95% confidence intervals: 0.41-0.53). In additional, the sex ratio in offspring to both groups of exposed fathers were significantly less than unexposed fathers of all ages (sex ratio: 0.557; 95% confidence interval: 0.50-0.62). The authors did not present data on age specific sex-ratio among the unexposed fathers, thus leaving the comparison incomplete. An additional analysis would have been more informative: the interaction of paternal age by paternal exposure level for sex ratio. Also, details of factors considered and controlled in the multivariable analyses were not presented, limiting the examination of this interesting study. This study presents intriguing data on the possible relationship of sex ratio and age at dioxin exposure.

Dimich-Ward (1996, 1998) and colleagues examined 23,829 production and maintenance workers employed in 11 sawmills in British Columbia between 1950 and 1985. These sawmills used dioxin-contaminated chlorophenate as a fungicide for lumber. These workers were linked with vital records on live and stillbirths for 1952-1988, obtaining data on the birth date and sex of the 19,675 children born to the workers after beginning employment. The sex ratio of the entire group was 51.6% male and 48.6% female, proportions consistent with those typically observed. Exposures in this study were estimated by 10 experienced workers, because no measurements were available. These estimated measures were then combined with cumulative hours worked during different time periods. The exposure breakdowns, however, were not used in examination of these data; the proportion of males and females were erroneously presented (reversed) in the

heading of a table. Then James (1997) tested these overall proportions, found them statistically significant, and stated that they supported his hypothesis. A correction of the headers (Dimich-Ward, 1998), stating that the numbers had been transposed, resulted in a retraction of James' letter (1998). This study does not contribute much to the discussion, given the lack of specific exposure data, either on dioxins or other potential exposures.

Michalek and co-workers (1998) examined the Operation Ranch Hand study group for differences in sex ratio. Men were grouped in one of four exposure categories (comparison [n=1254] and background [n=346] both less than 10 ppt, low [n=277], and high [n=280] with >79 ppt) based on serum blood levels in 1987 or 1992 extrapolated to the time of conception, and using a fixed 8.7 year half-life for dioxin. No maternal data on dioxin exposure were available, but the investigators suspected that mothers had "background" levels. The analyses looked at those children conceived within 1 month, 1 year, or 5 years, and any time post-service. Confidence intervals (95%) were calculated using the binomial distribution. No significant differences were observed in any analyses. The authors suggest that the findings in Seveso might be associated with maternal exposure.

Rogan et al. (1999) evaluated sex ratio in their population of Taiwanese children whose mothers were affected by exposure to dioxin-like compounds (PCBs and PCDFs) after consumption of contaminated cooking oil (Yu-Cheng). Health effects identified in this population included developmental delays and ectodermal effects in children born to affected mothers. Overall the proportion of males in live births (n=137) from 1978-1985 was 0.496. When the first year was eliminated, to examine only those births conceived at the time the oil was first sold (June 1998), the proportion of males was 0.508, not different from that number used as a comparison on other analyses.

Examination of 44 (of 59) primiparous mothers in a cotton-growing region in Kazakhstan (Hooper et al., 1999), showed that those living near a reservoir with agricultural runoff (zone A) had higher levels of dioxin in breast milk than those located >10 miles away (zone B). This information was then used to group all live births in the region occurring 2-8 weeks before the sampling period in 1997. Women in zone A (N = 17) had mean breast milk levels of 53 pg/g versus those in zone B (N = 24) with mean levels of 21 pg/g. When the data were examined by zone, or by TCDD level ($\geq$30 pg/g versus < 30 pg/g), statistically significant differences were not observed. The numbers were small, limiting the power, but in all the subgroups except zone B (45.8%), more males were born (proportions ranging from 54.5% to 70.6%).

More recently, NIOSH (Schnorr et al., in press 2001) has examined its occupational cohort study for altered sex ratio at birth. The study compared births of male workers' mates (292 births with <20 ppt TCDD; 104 with 20-254 ppt TCDD; 88 with 255-1119 ppt TCDD; and 60 with

1120+ ppt TCDD) to 647 births to never-exposed referents (<20 ppt TCDD). The exposed proportions range from 0.51 to 0.55; none were significantly different from the referent pregnancies (proportion=0.54), either in unadjusted or adjusted analyses (adjusted for maternal education and paternal race). The pregnancies in this occupational setting experienced higher paternal TCDD exposures than the environmental studies; even in the highest exposure group, while limited in size, did not experience a change from the referents (1120+ ppt TCDD: proportion=0.55; 95% CI: 0.49-0.61 versus <20 ppt TCDD: proportion=0.54; 95%CI: 0.52-0.56)

7.13.12.7.1. ***Comments.*** Many of these analyses provide limited data for one or more of the following reasons: limited exposure data, no or limited adjustment for other risk factors/confounders, assumption of a gold standard of 51.4% males (and not having comparison groups) or small numbers. The recent NIOSH analysis, of occupationally exposed (adult) men, with a broad range of exposure, an appropriate comparison group and adjustment of the analyses, did not observe any differences.

Sex ratio at birth was significantly depressed in a group of 17 children in zone A of Seveso in the years shortly following the industrial accident. This pattern disappeared a few years later. However, a recent expanded effort suggests that paternal age at the time of exposure may be a key factor. Sex ratio differences were not observed in other groups examined. If effects are restricted to offspring of fathers less than 19 years old, as suggested in the new Seveso study, the lack of effect elsewhere could be explained by the groups examined: maternal levels of dioxin in community studies or studies of men older than 19. The findings in the most recent Seveso study emphasize the need for more attention on male-mediated development effects, and the potential importance of exposures prior to and during puberty.

7.13.12.8. ***Growth, Malformations, and Infant Mortality***

In recent years, those investigating developmental outcomes have started looking at a variety of measures of prenatal and postnatal growth. Outcomes considered have included birth weight and size, intrauterine growth retardation (IUGR), and postnatal measures up to the age of 42 months. IUGR, also known as small-for-gestational-age, basically combines information on birth weight with the length of gestation. Children with low birth weight are not necessarily IUGR because of different expected birth weights at different gestational ages.

New analyses of growth of children in the smaller Dutch study (38 children, Pluim et al., 1997) have been made. Birth weight data were recorded at delivery by the obstetrician or midwife. Weight and length were recorded at 10 and 20 weeks during postnatal examination, and used to calculate the Quetelet index (weight/length^2). In addition to these measures, the

circumference of the head was measured (1, 11, and 26 weeks) and area of the liver determined by ultrasound (10 days and 11 weeks). No differences were found between low and high exposure for any of the growth measures (using Student's t-test).

The Rotterdam study also examined birth weight and growth (Patandin et al., 1998). Birth weight was only evaluated in relation to PCBs and so will not be discussed here. Postnatal growth was examined in relation to TEQs for dioxins, furans and PCBs in breast milk multiplied by weeks breast fed. Using multivariable regression, and controlling for other factors potentially related to growth, no significant differences were observed at 3 months. A statistically significant decrease in growth in length was observed (beta = -0.21, $p = 0.04$) with TEQ, but not with weight or head circumference between 3 and 7 months of age. No differences were observed between 7 to 18 months or 18 to 42 months.

Another study examined the effects of background levels of PCDD and PCDF levels and birth weight in all consecutive births from January-May 1987 in one maternity clinic in an urban area (Helsinki, Finland) and in a rural area (Kuopio province) (Vartiainen et al., 1998). Approximately 150 women were recruited from each area, with the commitment to provide a breast milk sample at 4 weeks postpartum, if still lactating. A total of 167 samples were obtained, with about a 50% response rate in Helsinki (77 women provided samples, representing 26% of births) and about 60% from Kuopio (90 women, representing 30% of births). TEQs of breast milk were significantly higher in the urban area (26.3 pg/g TEQ versus 20.1 pg/g TEQ in Kuopio province). Correlation analyses (Pearson's correlation) were significant for all births and all male births. Regression analyses of all children showed a decreasing relationship of birth weight with TEQ of milk (beta = -0.00228), which appeared to be primarily in male births (beta = -0.00302; versus in females, beta = -0.00107). Statistical significance of the regressions was not presented, nor were details on other characteristics included in the models. When restricted to examination of first-born children (N = 84), no significant relationships were observed.

One report examines placental AH receptor binding of TCDD in IUGR, preterm birth, and structural malformation (Okey et al., 1997). The study group, 86 births, included 21 preterm births, 20 with IUGR (8 of these were preterm), and 7 infants with structural malformations. The B_{max} (concentration of AH receptor sites for TCDD) and K_d (affinity for binding of TCDD) were not significantly different for the different pregnancy outcomes. Some modest increases were observed for B_{max} (and less so for K_d) with IUGR (N = 10) and structural malformations (N = 5) over normal deliveries (N = 23), but the power was limited by small numbers.

Michalek and colleagues (1998) examined IUGR in their study of the veterans of Operation Ranch Hand. The analyses included 2,082 liveborn, singleton births occurring during or after the father's service in Southeast Asia, for whom paternal serum measures of dioxin were

available. Of the 2,082, 859 were in the Ranch Hand group and 1,223 were comparisons. If serum dioxin levels in 1987 or 1992 were >10 pg/g lipid, the investigators modeled the father's level at the time of conception of the child. For those at or under 10 pg/g lipid, levels at conception were considered to be "background." Levels greater than 10 and less than 79 were "low," and above that were "high." Length of gestation and birth weight were obtained from labor and delivery records. Included births occurred between 1959 and 1992; the earliest births were from comparison subjects. No differences were observed in IUGR across the exposure groups. Small, nonsignificant increases were seen in preterm birth (<37 weeks gestation) for Ranch Hand background and high groups (RR = 1.4, 95% CI = 0.9-2.3 and RR = 1.3, 95% CI = 0.8-2.3, respectively). Significant increases were observed in these groups for neonatal death (within the first 28 days of life): RR = 3.2, 95% CI = 1.0-10.3 for background, and RR = 4.5, 95% CI = 1.5-14.0 for high (for the low group: RR = 1.5, 95% CI = 0.3-7.5). Most of the Ranch Hand deaths were due to short gestation and low birth weight, but only a third in the comparison group. While these numbers were relatively small, the proportions in the background and high groups were much higher: comparison: 3.7% of 54 preterm births; background: 25% of 20; low: 0% of 6; high: 31.3% of 16. An examination of these data using occupation, so as to include all births, not just those with serum measures, also showed elevated proportions of infant deaths in preterm births in the exposed versus the comparison group. However, the proportions did not follow the relative exposures observed among the categories.

7.13.12.9. *Comment*

At this time, the data relating growth measures and neonatal death are limited. For example, in one study, decrements in length (but not other measures of growth) were observed early, but disappeared with increasing age (Patandin et al., 1998). Some changes were observed in the Ranch Hand study for preterm birth and neonatal death, but these did not follow an exposure-response relationship (Michalek et al., 1998). The Finnish data are interesting because birth weight did decrease in males, with increasing TEQ, but the lack of detail on the statistical analyses makes interpretation difficult.

7.14. NONCANCER EFFECTS OF INGESTION OF RICE OIL CONTAMINATED WITH POLYCHLORINATED DIBENZOFURANS, QUATERPHENYLS, AND BIPHENYLS IN JAPAN (YUSHO) AND TAIWAN (YU-CHENG)

This section briefly reviews the noncancer effects observed in Yusho (Japan) and Yu-Cheng (Taiwan) victims, individuals exposed by ingestion to large concentrations of compounds structurally related to dioxins, namely polychlorinated dibenzofurans, quaterphenyls, and biphenyls. The history of each incident, the chemicals in question, and levels of exposure are described in this chapter. In addition, other reviews have summarized the numerous papers dedicated to Yusho and Yu-Cheng (Lü and Wong, 1984; Kuratsune, 1989; Rogan, 1989).

Reports describing effects among individuals who ingested the contaminated rice oil both in Taiwan and Japan are limited to acute rather than chronic effects. Studies have not comprehensively evaluated long-term effects even though over 30 years have passed since the Yusho incident and over 20 years since the Yu-Cheng incident and that serum levels of some contaminants are available for both populations. Recent epidemiologic studies have concentrated on the development of offspring of Yu-Cheng mothers. These children were exposed *in utero* at the time the contaminants were ingested, or were conceived after the poisoning and were exposed to residual contaminants transplacentally or through breast milk (Chen et al., 1992; Lai et al., 1993, 1994; Hsu et al., 1993; Guo et al., 1993,1994a,b, 1995a,b,1996; Chao et al., 1997; Yu 1994, 1998).

7.14.1. Acute Effects in Adults and Children Directly Exposed to Contaminated Rice Oil

In both groups, the most notable acute effects are dermatologic and neurologic signs and symptoms of fatigue, headaches, and gastrointestinal distress (nausea, vomiting, abdominal pain) (Kuratsune, 1989; Rogan, 1989).

7.14.1.1. *Yusho*

The initial recognition of Yusho occurred in 1968. As of 1983, a total of 2,060 individuals were identified as part of the Yusho population (Masuda et al., 1985). Five years after exposure ended, the mean concentrations of PCBs in the adipose tissue, liver, and blood of Yusho cases were 1.9 ppm, 0.08 ppm, and 6.7 ppb (Masuda et al., 1985), respectively, which were about twice the levels in the control group. Adipose tissue levels of PCDFs ranged from 6 to 13 ppb (Masuda et al., 1985). Sixteen years after exposure, mean PCQ level in adipose tissue of Yusho cases was 207 ppb, approximately 100 times the level in Japanese controls (Kashimoto et al., 1985).

In addition to the major health effects, other possible outcomes were examined. Effects observed shortly after exposure included elevated triglyceride levels and effects on female reproductive hormones, noted by changes in menstrual and basal body temperature patterns and lowered excretion of estrogens and pregnanediol in exposed women (Kuratsune, 1989). However,

fertility and other measures of reproductive function were not evaluated. Evidence of chronic bronchitis and respiratory infections still remained 14 years after exposure ended (Nakanishi et al., 1985). However, more than 10 years postexposure, PCB levels were not related to levels of serum triiodothyronine (T3), thyroxine (T4), and thyroxine-binding globulin (TBG) (Murai et al., 1987). Although the liver is the suspected target organ for halogenated hydrocarbons, and marked proliferation in the endoplasmic reticulum was observed, clinical evidence of liver damage, such as alterations in liver enzymes or liver disease, was not observed (Kuratsune, 1989).

Dermatologic effects were the most evident signs, characterized by hyperpigmentation of the nails, gingivae, and face, and by nail deformities, horny plugs, comedones, acneform eruptions, cysts, and other abnormal keratotic changes (Urabe and Asahi, 1985). Acneform eruptions were observed on the face, cheeks, auricles, retroauricular areas, inguinal regions, and external genitalia (Urabe and Asahi, 1985). More than 80% of Yusho cases experienced one or more dermatologic effects (Kuratsune, 1989), which diminished in severity over time (Urabe and Asahi, 1985).

Ophthalmologic effects were characterized by swelling and hypersecretion of the meibomian glands and pigmentary changes of the conjunctiva (Kuratsune et al., 1972). More than 80% of Yusho cases exhibited ocular changes, which, in some cases, appeared to persist 15 years after exposure ended (Kuratsune, 1989).

Thirty percent of the cases reported having at least one symptom consistent with neurologic involvement, such as limb parasthesia and spasms, weakness, headaches, and fatigue (Kuratsune, 1972). As summarized by Kuratsune (1989), Kuriowa et al. (1969) found mostly sensory deficits, identified through slowed nerve conduction velocities, in 23 cases. Follow-up of these cases indicated that the neurologic symptoms disappeared over time; however, conduction velocities were not repeated.

A number of studies examined the immune status of Yusho cases (Kuratsune, 1989). Significant decreases in mean IgA and IgM and increases in IgG were noted in 28 cases tested in 1970 ($p<0.05$) (Nakanishi et al., 1985). Within 2 years, mean levels of all three immunoglobulins returned to normal. Small increases in the percentage of CD4 cells, small decreases in the percentage of CD8 cells, and enhanced lymphocyte stimulation were also noted in Yusho cases (Nakanishi et al., 1985).

Studies of offspring of Yusho cases have been limited to descriptions of effects on newborns exposed *in utero*. An early description of 13 children born to exposed mothers noted two stillborn infants, one of whom was diffusely and deeply hyperpigmented (Rogan, 1982). Neonates described in other reports were darkly pigmented and had marked secretions of the conjunctival palpebra, gingival hyperplasia, hyperkeratosis, calcification of the skull, low birth weight, and natal teeth (Yamashita and Hayashi, 1985). The abnormal pigmentation disappeared

after 2 to 5 months. No other physical abnormalities (neurologic, cardiovascular, or malformations) were identified.

7.14.1.2. *Yu-Cheng*

The initial recognition of Yu-Cheng occurred in 1979. As of 1983, approximately 2,000 individuals were found to have been exposed to the contaminated rice oil. Within the first year of exposure, mean serum PCB, PCDF, and PCQ levels for 15 cases were 60 ppm (range 4-188 ppm), 0.14 ppb (range <0.005-0.27 ppb), and 19.3 ppb (range 0.9-63.8), respectively (Kashimoto et al., 1985). Analysis of PCB levels in 1980-1981 in 165 cases (mean 38 ppb, range 10-720) (Rogan, 1989) and in 1985 in 32 cases (mean 15.4 ppb, range 0.6-86.8) (Lundgren et al., 1988) suggested that some PCBs were being eliminated. It is not clear from the reports if the samples were drawn from distinctly different individuals or included some of the same individuals.

The ophthalmologic and dermatologic changes observed in Yu-Cheng cases were very similar in character and anatomical distribution to those noted in Yusho cases (Lü and Wu, 1985). In 89 cases followed for up to 17 months, dermatologic conditions of 38% of the cases improved, 54% remained the same, and 7% showed deterioration of their conditions (Lü and Wong, 1984).

Like Yusho cases, Yu-Cheng cases examined within 2 years of exposure for nerve function exhibited slowing of sensory nerve conduction. They also exhibited motor nerve slowing and mixed deficits (Chen et al., 1981, 1983, 1985; Chia and Chu, 1984). Twenty percent of a population of 27 individuals also had abnormal EEGs (Chia and Chu, 1984). However, the authors suggest that any correlation between PCB exposure and the abnormal EEGs may be spurious because of low PCB levels in the cerebrospinal fluid (0.5-2.3 ppb) (measured in four subjects), despite much higher blood PCB levels of 48-64 ppb. A sample of 28 individuals with peripheral neuropathy in 1980 was reexamined in 1982 and was found to have normal EEGs and some recovery of sensory and motor nerve conduction velocity (Chia and Chu, 1985).

In 1981, immunologic function was assessed on different subsets of Yu-Cheng cases and summarized by Lü and Wong (1984). In 30 cases compared with unexposed controls, both IgA and IgM were significantly decreased, while IgG did not differ from controls. In this same group, percentages of active T-cells and T-cells were significantly increased ($p<0.05$), while total lymphocyte count and percentage of B cells were unchanged. Significant increases in helper T-cells (T4) but not suppressor T-cells (T8) were also observed. In another group of cases, response to lymphocyte-stimulating mitogens was mixed and the findings unclear. In 143 cases, reaction to streptococci antigen appeared to be significantly ($p<0.05$) depressed relative to controls.

Alterations in porphyrin levels and liver enzymes have been identified as acute reactions to exposure to halogenated polycyclic hydrocarbons, including PCBs. Porphyrin levels were

measured in two exposed groups (Chang et al., 1980; Gladen et al., 1988). In 1980, statistically significant elevations in 24-hour urinary excretion of uroporphyrin (exposed = 41.23 μg ± 24.56; unexposed = 13.57 μg ± 11.76, $p<0.01$) and α-aminolevulinic acid (exposed = 1.002 mg ± 0.600; unexposed = 0.715 ± 0.337, $p<0.05$) were noted among 69 subjects (Chang et al., 1980). Coproporphyrin and porphobilinogen levels were increased in the exposed group but were not significantly elevated. The second study group was composed of 75 children born between June 1978 and March 1985 to mothers who ingested contaminated rice oil (Gladen et al., 1988). Spot urines were collected in 1985. Mean total porphyrin (exposed = 95.2 μg/L; unexposed = 80.7 μg/L) and coproporphyrin (exposed = 72.4 μ/L; unexposed = 59.8 μ/L) excretion was elevated in the exposed subjects, possibly because of extremely high levels (>200 μg/L) in eight exposed children and two controls (Rogan et al., 1988). However, no porphyria cutanea tarda, a severe form of porphyria, was observed in either group of children. Moderate, but statistically significant, increases were observed in AST and ALT levels in 23 cases tested 1 year after exposure (Lü and Wong, 1984). LDH and bilirubin levels were not significantly elevated. As in Yusho cases, triglyceride levels were significantly increased by approximately twice the level in unexposed controls.

Recently, a follow-up study interviewed 59% of 600 surviving women who were 30+ years of age and less than 60 years of age in 1993. Drawing from registration data, women who lived in the same areas in 1979 and were within 3 years of age of cases were selected for comparison. Of these, 312 of 594 (53%) participated. The low response rate meant that not all case respondents had matches in the controls, and the reverse. The authors chose not to maintain matching in the analyses (unpaired X2 and t-tests), which may affect the results reported. In comparisons of the Yu-Cheng women and their controls, Yu-Cheng women were more likely to have abnormal menstrual flow (16.6% vs 7.5%, p <0.068), to have had a stillborn infant since 1979 (4.2% vs 1.7%, p <0.05), to have had a child die before adolescence (10% vs 6.1%, p<0.05), and decided to limit childbearing for health reasons (6.9% vs 2.0%, p <0.05). On the last point, the Yu-Cheng women who did not decide to limit childbearing did not have a difference in family size from the controls.

7.14.1.3. *Effects Observed in Offspring of Yu-Cheng Cases*

A concerted effort has been made to evaluate the overall development of children exposed prenatally to contaminated rice oil ingested by their mothers. Two studies are currently being conducted: one to evaluate children and controls born between 1978 and 1985 (termed “early-born”), and the second to evaluate children born in 1985 and later. Controls were matched within 30 days of age (15 days if less than one year of age), neighborhood, sex, mother’s age within 3

years, and combined parental education (within 3 years). The purpose of the two studies was to determine if there are differences among children exposed *in utero* or shortly after birth by the mothers' ingestion of the contaminated rice oil and children born 7 to 12 years after direct exposure to either parent ended (July 1, 1985 to December 31, 1991). Only a small sample of these children (n=31) have had serum levels of PCB and PCDF determined, so all the analyses are based on broad groupings, reducing their ability to look at exposure levels and health endpoints. The studies comparing the Yu-Cheng children and controls typically analyzed data using paired t-tests, with out adjusting for other potential risk factors (except for the matching of controls).

In terms of musculoskeletal development, several studies have documented delays and abnormalities (Yu et al., 1991; Rogan, 1989; Chen et al., 1993; Guo et al., 1994a). In one of the first studies conducted in 1985, Rogan and colleagues (1988) examined 117 children born since the mothers' exposure in 1979 and 107 unexposed controls. In this study, babies of exposed mothers were consistently smaller and shorter at birth than controls and had similar characteristics: natal teeth, neonatal conjunctivitis, and pigmentation. Exposed mothers reported a mean birth weight 479 g lower than that reported by control mothers; no validation of these reports using medical records was undertaken. As older children, they exhibited a variety of signs and symptoms: fragile chipped teeth and gum hypertrophy, pigmented and deformed fingernails and toenails, and abnormal lung auscultation. In this same study, neurologic developmental assessments were also conducted to evaluate development (Yu et al., 1991). Forty-nine percent of Yu-Cheng children compared with 22% of controls were developmentally delayed in 32 of 33 developmental milestones, 12% had clinical evidence of developmental or psychomotor delays compared with 2% of controls, and 7% of Yu-Cheng children versus 3% of controls had speech problems. These delays were noted at all ages and persisted over 2 years of testing. Delay was greater in children of smaller size and in children who had exhibited neonatal symptoms of intoxication.

In 1991, the musculoskeletal development of 56 Yu-Cheng children (age range 6-10 years) and their matched controls was again assessed (Guo et al., 1994a). Only children born first after the mother's exposure were shorter in stature (-3.4 cm, $p = 0.02$) and had decreased lean muscle mass (-2.9 gm, $p = 0.04$) and soft tissue content (-5.3 gm, $p = 0.06$). Another examination of 110 Yu-Cheng children and 108 controls, ages 8 through 14 years, found Yu-Cheng girls to be significantly shorter than controls, matched to the exposed children by age, sex, maternal age, parents' combined educational level, occupation, and neighborhood (Guo et al., 1993). As measured by the Tanner scale, sexual maturation was not slower in Yu-Cheng boys or girls. However, Yu-Cheng boys aged 11-14 had significantly shorter penis length, but testicular and scrotal development did not differ from the controls. Penile length was not related to sexual development as measured by the Tanner scale.

With a validated and standard battery of tests, cognitive and behavioral development of Yu-Cheng offspring were studied yearly from 1985 through 1991. Throughout the testing period, Yu-Cheng children scored consistently lower in the Stanford Binet IQ (SB-IQ) and 4-5 points lower than controls (with the same matching criteria as the above study) in three subscales of the Wechsler Intelligence Scale for Children, Revised (WISC-R): verbal IQ (VIQ), with significant differences observed in 1990 and 1991; performance IQ (PIQ); with significant differences in 1987-1991; and full-scale IQ (FIQ), with significant differences in 1989-1991 (Chen et al., 1992; Lai et al., 1993; Lai et al., 1994). When the PIQ, VIQ, and FIQ were examined by the age of the child, significant deficits were observed for ages 6-8 (Lai et al., 1994). The Yu-Cheng children scored lower on the mental development index (MDI) and the psychomotor development scale (PDI) of the Bailey scale, with a significant difference at 2 years of age (Lai et al., 1994).

Yu-Cheng children are also reported to exhibit more health, habit, and behavior problems as reported by parents responding to the Rutter's scale, and to manifest higher activity based on teachers' responses to the Teacher's Activity Check List (Hsu et al., 1993). Rutter's Child Behavior Scale A was used to screen children for their likelihood for social, health and behavioral problems (Yu et al, 1994). The scale, validated in Britain of children ages 9-12, assesses these outcomes through a questionnaire-checklist on each of the three topic areas. This study compared the 113 of the 118 Yu-Cheng children born between July 1978 and June 1985 to exposed women who participated in the seven year follow-up to their matched controls. Only those three years old or older were included in this evaluation. The results showed that, at all ages, the exposed children scored 14-38% worse on mean Rutter scores than did the controls. All age groups, except the 10-12 year olds were significantly different. For the health subscores, all age groups, except 5 year olds and 10 year olds were significantly different; for habits, all were significantly different except for 8, 11, and 12 year olds; and for behavior subscores, ages 4, 6, 7, 8, and 9 were significantly different.

In addition to these developmental measures, Raven's Colored Progressive Matrices (CPM - used for those 6 to 8 years of age) and Standardized Progressive Matrices (SPM - used for 9 year olds) were used to assess cognitive development (Guo et al., 1995a). Analyses used Wilcoxin one-sample test for comparison of exposed children to their matched controls, and an examination of differences for offspring born longer after exposure, regression analysis was used on year of birth. Significant deficits were observed for children aged 6 through 8, with no pattern of differential effects with year of birth. When the results were examined by sex, the significant effects were found only in males.

In a follow-up of these children, a random sample of 27 case-comparison pairs were selected from those pairs whose case was between 7 and 12 years of age (Chen and Hsu, 1994). These children were assessed for neuropsychologic changes (including cognitive -- WISC-R and auditory event-related potentials -- P300), and neurophysiological changes (including pattern visual evoked potentials -- P-VEP, and short-latency somatosensory evoked potentials -- SSEP). The exposed children had significantly lower verbal and full-scale IQs (VIQ and FIQ) and P300 latencies at Cz and Pz. No differences were observed for P-VEP or for SSEP.

In October 1991, researchers began analysis of physical and cognitive development of 104 children whose mothers were exposed and 109 children whose fathers but not mothers were exposed, and of three matched controls born after 1985 (Guo et al., 1993). Like children born before 1985, the later-born children were shorter in stature and lower in weight than controls, although the authors indicate that the differences were no longer statistically significant. Yu-Cheng children are reported to have higher activity levels but do not have temperament, physical, habit, or behavioral problems. Overall, scores on all tests in paternally exposed children were similar to those of the controls. However, maternally exposed children scored lower on the SB-IQ and on all subscales of the WISC-R.

Another report (Guo et al., 1994b), probably of the same children (born between 1985 and 1991) gave more details: Local household registration were used to identify these children and two comparisons from the same neighborhood, age, sex, mother's age within three years, and combined parental education (within three years). Only one comparison was used in this report. Researchers began analysis of physical and cognitive development of 120 children of 79 exposed mothers, 75 children of 52 exposed fathers, and 4 children of exposed mothers and fathers. The children's development was assessed using the Chinese Child Developmental Inventory (CCDI). The CCDI is a modification of the Minnesota Child Developmental Inventory (MCDI), a tool used to evaluate children from six month to six years of age. The scales in the CCDI include gross and fine motor activity, language skill, comprehension, self-help activities and personal-social skills. Of these, children of Yu-Cheng mothers had significantly lower scores for self help and general development (a summary scale); no significant differences were observed in children of exposed fathers. When the children of exposed mothers were examined by gender in comparison to their controls, Yu-Cheng girls scored significantly lower on self help, general development and on conceptual comprehension. Other borderline differences were observed for fine motor activity and situational comprehension. No significant differences were observed for sons of exposed mothers. Rutter's Child Behavior Scale A was used to screen children for their likelihood for health and behavioral

problems. No significant differences were reported for this measure in these later born children (versus effects observed in those born before July 1985 - Yu et al., 1994).

A report examined middle ear abnormalities in 1993 in 110 Yu-Cheng children and 96 matched controls (Chao and Hsu, 1994), born before July 1985. Ages ranged from 8 to almost 16 years. Of the 220 tympanic membranes evaluated in the Yu-Cheng children, 49 were abnormal compared to 34 out of 192 tympanic membranes in the comparison group ($p<<0.01$). A second report, apparently resulting from these examinations (Chao et al., 1997), presented the same results with slightly different numbers: In this 44 of 103 children (206 tympanic membranes assessed) were abnormal in the Yu-Cheng children, versus 18 of 96 control children (192 tympanic membranes assessed) ($p<<0.01$). In this second report, 30 Yu-Cheng children with serum levels of PCBs and PCDFs measured in 1991 were compared: this group was divided into those with and without middle ear diseases. In analyses unadjusted for other factors, significantly higher serum levels of 1,2,3,4,7,8-hexachloro-dibenzofuran ($p=0.046$) and 2,3,4,7,8-pentachloro-dibenzofuran ($p=0.022$) were observed for Yu-Cheng children with disease compared to Yu-Cheng children without. Differences were not observed for serum PCB levels.

The authors attributed the association with middle ear disease with, at least in part, immunologic effects. Yu and colleagues (1998) recently published a report directly examining immunologic function in Yu-Cheng children. In this effort, 105 Yu-Cheng children of exposed mothers and 101 control children, born between July 1978 and June 1987, were assessed. The Yu-Cheng children had significantly elevated rates of influenza during the 6 months preceding the 1995 physical examination. Blood was drawn for immunologic analyses, and compared for the exposed and control children. Due to laboratory problems, 29 samples from exposure, and 22 from controls were examined. In this limited comparison, no significant differences were observed in leukocyte classification and immune markers.

7.14.1.4. *Mortality Among Yu-Cheng Population*

Cause-specific mortality through December 31, 1991, for the 2,000 Yu-Cheng poisoning cases was assessed (Yu et al., 1997). Eighty-three deaths were identified from among the 1,837 persons whose vital status was known. Compared to the age, gender, and calendar-time specific mortality rates of the Taiwan general population, the overall SMR was significantly lower among the Yu-Cheng population (SMR = 80 (95% CI = 70-100). However, mortality from chronic liver disease and cirrhosis was statistically significantly increased in the Yu-Cheng population (SMR = 2.7, 95%CI = 1.3, 4.9) (Yu et al., 1997).

7.14.1.5. *Comment*

Data from Yusho and Yu-Cheng strongly implicate direct ingestion of contaminated rice oil with numerous acute effects of the skin and peripheral and central nervous systems. The data on immunologic function suggest possible effects, but the numbers of subjects in the various studies were too small to determine an exposure-response relationship. Similarly, data on elevated triglyceride and liver enzyme levels are from a small number of cases and, therefore, the relationship between exposure and the effect is unclear. Furthermore, there are few data to evaluate the long-term effects of these very high exposures. Because many of the 4,000 individuals who were exposed in these two episodes were children, longitudinal studies would be invaluable in assessing the long-term health effects of these exposures.

One difficulty in evaluating the various reports relating to Yusho and Yu-Cheng is the inability to determine if the effects are generalizable to the entire exposed population. It may be that the cases reported in the literature were those who tended to have the severest signs and symptoms and probably had the highest body burden of contaminants. Some reports included small numbers of cases and controls, relative to the size of the exposed population, and others had no controls. Finally, while much work has gone into determining severe acute effect, it would be interesting to know what chronic, age, and gender-specific effects are now being exhibited in the approximately 4,000 individuals directly exposed to the contaminated rice oil.

Another difficulty is the presence of several chlorinated hydrocarbons in the contaminated oil, which results in uncertainty as to which contaminant or combination of contaminants is responsible for the noted effects. The data on the offspring of exposed Yu-Cheng mothers and fathers are fascinating and disturbing. It appears that parental exposure, specifically, maternal exposure, to PCBs, PCDFs, and PCQs is directly linked to *in utero* exposure of the fetus, affecting neurodevelopment, selective musculoskeletal, and possibly sexual development of the offspring. The ongoing assessment of development in the Yu-Cheng children will contribute to the understanding of long-term consequences of these exposures on the children's future quality of life and highlights the importance of parental exposures to their children’s well-being. Prospective studies of fertility and reproductive experience in these offspring would provide insight into possible intergenerational effects of exposure to these compounds.

7.15. SUMMARY

The data presented in this chapter describe nonmalignant effects in epidemiologic studies of populations with the potential for exposure to chemicals contaminated with 2,3,7,8-TCDD. The purpose of this review is to highlight the salient results of the studies and to assess whether the observed effect was related to exposure to 2,3,7,8-TCDD.

In summary, based on the results of two or more studies, recent evidence suggests that, in adults, chloracne, elevated GGT levels, and altered testosterone levels appear to be long-term consequences of exposure to 2,3,7,8-TCDD (Table 7-53). In contrast, multiple studies show possible acute effects but few chronic exposure-related effects for dermatologic endpoints other than chloracne, such as eyelid cyst, hypertrichosis, hyperpigmentation, actinic keratosis, and Peyronie's disease; for liver diseases such as cirrhosis, liver enlargement, and hepatic enzyme levels (LDH, AST, ALT, and D-glucaric acid) other than GGT; and for lipid concentrations, porphyrias, and thyroid function; as well as renal, neurologic, and pulmonary disorders. Although the available data are suggestive of an association between TCDD exposure and other adverse outcomes, circulatory and heart diseases, diabetes and glucose metabolism, reproductive and developmental outcomes, and immunologic disorders require further study before their respective relationships to 2,3,7,8-TCDD can be more definitively assessed.

In the best of circumstances when reviewing studies, it would be ideal if all studies examined the same endpoints in the same manner, had sufficient statistical power to detect truly positive findings, had good estimates of extent of exposure, and had consistent exposure-response relationships. In the absence of ideal situations, epidemiologists examine the evidence of studies using “six tenets of judgment” (Hatch and Stein, 1986; Hill, 1965) to assess the collective wisdom of the study results. These tenets are temporality (sequence of events); degree of exposure; strength, consistency, and specificity of association; and biological plausibility.

In evaluating many of the studies that examined the relationship between serum 2,3,7,8-TCDD and, in some cases, dioxins, furans, and PCBs, there are several common threads that bear noting. They will be discussed first to avoid repetition throughout the summary.

In terms of temporality, all studies reviewed in this chapter were conducted after the presumed exposure occurred. Some of the studies obtained exposure data at (approximately) relevant time for the outcomes (e.g., Dutch developmental studies of dioxins, furans, and PCBs) or shortly after the exposure, as in Seveso; others were conducted many years after the groups' last exposure to evaluate more chronic health outcomes. One dilemma in assessing the effect of past exposures is ascertaining whether an effect observed many years postexposure is due to the exposure itself or to an exposure or event that occurred during the intervening period. Another problem is determining what, in the analysis, the investigator considered the most important of the possible confounding exposures. Finally, restricting examination of events to those that occurred after the exposure does not in and of itself satisfy this time-order criterion. Several factors must be considered, such as the half-life of the contaminant in the body and the concentration at the time of the event. Consistency in the results of similarly designed studies of exposed populations should help strengthen the conclusion of an effect or no effect.

Determination of the extent of exposure throughout the studies was varied. When the risk of disease increases with the dose or gradient of exposure, the evidence for causation is strengthened. It should be emphasized that there are many possible dose-response patterns, which may result in different threshold levels for different endpoints. Because of the exposure misclassification bias present in most dioxin research, with the exception of a few studies, it is not valid to attempt to determine dose-response relationships. To summarize, six studies evaluated the relationship between nonmalignant effects and body levels of 2,3,7,8-TCDD (or a mixture of dioxins, furans, and PCBs): the Ranch Hand study of U.S. Air Force personnel (Roegner et al., 1991; Grubbs et al., 1995); the study of 50 Missouri residents (Stockbauer et al., 1988); the evaluation of the BASF accident cohort (Ott et al., 1994) and the cohort of Hamburg chemical workers (Flesch-Janys et al., 1995); the NIOSH study of 281 TCP production workers (Egeland et al., 1994); and the two Dutch developmental series of studies (Series 1: Huisman et al., 1995a,b; Koopman-Esseboom et al., 1994a-c, 1995a,b, 1996; Weisglas-Kuperus et al., 1995, 2000; Series 2: Pluim et al., 1992, 1993, 1994, 1996). In 1988, the workers in the NIOSH study had the highest serum 2,3,7,8-TCDD concentrations (mean = 220 pg/g) of these six study groups. Of these six sets of studies, only the Dutch developmental studies examined common environmental exposures. Because the Dutch series examined a variety of dioxins, furans and PCBs (see Table 7-21b), the data are not strictly comparable to the other five studies.

In U.S. Army veterans (Centers for Disease Control Veterans Health Studies, 1988), serum 2,3,7,8-TCDD levels were measured, but the levels were not used to examine dose-response relationships. In the Veterans Health Studies, more than 99% of the serum 2,3,7,8-TCDD levels of the sample of both Vietnam and non-Vietnam veterans were at the background level (4 pg/g). Therefore, comparisons were made between the two groups as a whole.

Serum levels of Seveso residents were obtained for a small proportion (N = 20) of the total number of residents of zone A within 1 year of the reactor release (Mocarelli et al., 1991). The data suggest that the levels may be related to a number of factors, including age (younger children were outside at the time of the release), whether the resident was inside or outside, ingestion of local produce, or number of days of residence in the area after the release, to name a few. The data suggest that the potential for substantial exposure was high for individuals residing in the area. The range of levels in the 20 zone A residents was 820 pg/g to 56,000 pg/g (median = 7,400 pg/g). Thirty years after the explosion, Landi et al. measured serum 2,3,7,8-TCDD in 62 individuals randomly selected from zones A, B and non-ABR (Landi et al., 1997). Geometric mean serum concentrations were 53.2 pg/g lipid in zone A; 11.0 pg/g lipid in zone B, and 4.9 pg/g lipid in zone non-ABR. These data suggest that elevated levels persist but are decreased from the original levels of 1976.

The majority of the remaining studies examined the differences between individuals identified as exposed or unexposed, or with or without chloracne. Most of these studies did not evaluate other parameters that might explain differences in effects between exposed and unexposed, for example, the length of exposure. However, one study assessed dose-response relationships based on a statistical algorithm of intensity and highest dose of TCDD exposure (Bond et al., 1989).

In terms of the magnitude (or strength) of the association, this criterion refers to the degree to which the measure of association (e.g., odds ratio or relative risk) exceeds the null value of 1. The stronger the association between exposure and effect, the more convincing is the argument for causation. There is no definitive cut point to numerically define a meaningful measure of association. Other factors, such as the prevalence of the exposure in the population, affect the significance of the measure.

A critical element that should always accompany the effect measure is a confidence interval. Placement of an interval around the measure enables quantitation of the result for a more meaningful interpretation. An odds ratio of 30 is quite impressive, but if the 95% confidence interval is 0.9-200, the magnitude of the association is less impressive.

If an association between a factor and a disease is demonstrated across a variety of studies employing different designs and different populations (consistency), then the argument for causation is strengthened. Replication of an association under different conditions decreases the likelihood that confounding is responsible for the observed association. Consistency is a powerful criterion for causation, but only when "the variables under test (exposure, outcomes) are similar enough" to justify the comparison of the various studies' findings (Hoffman et al., 1986).

It should also be determined a priori that each study included in the critical evaluation process is in adherence to basic epidemiologic principles governing study design and analysis. Deficient studies with suspect results should be excluded. While this is not to imply that such studies have no worth, as invaluable information has often been derived from those studies that improve on subsequent examinations of the issue, they have no place in the evaluation process. Unfortunately, in studies of 2,3,7,8-TCDD and effects in humans, the probability of exposure misclassification forces exclusion of much of the research to date.

Specificity refers to the uniqueness of the association between a factor and an outcome. If the relationship were absolute, then only factor X would be related to only effect Y. It is indeed rare to encounter this type of association, which renders this criterion generally less useful in the evaluation process.

Finally, according to the criterion of biological plausibility, the observed association between exposure and effect should be consistent with existing theory and information from other

scientific disciplines. Certainly one would feel more secure in the causation debate if the biological basis for an observed association could be explained. However, biological implausibility may simply reflect gaps in existing scientific knowledge that could explain the relationship.

7.15.1. Effects Having a Positive Relationship With Exposure to 2,3,7,8-TCDD

The following section describes those endpoints for which there is good evidence from two or more studies suggesting an effect of exposure to 2,3,7,8-TCDD.

7.15.1.1. *Chloracne*

7.15.1.1.1. *Temporality.* Chloracne is one of the best known of the medical consequences of exposure to 2,3,7,8-TCDD-contaminated substances. In general, it has been observed in most incidents where substantial exposure has occurred, particularly among TCP production workers (Goldman, 1972; May, 1973; Bleiberg et al., 1964; Bond et al., 1987; Suskind and Hertzberg, 1984; Moses et al., 1984; Zober et al., 1990) and Seveso residents (Reggiani, 1978; Caramaschi et al., 1981; Ideo et al., 1985; Mocarelli et al., 1986; Assennato et al., 1989). As previously stated, chloracne appears within several weeks to months from the time of exposure, often resolving after discontinuation of exposure (Moses et al., 1984; Suskind and Hertzberg, 1984), although for some it may remain for extended periods after exposure ended (Moses et al., 1984).

7.15.1.1.2. *Degree of exposure, consistency of the association.* The amount of exposure necessary for development of chloracne has not been resolved, but studies suggest that high exposure (both high acute and long-term exposure) to 2,3,7,8-TCDD increases the likelihood of chloracne, as evidenced by chloracne in TCP production workers and Seveso residents who have documented high serum 2,3,7,8-TCDD levels (Beck et al., 1989; Fingerhut et al., 1991a; Mocarelli et al., 1991; Neuberger et al., 1991) or in individuals who have a work history with long duration of exposure to 2,3,7,8-TCDD-contaminated chemicals (Bond et al., 1989). The absence of substantial chloracne in U.S. Army Vietnam veterans whose mean serum 2,3,7,8-TCDD levels were at background (4 pg/g) (Centers for Disease Control Vietnam Experience Study, 1988d) and U.S. Air Force Ranch Hands whose serum 2,3,7,8-TCDD levels fell intermediate to those of workers and Army Vietnam veterans (Roegner et al., 1991; Burton et al., 1997) suggests that there is a higher incidence of the disorder among those with higher serum 2,3,7,8-TCDD levels.

7.15.1.1.3. *Strength of the association.* In earlier studies, chloracne was considered to be a "hallmark of dioxin intoxication" (Suskind, 1985). However, only in two studies were risk

estimates calculated for chloracne. Both were studies of different cohorts of TCP production workers (Suskind and Hertzberg, 1984; Bond et al., 1989); one group was employed in a West Virginia plant, the other in a plant in Michigan. Of the 203 West Virginia workers, 52.7% ($p<0.001$) were found to have clinical evidence of chloracne, and 86.3% reported a history of chloracne ($p<0.001$) (Suskind and Hertzberg, 1984). None of the unexposed workers had clinical evidence or reported a history of chloracne. Among the Michigan workers, the relative risk for cases of chloracne was highest for individuals with the longest duration of exposure (≥60 months; RR = 3.5, 95% CI = 2.3-5.1), those with the highest cumulative dose of TCDD (based on duration of assignment across and within 2,3,7,8-TCDD-contaminated areas in the plant) (RR = 8.0, 95% CI = 4.2-15.3), and those with the highest intensity of 2,3,7,8-TCDD exposure (RR = 71.5, 95% CI=32.1-159.2) (Bond et al., 1989).

7.15.1.1.4. ***Specificity of the association.*** Chloracne is associated with exposure to other polyhalogenated chemicals, including dibenzofurans, PCBs, naphthalenes, and others (Taylor, 1979). The likelihood of exposure to other polyhalogenated chemicals in the populations studied is probably low, particularly among the Seveso children, whose exposure was to TCP reactant effluents that were primarily contaminated with 2,3,7,8-TCDD. The issue is more relevant in chemical workers, who by virtue of their occupation, have the potential for exposure to other chemicals. Yet, much of the documented chloracne appeared shortly after TCP reactor releases (Ashe and Suskind, 1950; Goldman, 1972; May, 1973) or during TCP or 2,4,5-T production (Bond et al., 1989), suggesting that 2,3,7,8-TCDD was the chloranegenic agent.

7.15.1.1.5. ***Biological plausibility.*** Animal studies have been effective in describing the relationship between 2,3,7,8-TCDD and chloracne, particularly in rhesus monkeys (McNulty, 1977; Allen et al., 1977; McConnell et al., 1978). Subsequent to exposure to 2,3,7,8-TCDD, monkeys developed chloracne and swelling of the meibomian gland, a modified sebaceous gland. The histologic changes in the meibomian gland are physiologically similar to those observed in human chloracne (Dunagin, 1984).

In summary, the evidence provided by the various studies convincingly states what is already presumed, that chloracne is a common sequela of high levels of exposure to 2,3,7,8-TCDD. More information is needed to determine the level and frequency of 2,3,7,8-TCDD exposure needed to cause chloracne and whether personal susceptibility plays a role in the etiology. Finally, it is important to recall that the absence of chloracne does not imply lack of exposure (Mocarelli et al., 1991).

7.15.1.2. ***Gamma Glutamyl Transferase (GGT) Levels***

7.15.1.2.1. ***Temporality, degree of exposure, and strength and consistency of association.*** There appears to be a consistent pattern of increased GGT levels among individuals exposed to 2,3,7,8-TCDD-contaminated chemicals. Elevated levels of serum GGT have been observed within a year after exposure in Seveso children (Caramaschi et al., 1981; Mocarelli et al., 1986) and 10 or more years after cessation of exposure among TCP and 2,4,5-T production workers (May, 1982; Martin, 1984; Moses et al., 1984; Calvert et al., 1992) and among Ranch Hands (Roegner et al., 1991; Grubbs et al., 1995). All of these groups had a high likelihood of substantial exposure to 2,3,7,8-TCDD. In addition, for those studies that evaluated dose-response relationships with 2,3,7,8-TCDD levels, the effect was observed only at the highest levels or categories of 2,3,7,8-TCDD.

In contrast, although background levels of serum 2,3,7,8-TCDD suggested minimal exposure to Army Vietnam veterans, GGT was increased, at borderline significance, among Vietnam veterans compared to non-Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988a). In addition, despite the increases observed in some occupational cohorts, other studies of TCP production workers from West Virginia or Missouri residents measured but did not report elevations in GGT levels (Suskind and Hertzberg, 1984; Webb et al., 1989).

7.15.1.2.2. ***Specificity.*** In clinical practice, GGT is often measured because it is elevated in almost all hepatobiliary diseases and is used as a marker for alcoholic intake (Guzelian, 1985). In individuals with hepatobiliary disease, elevations in GGT are usually accompanied by increases in other hepatic enzymes, e.g., AST and ALT, and metabolites, e.g., uro- and coproporphyrins. Significant increases in hepatic enzymes other than GGT and metabolic products were not observed in individuals whose GGT levels were elevated 10 or more years after exposure ended, suggesting that the effect may be GGT-specific. However, in the Seveso male children and those with chloracne (both sexes), ALT was significantly increased concomitantly with GGT within 1 year of the reactor release (Mocarelli et al., 1986; Caramaschi et al., 1981). In a longitudinal analysis, both enzymes returned to normal levels within 5 years after exposure (Mocarelli et al., 1986; Assennato et al., 1989). In the NIOSH cohort, elevated GGT levels occurred only among workers with both high 2,3,7,8-TCDD concentrations and lifetime alcohol consumption. Yet, no other enzymes were found to be outside of normal ranges. Likewise, GGT was the single enzyme found to be significantly elevated in Ranch Hands compared to nonexposed referents (Roegner et al., 1991; Grubbs et al., 1995).

These data suggest that in the absence of increases in other hepatic enzymes, elevations in GGT are associated with exposure to 2,3,7,8-TCDD, particularly among individuals who were

exposed to high 2,3,7,8-TCDD levels. The fact that investigators observed a decline in enzyme levels in Seveso children but a continued elevation in TCP workers may reflect (1) differences in how exposure occurred (i.e., acute but high doses in Seveso versus continuous or frequent long-term, medium to high doses in TCP workers), (2) differences in the metabolism of the maturing versus mature system, (3) the fact that children grow rapidly, thus "diluting" a peak exposure within that group, or (4) some combination of these.

7.15.1.2.3. ***Biological plausibility.*** The animal data with respect to 2,3,7,8-TCDD-related effects on GGT are sparse. Statistically significant changes in hepatic enzyme levels, particularly AST, ALT, and ALK, have been observed after exposure to 2,3,7,8-TCDD in rats and hamsters (Gasiewicz et al., 1980; Kociba et al., 1978; Olson et al., 1980). Only one study evaluated GGT levels (Kociba et al., 1978). Moderate but statistically nonsignificant increases were noted in rats fed 0.10 μg/kg 2,3,7,8-TCDD daily for 2 years, and no increases were observed in control animals.

Among humans, increased levels of GGT may suggest activity such as cholestases, liver regeneration, or drug or xenobiotic metabolism. In human adults, most of 2,3,7,8-TCDD is stored in the adipose tissue and has a half-life of approximately 7 years (Pirkle et al., 1989). Continued GGT activity in adults with serum 2,3,7,8-TCDD levels many times over background levels may reflect continuous, low-level metabolism of 2,3,7,8-TCDD.

In summary, GGT is the only hepatic enzyme examined that was found in a number of studies to be chronically elevated in adults exposed to high levels of 2,3,7,8-TCDD. The consistency of the findings in a number of studies suggests that the finding may reflect a true effect of exposure but for which the clinical significance is unclear. Long-term pathologic consequences of elevated GGT have not been illustrated by excess mortality from liver disorders or cancer, or in excess morbidity in the available cross-sectional studies.

It must be recognized that the absence of an effect in a cross-sectional study, for example, liver enzymes, does not obviate the possibility that the enzyme levels may have increased concurrent to the exposure but declined after cessation. The apparently transient elevations in ALT levels among the Seveso children suggest that hepatic enzyme levels other than GGT may react in this manner to 2,3,7,8-TCDD exposure.

7.15.1.3. ***Reproductive Hormones***

7.15.1.3.1. ***Strength and consistency of association.*** Levels of reproductive hormones have been measured with respect to exposure to 2,3,7,8-TCDD in three cross-sectional medical studies. Testosterone, LH, and FSH were measured in TCP and 2,4,5-T production workers (Egeland et al., 1994), in Army Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988d),

and in Ranch Hands (Thomas et al., 1990; Grubbs et al., 1995). The risk of abnormally low testosterone was two to four times higher in exposed workers with serum 2,3,7,8-TCDD levels above 20 pg/g than in unexposed referents (Egeland et al., 1994). In both the 1987 and 1992 examinations, mean testosterone concentrations were slightly, but not significantly higher in Ranch Hands (Roegner et al., 1991; Grubbs et al., 1995). FSH and LH concentrations were no different between the exposed and comparison groups. No significant associations were found between Vietnam experience and altered reproductive hormone levels (Centers for Disease Control Vietnam Experience Study, 1988d). Only the NIOSH study found an association between serum 2,3,7,8-TCDD level and increases in serum LH.

7.15.1.3.2. ***Specificity.*** The NIOSH study excluded from analysis participants who had conditions that might have influenced gonadotropin and/or testosterone levels: history of prostate cancer, thyroid or other hormone usage, or liver cirrhosis. Similarly, in the Ranch Hand study, individuals with orchiectomies or who were taking testosterone medication were excluded from the analysis of testosterone; no participants were excluded from the analyses of FSH. The CDC study of Vietnam veterans did not describe the exclusions.

7.15.1.3.3. ***Biological plausibility.*** In rats, 2,3,7,8-TCDD has been shown to decrease testosterone levels (Moore et al., 1985; Moore and Peterson, 1988; Mebus et al., 1987) through a decrease in testosterone synthesis (Kleeman et al., 1990) or by decreasing the production of pregnenolone from cholesterol (Ruangwies et al., 1991). In addition, 2,3,7,8-TCDD has been shown in rats to reduce the responsiveness of the pituitary to testosterone (Bookstaff et al., 1990a) and of the Leydig cells to LH stimulation (Moore et al., 1991).

The findings of the NIOSH and Ranch Hand studies are plausible given the pharmacological and toxicologicalal properties of 2,3,7,8-TCDD. A mechanism responsible for the effects may involve the ability of 2,3,7,8-TCDD to influence hormone receptors. The aryl hydrocarbon (Ah) receptor to which 2,3,7,8-TCDD binds can cross-talk with steroid hormone receptors in both structure and mode of action. Studies suggest that 2,3,7,8-TCDD modulates hormone receptors, including estrogens (Romkes and Safe, 1988; Romkes et al., 1987), prolactin, and its own Ah receptor (Poland and Glover, 1980; Morrow et al., 1986). However, the effect of 2,3,7,8-TCDD on testosterone receptors has not been evaluated.

In summary, the results from both the NIOSH and Ranch Hand studies are limited by the cross-sectional nature of the data and the type of clinical assessments conducted. However, the available data provide evidence that alterations in human male reproductive hormone levels are associated with serum 2,3,7,8-TCDD.

7.15.2. Possible Effects of Exposure to 2,3,7,8-TCDD or Mixtures of Dioxins, Furans, and PCBs

The following section describes outcomes that may be related to 2,3,7,8-TCDD exposure. Further research would assist in the final evaluation of the effects of dioxin for the following outcomes.

7.15.2.1. *Possible Adult Effects*

7.15.2.1.1. ***Lipid concentrations.*** Animal studies indicate that 2,3,7,8-TCDD is associated with generally increased serum cholesterol and serum triglyceride levels. The effect of exposure to 2,3,7,8-TCDD-contaminated chemicals on lipids is not consistent in the available epidemiology studies. Elevations in total cholesterol and triglyceride levels were reported after high 2,3,7,8-TCDD exposure in TCP workers (Pazderova-Vejlupkova et al., 1981; Martin, 1984) and laboratory workers (Oliver, 1975). Despite their very high exposure to 2,3,7,8-TCDD-contaminated chemicals, neither adults nor children from Seveso had lipid levels above the referent level. Risk factors such as dietary fat intake, familial hypercholesterolemia, alcohol consumption, and exercise, which also affect cholesterol and other lipid levels, may be factors that were not considered in these studies.

Ranch Hands and the NIOSH cohort continue to have marginally elevated lipid concentrations despite the extended length of time between exposure and testing (Grubbs et al., 1995; Calvert et al. 1995). These most recent data suggest that high exposure to 2,3,7,8-TCDD contaminated substances are not related to significantly increased lipid concentrations, specifically total cholesterol and triglycerides. Nevertheless, slight but chronic elevations in serum lipids may put an individual at increased risk for disorders such as atherosclerosis and other conditions affecting the vascular system.

7.15.2.1.2. ***Diabetes and fasting serum glucose levels.*** Diabetes mellitus is a heterogeneous disorder that is a consequence of alterations in the number or function of pancreatic beta cells responsible for insulin secretion and carbohydrate metabolism. Depending on its type, diabetes has been attributed to endogenous factors such as genetic predisposition, to autoimmune processes, and to exogenous factors such as viral infections (Yoon et al., 1987) and chemical exposures, notably a rat poison (Miller et al., 1978) and some medications (Wilson and LeDoux, 1989), environmental toxins (Diabetes Epidemiology Research International, 1987), age, obesity, reduced physical exercise, diet, socioeconomic status, increased insulin resistance by the beta cells, and possibly parity (Pareschi and Tomasi, 1987).

The long-term risk of developing diabetes or other alterations in glucose metabolism after exposure to 2,3,7,8-TCDD is not well addressed by the available toxicological data. The results of the animal studies suggest that glucose levels are altered, generally decreased, by short-term, high-dose exposure to 2,3,7,8-TCDD, and that the response may be species-specific. Studies of rats and rhesus monkeys showed consistent decreases in serum glucose levels after daily doses administered over 30 days (Zinkl et al., 1973) or after a single dose of 2,3,7,8-TCDD (McConnell et al., 1978a; Gasiewicz et al., 1980; Schiller et al., 1986; Ebner et al., 1988) (Table 7-54). In one study, glucose levels continued to drop up to 3 weeks postexposure (Gorski et al., 1990). Dose-related decreases were also noted in CD rats fed 0.1, 1.0, or 10.0 μg/kg daily for 30 days (Zinkl et al., 1973). In contrast, lower daily doses of approximately 0.004 μg/kg/day administered to guinea pigs over a 90-day period produced no significant changes in either glucose or insulin levels (DeCaprio et al., 1986). Wasting syndrome, observed in many species after high dose exposure to 2,3,7,8-TCDD, is hypothesized to be related to various changes in the glucose transport mechanism and is mediated through the Ah receptor. Differences among the results of human and most animal studies may, hypothetically, be due to a number of factors, such as the species studied, the length of the exposure and the short observation periods of the toxicology studies, the rate of insulin metabolism after 2,3,7,8-TCDD exposure, possible differential effects of 2,3,7,8-TCDD on the various types of islet cells, and the high, usually single, 2,3,7,8-TCDD dose administered to the animals. Additionally in humans, with some exceptions, onset of diabetes occurs later in life; unfortunately, these characteristics were not evaluated in the chronic toxicity studies. Long-term feeding studies to evaluate the relationship among glucose levels, the development of diabetes, and 2,3,7,8-TCDD dose would be helpful in assessing the effect of exposure on the physiologic integrity of the islet cells. In addition, such studies may identify other factors that affect either directly or indirectly the function of the islet cells and the effect of 2,3,7,8-TCDD on the glucose transport mechanism. More discussion of glucose transport system effects and their potential relationship to diabetes is found in Part II: Chapter 3. Acute Subchronic, and Chronic Toxicity, Section 3.5. Also, a study by Matsumura and his colleagues is underway to determine whether there is a biological basis for the association between type II diabetes mellitus and dioxin levels in Ranch Hand veterans (personal communication, J. Michalek, 2000).

The results of the Ranch Hand morbidity study are in direct contrast with the results of the NIOSH mortality study, which included workers who were more highly exposed, with greater frequency and severity of exposure. The prevalence of diabetes in the exposed, and referent groups was similar and there was no significant positive trend between prevalence of diabetes and serum glucose levels. What was notable is that 60% of the workers who had serum 2,3,7,8-TCDD concentrations >1,500 pg/g lipid at the time of the study met the case definition for diabetes, and

that the adjusted geometric mean glucose concentration measured at the time of the study was statistically significantly higher than referent levels only in the half-life adjusted TCDD category 1,860-30,000 pg/g lipid. However, the analysis of the mortality data by exposure score, found decreasing prevalence of mortality from diabetes with increasing exposure score. The exposure score incorporated duration and intensity of exposure, among other factors. This puzzling picture suggests that in the NIOSH cohort, diabetes may not be well correlated with serum 2,3,7,8-TCDD concentration or duration of exposure.

The studies of Seveso populations and the NIOSH and IARC occupational cohort found very modest and generally statistically nonsignificant increases in mortality from diabetes. The results of each of these studies should be evaluated within the context of the limitations of the studies. Although the Seveso study is limited by a short follow-up period (15 years), all of the studies have small numbers of deaths from diabetes. It is well known that diabetes has a complex etiology ranging from genetic susceptibility, viral infections, and chemical insult to physical states such as obesity or pregnancy. The various mortality studies that examined the relationship between rates of death from diabetes and 2,3,7,8-TCDD exposure evaluated no data on important confounders such as the body mass index of cases, family history of diabetes, or the relationship between year of onset of the diabetes and exposure to 2,3,7,8-TCDD or chlorophenols. In addition, although the studies have reasonably good estimates of exposure to 2,3,7,8-TCDD, misclassification of exposure for many subjects is possible.

The analyses by Longnecker and Michalek (2000) provide only weak evidence to support a causal relationship between very low levels of serum 2,3,7,8-TCDD and increased risk of diabetes or changes in serum glucose or insulin. The risk measures are generally not strong or statistically significant, nor do they increase monotonically with increasing dose.

The current epidemiologic and toxicological data to date do not support a strong relationship between exposure to 2,3,7,8-TCDD and diabetes or alterations in glucose metabolism. However, there is some evidence to suggest that, particularly at high doses, 2,3,7,8-TCDD may perturb glucose metabolism in some species, a fact which needs further exploration.

7.15.2.2. *Possible Postnatal Developmental Effects*

Given that postnatal developmental effects of dioxin have been studied only in one human population (with the exception of some of the thyroid measures), these studies are being place in the “potential” category. Additional studies in other groups are recommended, as well as followup of these findings over time to evaluate whether these changes are temporary, with no long-term health effects, or an early indication of chronic effects. All the effects in this section were part of one or both of the Dutch developmental studies. The exposures assessed here are different from

the more typical "dioxin" study: the first series of studies (in Amsterdam) examined dioxins and furans, while the second (in Rotterdam and Grongenin) examined dioxins, furans, and PCBs. Thus, any effects observed could be from one agent or some mixture. Even though the studies may have picked out certain exposures as statistically significant, this does not mean that other factors not selected are not associated. For example, in the Rotterdam/Grongenin studies, only PCBs were evaluated prenatally and at birth, but these values were significantly correlated with dioxins, furans, and PCBs collected about 2 weeks after delivery. Many of the findings in the Rotterdam/Grongenin studies were associated with *in utero* PCB exposures measured in maternal blood (IUPAC 118, 138, 153, 180). However, of these, only 118 is considered dioxin-like. As the technology improves to measure dioxin levels in smaller samples, direct measurements will help clarify the issues related to surrogate measures (e.g. PCBs).

7.15.2.2.1. ***Neurobehavioral effects.*** Of the various endpoints covered by the series of reports on the Dutch population, the most interesting findings related the neurobehavioral endpoints. Prechtl's Neurologic Optimality Scores (NOS) and the related postural tone cluster scores and reflex cluster scores were collected at 18 months of age (Huisman et al., 1995a) and, at 42 months of age, children were assessed for cognitive abilities using the Kaufman Assessment Battery for Children (K-ABC) and for verbal comprehension using the Reynell Language Developmental Scales (RDLS) (Patandin et al., 1999).

The NOS scores were somewhat arbitrarily divided at the median and compared to the individual dioxins, furans, and PCBs, as well as their summary measures (Huisman et al., 1995a). A number of the levels of the above agents in breast milk were associated with the NOS, while the prenatal PCBs were not (Table 7-46). Coplanar PCB TEQ was associated with hypotonia (measured through the posture tone cluster score). This observation of hypotonia and prenatal PCB exposure is consistent with Rogan et al. (1986). An evaluation of motor function was associated only with the prenatal PCB levels. Because of the small volume of materal and cord blood collected, dioxins and furans were not measured during the prenatal period. An interaction observed with paternal smoking suggests that this issue should be examined further by collecting postnatal materal smoking data.

Statistically significant deficits in K-ABC were associated with $\sum PCB_{\text{maternal blood}}$ for the entire group, and in RDLS only in the formula-fed children (Patandin et al., 1999). Importantly, the current body burdens in the 42-month-old children were not associated with any cognitive deficits. Statistically significant changes were not observed in the breast-fed children, possibly because of the higher SES status, parental education, and parental verbal IQs. Another possibility is the beneficial effect of breast feeding in general.

Even though studies of Yu-Cheng children are not directly comparable to the above studies, they also showed neurobehavioral delays: increased psychomotor delays (Yu et al., 1991) and lower scores on IQ tests (Chen et al., 1992; Lai et al., 1993; Guo et al., 1993; Chen and Hsu, 1994).

Many of the other outcomes in the Dutch population were "better" in those with exposure: fluency cluster score (Huisman et al., 1995b), mental development index -- MDI (Koopman-Esseboom et al., 1996), and visual recognition memory test at 7 months of age (Koopman-Esseboom et al., 1995b). This may be a result of the inherent benefit of breast feeding (and length of breast feeding) over formula for those measures, or may be due to the way women select to breast fed (e.g., higher SES women, parents with higher educational levels). As noted above, this later notion is supported in a recent report by Patandin et al. (1999).

Transplacental exposures of mice demonstate neurobehavioral effects of dioxin and dioxin-like compounds. These include effects on postural endpoints, motor function, visual abilities, and learning. Perinatally exposed monkeys showed a deficit in cognitive function. Exposures are presented by dietary levels or dose given, and thus are difficult to compare to the exposure measures used in human studies. More details on these studies are presented in Chapter 5 (Developmental and Reproductive Toxicity).

Endpoints varied in the above studies, as did the components and levels of the exposures, overall; in spite of this, the data suggest the relationship between dioxin and dioxin-like compounds and neurobehavioral outcomes. Examination of other human populations and long-term follow-up of these study groups will greatly benefit this database.

7.15.2.2.2. ***Thyroid function.*** Two series of studies, both in The Netherlands but conducted in different groups, have examined thyroid function (Pluim et al., 1993; Koopman-Esseboom et al., 1994c). The two reports did have a finding in common: both observed higher TSH at 3 months of age with higher TEQs. They both had significant findings for T4, but they were in opposite directions. All these findings, plus other changes found in the second report (an increase in T4/TBG and a decrease in free T4) strongly suggest that more work be done in this area. These findings suggest a possible shift in the distribution of thyroid hormones, and point out the need for collection of longitudinal data to assess the potential for long-term effects associated with these changes.

7.15.2.2.3. ***AST and ALT.*** One study looked at blood measures in 35 perinatally exposed children in The Netherlands (Pluim et al., 1994). AST, ALT, and platelets all varied with exposure (Table 7-26). Even though all but three children had values within "normal" ranges, the distributions of

has shifted (e.g., an increase in platelets), which could have some currently unknown/unrecognized short- or long-term effect on health.

7.15.3. Effects for Which Further Research Is Needed

The following section describes endpoints for which the animal data have demonstrated exposure-related effects, but the human data are inconclusive and require further study.

7.15.3.1. *Diseases of the Circulatory System*

In general, the results of the cohort mortality studies of TCP production workers were remarkably similar. For all of the early studies, the SMRs for diseases of the circulatory system (ICD-9: ICD 390-459) were approximately 100, meaning that the death rate in the exposed population was nearly the same as that in the general population, controlling for age, race, gender, and calendar year (Fingerhut et al., 1991b; Zober et al., 1990; Bueno de Mesquita et al., 1993; Bertazzi et al., 1989, 1992; Collins et al., 1993; Bond et al., 1989; Coggon et al., 1991). None of the SMRs above 100 were statistically significantly elevated.

In the only study of its kind, Flesch-Janys and colleagues (1995) estimated exposure to PCDDs, PCDFs, and total TEQs for all members of the Hamburg chemical worker cohort based on a sample of workers with either serum or adipose tissue exposure measurements. Mortality from all cardiovascular diseases, and specifically from ischemic heart disease, was related to increasing 2,3,7,8-TCDD concentrations. More striking was the positive relationship betweeen Total TEQs and cardiovascular disease as a whole.

Mortality from circulatory system diseases among Ranch Hands (SMR = 110, 95% CI = 60-150) (Michalek et al., 1990) and Australian Vietnam veterans (RR = 1.7, 95% CI = 0.9-3.0) (Fett, 1987b) was nonsignificantly elevated. In an update of the Ranch Hand data, the SMR for all circulatory diseases combined among all Ranch Hands was not elevated (SMR = 100, 95% CI = 70-130) (Michalek et al., 1998). However, a significant increase in cardiovascular mortality was noted in enlisted ground crew (SMR=150, 95% CI =1.0-2.2). There was a deficit of deaths from this cause among U.S. Army Vietnam veterans compared to non-Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988c). Elevated mortality from circulatory diseases among Seveso residents is considered by the authors to be the result of environmental stresses and possibly other risk factors rather than exposure to 2,3,7,8-TCDD (Bertazzi et al., 1989; Pesatori et al., 1998). The mortality pattern from circulatory and cardiovascular disease among the three zones does not suggest a pattern of effect. Perhaps further followup will clarify the story.

Diseases of the circulatory system, particularly heart disease, are the leading causes of death among populations of most developed nations. Leading risk factors include age, cigarette

smoking, elevated lipid levels, obesity, hypertension, diabetes, and physical inactivity (Smith et al., 1992). Among the studies that examined mortality from circulatory system diseases, none directly adjusted SMRs for known risk factors or attempted to evaluate jointly the contribution of known risk factors and 2,3,7,8-TCDD to the mortality rates (Fingerhut et al., 1991b; Zober et al., 1990; Bueno de Mesquita et al., 1993; Bertazzi et al., 1989, 1992; Collins et al., 1993; Bond et al., 1989; Coggon et al., 1991). More recent mortality studies attempted to control for known confounders; when possible, most used internal control groups. Still, the picture is inconsistent among the various cohorts. Therefore, given the strong contribution of these risk factors, it is not possible to rule out physical and personal risk factors in the etiology of diseases of the circulatory system and heart in these populations. However, the absence of a "healthy worker effect" for these causes of death suggests that future research be directed specifically at the relationship between circulatory and heart disease and exposure to 2,3,7,8-TCDD.

Cross-sectional morbidity studies have not found increases in the prevalence of circulatory or heart disease among TCP workers, Ranch Hands, or U.S. Army Vietnam veterans (Suskind and Hertzberg, 1984; Bond et al., 1987; Moses et al., 1984; Centers for Disease Control Vietnam Experience Study, 1988a; Calvert et al., 1998). In some cross-sectional studies, risk estimates were adjusted for some risk factors, depending on the study (Suskind and Hertzberg, 1984; Poland et al., 1971; Moses et al., 1984; Bond et al., 1983; Centers for Disease Control Vietnam Experience Study 1988a; Roegner et al., 1991). Ranch Hands were the only group to experience marginal differences in diastolic blood pressure, arrhythmias, and peripheral pulse abnormalities after adjusting for selected risk factors (Roegner et al., 1991).

The animal data suggest that at high levels of 2,3,7,8-TCDD, the vascular system, cardiac muscle, and valves and function may be affected by exposure (Kociba et al., 1978; Buu-Hoï et al., 1972; Brewster et al., 1988; Hermansky et al., 1988; Kelling et al., 1987; Canga et al., 1988). However, with the exception of the long-term feeding study (Kociba et al., 1978), the exposures in animals were single high doses and the human exposures (except Seveso) were chronic, medium to high doses.

In summary, the animal studies suggest that 2,3,7,8-TCDD causes pathologic changes that may lead to later circulatory system disease. However, long-term studies of mature and aged animals have not been carried out to evaluate these hypotheses and to correlate the results of the animal with the human studies. Few epidemiologic studies were designed to control for many of the risk factors known to cause circulatory system and heart disease, but a consistent absence of the healthy worker effect for circulatory disorders and heart disease in numerous mortality studies, and the positive relationship observed in one study between total TEQs and circulatory diseases, suggest the need for additional research in this area. These studies should also include methods to

quantify subject exposure to 2,3,7,8-TCDD and control of confounders related to circulatory diseases.

7.15.3.2. *Immunologic Effects*

The available epidemiologic studies on immunologic function in humans relative to exposure to 2,3,7,8-TCDD do not describe a consistent pattern of effects among the examined populations. Two studies of German workers, one exposed to 2,3,7,8-TCDD and the other to 2,3,7,8-tetrabrominated dioxin and furan, observed dose-related increases of complements C3 or C4 (Zober et al., 1992; Ott et al., 1994), while the Ranch Hands continue to exhibit elevations in IgA (Roegner et al., 1991; Grubbs et al., 1995). Other studies of groups with documented exposure to 2,3,7,8-TCDD have not examined complement components to any great extent or observed significant changes in IgA. Suggestions of immunosuppression in a small group of exposed workers as a result of a single test should be confirmed in other cohorts of similarly exposed populations (Tonn et al., 1996).

Comprehensive evaluation of immunologic status and function of the NIOSH, Ranch Hand, and Hamburg chemical worker cohorts found no consistent differences between exposed and unexposed groups for lymphocyte subpopulations, response to mitogen stimulation, or rates of infection (Halperin et al., 1998; Michalek et al., 1999; Jung et al., 1998; Ernst et al., 1998). However, in a single study, T-cell response to Inferon-γ in TCDD-exposed workers was negative in isolated peripheral blood mononuclear lymphocytes and positive in diluted whole blood (Ernst et al., 1998).

More comprehensive evaluations of immunologic function with respect to exposure to 2,3,7,8-TCDD and related compounds are necessary to assess more definitively the relationships observed in nonhuman species. Longitudinal studies of the maturing human immunologic system may provide the greatest insight, particularly because animal studies have found significant results in immature animals, and human breast milk is a source of 2,3,7,8-TCDD and other related compounds. Data from a longitudinal study of children (Weisglas-Kuperus et al., 1995, 2000) suggest long term effects. Additional follow-up and investigations of other populations would be informative.

7.15.3.3. *Adult Reproductive Outcomes*

7.15.3.3.1. ***Semen changes.*** The Vietnam Experience Study found a significant relationship between service in Vietnam and sperm abnormalities, whereas the Ranch Hand study did not confirm these results when exposure was defined by both cohort status and 2,3,7,8-TCDD levels.

However, the data on alterations in male reproductive hormone levels associated with occupational exposure to 2,3,7,8-TCDD emphasize that further research in these areas is required.

7.15.3.3.2. ***Endometriosis.*** Two published reports of infertility patients (Mayani et al., 1997; Pauwels et al., 1999) have raised the potential for an association between endometriosis and TCDD/TEQ exposure. These studies are small and of limited power. Studies of women from Seveso and New York State are currently underway and will add to the database on this outcome.

7.15.3.4. ***Developmental Effects***

This section includes all developmental effects except those postnatal developmental effects which are covered by outcome (thyroid, neurobehavioral outcomes, and AST and ALT). Outcomes related to fertility are also reported (e.g., time to pregnancy, birth rates, semen changes).

7.15.3.4.1. ***Consistency.*** A variety of study designs, including case-control, ecologic, cross-sectional, and historical cohort designs, have addressed the issue of 2,3,7,8-TCDD and reproductive effects in humans. Unfortunately, the different criteria for case definitions across studies make it difficult to compare the results. In addition, the method of case ascertainment for certain endpoints influences the rate of events observed. The Vietnam Experience Study substudies of veteran-reported birth defects compared with those identified through hospital records demonstrated that rates of self-reported outcomes differed by exposure status. Moreover, predictive value of self-reported events was poor in both cohorts. In contrast, rates of birth defects in the Ranch Hand study were similarly reported by the Ranch Hands and controls. Both groups underreported 7% of birth defects in children conceived prior to their SEA tour and 14% after their tour of duty.

7.15.3.4.2. ***Strength.*** No developmental effect measure greater than 2 was noted in any of these investigations. This is not surprising, given the limitations of the studies, particularly with regard to exposure misclassification. Therefore, the trends across these studies carry more import than "statistically significant" results.

7.15.3.4.3. ***Temporality, dose-response.*** Although these studies have restricted inclusion of reproductive events to those that occurred after exposure to 2,3,7,8-TCDD was suspected, no study has evaluated 2,3,7,8-TCDD levels at the time of the outcome. Determination of a 2,3,7,8-TCDD dose-response relationship with adverse reproductive outcomes would not be valid unless individual 2,3,7,8-TCDD levels were available. The recent Ranch Hand study estimated the

2,3,7,8-TCDD levels at the time of the developmental outcome, which is an important contribution toward understanding this phenomenon. However, with regard to early losses, this analysis would not be able to address those occurring very early in gestation, before recognition of the pregnancy by the woman or her physician, or their subsequent effect on identification of adverse outcomes identified later in pregnancy or at birth.

7.15.3.4.4. ***Biological plausibility.*** A growing body of animal research described in Chapter 5 lends biological plausibility to the association between dioxin and most of the reproductive endpoints evaluated in these studies, with the notable exception of molar pregnancies. There is growing evidence that dioxin affects testis and accessory gland weight, testicular morphology, spermatogenesis, and fertility in males. A model for a paternally mediated dioxin effect on congenital malformations has not been reported; however, increased interest in this area (Olshan and Mattison, 1994) may yield more information on this topic. Among female animals, the primary reproductive endpoints that have been examined include decreased fertility and pregnancy loss.

The mechanism by which 2,3,7,8-TCDD causes adverse reproductive and developmental effects has not been well described, although considerable insight has been gained from research focusing on the Ah receptor. Although the Ah receptor has been linked with birth defects in several mouse strains, it appears that the mechanism of effect may be dependent on the outcome evaluated, as well as other dioxin congeners to which the population is exposed. Clearly, these relationships in humans have not been adequately investigated.

The discovery in the Times Beach, Missouri; CDC; and Ranch Hand studies that self-reported dioxin exposure and exposure indices developed from the analyses of 2,3,7,8-TCDD in soil and military records are poorly correlated with serum 2,3,7,8-TCDD levels aids in understanding the inconsistencies of the research to date regarding 2,3,7,8-TCDD and effects. Thus, because of the likelihood of exposure misclassification in those studies lacking direct measures of exposure, the findings have historically been severely limited.

7.15.3.4.5. ***Spontaneous abortions.*** Miscarriages were investigated in several studies with different designs and varied patterns of parental exposure. Events were generally ascertained by self- or spousal report. When case ascertainment was through medical records, such as in the Ranch Hand study or the Vietnamese investigations, the events are by definition restricted to those miscarriages that were clinically recognized.

Research in the area of pregnancy loss indicates that 30%-50% of all conceptions are lost prior to or during implantation (Hertig et al., 1959). The rate of loss between implantation and

expected first menstrual period ranges from 22% to 30% (Wilcox et al., 1988; Sweeney et al., 1988). Thus it is clear that restriction of the examination of pregnancy loss to those events that are ascertained through medical records, or even self-reports, results in excluding a large proportion of the outcome of interest. In studies of environmental factors and spontaneous abortion where information is lacking concerning conception, "the conflation of different doses with different effects can mislead" (Kline et al., 1989). Because of these discrepancies, it would not be meaningful to pool the results of the research on the association between dioxin exposure and miscarriage to judge the "consistency" of the association.

Overall, it must be acknowledged that the data compiled to date are inadequate to address this issue. To simply enumerate and compare the number of "positive" versus "negative" studies to ascertain consistency in the research would be inappropriate. The reasons for this have been described above in detail, with emphasis on the high (40%-50%) exposure misclassification that has been documented in the majority of these investigations, the small sample sizes evaluated, lack of data on dioxin levels at the time of conception, and the unknown impact of early pregnancy loss on identification of birth defects. The animal and human evidence for a 2,3,7,8-TCDD-pregnancy loss relationship is sufficiently suggestive to warrant further investigation. Several studies of various designs and populations have demonstrated weak but consistent associations (Reggiani, 1978; Hatch, 1984b; Constable and Hatch, 1985; Huong et al., 1989; Phuong et al., 1989a; Stellman et al., 1988), whereas others have not (Townsend et al., 1982; Smith et al., 1982; Cutting et al., 1970; Kunstadter, 1982; Report to the Minister for Veterans' Affairs, 1983; Stockbauer et al., 1988; Erickson et al., 1984; Centers for Disease Control Vietnam Experience Study, 1989). (These studies include those that should be restricted to the assessment of military service in Vietnam and reproductive events.) Only two studies, one an analysis of the Ranch Hand developmental data (Wolfe et al., 1995) and the other a recent analysis of the NIOSH cohort (Schnorr et al., in press 2001), have used biological measurements and estimated the 2,3,7,8-TCDD levels present around the time of conception. The first study did note a modest increase in recognized spontaneous abortions and stillbirths but only at the low, but not the high, level. The Ranch Hand study leaves several questions unanswered, including the determination of a dose-response level and the impact of very early pregnancy losses on rates of recognized fetal death and birth defects that survive long enough to be "counted." The NIOSH study did not observe increases at any level.

7.15.3.4.6. ***Congenital malformations/birth defects.*** The confusing evidence regarding the relationship between dioxin exposure and birth defects suffers not only from the limitations described above for the studies of miscarriage but also from the lack of power to evaluate specific types of malformations. To increase the power to detect a potential relationship, the studies have

combined all birth defects together and calculated an odds ratio for total birth defects. Given evidence for etiologic heterogeneity among subgroups of birth defects (Khoury, 1989), it is probable that this approach dilutes the effect measure.

These studies also should more carefully examine type of parental exposure, i.e., paternal, maternal, or both. Timing of exposures and potential biological mechanisms for birth defects are different for maternal and paternal exposures. The field of paternally mediated effects is rather new, but future research may assist in the interpretation of these results (Olshan and Mattison, 1994). If dioxin exposure is related to malformations among the offspring conceived after paternal service in Vietnam, then the effect must occur either premeiotically, or anew with continuing circulating levels of TCDD. Some animal studies have found that spermatogonia and spermatocytes (premeiotic spermatogenic cells) were able to repair DNA after exposure to toxic agents, whereas spermatids and spermatozoa did not have this capability (Lee and Dixon, 1978).

A few studies (Hatch, 1984a; Constable and Hatch, 1985; Huong et al., 1989; Phuong et al., 1989a), including those investigations of the Yusho and Yu Cheng incidents (Rogan, 1982; Yen et al., 1989; Rogan et al., 1988) have suggested an association. Many studies have failed to find a relationship between dioxin and birth defects (Townsend et al., 1982; Smith et al., 1982; Cutting et al., 1970; Kunstadter, 1982; Stockbauer et al., 1988; Erickson et al., 1984; Centers for Disease Control Vietnam Experience Study, 1989; Mastroiacovo et al., 1988). Again, however, in view of the serious limitations of these studies of 2,3,7,8-TCDD and developmental and reproductive events, it must be concluded that the relationship between paternal dioxin exposure and congenital malformations remains unknown. However, if military service in Vietnam is the exposure of interest, there is only modest evidence to support an association with birth defects. Most of the data on grouped birth defects have very small numbers.

7.15.3.4.7. ***Dental effects.*** Finnish investigators examined the association of enamel hypomineralization of permanent first molars in 6-7-year-old children and TEQ exposure through breast feeding (these teeth develop during the first 2 years of life) (Alaluusua et al., 1996; Alaluusua et al., 1999). These data present interesting findings. Unfortunately, the presentation of the results is incomplete, so the potential biological significance cannot be assessed. This would be an interesting outcome to examine in additional studies.

7.15.3.4.8. ***Sex ratio at birth.*** Sex ratio at birth was significantly depressed in a group of 17 children in zone A, Seveso, in the years shortly following the industrial accident. This pattern disappeared a few years later. However, a recent expanded effort suggests that paternal age at the time of exposure may be a key factor. Sex ratios differences were not observed in other groups

examined. If effects are restricted to offspring to fathers less than 19 years old, as suggested in the new Seveso study, the lack of effect elsewhere could be explained by the groups examined: maternal levels of dioxin in community studies or studies of men older than 19. The findings in the most recent Seveso study emphasize the need for more attention on male-mediated development effects, and the potential importance of exposures prior to and during puberty. Because this outcome can easily be collected in studies of developmental effects, more thorough examination of this outcome could be useful.

7.15.3.4.9. ***Growth measures.*** Growth measures include endpoints such as intrauterine growth retardation (IUGR), low birth weight, and postnatal growth. Available evidence does not support an association between paternal dioxin level and low birth weight (Centers for Disease Control Vietnam Experience Study, 1989; Wolfe et al., 1992b, 1995; Michalek et al., 1998). In the Rotterdam study, decrements in length (but not other measures of growth) were observed early (months 3-7 postnatally) and associated with PCB levels, but disappeared with increasing age (Patandin et al., 1998). The Finnish data (Vartiainen et al., 1998) are interesting because birth weight did decrease in males with increasing TEQ, but the lack of detail on the statistical analyses makes interpretation difficult.

7.15.3.4.10. ***"Miscellaneous" endpoints.*** Additional reproductive outcomes that were evaluated in a subset of the studies include molar pregnancies (in the Vietnamese studies), neonatal and infant death, and childhood cancer and mortality. Mainly because of small sample sizes, it is difficult to reach conclusions regarding neonatal, infant, and child mortality and childhood cancers. Recently, the increased risk for neonatal death observed in the Ranch Hand study, the only study with individual TCDD levels, was investigated. Some changes were observed in the Ranch Hand study for preterm birth and neonatal death, but these did not follow an exposure-response relationship (Michalek et al., 1998).

Conclusion

In conclusion, the research to date has been successful in resolving some confusion surrounding the evidence for an association of dioxin exposure and various developmental and reproductive endpoints in humans. High occurrence of exposure misclassification, differences in case definitions across studies, and small sample sizes have severely limited the power of these studies to address these questions. Additional research that includes a measure of dioxin level at the time of conception for both the father and mother is necessary if the effect of dioxins on the spectrum of reproductive outcomes is to be understood.

7.15.4. Acute Effects

The following section reviews endpoints that were described in groups shortly after exposure to 2,3,7,8-TCDD but were not observed as chronic effects in studies conducted many years after exposure ceased. Also reviewed are endpoints observed as long-term effects in single studies.

7.15.4.1. *Dermatologic Conditions Other Than Chloracne*

Dermatologic conditions other than chloracne, such as hyperpigmentation, hypertrichosis, and eyelid cysts, have been related to exposure to 2,3,7,8-TCDD in early case reports (Ashe and Suskind, 1950; Suskind et al., 1953; Bleiberg et al., 1964; Poland et al., 1971; Bauer et al., 1961; Goldman, 1972; Jirasek et al., 1974; Oliver, 1975). However, these conditions may have been acute effects of 2,3,7,8-TCDD exposure that resolved over time or may be residual effects of chloracne, because they appear to occur more frequently in individuals with persistent chloracne (Suskind and Hertzberg, 1984). These conditions were not observed in studies in which the cohorts were examined years after cessation of exposure, in individuals with the potential for high exposure, or in those with high adipose or serum 2,3,7,8-TCDD levels (Moses et al., 1984; Webb, 1989; Roegner et al., 1991; Burton et al. 1998).

Actinic keratosis, Peyronie's disease, and basal cell carcinoma may not be associated with 2,3,7,8-TCDD. All three conditions were observed in only one study (Suskind and Hertzberg, 1984; Lathrop et al., 1984) and were not observed in studies of individuals with similar potential for exposure (Ott et al., 1994).

7.15.4.2. *Liver Enzymes Other Than GGT and Hepatomegaly*

A number of studies reported elevated liver enzymes, particularly AST and ALT, among individuals who were being exposed at the time of the measurement (May, 1973) or whose exposure was within a few years of the measurement (Jirasek et al., 1974; Mocarelli et al., 1986; Caramaschi et al., 1981). Follow-up studies or longitudinal analyses of exposed cohorts suggest that the increase in enzyme level resolves over time (Mocarelli et al., 1986; Assennato et al., 1989; Pazderova-Vejlupkova et al., 1981; May, 1982). In studies of exposed populations tested many years after exposure ceased, levels of AST and ALT were within normal range (Calvert et al., 1992; Webb et al., 1989; Roegner et al., 1991; Suskind and Hertzberg, 1984; Moses et al., 1984).

D-glucaric acid was tested in a number of 2,3,7,8-TCDD-exposed populations as an indicator of enzyme induction (Ideo et al., 1985; Martin, 1984; Roegner et al., 1991; Calvert et al., 1992). Shortly after the TCP reactor release, D-glucaric acid levels in Seveso children were elevated (Ideo et al., 1985). No other studies of exposed groups tested 5 to 37 years after exposure ceased found elevations of this enzyme (Martin, 1984; Roegner et al., 1991; Calvert et al., 1992).

These data suggest that certain hepatic enzymes are increased as a response to high, exogenous exposure to 2,3,7,8-TCDD. Once the exposure ends, the enzyme levels seem to decrease over time, as observed in the Seveso populations (Mocarelli et al., 1986; Ideo et al., 1985). Additional evidence of the acute nature of AST, ALT, and D-glucaric acid elevations is demonstrated by the lack of such increases in studies of highly exposed groups conducted long after exposure ceased (Calvert et al., 1992; Roegner et al., 1991; Grubbs et al., 1995; Martin, 1984).

As in the case of ALT, AST, and D-glucaric acid, hepatomegaly appears to be a condition reported in case reports after high exposure to 2,3,7,8-TCDD-contaminated chemicals, particularly among TCP production workers examined after a TCP reactor explosion and among Seveso residents (Ashe and Suskind, 1950; Suskind et al., 1953; Jirasek et al., 1974; Reggiani, 1980a). Later studies conducted after exposure ceased failed to find excess dose-related hepatomegaly in the exposed populations (Bond et al., 1983; Suskind and Hertzberg, 1984; Moses et al., 1984; Calvert et al., 1992; Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991; Webb et al., 1989; Hoffman et al., 1986). However, the absence of an effect in cross-sectional studies does not confirm the lack of an effect in the past.

7.15.4.3. *Pulmonary Disorders*

Early case reports suggest that exposure to 2,3,7,8-TCDD chemicals may cause temporary respiratory irritation (Zack and Suskind, 1980) and tracheobronchitis (Goldman, 1972). The data from two cross-sectional medical studies provide weak evidence of slightly decreased lung function among exposed individuals (Suskind and Hertzberg, 1984; Roegner et al., 1991). In these studies, the effects may be due more to smoking (Roegner et al., 1991) or to a substantial age difference between the exposed and unexposed groups (Suskind and Hertzberg, 1984). One study of highly exposed TCP production workers found no relationship between serum 2,3,7,8-TCDD levels and chronic obstructive pulmonary disease, bronchitis, or decreased pulmonary function (Calvert et al., 1992).

In conclusion, case reports indicate that intense acute exposure to 2,3,7,8-TCDD can produce respiratory irritation. However, the findings from controlled epidemiologic studies conducted many years after exposure do not convincingly support an association between 2,3,7,8-TCDD exposure and chronic effects on the respiratory system.

7.15.4.4. *Neurologic Disorders*

The results of case reports and epidemiologic studies demonstrate that exposure to 2,3,7,8-TCDD-contaminated materials is associated with symptoms referable to the central and peripheral nervous systems shortly following exposure and, in some cases, lasting many years

(Filippini et al., 1981; Ashe and Suskind, 1950; Moses et al., 1984). Overall, however, the neurologic status of workers, community residents, and Vietnam veterans exposed to 2,3,7,8-TCDD and evaluated from 5 to 37 years after last exposure appears to be normal (Centers for Disease Control of Vietnam Experience Study, 1988a; Lathrop et al., 1984; Sweeney et al., 1993). The data suggest that, although exposure to 2,3,7,8-TCDD may have been extensive as in exposed workers, Ranch Hands, and Seveso residents, the effects described in case reports may have been transient (Filippini et al., 1981; Lathrop et al., 1984; Centers for Disease Control Vietnam Experience Study, 1988a,b; Assennato et al., 1989; Alderfer et al., 1992; Sweeney et al., 1993). The findings of recent studies suggest that in adults there are no long-term neurologic effects caused by even high exposure to 2,3,7,8-TCDD-contaminated materials, but there is very little information with which to examine the effects of exposure on the developing human neurologic system.

7.15.4.5. *Porphyrias*

In rats and mice, exposure to 2,3,7,8-TCDD has been clearly shown to alter porphyrin metabolism (Goldstein et al., 1973; Smith et al., 1981; Jones and Chelsky, 1986; DeVerneiul et al., 1983; Cantoni et al., 1981; Goldstein et al., 1982). Whether 2,3,7,8-TCDD is associated with porphyrin changes in humans, particularly PCT, is a subject of unresolved debate. It has been suggested that the PCT and elevated urinary porphyrins observed in the New Jersey and Czechoslovakian workers during the years of operation of the plants were the result of exposure to hexachlorobenzene, which was produced at the same time as TCP (Pazderova-Vejlupkova et al., 1981; Jones and Chelsky, 1986). These statements have not been corroborated with strong studies. In the follow-up studies, urinary porphyrin levels of these TCP production workers were not elevated (Pazderova-Vejlupkova et al., 1981; Poland et al., 1971) or did not differ from levels in the control group (Calvert et al., 1993). Doss et al. (1984) also described transient elevations in coproporphyrins among 22 Seveso residents exposed to 2,3,7,8-TCDD.

Because 2,3,7,8-TCDD is a porphyrigen in rats and mice, it has been of interest to determine whether exposures may have contributed to the observed changes in porphyrin levels in human populations. The NIOSH study could not address the question of etiology or transient porphyria, but it did not find porphyria in highly exposed workers many years after their occupational exposure.

However, although porphyria was not found after 2,3,7,8-TCDD exposure, it may be an outcome of exposure to PCBs when there is a co-exposure to chlorophenols, as suggested by the study of pentachlorophenol workers (Hryhorczuk et al., 1998). Further research is recommended to examine these findings.

7.15.4.6. *Thyroid Function*

Many effects of 2,3,7,8-TCDD exposure in animals resemble signs of thyroid dysfunction or significant alterations of thyroid-related hormones. In the few human studies that examined the relationship between 2,3,7,8-TCDD exposure and hormone concentrations in adults, the results are mostly equivocal (Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991; Grubbs et al., 1995; Suskind and Hertzberg, 1984). However, concentrations of thyroid binding globulin (TBG) appear to be positively correlated with current levels of 2,3,7,8-TCDD in the BASF accident cohort (Ott et al., 1994). Little additional information on thyroid hormone levels has been reported for production workers and none for Seveso residents, two groups with documented high serum 2,3,7,8-TCDD levels.

Table 7-21b. Overview of biologic measurements in Dutch studies of postnatal developmental effects

Compound	IUPAC[a]	TEF[b]	Rotterdam/ Groningen[c] Breast milk [plasma/ cord blood][d]	Amsterdam[e] Breast milk	Compound	IUPAC	TEF	Rotterdam/ Groningen Breast milk [plasma/ cord blood]	Amsterdam Breast milk
PCDDs					**PCDFs (cont.)**				
2,3,7,8-TCDD	48	1	4.0	3.8	1,2,3,4,7,8-HXCDF	118	0.1	6.6	7.0
1,2,3,7,8-PECDD	54	0.5	10.6	10.6	1,2,3,6,7,8-HXCDF	121	0.1	5.7	6.2
1,2,3,4,7,8-HXCDD	66	0.1	8.7	1.3	1,2,3,7,8,9-HXCDF	124	0.1	3.6	3.2
1,2,3,6,7,8-HXCDD	67	0.1	47.4	49.1	2,3,4,6,7,8-HXCDF	130	0.1	0.3	BDL
1,2,3,7,8,9-HXCDD	70	0.1	6.7	6.5	1,2,3,4,6,7,8-HPCDF	131	0.01	7.9	6.1
1,2,3,4,6,7,8-HPCDD	73	0.01	63.2	54.3	1,2,3,4,7,8,9-HPCDF	134	0.01	0.2	BDL
1,2,3,4,6,7,8,9-OCDD	75	0.001	799.6	297.5	1,2,3,4,6,7,8,9-OCDF	135	0.001	2.2	1.3
PCDFs					**Planar PCBs**				
2,3,7,8-TCDF	83	0.1	0.8	2.0	3,3',4,4'-PCB	77	0.0005	19.3	
1,2,3,7,8-PECDF	94	0.05	0.3	0.2	3,3',4,4',5-PCB	126	0.1	152.0	
2,3,4,7,8-PECDF	114	0.5	22.7	21.9	3,3',4,4',5,5'-PCB	169	0.01	84.3	

Table 7-21b. Overview of biologic measurements in Dutch studies of postnatal developmental effects (continued)

Compound	IUPAC[a]	TEF[b]	Rotterdam/ Groningen[c] Breast milk [plasma/ cord blood][d]	Amsterdam[e] Breast milk
Nonplanar PCBs				
2,4,4'	28		12.1	
2,2',5,5'	52		2.6	
2,3',4,4'	66		11.6	
2,3',4',5	70		18.5	
2,2',4,4',5	99		19.7	
2,2',4,5,5'	101		1.5	
2,3,3',4,4'	105[f]	0.0001	9.4	
2,3',4,4',5	118[f]	0.0001	35.5 [0.16/0.04]	
2,2',3,3',4,4'	128		4.0	
2,2',3,4,4',5	137		16.8	
2,2',3,4,4',5'	138		129.9 [0.60/0.13]	
2,3,4,5,2',5'	141		1.1	
2,2',3,5,5',6	151		0.9	
2,2',4,4',5,5'	153		186.3 [0.91/0.18]	
2,3,3',4,4',5	156[f]	0.0005	21.0	
2,2',3,3',4,4',5	170[g]	0.0001	37.1	
2,2',3,3',4',5,6	177		6.3	
2,2',3,4,4',5,5'	180[g]	0.00001	76.8 [0.54/0.10]	
2,2',3,4,4',5',6	183		12.2	
2,2',3,4',5,5',6	187		20.0	
2,2',3,3',4,4',5,5'	194		8.6	
2,2',3,3',4,4',5,6	195		2.9	
2,2',3,3',5,5',6,6'	202		0.9	

[a] IUPAC: International Union of Pure and Applied Chemistry.
[b] TEF:Toxic Equivalence Factor (WHO, 1993).
[c] Measurements for this series of studies from Koopman-Esseboom et al., 1994.
[d] Mean values: breast milk in pg/g fat, blood in ng/g plasma.
[e] Measurements for this series of studies from Pluim et al., 1994.
[f] Mono-ortho PCBs.
[g] Di-ortho PCBs.

Table 7-22. Serum 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) levels in Seveso residents with chloracne and adipose tissue levels of 2,3,7,8-TCDD and hexachlorinated (HxCDD) dioxins in German chemical workers

Author	Population	2,3,7,8-TCDD level (pg/g)[a]	HxCDD level (pg/g)[a]	Year of chloracne diagnosis	Half-life extrapolated 2,3,7,8-TCDD[b]	Half-life extrapolated HxCDD[b]
Beck et al., 1989	Chemical workers[c]	174	247	1955	750	10,000
		99	166	1955	4,010	6,720
		147	5,101	1963	2,350	81,620
		61	172	1957(?)	2,470	6,970
		50	517	1969	380	3,940
		16	58	1955	650	2,360
		1,280	1,019	1978	3,380	2,690
		49	3,442	1974(?)	210	14,760
		50	9,613	1972(?)	260	50,740
		2,252	3,087	1984	2,850	3,910
		158	1,191	1977(?)	460	3,490
		6	283	1970	40	1,970
Mocarelli et al., 1991	Seveso residents	56,000[d]	—	1976-1977		
		27,800	—	1976-1977		
		26,400	—	1976-1977		
		15,900	—	1976-1977		
		12,100	—	1976-1977		
		17,300	—	1976-1977		
		7,420	—	1976-1977		
		1,690	—	1976-1977		
		828	—	1976-1977		

[a]pg/g of lipid (parts per trillion).
[b]Half-life extrapolation calculated by authors (Beck et al., 1989) using the formula $C_o = C_{t_n} \times 2^n$ where C_o = original concentration of 2,3,7,8-TCDD or HxCDD, C_{t_n} = concentration at time t, n = number of half-life periods, and t = half-life period of 5.8 years. Exposures occurred between 1949 and 1986.
[c]Measured in 1984.
[d]Measured in 1976.

Table 7-23. Mean serum levels of gamma glutamyl transferase (GGT) among Seveso and Missouri residents, TCP production workers, BASF accident cohort, and Vietnam veterans

Author	Population	Exposed			Unexposed		
		N	Mean level[a]	(SD)	N	Mean level[a]	(SD)
	Seveso residents						
Mocarelli et al., 1986	(1977) (Boys)	52[b]	9.73	(4.72-20.04)	42[b]	7.28	(3.71-14.26[c])
	(Girls)	32[b]	9.10	(3.62-22.85)	43[b]	8.05	(2.99-21.68)
	(1982) (Boys)	106[b]	9.70	(4.29-21.93)	138[b]	8.99	(4.45-18.20)
	(Girls)	117[b]	9.04	(4.25-19.23)	140[b]	8.59	(3.85-19.14)
Assennato et al., 1989	1976	193[d]	11.94	(16.80)	—[e]	—	—
	1982	152[d]	11.67	(7.94)	123[f]	11.23	(7.41)
	1984	142[d]	10.53	(7.07)	196[f]	11.19	(7.05)
	1985	141[d]	10.57	(7.38)	167[f]	10.94	(4.68)
	Missouri residents						
Webb et al., 1989	<20[g]	16	24.0	(15.5)	—	—	—
	20-60	13	17.7	(7.23)	—	—	—
	>60	12	32.8	(23.90)	—	—	—
Hoffman et al., 1986	Missouri residents in Quail Run Mobile Home Park	140	30.0[h]	(88.3)	141	20.1	(23.4)
May, 1982	TCP production workers in Great Britain	41	39.0	—	31	27.7[c]	—
Martin, 1984	TCP production workers in Great Britain	41	40.0	(14-91)	120	32.0	(11-90)
Moses et al., 1984	TCP and 2,4,5-T production workers in West Virginia	22[d]	26.3[c]	(27.0)	9[f]	17.4	(11.0)
Calvert et al., 1992	TCP and 2,4,5-T production workers in Missouri and New Jersey	280	58.5[c,i]	(73.7)	259	47.4	(41.1)

Table 7-23. Mean serum levels of gamma glutamyl transferase (GGT) among Seveso and Missouri residents, TCP production workers, BASF accident cohort, and Vietnam veterans (continued)

Author	Population	Exposed			Unexposed		
		N	Mean level[a]	(SD)	N	Mean level[a]	(SD)
Ott et al., 1993b	BASF accident cohort	133	30.5	(58.4)	6,708	29.9	(43.5)
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army ground troops	2,490	43.2[j]		1,972	41.1[k]	—
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown ≤10[l,m]	338	31.49[c,o]	—	777	34.64[o]	—
	Low 15-≤33.3[l,m]	191	38.28[c,o]	—			
	High >33.3[l,m]	182	40.82[c,o]	—			
Grubbs et al., 1995	U.S. Air Force Ranch Hand personnel						
	Background [l,m,n]	362	32.60[o]	—	1025	34.34[o]	—
	Low [l,m,n]	251	36.99[c,o]	—			
	High [l,m,n]	502	37.67[c,o]	—			

[a]Units = U/L.
[b]Number of samples.
[c]$p < 0.05$.
[d]Chloracne.
[e]No data for controls in 1976.
[f]No chloracne.
[g]Adipose tissue level of 2,3,7,8-TCDD in pg/g of lipid.
[h]% abnormal: exposed = 3.6; unexposed = 3.6.
[i]% abnormal: exposed = 10.7; unexposed = 5.0; OR=227 (95% CI=1.17, 4.39).
[j]Geometric mean.
[k]% Abnormal (Vietnam veterans, 5.5%; non-Vietnam veterans, 4.4; OR=1.3, 95% CI=1.0-1.8).
[l] Serum 2,3,7,8-TCDD level in pg/g of lipid.
[m]Contrasted to unexposed comparison population.
[n]Background: current dioxin ≤10 pg/g of lipid.
Low: current dioxin > 10 pg/g of lipid, 10 pg/g< initial dioxin ≤143 pg/g of lipid.
High: current dioxin > 10 pg/g of lipid, 10 pg/g< initial dioxin >143 pg/g of lipid.
[o]Adjusted mean.

Table 7-24. Logistic regression model for an out-of-range serum gamma-glutamyl transferase[a] (GGT) level using the categorical TCDD[b] exposure measure[c]

Variable	Beta	Standard error of the estimate	X^2	*p*
Intercept	-4.12	0.50	67.01	<0.001
Exposure (worker = 1, referent = 0)	0.37	0.43	0.74	0.195
Per alcohol-year	-2.4×10^{-6}	2.6×10^{-3}	0.00	0.999
Current alcohol drinker (yes = 1, no = 0)[d]	0.90	0.42	4.70	0.030
Alcohol-years/exposure interaction[e,f]	7.2×10^{-3}	3.5×10^{-3}	4.24	0.039
Triglyceride level	3.8×10^{-3}	1.1×10^{-3}	11.94	<0.001

[a]Reference value: 96 IU/L; a level was considered out of range if it exceeded the reference value. Reference values were defined as the 95th percentile for the referent cohort.
[b]TCDD = 2,3,7,8-tetrachlorodibenzo-*para*-dioxin.
[c]N=536 observations.
[d]The results from this logistic regression analysis change little when this term is dropped from the model.
[e]Interaction between alcohol-years and exposure.
[f]Exposure odds ratios (ORs) for an abnormal γ-glutamyltransferase level among workers by selected alcohol-year levels, adjusting for all variables in the model, are as follows: OR=2.96 (95% confidence interval [CI]=1.34-6.54) for 100 alcohol-years; OR=1.79 (95% CI=0.81-3.97) for 30 alcohol-years; OR=1.45 (95% CI=0.63-3.36) for 1 alcohol-year; and OR=1.44 (95% CI=0.62-3.34) for 0 alcohol-years.

Source: Calvert et al., 1992b. Used with permission from the author.

Table 7-25. Serum alanine aminotransferase (ALT) among Seveso children and Missouri residents, TCP production workers, BASF accident cohort, and Vietnam veterans

Author	Population	Exposed N	Exposed Mean level[a]	Exposed (SD)	Unexposed N	Unexposed Mean level[a]	Unexposed (SD)
Mocarelli et al., 1986	Seveso children						
	1977 Boys	46[b]	12.25[c]	(7.14-20.99)	45[b]	9.33[c]	(3.73-23.33)
	Girls	100	12.97	(6.68-25.18)	141	11.99	(5.51-26.12)
	1982 Boys	33	10.74	(5.09-22.65)	39	10.74	(5.09-22.65)
	Girls	119	12.27	(6.5 -23.17)	136	12.19	(6.46-23.01)
Caramaschi et al., 1981	Seveso children	141[d]	3.5[e,c]		0[f]	0[e]	
Moses et al., 1984	TCP production workers; 10-30 years postexposure	105[d]	15.9	(13.0)	101[f]	15.7	(13.0)
Calvert et al., 1992	TCP production workers; 15-37 years postexposure	280[g]	33.8	(22.6)	259	33.0	(21.2)
Ott et al., 1994	BASF accident cohort	133	14.8	(8.4)	6,721	15.1	(10.0)
Hoffman et al., 1986	Missouri residents in Quail Run Mobile Home Park	134	—[h]		135	—[h]	
Webb et al., 1989	Missouri residents						
	<20[i]	16	22.7	(2.76)			
	20-60	12	22.5	(2.39)			
	>60	12	23.3	(3.06)			
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army ground troops	2,490	26.4[j,k]		1,972	25.8	
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown ≤10[l]	338[m]	19.16	—	777	20.34	
	Low 15-≤33.3[l]	19[m] 1	20.83	—	—	—	
	High >33.3[l]	182[c,m]	20.09	—	—	—	
Grubbs et al., 1995	U.S. Air Force Ranch Hand personnel						
	Background[l]	367[m]	26.25	—	1,025	27.56	
	Low[l]	254[m]	27.47	—			
	High[l]	254[m]	27.09	—			

[a]ALT; units U/L.
[b]Number of samples.
[c]$p<0.05$.
[d]Chloracne.
[e]% abnormal.
[f]No chloracne.
[g]% abnormal: workers 4.3; unexposed 5.0; OR=0.85 (95% CI=0.38, 1.89).
[h]% abnormal: exposed 6.0; unexposed 3.0.
[i]Adipose 2,3,7,8-TCDD level in pg/g of lipid.
[j]Geometric mean.
[k]% abnormal; Vietnam veterans, 5.3; non-Vietnam veterans, 4.4; OR=1.2, 95% CI=0.9-1.5.
[l]Serum 2,3,7,8-TCDD in pg/g of lipid.
Background: current dioxin≤ 10 pg/g of lipid.
Low: current dioxin > 10 pg/g of lipid, 10 pg/g< initial dioxin ≤143 pg/g of lipid.
High: current dioxin > 10 pg/g of lipid, 10 pg/g< initial dioxin >143 pg/g of lipid.
[m]Adjusted mean.

Table 7-26. Blood measures and cumulative dioxin-furan TEQs from 11 weeks of breast feeding (Pluim et al., 1994)

Blood measure	Correlation coefficient	*p*-value
ALT	0.40	0.02
AST	0.44	0.009
Platelets	-0.48	0.011

Table 7-27. Mean D-glucaric acid levels among Seveso residents, TCP production workers, and Vietnam veterans

Author	Population	Exposed			Unexposed		
		N	Mean	(SD)	N	Mean	(SD)
Ideo et al., 1985	Seveso adults Levels measured in 1978 (Zone B)	117	27.1[a,b,c]	—	127	19.8[a,b,d]	—
	Seveso children Zone A, 1976	14[e]	39[a,b,c]	—	17[f]	20.5[b]	—
	Zone B						
	1979	26.8	—[g]	—	—	—	—
	1980	17.0	—	—	—	—	—
May, 1982	TCP production workers in Great Britain	41	2.07[h]	—	31	1.52[h]	—
Martin, 1984	TCP production workers in Great Britain	39	2.09[c]	(0.7-7.9)	126	1.59	(0.8-8.3)
Calvert et al., 1992	TCP and 2,4,5-T production workers in Missouri and New Jersey	273	14.1[i]	(11.1)	256	13.2[i]	(7.9)
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown ≤10[j]	317	13.99[k,l]	—	727	14.11[k]	—
	Low 15-≤33.3	180	14.43[l]	—	—	—	—
	High >33.3	173	15.22[l]	—	—	—	—

[a]Units: μmol/g of creatinine.
[b]Median level.
[c]$p < 0.05$.
[d]Residents of unexposed community.
[e]Skin lesions.
[f]No skin lesions.
[g]d-glucaric acid levels measured in 1979 were significantly higher than levels measured in 1980 ($p < 0.05$), no data presented.
[h]d-glucaric acid/creatinine ratio.
[i]Units = μg/g of creatinine.
[j]Serum 2,3,7,8-TCDD levels in pg/g of lipid.
[k]Units = μM.
[l]Contrasted to unexposed comparison population.
[m]Adjusted mean.

Table 7-28. Mean total cholesterol levels among Seveso and Missouri residents, TCP production workers, BASF accident cohort, and Vietnam veterans

Author	Population	Exposed			Unexposed		
		N	Mean[a] level	(SD)	N	Mean[a] level	(SD)
Mocarelli et al., 1986	Seveso children 1977 1982	 16[b] 182[b]	 4.62 4.48	 3.26-5.98 2.47-5.99	 28[b] 250[b]	 4.45 4.41	 (3.12-5.77) (2.99-5.83)
Caramaschi et al., 1981	Seveso children	138	15.2[c,d]	—	120	12.5[c,e]	—
Assennato et al., 1989	Seveso residents 1976 1982 1984 1985	 193[d] 152[d] 142[d] 141[d]	 4.78 4.06 4.09 4.14	 (0.99) (0.80) (0.88) (0.91)	 —[f] 123[e] 196[e] 167[e]	 — 4.14 4.12 4.13	 — (0.77) (0.86) (0.78)
May, 1982	TCP production workers in Great Britain	41	5.97	—	31	6.6	—
Martin, 1984	TCP production workers in Great Britain	39	6.02[g]	—	126	5.6	—
Poland et al., 1971	TCP production workers in New Jersey	71	6.12	(0.82)	—	—	—
Moses et al., 1984	TCP production workers in West Virginia	105[d]	5.38	(0.88)	101[e]	5.37	(0.85)
Suskind and Hertzberg, 1984	TCP production workers in West Virginia	200	5.46	(0.07)	163	5.28	(0.08)
	TCP production workers: chloracne vs. never chloracne	105[d]	5.44	(0.08)	28[e]	5.30	(0.18)
Ott et al., 1994	BASF accident cohort	135	6.14[m]	(1.01)	6,581	6.37[m]	(1.17)
Calvert et al., 1996	TCP and 2,4,5-T production workers in Missouri and New Jersey	278	5.7[n]	—	259	5.6[n]	—

Table 7-28. Mean total cholesterol levels among Seveso and Missouri residents, TCP production workers, BASF accident cohort, and Vietnam veterans (continued)

Author	Population	Exposed			Unexposed		
		N	Mean[a] level	(SD)	N	Mean[a] level	(SD)
Hoffman et al., 1986	Missouri residents in Quail Run Mobile Home Park	142	4.97[g]	(0.96)	148	5.2	(1.09)
Webb et al., 1989	Missouri residents						
	<20[h]	16	5.88	(1.10)	—	—	—
	20-60[h]	12	6.60	(0.93)	—	—	—
	>60[h]	12	6.76	(0.97)	—	—	—
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	5.43[i,j]	—	1,972	5.36	—
Roegner et al., 1991	Air Force Ranch Hand personnel						
	Unknown ≤10[k]	338[l]	5.53[p]	—	777	5.51	—
	Low 15-≤33.3[k]	191	5.55[p]	—	—	—	—
	High >33.3[k]	182	5.68[g,p]	—	—	—	—
Grubbs et al., 1995	U.S. Air Force Ranch Hand personnel						
	Background[o]	362	5.71[p]	—	1,025	5.69	—
	Low [o]	251	5.68[p]	—			
	High [o]	251	5.76[p]	—			

[a]Units = mmol/L.
[b]Number of samples.
[c]% abnormal.
[d]Chloracne.
[e]No chloracne.
[f]No data for controls in 1976.
[g]$p<0.05$.
[h]Adipose tissue levels of 2,3,7,8-TCDD in pg/g of lipid.
[i]Geometric mean.
[j]% abnormal: Vietnam veterans, 5.1; non-Vietnam veterans, 4.7; OR=1.1, 95% CI=0.8-1.5.
[k]Serum 2,3,7,8-TCDD levels in pg/g of lipid.
[l]Contrasted to unexposed comparisons.
[m]Adjusted for age, body mass index, smoking history.
[n]Adjusted for age, body mass index, smoking, gender.
[o]Serum 2,3,7,8-TCDD levels in pg/g of lipid.
Background: current dioxin ≤10 pg/g of lipid.
Low: current dioxin > 10 pg/g of lipid, 10 pg/g< initial dioxin ≤143 pg/g of lipid.
High: current dioxin > 10 pg/g of lipid, 10 pg/g< initial dioxin >143 pg/g of lipid
[p]Adjusted mean.

Table 7-29. Mean triglyceride levels among Seveso and Missouri residents, TCP production workers, BASF accident cohort, and Vietnam veterans

Author	Population	Exposed		Unexposed	
		N	Mean (SD)	N	Mean (SD)
Mocarelli et al., 1986	Seveso children 1977 1982	 38[b] 207[b]	 0.97 (0.60-1.50) 0.91 (0.52-.60)	 36[b] 257[b]	 0.95 (0.63-1.51) 0.86 (0.47-1.56)
Assennato et al., 1989	Seveso residents 1976 1982 1984 1985	 193 152 142 141	 0.99 (0.43) 0.87 (0.40) 0.94 (0.59) 0.84 (0.44)	 — 123 196 167	 — — 0.85 (0.37) 0.88 (0.46) 0.87 (0.55)
May, 1982	TCP production workers, Great Britain	41[d]	2.03 —	31[e]	1.83 —
Martin, 1984	TCP production workers, Great Britain	39[d]	1.97[f] (0.4-4.0)	126[e]	1.41 (0.3-3.2)
Moses et al., 1984	TCP production workers, West Virginia	93[d]	1.69[g] (1.26)	93[e]	1.46 (0.73)
Suskind and Hertzberg, 1984	TCP production workers, West Virginia	200	1.65 (0.08)	163	1.76 (0.08)
Ott et al., 1994	BASF accident cohort	135	1.91[h] (1.19)	4,471	1.97[h] (1.65)
Calvert et al., 1996	TCP and 2,4,5-T production workers, Missouri and New Jersey	273	1.20[i] —	259	1.15[i] —
Hoffman et al., 1986	Missouri residents in Quail Run Mobile Home Park	141	1.07 (0.73)	146	1.19 (1.07)
Webb et al,. 1989	Missouri residents <20[h] 20-60[h] >60[h]	 16 12	 2.17 (2.08) 1.81 (1.19)	 — —	 — — — —
		12	2.69 (1.06)	—	— —

Table 7-29. Mean triglyceride levels among Seveso and Missouri residents, TCP production workers, BASF accident cohort, and Vietnam veterans (continued)

Author	Population	Exposed		Unexposed	
		N	Mean (SD)	N	Mean (SD)
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1.06[j,k] —	1,972	1.05
Roegner et al., 1991	Air Force Ranch Hand personnel Unknown ≤10[k] Low 15-≤33.3[k] High >33.3[k]	 338[l] 191 182	 1.02[f,m,o] — 1.37[f,m,o] — 1.35[f,m,o] —	777	1.16 —
Grubbs et al., 1995	U.S. Air Force Ranch Hand personnel Background Low [l] High [l]	 362 191 182	 1.43[m,n] — 1.49[m,n] — 1.35[m,n] —	1,025	1.47

[a]Units = mmol/L.
[b]Number of samples.
[c]Adipose tissue 2,3,7,8-TCDD levels in pg/g of lipid.
[d]Chloracne.
[e]No chloracne.
[f]$p<0.01$.
[g]$p=0.056$.
[h]Adjusted for age, body mass index, smoking history.
[i]Adjusted for age, body mass index, smoking, gender.
[j]% abnormal: Vietnam veterans, 4.7; non-Vietnam veterans, 5.3; OR=0.9, 95% CI=0.7-1.2.
[k]Geometric mean.
[l]Serum 2,3,7,8-TCDD levels in pg/g of lipid.
[m]Contrasted to unexposed comparison group.
[n]Serum 2,3,7,8-TCDD levels in pg/g of lipid.
Background: current dioxin ≤ 10 pg/g of lipid.
Low: current dioxin > 10 pg/g of lipid, 10 pg/g< initial dioxin ≤143 pg/g of lipid.
High: current dioxin > 10 pg/g of lipid, 10 pg/g< initial dioxin >143 pg/g of lipid.
[o]Adjusted mean.

Table 7-30. Levels of thyroxine-binding globulin (TBG), thyroxine (T4), free thyroxine (FT4), or T4/TBG

Outcome	Author	Population	Exposed[a]			Unexposed[b]		
			N	Mean level	Standard deviation	N	Mean level	Standard deviation
Nursing infants								
Total T4 (nmol/L)								
	Pluim et al., 1992 Pluim et al., 1993	Neonates; Amsterdam, The Netherlands						
		At birth/cord blood	15	134.3	4.8[d]	18	122.5	4.1[d]
		1 wk postnatal	19	178.7[c]	5.5	19	154.5	6.3
		11 wks postnatal	16	122.2[c]	3.0	18	111.1	4.0
	Koopman-Esseboom, 1994	Neonates; Rotterdam, The Netherlands						
		2nd wk postnatal	39	159.9[c]	31.6	39	177.5	39.2
Free T4 (nmol/L)								
	Koopman-Esseboom, 1994	Neonates; Rotterdam, The Netherlands						
		2nd wk postnatal	39	23.0[c]	3.3	39	24.6	3.5
TBG (nmol/L)								
	Pluim et al., 1992 Pluim et al., 1993	Neonates; Amsterdam, The Netherlands						
		At birth/cord blood	15	589.5	30.5[d]	18	520.1	27.2[d]
		1 wk postnatal	19	546.2	19.1	19	532.6	16.3
		11 wks postnatal	16	500.7	13.0	18	519.0	29.4

Table 7-30. Levels of thyroxine-binding globulin (TBG), thyroxine (T4), free thyroxine (FT4), or T4/TBG (continued)

Outcome	Author	Population	Exposed[a]			Unexposed[b]		
			N	Mean level	Standard deviation	N	Mean level	Standard deviation
Nursing infants (continued)								
T4/TBG								
	Pluim et al., 1992 Pluim et al., 1993	Neonates; Amsterdam, The Netherlands						
		At birth/cord blood	15	0.232	0.008[d]	18	0.240	0.007[d]
		1 wk postnatal	19	0.332[c]	0.011	19	0.291	0.009
		11 wks postnatal	16	0.247[c]	0.009	18	0.220	0.008
BASF accident cohort								
TBG (mg/L)	Ott et al., 1994	BASF cohort	131	12.7	3.2	141	12.7	2.9
T4 (µg/dL)	Ott et al., 1994	BASF cohort	131	7.8	1.9	141	8.3	1.5
T4/TBG (mU/L)	Ott et al., 1994	BASF cohort	131	6.3	1.3	141	6.7	1.6

[a]High-exposure group: 29.2-62.7 ng toxic equivalents/kg (dioxin-furan TEQ per kg milk fat) (Pluim et al, 1992 and 1993); >30.75-76.43 pg dioxin-furan-TEQ/g fat (Koopman-Esseboom et al., 1994).
[b]Low-exposure group: 8.7-28 ng TEQ/kg (Pluim et al, 1992 and 1993); 12.44-30.75 pg dioxin-furan TEQ/g fat (Koopman-Esseboom et al., 1994).
[c]$p<0.05$ compared to the unexposed group.
[d]Standard error of the mean.

Table 7-31. Levels of triiodothyronine percent (T3%) uptake or free thyroxine index in Vietnam veterans

Author	Population	Exposed		Adjusted RR (95% CI)	Unexposed	
		N	Mean level		N	Mean level
T3% uptake						
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel				772	30.7
	Unknown ≤10[a]	338	30.7	1.1 (0.6, 2.1)		
	Low 15-≤33.3[a]	194	30.4	0.9 (0.4, 2.2)		
	High >33.3[a]	181[b]	30.0	0.5 (0.1, 1.5)		
	All Ranch Hand vs. all comparisons	937	30.6	1.14 (0.7-1.8)	1,198	30.6
Free thyroxine index						
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	2.2[c]	Adjusted OR (95% CI) 1.2 (0.9,1.5)	1,972	2.2[c]

[a]Serum 2,3,7,8-TCDD in pg/g of lipid.
[b]$p<0.05$ comparison of veterans at background level with ≥33.3 pg/g TCDD.
[c]Geometric mean.

Table 7-32. Levels of thyroid-stimulating hormone (TSH) in Vietnam veterans

Author	Population	Exposed		Adjusted RR (95% CI)	Unexposed	
		N	Mean level[a]		N	Mean level[a]
Vietnam veterans						
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1.6[b]	Adjusted OR (95% CI) 2.0 (0.9-4.3)	1,972	1.6[b]
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel, 1988					
	Unknown ≤10[c]	338	0.948	1.45 (0.62-3.40)	616	0.920
	Low 15-≤33.3[c]	194	0.978	0.88 (0.24-3.02)		
	High >33.3[c]	181	1.026[d]	2.15 (0.80-5.79)		
Grubb et al., 1995	U.S. Air Force Ranch Hand personnel, 1992					
	Comparison ≤10[e]				1,027	1.58
	Background RH ≤10[e]	365	1.64	0.88 (0.40-1.92)		
	Low 10-≤143[e]	254	1.60	0.50 (0.15-1.67)		
	High >143[e]	255	1.64	1.72 (0.78-3.80)		
	Low + High	509	1.62	1.06 (0.53,2.15)		

[a]Units: μU/mL.
[b]Geometric mean.
[c]Serum 2,3,7,8-TCDD level in pg/g (ppt) of lipid.
[d]$p < 0.05$ compared to the unexposed group.
[e]Comparisons: current dioxin ≤10 ppt.
Background (Ranch Hand): current dioxin ≤10 ppt.
Low (RH): current > 10 ppt, 10 ppt < initial dioxin ≤143 ppt.
High (RH): current > 10 ppt, initial dioxin > 143.

Table 7-33. Levels of thyroid-stimulating hormone (TSH) in nursing infants and BASF accident cohort

Author	Population	Exposed			Unexposed		
		N	Mean level[a]	SD	N	Mean level[a]	SD
Nursing infants							
Pluim et al., 1992 Pluim et al., 1993	Neonates; Amsterdam, The Netherlands						
	At birth/cord blood	11[b]	11.9	1.9[c]	14[d]	10.4	1.3[c]
	1 wk postnatal	11	2.56	0.41	15	2.93	0.41
	11 wks postnatal	12	2.50[e]	0.26	18	1.81	0.19
Koopman-Esseboom, 1994	Neonates; Rotterdam, The Netherlands						
	At birth/cord blood	[f]	11.6[e]	8.0		8.5	6.0
	2nd wk postnatal	39[b]	2.6[e]	1.5	39[d]	1.9	0.8
	3 months	39	2.3[e]	1.0	39	1.6	0.6
BASF accident cohort							
Ott et al., 1993b,1994	BASF chemical workers	130	1.19	0.9	—[g]	—	—

[a]Units: µU/mL.
[b]High-exposure group: 29.2-62.7 ng toxic equivalents/kg (TEQ per kg milk fat) (Pluim et al., 1992 and 1993); >30.75-76.43 pg dioxin-furan-TEQ/g fat (Koopman-Esseboom et al., 1994).
[c]Standard error of the mean.
[d]Low-exposure group: 8.7-28 ng TEQ/kg (Pluim et al., 1992 and 1993); 12.44-30.75 pg TEQ/g fat (Koopman-Esseboom et al., 1994).
[e]$p<0.05$ compared to low-exposure group.
[f]Total for both high and low = 75.
[g]No referent values.

Table 7-34. CD4/CD8 ratios in Missouri residents, Vietnam veterans, and BASF accident cohort

Author	Population	Exposed			Unexposed		
		N	Mean level (SD)	Ratio	N	Mean level (SD)	Ratio
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown ≤10[a]	126	1.72	—	301	1.89	—
	Low 15-≤33.3[a]	72	1.91				
	High >33.3[a]	72	1.99				
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1.8[b]	OR < reference range 0.9 OR > reference range 1.1	1,972	1.8[b]	—
Hoffman et al., 1986	Missouri residents	135	1.9 (0.8)	% abnormal 8.2	142	1.9 (0.6)	% abnormal 6.3
Webb et al., 1989	Missouri residents						
	<20[c]	16	2.0 (0.7)	—	—	—	—
	20-60[c]	12	2.1 (1.0)				
	>60[c]	12	1.4 (0.7)				
Ott et al., 1994	BASF accident cohort	132	1.6 (0.94)	—	42[d]	1.5 (0.6)	—
Zober et al., 1992	BASF personnel exposed to TBDD and TBDF[e]	21	1.6 (0.5)	—	42	1.5 (0.6)	—
Grubbs et al., 1995	U.S. Air Force Ranch Hand personnel						
	Background[f]	139	1.50	—	399	1.48	—
	Low	94	1.58				
	High	106	1.57				

[a]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[b]Geometric mean.
[c]Adipose 2,3,7,8-TCDD level in pg/g of lipid.
[d]From Zober et al., 1992.
[e]TBDD = 2,3,7,8-tetrabrominated dibenzo-*p*-dioxins and TBDF = 2,3,7,8-tetrabrominated dibenzofurans.
[f]Comparison: current dioxin ≤10 pg/g of lipid.
Background: current dioxin > 10 pg/g.
Low: current dioxin > 10 pg/g, 10 pg/g < initial dioxin ≤ 143 pg/g.
High: current dioxin > 10 pg/g, 10 pg/g < initial dioxin > 143 pg/g.

Table 7-35. Total lymphocytes in 2,4,5-T production workers, Missouri residents, Vietnam veterans, and BASF accident cohort

		Exposed			Unexposed		
Author	**Population**	**N**	**Mean level[a] (SD)[b]**	**Ratio**	**N**	**Mean level (SD)**	**Ratio**
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown ≤10[c]	127	1,954 —[d]	—	301	1,972 —	
	Low 15-≤33.3[c]	73	2,011 —				
	High >33.3[c]	74	2,032 —				
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1,973 —	OR<reference range 1.0 OR>reference range 1.2	1,972	1,936 —	
Hoffman et al., 1986	Missouri residents	135	2,465 (724)	—	142	2,311(634)	
Webb et al., 1989	Missouri residents			% Lymphocytes			
	<20[e]	16	2,200 (830)	32			
	20-60[e]	12	2,300 (600)	32	—	—	
	>60[e]	12	2,200 (720)	28			
Jennings et al., 1988	2,4,5-T production workers exposed 17 yrs prior to the study	18	1,980 (840)	—	15	2,020 (470)	
Ott et al., 1994	BASF accident cohort	133	1,978.3 (805)	% Lymphocytes 33.4 (9.4)	42	2,267.6 (837.5)	% Lymphocytes 36 (12.4)
Zober et al., 1992	BASF personnel exposed to TBDD[g] &TBDF[g]	21	2,179.5 (678)	% Lymphocytes 33.4 (8.4)	42	2,267.6 (837.5)	% Lymphocytes 36 (12.4)
Grubbs et al., 1995	U.S. Air Force Ranch Hand personnel						
	Background[h]	141	2,066.7[i]	—	400	2,022.4	—
	Low[h]	95	1,988.6				
	High[h]	108	2,034.4				

[a]Units: counts/mm^3.
[b]SD = standard deviation.
[c]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[d]— = data not presented.
[e]Adipose 2,3,7,8-TCDD level in pg/g of lipid.
[f]From Zober et al., 1992.
[g]TBDD = 2,3,7,8-Tetrabrominated dibenzo-*p*-dioxins; TBDF = 2,3,7,8-Tetrabrominated dibenzofurans.
[h] Comparison: current dioxin ≤10 pg/g of lipid.
Background: current dioxin > 10 pg/g.
Low: current dioxin > 10 pg/g, 10 pg/g<initial dioxin ≤143 pg/g.
High: current dioxin > 10 pg/g, 10 pg/g<initial dioxin > 143 pg/g.

[i]Adjusted mean.

Table 7-36. B1 levels in production workers, 2,4,5-T Missouri residents, Vietnam veterans, BASF accident cohort, and extruder personnel

Author	Population	Exposed			Unexposed		
		N	Mean level[a] (SD)	Ratio	N	Mean level[a] (SD)	Ratio
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel Unknown ≤10[b] Low 15-≤33.3[b] High >33.3[b]	 127 71 73	 176 — 183 — 191 —	—	301	172 —	—
Grubbs et al., 1995	U.S. Air Force Ranch Hand personnel Backgound[e] Low [e] High [e]	 140 95 106	 245[f] — 224 — 220 —	— — —	400	214 —	—
CDC Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	240[c]	OR<ref. range 1.1 OR>ref. range 1.2	1,972	230[c] —	—
Webb et al., 1989	Missouri residents <20[d] 20-60[d] >60[d]	 16 12 12	 190 (865) 189 (983) 171 (573)	% B1 cells 9.1 8.3 7.8	—	—	—
Jennings et al., 1988	2,4,5-T production workers exposed 17 yrs prior to the study	18	210 (110)	—	15	160 (80)	—
Ott et al., 1994	BASF accident cohort	133	10.4[e] (6.0)	—	42	12.3[e,f] (5.1)	—
Zober et al., 1992	BASF personnel exposed to TBDD[g] and TBDF[g]	21	276.3 (156)	% B cells 12.5 (4.4)	42	286.4 (199.3)	12.3 (5.1)

[a]Units: cells/mm^3.
[b]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[c]Geometric mean.
[d]Adipose 2,3,7,8-TCDD level in pg/g of lipid.
[e]% B1 cells.
[f]From Zober et al., 1992.
[g]TBDD = 2,3,7,8-tetrabrominated dibenzo-*p*-dioxin.
TBDF = 2,3,7,8-tetrabrominated dibenzofuran.

Table 7-37. CD4 levels in production workers, Missouri residents, Vietnam veterans, and BASF chemical workers

Author	Population	Exposed			Unexposed		
		N	Mean level[a] (SD)	Ratio	N	Mean level (SD)	Ratio
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel Unknown ≤10[b] Low 15-≤33.3[b] High >33.3[b]	 127 72 72	 867 — 945 — 929 —		301	907 —	—
Grubbs et al., 1995	U.S.A.F. Ranch Hand personnel Background[g] Low High	 141 95 108	 961[h] — 917 — 962 —	—	403	923 —	—
CDC Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1,020[c] —	OR<ref. range 1.0 OR>ref. range 1.4	1,972	990[c] —	—
Hoffman et al., 1986	Missouri residents	135	1,021 (353)	% Abnormal 0.7	142	1,033 (346)	% Abnormal 0.0
Webb et al., 1989	Missouri residents <20[d] 20-60[d] >60[d]	 16 12 12	 1,084 (485) 1,198 (391) 963 (403)	% T4 cells 48 51 42	—	—	—
Jennings et al., 1988	2,4,5-T production workers exposed 17 yrs prior to the study	18	950 (340)	—	15	1,040 (290)	—
Tonn et al., 1996	2,4,5-TCP production & maintenance workers	11	—	% of total 47.6 (8.1)	10	—	% of total 48.5 (10.6)

Table 7-37. CD4 levels in production workers, Missouri residents, Vietnam veterans, and BASF chemical workers (continued)

Author	Population	Exposed			Unexposed		
		N	Mean level[a] (SD)	Ratio	N	Mean level (SD)	Ratio
Ott et al., 1994	BASF chemical workers	133	—	% T4 cells 42.5 (10.4)	42[e]	—	% T4 cells 45.1 (8.9)
Zober et al., 1992	BASF personnel exposed to TBDD[f] and TBDF[f]	21	973.3 (381.9)	% CD4 44.8 (6.1)	42	1,032 (444.6)	% CD4 45.1 (8.9)

[a]Units: counts/mm^3.
[b]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[c]Adipose 2,3,7,8-TCDD level in pg/g of lipid.
[d]Adipose 2,3,7,8-TCDD level in pg/g of lipid.
[e]From Zober et al., 1992.
[f]TBDD = 2,3,7,8-tetrabrominated dibenzo-*p*-dioxins;
TBDF = 2,3,7,8-tetrabrominated dibenzofurans.

Table 7-38. CD8 levels in 2,4,5-T production workers, Missouri residents, Vietnam veterans, and BASF accident cohort

Author	Population	Exposed			Unexposed		
		N	Mean level[a] (SD)	Ratio	N	Mean level[a] (SD)	Ratio
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel Unknown ≤10[b] Low 15-≤33.3[b] High >33.3[b]	 126 71 73	 485 — 465 — 475 —		301	473 —	—
Grubbs et al., 1995	U.S. Air Force Ranch Hand personnel Background[h] Low High	 140 95 106	 645 — 606 — 618 —	—	400	634 —	—
CDC Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	560[c] —	OR<ref. range 1.0 OR>ref. range 0.9	1,972	550 —	—
Hoffman et al., 1986	Missouri residents	135	592 (223)	% Abnormal 1.5	142	578 (198)	% Abnormal 0.0
Webb et al., 1989	Missouri residents <20[d] 20-60[d] >60[d]	 16 12 12	 562 (215)[e] 645 (225) 807 (381)	% T8 cells 26 28 35			
Jennings et al., 1988	2,4,5-T production workers exposed 17 yrs prior to the study	18	630 (280)	—	15	590 (230)	—
Ott et al., 1994	BASF accident cohort	132	—	% T8 cells 31.9 (10.4)	42[f]	—	% T8 cells 32.0 (7.1)
Zober et al., 1992	BASF personnel exposed to TBDD[g] and TBDF[g]	21	665 (282.6)	% CD8 30.8 (7.9)	42	717.2 (282.4)	% CD8 32.0 (7.1)

[a]Units: counts/mm^3.
[b]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[c]Geometric mean.
[d]Adipose 2,3,7,8-TCDD level in pg/g of lipid.
[e]$p < 0.05$.
[f]From Zober et al., 1992.
[g]TBDD = 2,3,7,8-tetrabrominated dibenzo-*p*-dioxins; TBDF = 2,3,7,8-tetrabrominated dibenzofurans.

Table 7-39. IgG levels in Missouri residents, Vietnam veterans, and BASF accident cohort

Author	Population	Exposed			Unexposed	
		N	Mean level[a] (SD)	Ratio	N	Mean level[a] (SD)
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel Unknown ≤10[b] Low 15-≤33.3[b] High >33.3[b]	 335 190 175	 1,087 — 1,122 — 1,122 —	 —	 757	 1,120 —
Grubbs et al., 1995	U.S. Air Force Ranch Hand personnel Background[h] Low[h] High[h]	 364 243 251	 1,126[i] — 1,111 — 1,115 —	 —	 1,035	 1,1139 —
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1,078[c]	OR < reference range 1.0 OR > reference range 1.0	1,972	1,077[c]
Webb et al., 1989	Missouri residents <20[d] 20-60[d] >60[d]	 16 12 12	 1,064[e] (273) 1,146 (193) 1,151 (223)	—	—	—
Ott et al., 1994	BASF accident cohort	132	1,199[f] (226)	—	194	1,182.6 (310.0)
Zober et al., 1992	BASF personnel exposed to TBDD[g] and TBDF[g]	21	1,057.7 (199.0)	—	42	1,102.9 (207.1)

[a]Units: mg/dL.
[b]Serum 2,3,7,8-TCDD in pg/g of lipid.
[c]Geometric mean.
[d]Adipose 2,3,7,8-TCDD in pg/g of lipid.
[e]$p < 0.05$ trend.
[f]Significant positive relationship between IgG and current 2,3,7,8-TCDD level and back-extrapolated 2,3,7,8-TCDD ($p < 0.01$).
[g]TBDD = 2,3,7,8-tetrabrominated dibenzo-*p*-dioxins; TBDF = 2,3,7,8-tetrabrominated dibenzofurans.
[h] Comparison: current dioxin ≤10 pg/g lipid.
Background: current dioxin > 10 pg/g.
Low: current dioxin > 10 pg/g, 10 pg/g<initial dioxin ≤143 pg/g.
High: current dioxin > 10 pg/g, 10 pg/g<initial dioxin > 143 pg/g.
[i] Adjusted mean.

Table 7-40. IgM levels in Missouri residents, Vietnam veterans, BASF accident cohort, and extruder personnel

Author	Population	Exposed			Unexposed	
		N	Mean level[a] (SD)	Ratio	N	Mean level[a] (SD)
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel Unknown ≤10[b] Low 15-≤33.3[b] High >33.3[b]	 335 190 175	 107 — 96 — 106 —	—	757	103
Grubbs et al., 1995	U.S. Air Force Ranch Hand personnel Background[g] Low[g] High[g]	 365 253 251	Data not presented		1,035	Data not presented
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	121[c] —	OR< reference range 1.0 OR> reference range 1.0	1,972	121[c]
Webb et al., 1989	Missouri residents <20[d] 20-60[d] >60[d]	 16 12 12	 128(89) 157(57) 114(44)	—	—	—
Ott et al., 1994	BASF accident cohort	132	139.6 (65.1)	—	192	134.7 (70)
Zober et al., 1992	Personnel exposed to TBDD[e] and TBDF[e]	21	142[f](52.6)	—	42	114.7[f] (46.5)

[a]Units: mg/dL.
[b]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[c]Geometric mean.
[d]Adipose 2,3,7,8-TCDD level in pg/g of lipid.
[e]TBDD = 2,3,7,8-tetrabrominated dibenzo-*p*-dioxins; TBDF = 2,3,7,8-tetrabrominated dibenzofurans.
[f]p=0.04.
[g]Comparison: current dioxin ≤10 pg/g of lipid.
Background: current dioxin > 10 pg/g.
Low: current dioxin > 10 pg/g, 10 pg/g<initial dioxin ≤143 pg/g.
High: current dioxin > 10 pg/g, 10 pg/g<initial dioxin > 143 pg/g.

Table 7-41. Levels of natural killer cells in Missouri residents, Vietnam veterans, and extruder personnel

Author	Population	Exposed		Unexposed	
		N	Mean level (SD)	N	Mean level (SD)
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel Unknown ≤10[a] Low 15-≤33.3[a] High >33.3[a]	 126 70 72	 455[b,c] 378 386	291	414[b]
Grubbs et al., 1995	U.S. Air Force Ranch Hand personnel Background[g] Low[g] High[g]	139[h] 94 106	CD16+CD56 242 — 219 — 237 —	399	248
Tonn et al., 1996	2,4,5-TCP production and maintenance workers	11	%CD56 5.4 (1.9)	10	%CD56 5.6 (1.6)
Jennings et al., 1988	2,4,5-T production workers exposed 17 yrs prior to the study	18	400[e] (210)[d]	15	590[e](230)
Zober et al., 1992	BASF personnel exposed to TBDD[f] and TBDF[f]	21	280.5 (175.2)	42	250.8(149.9)

[a]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[b]Units: cpm.
[c]Net response.
[d]$p<0.05$.
[e]Units: $10^3/mm^3$.
[f]TBDD = 2,3,7,8-tetrabrominated dibenzo-*p*-dioxins;
TBDF = 2,3,7,8-tetrabrominated dibenzofurans.
[g]Comparison: current dioxin ≤10 pg/g of lipid.
Background: current dioxin > 10 pg/g.
Low: current dioxin > 10 pg/g, 10 pg/g<initial dioxin ≤143 pg/g.
High: current dioxin > 10 pg/g, 10 pg/g<initial dioxin > 143 pg/g.
[h]Adjusted mean.

Table 7-42. Developmental immunologic outcomes in a study of Dutch Children

Outcome	Exposure(s)	Correlation coefficient	*p*-value
Weisglas-Kuperus et al., 1995			
Monocytes At 3 months:	Total TEQ Dioxin-furan TEQ Mono-ortho PCB TEQ Di-ortho PCB TEQ	-0.64 -0.55 -0.67 -0.51	≤ 0.01 ≤ 0.01 ≤ 0.01 ≤ 0.05
Granulocytes At 3 months:	Total TEQ	-0.47	≤ 0.05
TCR$\gamma\delta^+$ T-cells At birth:	Total TEQ Dioxin-furan TEQ	0.50 0.57	≤ 0.05 ≤ 0.01
TCR$\alpha\delta^+$ T-cells at 18 months:	Total TEQ Dioxin-furan TEQ Di-ortho PCB TEQ	0.57 0.71 0.61	≤ 0.05 ≤ 0.01 ≤ 0.05
$CD3^+$ $CD8^+$ cells at 18 months:	Total TEQ Dioxin-furan TEQ Planar PCB TEQ Di-ortho PCB TEQ	0.65 0.80 0.71 0.68	≤ 0.05 ≤ 0.01 ≤ 0.01 ≤ 0.05
Weisglas-Kuperus et al., 2000			
Lymphocytes at 42 months	Total Maternal PCB Total Cord PCB	0.25 0.22	0.02 0.05
CD3+ at 42 months	Total Maternal PCB Total Cord PCB	0.25 0.21	0.02 0.07
CD3+CD8+ at 42 months	Total Maternal PCB Total Cord PCB	0.27 0.24	0.01 0.04
CD4+CD45RO+ at 42 months	Total Maternal PCB Total Cord PCB	0.25 0.26	0.02 0.02
TCR$\alpha\beta^+$ T-cells at 42 months	Total Maternal PCB Total Cord PCB	0.25 0.20	0.02 0.08
CD3+HLA-DR+ at 42 months	Total Maternal PCB Total Cord PCB	0.26 0.31	0.02 0.005

Table 7-43. Case reports of psychological and neurologic effects among individuals exposed to 2,3,7,8-TCDD-contaminated materials

Author	Exposure	Population	Evaluation	Findings
Ashe and Suskind, 1950 West Virginia	Precursor and reaction products of TCP process TCP,[a] NaOH,[b] 2,4,5-T[c]	4 employees involved in cleanup after 1949 explosion or worked with equipment used prior to explosion; studied 6 mos and 1 yr after explosion	Clinical history and examination	Headaches Insomnia Social withdrawal Nervousness Fatigue Crying spells Numbness in feet Decreased libido, muscle strength, sensory ability, myelin sheath and fiber destruction of sural nerve
Suskind et al., 1953 West Virginia	TCP 2,4,5-T	36 workers with chloracne after TCP reactor explosion 25 persons exposed during production of TCP and 2,4,5-T 1948-1953	Clinical history and examination	Fatigue Nervousness Irritability Reduced libido Back and limb pain Vertigo Paresthesia
Baader and Bauer, 1951 Nordheim-Westfalen, West Germany	PCP[d] TCP	10 men in PCP production experimental TCP production	Record review case history	Self-reported: pain and weakness Paresthesia and pain in gluteal and femoral region

Table 7-43. Case reports of psychological and neurologic effects among individuals exposed to 2,3,7,8-TCDD-contaminated materials (continued)

Author	Exposure	Population	Evaluation	Findings
Bauer et al., 1961 Hamburg, West Germany	TCP 2,4,5-T	31 men in TCP and 2,4,5-T production with residual chloracne, continual "neuromuscular weakness," vasovagal disturbances, psychopathological disturbances" 5 yrs after first exam	Clinical history and examination Hamburg-Wechsler Test Rorschach Psychogram	Reduced libido Headaches, dizziness Decreased libido Irritability Depression Sleep disturbances Anorexia Paresthesia Tremor Muscle weakness Hamburg-Wechsler indicative of acquired decrease in mental efficiency Rorschach Psychogram indicative of decreased emotional reactivity, slowed thinking process for concentration
Goldman, 1972 Ludwigshafen, West Germany	TCP 2,4,5-T	42 BASF workers with chloracne after TCP reactor explosion in 1953	Clinical history and examination	Neurasthenia

Table 7-43. Case reports of psychological and neurologic effects among individuals exposed to 2,3,7,8-TCDD-contaminated materials (continued)

Author	Exposure	Population	Evaluation	Findings
Jirasek et al., 1974 Spolano, Czechoslovakia	TCP 2,4,5-T PCP, HCB[e]	55 workers in TCP and 2,4,5-T production Followed: 1969-1973	Clinical examination history and examination	35 patients with "neurasthenic or depressive syndrome"
Oliver, 1975 England	Pure 2,3,7,8-TCDD	3 scientists synthesis of 2,3,7,8-TCDD	Not described	Subject A: Fatigue and headache Subject B: Headaches Loss of vigor and fatigue Irritability and anger Poor concentration Subject C: Loss of concentration (apathy and fatigue) "Loss of energy and drive" Some difficulty sleeping

Table 7-43. Case reports of psychological and neurologic effects among individuals exposed to 2,3,7,8-TCDD-contaminated materials (continued)

Author	Exposure	Population	Evaluation	Findings
Pazderova-Vejlupkova et al., 1981 Spolano, Czechoslovakia	TCP 2,4,5-T PCP, HCB	4-year followup of 44 production workers	Clinical psychiatric examination	Psychiatric changes at start of "intoxication" 83% with neurotic symptoms; neurasthenia syndromes with depressive component; depressive syndrome with endogenous component (N=55) 16% with pseudoneurasthenia syndrome and CNS arteriosclerosis 3% with no psychiatric signs or symptoms Psychiatric changes in exposed individuals at followup (1973-1979) 58% with neurotic symptoms and no depressive or anxiety symptoms 18% with severe pseudoneurasthenia and signs of dementia (usually in patients over 50 years old but dementia occurred in 30-year-old patient)

Table 7-43. Case reports of psychological and neurologic effects among individuals exposed to 2,3,7,8-TCDD-contaminated materials (continued)

Author	Exposure	Population	Evaluation	Findings
Pazderova-Vejlupkova et al., 1981 (cont.)				24% with no psychiatric signs or symptoms.
Kimmig and Schultz, 1957b Hamburg, Germany	TCP 2,4,5-T	31 production workers with chloracne (1953-1954)	Clinical history and examination	Tiredness (N=3) Headaches (N=5)
Poland et al., 1971 New Jersey	TCP 2,4,5-T 2,4-D[f] HCB TCP reactor explosion in 1960 Daily exposure 1951-1969	73 male workers in production, maintenance, office areas	Noted histories of smoking, alcohol, medications MMPI[g] given to 52 production and 17 unexposed administrative staff	Headaches (N=8) Severity of acne significantly correlated with high score on the mania scale of MMPI.

[a]TCP = 2,4,5-trichlorophenol.
[b]NaOH = sodium hydroxide.
[c]2,4,5-T = 2,4,5-trichlorophenoxyacetic acid.
[d]PCP = pentachlorophenol.
[e]HCB = hexachlorobenzene.
[f]2,4-D = 2,4-dichlorophenoxyacetic acid.
[g]Minnesota Multiphasic Personality Inventory.

Table 7-44. Cross-sectional studies of psychological and neurologic effects among residents of Missouri and Seveso exposed to 2,3,7,8-TCDD-contaminated materials

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Webb et al., 1989 Missouri	2,3,7,8-TCDD-contaminated waste oil 1971	68 volunteers in State Dioxin Registry; estimated exposure to 20-100 ppb TCDD for 2 yrs or 100 ppb for 6 months	36 volunteers in State Dioxin Registry with no history of exposure to 2,3,7,8-TCDD	Missouri Dioxin Health Studies. Progress Report (1983)	No differences in neurologic exam or in feeling of pins-needles; loss of sensation in extremities, tingling in fingers and toes, diminished VIB 256, diminished VIB 64, diminished pin sensation, diminished thermal sensation (PRR=2%)[a]
Pocchiari et al., 1979 Seveso, Italy	TCP TCP reactor accident	446 residents of Seveso and Meda, Italy	255 residents of nearby towns without contamination	Not detailed but included clinical exam and NCV[b]	1977: neurologic damage in 6.7% of Seveso residents and in 1.2% unexposed residents 1978: neurologic damage in 11.7% of Seveso residents
	July 1976	200 workers from Icmesa plant	None	Exam, EMG[c], NCV	Workers: 4% neuropathic clinical signs exam, EMG, NCV

Table 7-44. Cross-sectional studies of psychological and neurologic effects among residents of Missouri and Seveso exposed to 2,3,7,8-TCDD-contaminated materials (continued)

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Filippini et al., 1981 Seveso, Italy	See description above	308 residents of Seveso, Italy	305 residents of nearby towns without contamination	Symptoms: pain, tingling, numbness Sensory review Muscle tone and strength NCV of ulnar and peroneal nerves	Elevated PRR for peripheral neuropathy in Seveso residents with indicators of exposure (high GGT, ALT, AST, or chloracne) (PRR=2.8, 95% CI=1.2-6.5) Seveso residents with predisposing factors (alcohol or inflammatory disease) (PRR=2.6, 95% CI=1.2-5.6)

[a]Prevalence risk ratio.
[b]Nerve conduction velocity.
[c]Electromyography.

Table 7-45. Cross-sectional studies of psychological and neurologic effects among TCP production workers and Vietnam veterans exposed to 2,3,7,8-TCDD-contaminated materials

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Moses et al., 1984 West Virginia	TCP[a] 2,4,5-T[b] 1949 TCP reactor explosion	226 workers invited based on union record of employment in production of TCP or 2,4,5-T Definition of exposure = "current or history of chloracne"	Workers without chloracne (N=109) Conduction velocities	Review of symptoms (ROS) obtained by examining physician	Significant diff. with and without chloracne for: insomnia; decreased libido, erection, or ejaculation; no change for fatigue, irritability, nervousness, depression, or personality changes. Decreased pinprick sensation in 18.3% with chloracne; no decreased pinprick sensation without chloracne
Suskind and Hertzberg, 1984 West Virginia	TCP 2,4,5-T	204 workers or maintenance workers in TCP or 2,4,5-T dept.	163 workers never in TCP or 2,4,5-T Mean age of exposed population was younger (56.7 vs. 46.2) ($p<0.0001$).	Interviews and clinical examination	Decreased libido was more frequent among exposed by Mantel-Haenszel Chi by age. Nervousness/depression/ anxiety not increased in exposed (sample size sufficient to detect a twofold increase)

Table 7-45. Cross-sectional studies of psychological and neurologic effects among TCP production workers and Vietnam veterans exposed to 2,3,7,8-TCDD-contaminated materials (continued)

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Sweeney et al., 1993 Alderfer et al., 1992 Missouri and New Jersey	TCP 2,4,5-T	280 production workers with daily exposure New Jersey 1951-1969 Missouri 1968-1972	261 unexposed community residents matched for age, race, gender	Symptom and medical history; neurologic examination; neurophysiologic tests of vibration and thermal sensitivity; Beck Depression Scale; SCL-90-R	No significant difference for symptoms or any examination variable No significant difference in mood disorders
Lathrop et al., 1984	Vietnam service 1962-1971	1,208 Ranch Hands assigned to aerial spraying herbicides and insecticides Republic of Vietnam	1,238 men who flew cargo missions in Southeast Asia Matched by month of birth, race, and occupational code	Self-report of psychological or emotional illness Diagnostic Interview Schedule (modified) Self-reported depression	No difference Significantly more fatigue, anger, erosion, anxiety, closest for high school-educated Ranch Hands; no difference for college-educated Greater for Ranch Hands

Table 7-45. Cross-sectional studies of psychological and neurologic effects among TCP production workers and Vietnam veterans exposed to 2,3,7,8-TCDD-contaminated materials (continued)

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Lathrop et al., 1984 (cont.)				Cornell Index (self-administered inventory of neuropsychiatric symptoms) (psychophysiologic)	After adjustment for education 4/10 parameters abnormal (nervousness, anxiety, startle, psychosomatic, gastrointestinal system); abnormal parameters inversely related to education level.
				MMPI	High school-educated Ranch Hands showed significant deficits on scales for hypochondria, masculinity/femininity; mania/hypomania but comparisons show more denial; MMPI scores influenced by education ($p<0.01$)
				Halstead-Reitan Battery	No impairment in Ranch Hands
				Wechsler Adult Intelligence Scale (WAIS)	Scores related to educational level

Table 7-45. Cross-sectional studies of psychological and neurologic effects among TCP production workers and Vietnam veterans exposed to 2,3,7,8-TCDD-contaminated materials (continued)

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Roegner et al., 1991	Vietnam service	720 USAF Ranch Hands; serum 2,3,7,8-TCDD levels were measured (lipid adjusted)	779 who flew cargo missions in Southeast Asia; serum 2,3,7,8-TCDD levels were measured	Self-report	No significant mental, emotional, or sleep disorders
				SCL-90-R[c]	No significant differences with increasing serum levels
				Cornell Medical Index (CMI)	Significantly higher mean schizoid and schizotypal scores in Ranch Hands over 33.3 pg/g 2,3,7,8-TCDD, but they did not relate to similar scales in the SCL-90-R
				Neurologic exam	Overall, no consistent relationship between neurologic abnormalities and TCDD level.

[a]TCP = 2,4,5-trichlorophenol.
[b]2,4,5-T = 2,4,5-trichlorophenoxyacetic acid.
[c]SCL-90-R: Symptom Checklist Revised.

Table 7-46. Studies of neurologic and behavioral effects among Dutch infants

Author	Exposure	Study groups	Evaluation	Findings N	Mean(SD)	Formula-fed N	Mean (SD)
Koopman-Essebaum et al., 1996	Total PCB-dioxin-furan TEQ from breast milk	Exposed: 105 breast-fed infants and their mothers	From Bayley Scales of Infant Development: Psychomotor Dev. Index (PDI) of infant at				
		Not exposed: 102 bottle-fed infants and their mothers	3 months,	99	118 (12)	100	117 (12)
			7 months, and	105	115 (15)[a]	102	111 (13)
			18 months of age	105	110 (17)	101	108 (14)
		All in the Rotterdam area	Mental Development Index (MDI) of infant at				
			3 months,	101	128 (31)	100	126 (13)
			7 months, and	105	115 (11)[a]	102	112 (9)
			18 months of age	105	113 (18)[d]	102	107 (17)
	CATEGORIES for total TEQ[b]: "Low" 3 mos.= 168-617 7+18 mos. = 168-769 "Med." 3 mos. = 618-810 7+18 mos. = 770-1,289 "High" 3 mos = 811-1,860 7+18 mos = 1,290-4,340	N=182		Regression analysis Coefficient	Std. error		
			PDI at 7 months[c]				
			Medium exposure	-9.5[d]	3.9		
			High exposure	-7.7	4.9		
			Breast feeding -duration	6.9[d]	2.3		
	$\sum PCB_{maternal\ blood}$ -- ng/g[e]	N=198	PDI at 3 months				
			Ln($\sum PCB_{plasma}$)	-4.8[a]	2.0		
			Breast feeding -duration	0.91	0.91		
		N=206	MDI at 7 months				
			Ln($\sum PCB_{plasma}$)	2.0	1.7		
			Breast feeding -duration	2.0[a]	0.9		

Table 7-46. Studies of neurologic and behavioral effects among Dutch infants (continued)

Author	Exposure	Study groups	Evaluation	Findings			
				N	Mean(SD)	Formula-fed N	Mean (SD)
Koopman-Essebaum et al., 1995b	Total PCB-dioxin-furan TEQ from breast milk	Exposed: 105 breast-fed infants and their mothers Not exposed: 102 bottle-fed infants and their mothers All in Rotterdam	From Fagan Test of Infant Intelligence: Visual Recognition Memory Test 3 months of age 7 months of age	105 105	61.5 (9.0) 59.9 (5.9)[c]	101 102	62.2 (10.7) 57.3 (5.9)
	CATEGORIES for total TEQ[b]: Low: 3 mos.= 168-617 7 mos. = 168-769 Med.: 3 mos. = 618-810 7 mos. = 770-1,289 High: 3 mos. = 811-1860 7 mos. = 1,290-4,340 $\sum PCB_{plasma}$ -- ng/g[e]	N=182 N = 181	Visual Recognition Memory Test at 7 months[c] Medium exposure High exposure Breast feeding -duration or Ln($\sum PCB_{plasma}$) Breast feeding -duration	Regression analysis Coefficient		Std. error	
					4.4[d] 4.6[a] -0.46 -0.50 1.63[d]		1.7 2.2 1.0 1.07 0.56
Koopman-Essebaum et al., 1995a submitted for publication	PCB-dioxin-furan TEQ from breast milk and thyroid status	Exposed: Breast-fed infants 104 in Groningen 105 in Rotterdam Comparison: Bottle-fed infants 107 in Groningen 102 in Rotterdam	Prechtl neurologic exam (2 wk):23 neuro. abnorm. 394 neurologically normal $\sum PCB_{cord\ blood}$[e] $\sum PCB_{maternal\ blood}$ Dioxin-furan TEQ Planar PCB TEQ Mono-ortho PCB TEQ Di-ortho PCB TEQ Total PCB-dioxin-furan TEQ	Neuro. Normal N	Mean (SD)	Neuro. Abnormal N	Mean (SD)
				352	0.5 (0.3)	20	0.4 (0.1)
				393	2.2 (0.9)	21	2.0 (0.6)
				189	30.0 (10.2)	12	25.4 (7.9)
				194	16.3 (7.4)[a]	12	11.7 (3.8)
				189	14.8 (5.3)	12	12.6 (4.6)
				189	4.4 (2.2)	12	3.7 (1.5)
				182	65.3 (21.6)[a]	12	52.5 (15.6)

Table 7-46. Studies of neurologic and behavioral effects among Dutch infants (continued)

Author	Exposure	Study groups	Evaluation	Findings	
				Odds ratio	95% confidence interval
Huisman et al., 1995a	Dioxins, dibenzo-furans, and PCBs in breast milk (No effects observed in maternal or cord blood)	Exposed: Breast-fed infants 104 in Groningen 105 in Rotterdam Comparison: Bottle-fed infants 107 in Groningen 102 in Rotterdam	Prechtl's exam at 10-21 days: Neurologic Optimality Score (NOS) ORs from logistic regression Total PCB-dioxin-furan TEQ[a] Dioxin-furan TEQ D54 (TEF=0.5) D66 (TEF=0.1) D67 (TEF=0.1) D70 (TEF=0.1) D73 (TEF=0.01) F83 (TEF=0.1) F114 (TEF=0.5) Planar PCB TEQ PCB169 (TEF=0.01) Non-planar PCB PCB70 PCB 99 PCB118 (TEF=0.0001) PCB138 PCB153 PCB156 (TEF=0.0005) PCB170 (TEF=0.0001) PCB177 PCB183 PCB187 $\sum PCB_{milk}$ Mono-ortho PCB TEQ Di-ortho PCB TEQ	 3.21 3.12 2.23 1.64 3.03 1.23 1.61 1.31 2.48 1.67 2.33 1.88 2.01 2.21 2.73 2.76 2.55 2.33 1.96 2.32 1.87 2.93 2.78 2.38	 1.37-7.48 1.36-7.18 1.10-4.52 1.08-2.50 1.34-6.81 1.03-1.48 1.01-2.56 1.04-1.66 1.16-5.32 0.97-2.87 1.13-4.80 1.29-2.72 1.19-3.40 1.24-3.95 1.40-5.35 1.39-5.48 1.31-4.95 1.19-4.56 1.14-3.39 1.21-4.46 1.07-3.28 1.45-5.90 1.40-5.59 1.21-4.71

Table 7-46. Studies of neurologic and behavioral effects among Dutch infants (continued)

Author	Exposure	Study groups	Evaluation	Findings: Coefficient (std error)	Findings: *p*-value
Huisman et al., 1995b	$\sum PCB_{cord\ blood}$ No effects observed with breast milk	Exposed: Breast-fed infants 104 in Groningen 105 in Rotterdam Comparison: Bottle-fed infants 107 in Groningen 102 in Rotterdam	Neurologic development in 18-month-old children: Neurologic optimality score Log($\sum PCB_{cord}$/0.8) Paternal smoking Log($\sum PCB_{cord}$/0.8) × paternal smoking Above analysis presented by paternal smoking status: Children w/nonsmoking fathers Children w/smoking fathers Fluency cluster score Log($\sum PCB_{cord}$) Breast (0) vs bottle (1) fed	 -0.149 (0.049) -0.402 (0.130) 0.200 (0.078) -0.149 -0.051 -0.295 (0.175) -0.450 (0.177)	 0.003 0.002 0.011 0.003 0.402 0.093 0.012

[a] $p<0.05$.
[b] pg PCB-dioxin-furan TEQ/g fat times weeks breast feeding.
[c] overall p for high + medium = 0.05.
[d] $p<0.01$.
[e] $\sum PCB_{maternal\ blood}$ (ng/g) -- maternal blood level sometime during 36-40 weeks of gestation. This and cord blood are measured in ng/g plasma.
[f] All summary logistic results presented; for individual chemicals, only those with ORs significantly greater than 1 are presented.

Table 7-47. Mortality from disease of the circulatory systems in populations exposed to 2,3,7,8-TCDD

Author	Population	Outcomes	No. of deaths	SMR	95% CI	Cohort size	Years of followup
Steenland et al., 1999	2,4,5-TCP and 2,4,5-T production workers, USA	Ischemic heart disease (ICD 410-414) Cerebrovascular disease (ICD 430-438)	456 92[2] 69	109 RR=1.75[a] 117[b] 96	100-120 1.07-2.87[a] 94-144[b] 74-121	5,132 608[b] 5,132	1942-1993
Hooiveld et al. 1998	2,4,5-TCP, 2,4,5-T, MCPA production workers, The Netherlands	Circulatory system (ICD390-459) Ischemic heart disease (ICD 410-414) Cerebrovascular disease (ICD 430-438)	45 16/(45) 33 10/(33) 9 3/9	100 RR=1.4[c] RR=1.5[d Medium] RR=1.5[d High] 120 RR=1.8[c] RR=1.5[d Medium] RR=2.3[d High] 140 RR=0.6[c] RR=2.0[d Medium] RR=0.8[d High]	80-140 0.8-2.5[c] 0.8-2.8[d] 0.8-2.9[d] 80-160 0.9-3.6[c] 0.7-3.6[d] 0.5-8.2[d] 60-260 0.4-5.1[c] 0.5-8.2[d] 0.2-4.1[d]	562 549 (males) 562 549 (males) 562 549 (males)	1955-1991
Vena et al., 1998	IARC expanded international cohort; 36 cohorts from 12 countries; workers employed in the production of phenoxyacid herbicide and chlorophenol & sprayers	Circulatory system (ICD 390-459) Ischemic heart disease (ICD 410-414) Cerebrovascular disease (ICD 430-438)	1,738 1,170[e] 1,151[f] 1,179 789[e] 1,151[f] 254 162[e] 1,151[f]	91 94[e] RR=1.51[f] 92 97[e] RR=1.67[f] 254 84[e] RR=1.54[f]	87-95 88-99[e] 1.17-1.96[f] 87-98 90-104[e] 1.23-2.26[f] 76-97 71-98[e] 0.83-2.88[f]	21,863 13,831	Varies by cohort 1939-1992

Table 7-47. Mortality from disease of the circulatory systems in populations exposed to 2,3,7,8-TCDD (continued)

Author	Population	Outcomes	No. of deaths	SMR	95% CI	Cohort size	Years of followup
Ott and Zober, 1996a	2,4,5,-T production workers or involved in cleanup after a TCP reactor release (Germany)	Diseases of the circulatory system (ICD not reported) TCDD[h] <0.1 µg/kg bw TCDD 0.1-0.99 µg/kg bw TCDD >1 µg/kg bw Ischemic heart disease TCDD <0.1 µg/kg bw TCDD 0.1-0.99 µg/kg bw TCDD >1 µg/kg bw Noncancer deaths: all causes Diseases of the circulatory system	37[g] 13 11 12 16 7 4 5 92 37	80 80 100 80 70 90 70 60 RR=1.03[i] RR=0.93[i]	60-120 40-140 50-170 40-120 40-110 30-180 20-170 20-130 0.88-1.22 0.70-1.24	243 males 4 females	1953-1992
Michalek et al., 1998	US Air Force Ranch Hands	Circulatory diseases (ICD 380-459) Pilots & navigators Enlisted flight engineers Administrative officers Enlisted ground personnel	39 12 8 0 24	100 90 30 — 150	70-130 50-160 — — 100-220	1,261 veterans 19,080 comparisons	1962-1993

Table 7-47. Mortality from disease of the circulatory systems in populations exposed to 2,3,7,8-TCDD (continued)

Author	Population	Outcomes	No. of deaths	SMR	95% CI	Cohort size	Years of followup
Pesatori et al., 1998	Residents of Seveso, Italy	Circulatory sys (ICD 390-459)		Rel. risk		PYAR[j]	1976-1991
		Zone A: males	5	1.6	1.1-2.5	5,541	
		Zone B	18	0.9	0.7-1.1	42,219	
		Zone R	126	1.1	1.0-1.2	271,483	
		Zone A: females	0	1.0	0.6-1.7	5,975	
		Zone B	15	1.0	0.8-1.2	41,391	
		Zone R	133	1.1	1.0-1.2	271,483	
		Ischemic heart disease (ICD 410-414)					
		Zone A: males	9	1.5	0.8-2.9		
		Zone B	36	0.8	0.6-1.2		
		Zone R	316	1.1	0.9-1.2		
		Zone A: females	1	0.3	0.0-2.0		
		Zone B	24	1.1	0.7-1.6		
		Zone R	210	1.0	0.9-1.2		
		Chronic ischemic heart disease (ICD 412, 414)					
		Zone A: males	5	3.0	1.2-7.3		
		Zone B	18	1.3	0.8-2.1		
		Zone R	126	1.4	1.1-1.7		
		Zone A: females	0	—	—		
		Zone B	15	1.3	0.8-2.2		
		Zone R	133	1.3	1.0-1.5		
		Chronic rheumatic heart disease (ICD 390-398)					
		Zone A: females	3	15.8	4.9-50.4		
		Zone B	0	—	—		
		Zone R	11	1.2	0.6-2.3		
		Cerebrovascular disease (ICD 430-438)					
		Zone A: males	5	1.5	0.6-3.7		
		Zone B	30	1.2	0.8-1.7		
		Zone R	190	1.1	0.9-1.3		
		Zone A: females	2	0.5	0.1-2.0		
		Zone B	26	1.0	0.7-1.5		
		Zone R	258	1.2	1.0-1.3		

Table 7-47. Mortality from disease of the circulatory systems in populations exposed to 2,3,7,8-TCDD (continued)

[a]Rate ratio calculated using Cox regression for cumulative exposure categories compared to an internal control group. Rate ratio for 7th septile exposure category.
[b]Chloracne cohort.
[c]Relative risks for 549 males, calculated using Poisson regression and using an internal control group.
[d]Relative risks calculated using Poisson regression adjusted for age, calendar period at end of followup, and time since first exposure/employment, comparing workers with estimated low $TCDD_{max}$=7.1 ppt (lipid adjusted) to workers with estimated medium $TCDD_{max}$=7.7 ppt to124.1 ppt (lipid adjusted) and estimated high $TCDD_{max}$=124.2 to 7,307.5 ppt (lipid adjusted).
[e]Workers exposed to TCDD or higher chlorinated dioxins.
[f]RR=relative risk calculated using Poisson regression, adjusted for age, gender, calendar period, employment status and years since first exposure and duration of exposure to phenoxy herbicides or chlorophenols. Comparison of workers exposed to TCDD or higher chlorinated dioxins to nonexposed workers.
[g]Expected deaths based on age, sex, calendar-period-specific death rates of the former West Germany, 1952-1992.
[h]Estimated 2,3,7,8-TCDD concentration based on 138 employees.
[i]Rate ratio calculated using Cox regression; TCDD μg/kg bw forced into model.
[j]PYAR: Person years at risk.

Table 7-48. Results of studies examining the effect of dioxin on reproductive and developmental outcomes in humans, 1984-1992

Author	Exposed group	Control group	Type of exposure	Data source: exposure/ outcome	Outcome	Outcome in exp. (N)	Outcome in unexp. (N)	OR	95% CI
Smith et al., 1982	548 male pesticide applicators who sprayed 2,4,5-T and other pesticides	441 agricultural contractors	Spraying of 2,4,5-T	Mailed survey/mailed survey	Total births	1,172	1,122		
					Congenital defect	13	9	1.19[a]	0.58-2.45
					Miscarriage[b]	43	40	0.89	0.61-1.30
					Stillbirth	3	0	—	—
Townsend et al., 1982	370 male chemical workers exposed to 2,4,5-T only and their spouses	345 employees not exposed to 2,4,5-T and their spouses	Working with chlorophenol processes	Interviewer-administered questionnaire/ interviewer-administered questionnaire	# Conceptions	418	2,031	—[c]	
					All fetal deaths	50	246	1.02	0.71-1.47
					Stillbirth	7	33	0.97	0.38-2.36
					Spontaneous abortions	43	213	0.96	0.65-1.42
					Infant deaths	6	39	0.82	0.30-2.09
					Health defects	32	155	0.93	0.60-1.43
					Congenital malformation	21	87	1.08	0.63-1.8
	Male chemical workers exposed to any dioxins				Total conceptuses	737	2,031		
					All fetal deaths	100	246	1.03[a]	0.78-1.37
					Stillbirth	15	33	1.06	0.54-2.09
					Spontaneous abortions	85	213	1.03	0.77-1.39
					Infant deaths	9	39	0.63	0.27-1.39
					Health defects	52	155	0.85	0.60-1.21
					Congenital malformation	30	87	0.85	0.53-1.35

Table 7-48. Results of studies examining the effect of dioxin on reproductive and developmental outcomes in humans, 1984-1992 (continued)

Author	Exposed group	Control group	Type of exposure	Data source: exposure/ outcome	Outcome	Outcome in exp. (N)	Outcome in unexp. (N)	OR	95% CI
Mastroia-covo et al., 1988	2,900 births in zones A, B, and R, Seveso, Italy, 1977-1981	12,391 births in study area outside zones A, B, and R	TCDD cloud released from chemical plant accident	TCDD soil analysis/ Seveso Congenital Malformations Registry	Total birth def. Multiple birth defects Syndromes Major birth defects Minor birth defects	137 10 5 67 70	605 38 29 343 262	0.97 1.12 0.74 0.83 1.14	0.83-1.13 0.63-2.02 0.33-1.63 0.67-1.04 0.92-1.42
Stehr et al., 1986	68 persons residing in areas of high TCDD conc.	36 persons with no known contact with contam. soil	Contact with TCDD-contam. soil	EPA soil analyses for TCDD/ interview	Infert. (males) Impotence Infert.(females)	— — —	— — —	— — —	NS NS NS
Hoffman and Stehr-Green, 1989	154 residents of Quail Run Mobile Home Park	155 residents nonexposed mobile home park	Contact with soil sprayed with TCDD for dust control	EPA soil analyses for TCDD/ interview	Fetal deaths Spontaneous abortions Congenital malformations	— — —	— — —	— — —	NS NS NS
Stockbauer et al., 1988	402 pregnancies to exposed mothers	804 pregnancies to unexposed mothers (matched on maternal age and race, hospital and year of birth, plurality)	Contact with soil sprayed with TCDD for dust control	EPA soil analyses for TCDD/vital statistics and hospital records	Birth defects-all Major birth defects Multiple birth defects Fetal deaths Infant deaths Perinatal deaths Low birth wt. Very low birth weight IUGR	17 15 2 4 5 6 27 1 14	42 35 11 5 5 9 36 4 26	0.78 0.84 0.34 1.60 2.00 1.33 1.59 0.50 1.09	0.40-1.47 0.40-1.66 0.03-1.65 0.32-7.43 0.46-8.69 0.39-4.20 0.89-2.81 0.01-5.05 0.50-2.28
Erickson et al., 1984	4,929 infants from the Met. Atlanta Congenital Defects Program	3,029 infants from Georgia vital statistics records	Vietnam military service	Self-reported, and Exposure Opportunity Index/birth defects registry and vital statistics	Total birth defects (96 subcategories also examined)	428	4,387	0.97	0.83-1.14

Table 7-48. Results of studies examining the effect of dioxin on reproductive and developmental outcomes in humans, 1984-1992 (continued)

Author	Exposed group	Control group	Type of exposure	Data source: exposure/ outcome	Outcome	Outcome in exp. (N)	Outcome in unexp. (N)	OR	95% CI
CDC, 1988d, 1989	7,924 Vietnam veterans	7,364 non-Vietnam veterans	Vietnam military service	Military records/self-reports	Total birth def. Spontaneous abortion Stillbirth Low birth wt. Childhood ca Sperm abnormalities: concentration motility morphology	826 1,566 126 100 25 51 91 51	590 1,190 131 87 17 20 58 29	1.3 1.3 0.9 1.1 1.5 2.3 1.2 1.6	1.2-1.4 1.2-1.4 0.7-1.1 0.8-1.4 0.8-2.8 1.2-4.3 0.8-1.8 0.9-2.8
Substudy #1, CDC 1988d, 1989	1,791 offspring of Vietnam veterans	1,575 offspring of non-Vietnam veterans	Vietnam military service	Military records/self-report and hospital records verification	Total birth def Low birth wt Perinatal mort. Suspected birth defects	130 51 58 21	112 37 54 21	1.0 1.1 1.0 0.9	0.8-1.4 0.7-1.8 0.7-1.5 0.5-1.7
Substudy #2, CDC 1988d, 1989	127 offspring of Vietnam veterans	94 offspring of non-Vietnam veterans	Vietnam military service	Military records/self-report and hospital record verification	Cerebrospinal malformations	26	12	1.8	0.8-4.0
Stellman et al., 1988	2,858 Vietnam veterans	3,933 non-Vietnam veterans	Vietnam military service	Survey/ survey	Difficulty conceiving 349 Time to conception Birth weight — Spontaneous abortion 231	363 4.4 yrs — 195	4.4 yrs	1.2 — — 1.3	NS NS NS 1.4-2.0
Aschengrau and Monson, 1989	201 spontaneous abortion cases at Boston Hospital for Women	1,119 full-term births at Boston Hospital for Women	Vietnam military service	Military records/ hospital records	Spontaneous abortion	8	44	0.9	0.42-1.9
Aschengrau and Monson, 1990	966 infants with late adv. preg. outcomes at Boston Hospital for Women	998 normal term infants at Boston Hospital for Women	Vietnam military service	Military records Hosp. records	Total birth def. ≥1 Major malf. Minor malf Stillbirths Neonatal deaths	55 18 11 5 3	656 151 189 51 36	1.3 1.8 0.9 1.5 1.2	0.9-1.9 1.0-3.1 0.5-1.7 0.4-3.9 0.2-4.2

Table 7-48. Results of studies examining the effect of dioxin on reproductive and developmental outcomes in humans, 1984-1992 (continued)

Author	Exposed group	Control group	Type of exposure	Data source: exposure/ outcome	Outcome	Outcome in exp. (N)	Outcome in unexp. (N)	OR	95% CI
Wolfe et al., 1992b	2,533 conceptions among 791 Ranch Hand personnel	2,074 conceptions among 768 non-Ranch Hand personnel	Spraying/ handling of Agent Orange	Serum TCDD levels/ hospital and medical records	Total birth def. ≤10[k] 15-≤33.3 >33.3	 202.1[l] 293.1 193.8	208.0	 0.96[m,n] 1.58 0.92	 0.69-1.34 1.10-2.27 0.64-1.32
					Nervous system anomalies ≤10[k] 15-≤33.3 >33.3	 0.0[l] 5.7 13.2	3.1	 — 1.88[m,o] 4.37[m,o]	 0.20-18.3 0.87, 21.8
					Respiratory sys. anomalies ≤10[k] 15-≤33.3 >33.3	 7.1[l] 5.7 4.4	2.0	 3.5[m,o] 2.83 2.17	 0.49-25.0 0.26-31.4 0.20-24.0
					Digestive system anomalies ≤10[k] 15-≤33.3 >33.3	 21.3[l] 34.5 17.6	24.5[l]	 0.83 1.30 0.64	 0.31-2.23 0.48-3.51 0.21-1.91
					Genital anomalies ≤10[k] 15-≤33.3 >33.3	 3.5[l] 51.7 13.2	18.3[l]	 0.19 2.92 0.72	 0.03-1.43 1.29-6.61 0.21-2.46
					Urinary system anomalies ≤10[k] 15-≤33.3 >33.3	 14.2[l] 34.5 22.0	12.2[l]	 1.16 2.88 1.82	 0.37-3.63 1.07-7.79 0.63-5.22
					Musculoskeletal deformities ≤10[k] 15-≤33.3 >33.3	 120.6[l] 143.7 105.7	134.6[l]	 0.88[m,n] 1.08 0.76	 0.59, 132 0.68-1.71 0.48-1.21

Table 7-48. Results of studies examining the effect of dioxin on reproductive and developmental outcomes in humans, 1984-1992 (continued)

Author	Exposed group	Control group	Type of exposure	Data source: exposure/ outcome	Outcome	Outcome in exp. (N)	Outcome in unexp. (N)	OR	95% CI
					Anomalies of the skin				
					≤10[k]	17.7[l]	21.4[l]	0.76	0.25-2.26
					15-≤33.3	34.5		1.83	0.72-4.70
					>33.3	8.8		0.46	0.11-1.95
					Circulatory system and heart anomalies				
					≤10[k]				
					15-≤33.3	14.2[l]	16.3	0.85	0.27-2.69
					>33.3	46.0		2.16	0.81-5.74
						8.8		0.32	0.04-2.52
Wolfe et al., 1995	1,006 conceptions among 454 Ranch Hand personnel	1,235 conceptions among 570 non-Ranch Hand personnel	Spraying/ handling of Agent Orange	Serum TCDD levels/hospital and medical records	Spontaneous abortion:				
					Comparisons		172	1	
					Background	57		1.1	0.8-1.5
					RH Low	56		1.3	1.0-1.7
					RH High	44		1.0	0.7-1.3
					Stillbirth:				
					Comparisons		13	1	
					Background	7		1.8	0.7-4.5
					RH Low	6		1.8	0.7-4.7
					RH High	1		0.3	0.0-2.3
					Developm'tal delays:				
					Comparisons			1	
					Background	24	71	1.2	0.8-1.8
					RH Low	26		1.5	1.0-2.3
					RH High	21		1.1	0.7-1.7
					Major birth defects:				
					Comparisons			1	
					Background	17	56	1.1	0.6-1.8
					RH Low	23		1.7	1.1-2.7
					RH High	19		1.2	0.8-2.1

[a]Relative risk.
[b]Rate: 86/1,000 births in applicators; 93/1,000 births in agricultural contractors (controls).
[c]Adjusted for mother's age at time of birth, birth control methods, labor and delivery complications, medical conditions and medications during pregnancy, smoking and alcohol use during pregnancy, high job risk, and gravidity.
[d]Rate: zone A: 0; zone B: 57.5/1,000 births; zone R: 45.1/1,000 births; zone non-ABR: 48.8/1,000 births.
[e]90% CI.
[f]Zones A and B.
[g]Zones A and B vs. non-ABR.
[h]Controls matched on maternal age, race, hospital of birth, plurality, and year of birth.
[i]Odds ratio adjusted for veteran's age at birth, year of entry in army, enlistment status, general technical test score, military occupational specialty, years between entry and birth, maternal age, and gravidity.[j]Children born after the father was stationed in Southeast Asia (SEA).
[k]Logit (p) = $\beta_0 + \beta_1 d_1 + \beta_2 d_2 + \beta_3 d_3$, where p = probability of an adverse reproductive outcome; d_1, d_2, d_3 are indicators for the dioxin categories: Unknown (Ranch Hands with up to 10 pg/g current dioxin), Low (Ranch

Hands with more than 15 pg/g of lipid and up to 33.3 pg/g of lipid current dioxin), and High (Ranch Hands with more than 33.3 pg/g of lipid current dioxin).

[l]Rate/1000 of abnormals.

[m]Unadjusted.

[n]Adjusted analysis not statistically significant.

[o]No adjusted analysis: total defects <10.

[p]Comparions: ≤10 ppt; Ranch Hand (RH) Background ≤ 10; RH LOW with current level> 10 ppt and estimated initial level≤≤110ppt; and RH HIGH with current level> 10 ppt and estimated initial level > 110 ppt.

Table 7-49. 2,3,7,8-TCDD levels (pg/g of lipid) for selected populations

Author	Study population	Specimen	Range	Mean	Median
Mocarelli et al., 1991	Seveso, Italy residents: 10 Zone A 10 former Zone A 10 non-ABR Zone	Serum	 828 - 56,000 1770 - 10,400 nd-137	 19,144 5,240 —	 14,000 4,540 —
Patterson et al., 1986b	39 Missouri residents with history of TCDD exposure 57 Missouri residents with no known TCDD exposure	Adipose tissue	2.8 - 750 1.4 - 20.2	79.7 7.4	17.0 6.4
Smith et al., 1992	9 New Zealand pesticide applicators 9 controls	Serum	3.0 - 131.0 2.4 - 11.3	53.3 5.6	37.6 9.3
CDC, 1988	646 Vietnam ground combat troops with service in heavily sprayed areas 97 non-Vietnam veterans	Serum	nd - 45 nd - 15	4.2 4.1	3.8 3.8
Kahn, 1988	10 "heavily exposed" Vietnam veterans 10 Vietnam veterans with "little or no" exposure 7 non-Vietnam veterans	Blood (per lipids) Adipose tissue Blood (per lipids) Adipose tissue Blood (per lipids) Adipose tissue	— — — — — —	46.3 41.7 6.6 5.1 4.3 3.2	25.1 15.4 5.3 5.4 3.9 3.5
Schecter et al., 1989	26 Vietnam veterans	Adipose tissue	nd - 11	5.8	—

Table 7-49. 2,3,7,8-TCDD levels (pg/g of lipid) for selected populations (continued)

Author	Study population	Specimen	Range	Mean	Median
Kang, 1991	36 Vietnam veterans 79 non-Vietnam veterans 80 civilians	Adipose tissue	— — —	13.4 12.5 15.8	10.0 11.4 11.8
Roegner et al., 1991	872 Ranch Hands 1,060 controls	Serum	0-617.8 0-54.8	— —	12.8 4.2
Phuong, 1989b	Vietnamese populations: 9 OB/GYN patients from a South Vietnam hospital	Adipose tissue	nd - 103	23	11.3

Table 7-50. Odds ratios for selected categories of birth defects for the telephone interview and hospital records study in the Vietnam experience study, 1989

Birth defects	Vietnam veterans rate/1,000	Controls rate/1,000	OR	95% CI
Telephone interview				
Total	64.6	49.5	1.3	1.2-1.4
Hospital records study				
Total	72.6	71.1	1.0	0.8-1.4
Major	28.5	23.5	1.1	0.7-1.8
Minor	32.4	34.3	1.00	0.7-1.5

Adapted from the Centers for Disease Control Vietnam Experience Study, 1988d.

Table 7-51. Results of the misclassification analyses for birth defects in the hospital records substudy, Vietnam experience study, 1989

Vietnam veterans		Non-Vietnam veterans	
PPV	24.8%	PPV	32.9%
NPV	95.2%	NPV	95.8%
Sensitivity	27.1%	Sensitivity	30.3%
Specificity	94.7%	Specificity	96.2%
% Agreement	90.6%	% Agreement	92.4%
Kappa index	20.9%	Kappa index	27.6%

PPV = Positive predictive value.
NPV = Negative predictive value.
Adapted from the Centers for Disease Control Vietnam Experience Study, 1989.

Table 7-52. Rates of miscarriage (per 1,000) by pre- and post-Vietnam tour status and time since tour of duty, among 1,475 Ranch Hands with >10 pg/g serum dioxin, Ranch Hand study, 1992[a]

Time of conception	Time since tour (years)	Miscarriage rate per 1000 (no./n) by current dioxin level			*p*-value
		10-14.9 pg/g	15-33.3 pg/g	>33.3 pg/g	
Pre-tour	≤18.6	142.0 (23/162)	146.8 (32/218)	48.8 (2/41)	0.014[b]
	>18.6	123.9 (14/113)	159.4 (33/207)	166.7 (16/96)	
Post-tour	≤18.6	92.1 (7/76)	136.6 (22/161)	168.5 (15/89)	
	>18.6	237.3 (14/59)	198.6 (29/146)	121.5 (13/107)	

[a]Adapted from Wolfe et al., 1992b.
[b]Comparison of pre- and post-tour data.

Table 7-53. Summary of effects observed in adults exposed to 2,3,7,8-TCDD

System	Acute	Chronic
Dermatologic	Conjunctivitis Red and irritated eyes Blepharitis	chloracne
Liver	Temporary enlargement	—[a]
Liver enzymes	↑ GGT ↑ AST ↑ ALT ↑ D-glucaric acid excretion	↑ GGT
GI other than liver	+\- RUQ[c] pain Loss of appetite Nausea	—
Urinary porphyrins	+/-[b] PCT Uroporphyrin Urobilinogen Coproporphyrin	—
Lipids	↑	—
Cholesterol	↑	—
Triglycerides	↑	—
Thyroid	↑ T_4 ↑ T_4/TBG in some studies	—
Diabetes	No data	+/-
Immune	No data	+/- ↑NK cells +/- ↑IgA
Neuro	+/- ↓ libido ↑ irritability ↑ nervousness ↓ pin prick sensation	—
Circulatory	—	+/-
Pulmonary	Irritation	—
Renal	—	—
Reproductive hormones	No data	+/- LH in ♂ +/- FSH in ♂ ↓ testosterone in ♂
Chromosome	+/-	no data

[a]No effect noted.
[b]Some positive and some negative studies.
[c]Right upper quadrant.

Table 7-54. Effects of exposure to 2,3,7,8-TCDD on serum glucose levels in nonhuman mammalian species

Author	Species	Route	Dose (μ/kg)	Duration	Percentage of serum glucose levels in control animals[a]
Zinkl et al., 1993	CD rat	oral	0.1 1.0 10.1	1x/day for 30 days	91[b] 71 51
Gasiewicz et al., 1980	Rats	ip (TPN) ip (chow-fed)	100[c]	1 time	29[d] 51[d]
Schiller et al., 1986	Fischer rat	gavage	30 60 90 180[c] 270[c] 360[c]	1 time	74[d,e] 54[d,e] 30[d,e] 43[d,e] 38[d,e] 39[d,e]
Gorski et al., 1990	Sprague-Dawley rat	ip	125[c]	1 time	day 4 75[b,d] day 8 67[b,d] day 16 50[b,d] day 21 31[b,d]
McConnell et al., 1978a	Rhesus monkey	gavage	70 350[c]	1 time	(decreased)[b,d]
DeCaprio et al., 1986	Hartley guinea pig	oral	0.01 0.06 0.44	90 days	NC[b,e,f] NC[b,e,f] NC[b,e,f]
Ebner et al., 1988	New Zealand rabbits	ip	1 50	1 time	NC 15 min NC 1 hour 125[d,e] 2 day 87 10 day 80[d,e]

[a]Relative to control values.
[b]Female animals.
[c]Lethal dose.
[d]Significantly different from controls.
[e]Male animals.
[f]Data not displayed.
ip = intraperitoneal.
TPN = total parenteral nutrition.
NC = no change.

REFERENCES FOR CHAPTER 7, PART B

Air Force Health Study. (1995) An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides. 1992 Followup examination results. 10 vols. Brook AFB, TX: Epidemiologic Research Division, Armstrong Laboratory.

Air Force Health Study. (2000) An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides. 1997 Followup examination results. Brook AFB, TX: Air Force Research Laboratory. AFRL-HE-BR-TR-2000-02.

Alaluusua, S; Lukinmaa, P-L; Vartiainen, T; et al. (1996) Polychlorinated dibenzo-p-dioxins and dibenzofurans via mother's milk may cause developmental defects in the child's teeth. Environ Toxicol Pharmacol 1:193-197.

Alaluusua, S; Lukinmaa, P-L; Torppa, T; et al. (1999) Developing teeth as biomarker of dioxin exposure. The Lancet 353:206.

Albro, PW; Corbett, JT; Harriss, M; et al. (1978) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on lipid profiles in tissue of the Fischer rat. Chem Biol Interact 23:315-330.

Alderfer, R; Sweeney, M; Fingerhut, M; et al. (1992) Measures of depressed mood in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Chemosphere 25:247-250.

Allen, JR; Carstens, LA. (1967) Light and electron microscopic observations in Macaca mulatto monkeys fed toxic fat. Am J Vet Res 28:1513-1526.

Allen, JR; Barsotti, DA; van Miller, JP; et al. (1977) Morphological changes in monkeys consuming a diet containing low levels of 2,3,7,8-tetrachlorodibenzodioxin. Food Cosmet Toxicol 15:401-410.

American Thoracic Society. (1962) Chronic bronchitis, asthma, and pulmonary emphysema. A statement by the Committee on Diagnostic Standards for Nontuberculous Respiratory Diseases. Am Rev Respir Dis 85:762-768.

Aschengrau, A; Monson, RR. (1989) Paternal military service in Vietnam and risk of spontaneous abortion. J Occup Med 7:618-623.

Aschengrau, A; Monson, RR. (1990) Paternal military service in Vietnam and risk of late adverse pregnancy outcomes. Am J Publ Health 10:1218-1224.

Ashe, WF; Suskind, RR. (1950) Reports on chloracne cases, Monsanto Chemical Co., Nitro, West Virginia, October 1949 and April 1950. Cincinnati, OH: Department of Environmental Health, College of Medicine, University of Cincinnati (unpublished).

Asp, S; Riihimäki, V; Hernberg, S; et al. (1994) Mortality and cancer morbidity of Finnish chlorophenoxy herbicide applicators: an 18-year prospective follow-up. Am J Ind Med 26:243-253.

Assennato, G; Cervino, D; Emmet, E; et al. (1989) Follow-up on subjects who developed chloracne following TCDD exposure of Seveso. Am J Ind Med 16:119-125.

Baader, EW; Bauer, HJ. (1951) Industrial intoxication due to pentachlorophenol. Ind Med Surg 20:286-290.

Bastomsky, GH. (1977) Enhanced thyroxine metabolism and high uptake goiter in rats after single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Endocrinology 101:292-296.

Bauer, H; Schulz, K; Spiegelburg, W. (1961) Industrial poisoning in the manufacture of chlorophenol compounds. Arch Gewerbepath Gewerbehyg 18:538-555.

Beck, H; Eckart, K; Mathar, W; et al. (1989) Levels of PCDD's and PCDF's in adipose tissue of occupationally exposed workers. Chemosphere 18:507-516.

Becklake, MR. (1985) Chronic airflow limitation: its relationship to work in dusty occupations. Chest 88:608-617.

Bertazzi, PA; Zocchetti, C; Pesatori, AC; et al. (1989) Ten-year mortality study of the population involved in the Seveso incident in 1976. Am J Epidemiol 129:1187-1199.

Bertazzi, PA; Zocchetti, C; Pesatori, AC; et al. (1992) Mortality of a young population after accidental exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Int J Epidemiol 21:118-123.

Biscanti, L; Bonetti, F; Caramaschi, F; et al. (1979) Experience of the accident of Seveso. Proceedings of the 6th European Teratology Conference, Akademiai Kiado, Pub.

Bleiberg, J; Wallen, M; Brodkin, R; et al. (1964) Industrially acquired porphyria. Arch Dermatol 89:793-797.

Bombick, DW; Matsumura, F; Madhukar, BV. (1984) TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) causes reduction in the low density lipoprotein (LDL) receptor activities in the hepatic plasma membrane of guinea pig and rat. Biochem Biophys Res Commun 118:548-554.

Bond, GG; Ott, MG; Brenner, FE; et al. (1983) Medical and morbidity surveillance findings among employees potentially exposed to TCDD. Br J Ind Med 40:318-324.

Bond, GG; Cook, RR; Brenner, FE; et al. (1987) Evaluation of mortality patterns among chemical workers with chloracne. Chemosphere 16:2117-2121.

Bond, GG; McLaren, EA; Brenner, FE; et al. (1989) Incidence of chloracne among chemical workers potentially exposed to chlorinated dioxins. J Occup Med 31:771-774.

Bookstaff, RC; Kamel, F; Moore, RW; et al. (1990a) Altered regulation of pituitary gonadotropin-releasing hormone (GnRH) receptor number and pituitary responsiveness to GnRH in 2,3,7,8-tetrachlorodibenzo-p-dioxin-treated male rats. Toxicol Appl Pharmacol 105:78-92.

Bookstaff, RC; Moore, RW; Peterson, RE (1990b) 2,3,7,8-tetrachlorodibenzo-p-dioxin increases the potency of androgens and estrogens as feedback inhibitors of luteinizing hormone secretion in male rats. Toxicol Appl Pharmacol 104:212-224.

Brewster, DW; Matsumura, F; Akera, T. (1987) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on guinea pig heart muscle. Toxicol Appl Pharmacol 89:408-417.

Bueno de Mesquita, HB; Doornbos, G; van der Kuip, DAM; et al. (1993) Occupational exposure to phenoxy herbicides and chlorophenols and cancer mortality in The Netherlands. Am J Ind Med 23:289-300.

Burton, JE; Michalek, JE; Rahe; AJ. (1998) Serum dioxin, chloracne, and acne in veterans of Operation Ranch Hand. Arch Environ Health 53:199-204.

Buu-Hoï, NP; Chanh, PH; Sesqué, G; et al. (1972) Enzymatic functions as targets of the toxicity of "dioxin" (2,3,7,8-tetrachlorodibenzo-p-dioxin). Naturwissenshaften 59:173-174.

Calvert, GM; Sweeney, MH; Morris, JA; et al. (1991) Evaluation of chronic bronchitis, chronic obstructive pulmonary disease (COPD) and ventilatory function among workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Am Rev Respir Dis 144:1302-1306.

Calvert, GM; Hornung, RW; Sweeney, MH; et al. (1992) Hepatic and gastrointestinal effects in an occupational cohort exposed to 2,3,7,8-tetrachlorodibenzo-para-dioxin. JAMA 267:2209-2214.

Calvert, GM; Sweeney; MH; Fingerhut, MA; et al. (1994) Evaluation of porphyria cutanea tarda in U.S. workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Am J Ind Med 25:559-571.

Calvert, GM; Wille, KK; Sweeney, MH; et al. (1996) Evaluation of serum lipid concentrations among U.S. workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch Environ Health 51:100-107.

Calvert, GM; Wall, DK; Sweeney, MH; et al. (1998) Evaluation of cardiovascular outcomes among U.S. workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ Health Perspect 106 Suppl 2:635-643.

Calvert, GM; Sweeney MH; Deddens, J; et al. (1999) Evaluation of diabetes mellitus, serum glucose, and thyroid function among United States workers exposed to 2,3,7,8-tetrachlorodibenzo-p- dioxin. Occup Environ Med 56:270-276.

Cam, C; Nigogosyan, G. (1963) Acquired toxic porphyria cutanea tarda due to hexachlorobenzene. JAMA 183:88-91.

Canga, L; Levi, R; Rifkind, A. (1988) Heart as a target organ in 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity: decreased β-adrenergic responsiveness and evidence of increased intracellular calcium. Proc Natl Acad Sci USA 85:905-909.

Cantoni, L; Salmona, M; Rizzardini, M. (1981) Porphyrogenic effect of chronic treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin in female rats. Dose-effect relationship following urinary excretion of porphyrins. Toxicol Appl Pharmacol 57:156-163.

Caramaschi, F; Del Caino, G; Favaretti, C; et al. (1981) Chloracne following environmental contamination by TCDD in Seveso, Italy. Int J Epidemiol 10:135-143.

Centers for Disease Control Veterans Health Studies. (1988) Serum 2,3,7,8-tetrachlorodibenzo-p-dioxin levels in U.S. Army Vietnam-era veterans. JAMA 260:1249-1254.

Centers for Disease Control Vietnam Experience Study. (1988a) Health status of Vietnam veterans. II. Physical health. JAMA 259:2708-2714.

Centers for Disease Control Vietnam Experience Study. (1988b) Health status of Vietnam veterans. I. Psychosocial characteristics. JAMA 259:2701-2707.

Centers for Disease Control Vietnam Experience Study. (1988c) Postservice mortality among Vietnam veterans. JAMA 257:790-795.

Centers for Disease Control Vietnam Experience Study. (1988d) Health status of Vietnam veterans. III. Reproductive outcomes and child health. JAMA 259:2715-2719.

Centers for Disease Control Vietnam Experience Study. (1989) Health status of Vietnam veterans. Volume V: Reproductive outcomes and child health. Atlanta: CDC.

Chang, KJ; Lu, FJ; Tung, TC; et al. (1980) Studies on patients with polychlorinated biphenyl poisoning. 2. Determination of urinary coproporphyrin, uroporphyrin, δ aminolevulinic acid and porphobilinogen. Res Commun Chem Pathol Pharmacol 30:547-554.

Chao, W-Y; Hsu, C-C. (1997) Middle ear abnormalities in children exposed prenatally to polychlorinated biphenyls and polychlorinated dibenzofurans. Arch environ Health 52:257-262..

Chen, CJ; Shen, RL. (1981) Clin Med (Taipei) 7:66-70.

Chen, PH; Hites, RA. (1983) Polychlorinated biphenyls and dibenzofurans retained in the tissues of a deceased patient with Yu-Cheng in Taiwan. Chemosphere 12:1507-1516.

Chen, RC; Chang, YC; Chang, KJ; et al. (1981) Peripheral neuropathy caused by chronic polychlorinated biphenyls poisoning. J Formosan Med Assoc 80:47-54 (in English; Chinese summary).

Chen, RC; Chang, YC; Tung, TC; et al. (1983) Neurological manifestations of chronic polychlorinated biphenyls poisoning. Proc Natl Sci Counc ROC (A) 7:87-91 (in English; Chinese summary).

Chen, RC; Tang, SY; Miyata, H; et al. (1985) Polychlorinated biphenyl poisoning: correlation of sensory and motor nerve conduction, neurologic symptoms, and blood levels of polychlorinated biphenyls, quaterphenyls and dibenzofurans. Environ Res 37:340-348.

Chen, YCJ; Guo, YLL; Hsu, CC. (1992) Cognitive development of children prenatally exposed to polychlorinated biphenyls (Yu-Cheng children) and their siblings. J Formosan Med Assoc 91:704-707.

Chen, YC; Guo, YL; Yu, ML; et al. (1993) Physical and cognitive development of Yu-Cheng children born after year 1985. Presented at: the 13th International Symposium on Chlorinated Dioxins and Related Compounds; September 20-24, 1993; Vienna, Austria.

Chen, YC; Hsu, CC. (1994) Effects of prenatal exposure to PCBs on the neurological function of children: A neuropsychological and neurophysiological study. Develop Med Child Neurol 36:312-320.

Chia, LG; Chu, FL. (1984) Neurological studies on polychlorinated biphenyl (PCB)-poisoned patients. Am J Ind Med 5:117-126.

Chia, LG; Chu, FL. (1985) A clinical and electrophysiological study of patients with polychlorinated biphenyl poisoning. J Neurol Neurosurg Psych 48:894-901.

Coggon, D; Pannett, B; Winter, P. (1991) Mortality and incidence of cancer at four factories making phenoxy herbicides. Br J Ind Med 48:173-178.

Commonwealth Department of Veterans' Affairs. (1998a) Morbidity of Vietnam veterans: A study of the health of Australia's Vietnam veteran Community. Volume 1: Male Vietnam veterans survey and community compariosn outcomes. Canberra: Commonwealth Department of Veteran's Affairs.

Commonwealth Department of Veterans' Affairs. (1998b) Morbidity of Vietnam veterans: A study of the health of Australia's Vietnam veteran Community. Volume 2: Female Vietnam veterans survey and community compariosn outcomes. Canberra: Commonwealth Department of Veteran's Affairs.

Collins, JJ; Strauss, ME; Levinkas, GJ; et al. (1993) The mortality experience of workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin in a trichlorophenol process accident. Epidemiology 4:7-13.

Constable, JD; Hatch, MC. (1985) Reproductive effects of herbicide exposure in Vietnam: recent studies by the Vietnamese and others. Teratogen Carcinogen Mutagen 5:231-250.

Courtney, KD. (1976) Mouse teratology studies with chlorodibenzo-p-dioxins. Bull Environ Contam Toxicol 16:674-681.

Courtney, KD; Moore, JA. (1971) Teratology studies with 2,4,5-T and 2,3,7,8-TCDD. Toxicol Appl Pharmacol 20:396-403.

Creso, E; DeMarino, V; Donatelli, L; et al. (1978) Effette neuropsicofarmacologici deila TCDD. Boll Soc It Sper 54:1592-1596.

Crow, K. (1978) Chloracne: the chemical disease. New Scientist 78(11):78-80.

Cutting, RT; Phuoc, TH; Ballo, J; et al. (1970) Congenital malformations, hydatidiform moles, and stillbirths in the Republic of Vietnam 1960-1969. Washington, DC: U.S. Government Printing Office.

DeCaprio, AP; McMartin, DN; O'Keefe, PW; et al. (1986) Subchronic oral toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the guinea pig: comparisons with a PCB-containing transformer fluid pyrolysate. Fundam Appl Toxicol 6:454-463.

DeVerneuil, H; Sassa, S; Kappas, A. (1983) Effects of polychlorinated biphenyl compounds, 2,3,7,8-tetrachlorodibenzo-p-dioxin, phenobarbital and iron on hepatic uroporphyrinogen decarboxylase. Biochem J 214:145-151.

Diabetes Epidemiology Research International. (1987).

Dimich-Ward, H; Hertzman, C; Teschke, K; et al. (1996) Reproductive effects of paternal exposure to chlorophenate wood preservatives in the sawmill industry. Scand J Work Environ Health 22:267-73. Amended and corrected in Scand J Work Environ Health 24:416.

Doss, M; Sauer, H; Von Tiepermann, R; et al. (1984) Development of chronic hepatic porphyria (porphyria cutanea tarda) with inherited uroporphyrinogen decarboxylase deficiency under exposure to dioxin. Int J Biochem 16:369-373.

Dunagin, WG. (1984) Cutaneous signs of systemic toxicity due to dioxins and related chemicals. J Am Acad Dermatol 10(4):688-700.

Ebner, K; Brewster, DW; Matsumura, F. (1988) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on serum insulin and glucose levels in the rabbit. J Environ Sci Health B23:27-438.

Egeland, GM; Sweeney, MH; Fingerhut, MA; et al. (1994) Total serum testosterone and gonadotropins in workers exposed to dioxin. Am J Epidemiol 139:272-281.

Elovaara, E; Savolainen, H; Parkki, MG; et al. (1977) Neurochemical effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Wistar and Gunn rats. Res Commun Chem Pathol Pharmacol 18(3):487-494.

England, JF. (1981) Herbicides and coronary ectasia [letter]. Med J Australia 1:140.

Erickson, JD; Mulinare, J; McClain, PW; et al. (1984) Vietnam veterans' risks for fathering babies with birth defects. JAMA 252:903-912.

Eriksson, M; Hardell, L; Adam, H. (1990) Exposure to dioxins as a risk factor for soft tissue sarcoma: a population-based case-control study. J Natl Cancer Inst 82:486-490.

Ernst, M; Flesch-Janys, D; Morgenstern, I; et al. (1998) Immune cell functions in industrial workers after exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin: dissociation of antigen-specific T-cell responses in cultures of diluted whole blood and of isolated peripheral blood mononuclear cells. Environ Health Perspect 106 Suppl 2:701-705.

Evans, GR; Webb, KB; Knutsen, AP; et al. (1988) A medical follow-up of the health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch Environ Health 43:273-278.

Fee, DC; Hughes, BM; Tiernan, TO. (1975) Analytical methods for Herbicide Orange, Vol. II: Determination of origin of USAF stock. USAFARL 75-00110, Vol. II.

Fett, MJ; Adena, MA; Cobbin, DM; et al. (1987) Mortality among Australian conscripts of the Vietnam conflict era. I. Causes of death. Am J Epidemiol 125:878-884.

Filippini, G; Bordo, B; Crenna, P; et al. (1981) Relationship between clinical and electrophysiological findings and indicators of heavy exposure to 2,3,7,8-tetrachlorodibenzo-dioxin. Scand J Work Environ Health 7:257-262.

Fingerhut, MA; Sweeney, MH; Patterson, DG; et al. (1989) Levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the serum of U.S. chemical workers exposed to dioxin contaminated products: interim results. Chemosphere 19:835-840.

Fingerhut, MA; Halperin, WE; Marlow, DA. (1991a) Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. New Engl J Med 324:212-218.

Fingerhut, MA; Halperin, WE; Marlow, D; et al. (1991b) Mortality among U.S. workers employed in the production of chemicals contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Cincinnati, OH: U.S. Department of Health and Human Services, National Institute for Occupational Safety and Health. NTIS# PB 91-125971.

Flesch-Janys, D; Berger, J; et al. (1992) Quantification of exposure to dioxins and furans in a cohort of workers of a herbicide producing plant in Hamburg FRG. Chemosphere 25:1021-1027.

Flesch-Janys, D; Berger, J; Gurn, P; et al. (1995) Exposure to polychlorinated dioxins and furans (PCDD/F) and mortality in a cohort of workers from a herbicide-producing plant in Hamburg, Federal Republic of Germany. Am J Epidemiol 142:1165-1167.

Fox, AJ; Collier, PF. (1976) Low mortality rates in industrial cohort studies due to selection for work and survival in the industry. Br J Prev Soc Med 30:225-230.

Gasiewicz, TA; Neal, RA. (1979) 2,3,7,8-Tetrachlorodibenzo-p-dioxin tissue distribution, excretion, and effects on clinical chemical parameters in guinea pigs. Toxicol Appl Pharmacol 51:329-339.

Gasiewicz, TA; Holscher, MA; Neal, RA. (1980) The effect of total parenteral nutrition on the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Toxicol Appl Pharmacol 54:469-488.

Giavinni, E; Prati, M; Vismara, C. (1983) Embryotoxic effects of 2,3,7,8-tetrachlordibenzo-p-dioxin administered to female rats before mating. Environ Res 31:105-110.

Gladen, BC; Rogan, WJ; Ragan, NB; et al. (1988) Urinary porphyrins in children exposed transplacentally to polyhalogenated aromatics in Taiwan. Arch Environ Health 43:54-58.

Goldman, PJ. (1972) Critically acute chloracne caused by trichlorophenol decomposition products. Arbeitsmed. Sozialmed. Arbeitshygiene 7:12-18.

Goldstein, JA; Hickman, P; Bergman, H; et al. (1973) Hepatic porphyria induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Res Com Chem Path Pharmacol 6:919-928.

Goldstein, JA; Linko, P; Bergman, H. (1982) Induction of porphyria in the rat by chronic versus acute exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Biochem Pharmacol 31:1607-1613.

Gorski, JR; Weber, LWD; Rozman, K. (1990) Reduced gluconeogenesis in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-treated rats. Arch Toxicol 64:66-71.

Greig, JB; Jones, G; Butler, WH; et al. (1973) Toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Food Cosmet Toxicol 11:585-595.

Grubbs, WD; Wolfe, WH; Michalek, JE; et al. (1995) Air Force Health Study: An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides. Report number AL-TR-920107.

Guo, YL; Lai, TJ; Ju, SH; et al. (1993) Sexual developments and biological findings in Yu-Cheng children. Presented at: 13th International Symposium on Chlorinated Dioxins and Related Compounds (Dioxin '93); September 20-24, 1993; Vienna, Austria.

Guo, YL; Lin, CJ; Yao, WJ; et al. (1994a) Musculoskeletal changes in children prenatally exposed to polychlorinated biphenyls and related compounds (Yu-Cheng Children). J Toxicol Environ Health 41:83-93.

Guo YL; Chen, YC,; Yu, ML; Hsu, CC. (1994b) Early development of Yu-Cheng children born seven to twelve years after the Taiwan PCB outbreak. Chemosphere 29: 2395-2404.

Guo, YL; Lai, TJ; Chen, SJ; Hsu, CC. (1995a) Gender-related decrease in Raven's progressive matrices scores in children prenatally exposed to polychlorinated biphenyls and related contaminants. Bull Environ Contam Toxicol 55:8-13.

Guo YL; Lambert, GH; Hsu, CC. (1995b) Growth abnormalities in the population exposed in utero and early postnatally to polychlorinated biphenyls and dibenzofurans. EHP 103(6):117-122.

Gupta, BN; Vos, JG; Moore, JA; et al. (1973) Pathologic effects of 2,3,7,8-TCDD in laboratory animals. Environ Health Perspect 5:125-140.

Guzelian, PS. (1985) Clinical evaluation of liver structure and function in humans exposed to halogenated hydrocarbons. Environ Health Perspect 60:159-164.

Halperin, W; Kalow, W; Sweeney MH; et al. (1995) Induction of P-450 in workers exposed to dioxin. Occup Environ Med 52:86-91.

Halperin, W; Vogt, R; Sweeney, MH; et al. (1998) Immunological markers among workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Occup Environ Med 55:742-749.

Hashiguchi, I; Toriya, Y; Anan, H; et al. (1995) An epidemiologic examination on the prevalence of the periodontal diseases and oral pigmentation in Yusho patients. Fukuoka Igaku Zasahi 86:256-260.[English Abstract].

Hatch, M. (1984a) Herbicide exposure and reproduction -- an overview. In: Herbicides and war: the long-term ecological and human consequences. Westing, AH, ed. Philadelphia: Taylor and Francis.

Hatch, M. (1984b) Reproductive effects of the dioxins. In: Public health risks of the dioxins. Lowrance, WW, ed. California: William Kaufmann; pp. 255-275.

Hatch, MC; Stein, ZA. (1986) Agent Orange and risks to reproduction: the limits of epidemiology. Teratogenesis Carcinogen Mutagen 6:185-202.

Henriksen, GL; Ketchum, NS; Michalek, JE; et al. (1997) Serum dioxin and diabetes mellitus in veterans of Operation Ranch Hand [see comments]. Epidemiology 8:252-258.

Henriksen, GL; Michalek, JE; Swaby, JA; et al. (1996) Serum dioxin, testosterone, and gonadotropins in veterans of Operation Ranch Hand. Epidemiology 7:352-357.

Henry, EC; Gasiewicz, TA. (1987) Changes in thyroid hormones and thyroxine glucuronidation in hamsters compared with rats following treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 89:165-174.

Hermansky, SJ; Holcslaw, TL; Murray, WJ; et al. (1988) Biochemical and functional effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the heart of female rats. Toxicol Appl Pharmacol 95:175-184.

Hertig, AT; Rock, J; Adams, EC (1959) Thirty-four fertilized human ova, good, bad, and indifferent, recovered from 210 women of known fertility: a study of biologic wastage in early human pregnancy. Pediatrics 23:202-211.

Hill, AB. (1965) The environment and disease: association or causation. Proc R Soc Med 58:295-300.

Hirota, Y; Hirohata, T; Kataoka, K; et al. (1993). Laboratory findings in the medical examination of chronic Yusho (PCB poisoning) patients with special reference to blood PCB and serum triglyceride. Fukuoka Igaku Zasahi 84:287-293.[English Abstract].

Hirota, Y; Hirohata, T; Kataoka, K; et al. (1995). Blood polychlorinated biphenyls and manifestations of symptoms in chronic Yusho patients. Fukuoka Igaku Zasahi 86:247-255.[English Abstract].

Hoffman, RE; Stehr-Green, PA. (1989) Localized contamination with 2,3,7,8-tetrachlorodibenzo-p-dioxin: the Missouri episode. In: Kimbrough, R.D.; Jensen, A.A., eds. Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins, and related products. New York: Elsevier, pp. 471-483.

Hoffman, RE; Stehr-Green, PA; Webb, KB; et al. (1986) Health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. JAMA 255:2031-2038.

Homberger, E; Reggiani, G; Sambeth, J; et al. (1979) Seveso accident: its nature, extent and consequences. Report from Givaudan Research Company Ltd. and F. Hoffmann-La Roche & Co. Ltd.

Hooiveld, M; Heederik, DJ; Kogevinas M; et al. (1998) Second follow-up of a Dutch cohort occupationally exposed to phenoxy herbicides, chlorophenols, and contaminants. Am J Epidemiol 147:891-901.

Hooper, K; Chuvakova, T; Cheng Y-Y. (1999) Sex ratio of infants in a TCDD-contaminated region in southern Kazakhstan. Organohalogen Compounds 44:389-392.

Hryhorczuk, DO; Wallace, WH; Persky, V; et al. (1998) A morbidity study of former pentachlorophenol-production workers. Environ Health Perspect 106:401-408.

Hsu, CC; Hu, HF; Lai, TJ; et al. (1993) Behavioral development of Yu-Cheng children as compared to their matched controls. Presented at: 13th International Symposium on Chlorinated Dioxins and Related Compounds (Dioxin '93); September 20-24, 1993; Vienna, Austria.

Huisman, M; Koopman-Esseboom, C; Lanting, CI; et al. (1995a) Neurological condition in 18-month-old children perinatally exposed to polychlorinated biphenyls and dioxins. Early Hum Dev 43:165-176.

Huisman, M; Koopman-Esseboom, C; Fidler, V; et al. (1995b) Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. Early Hum Dev 41(2):111-127.

Huong, LD; Phuong, NTN; Thuy, TT; et al. (1989) An estimate of the incidence of birth defects, hydatidiform mole and fetal death *in utero* between 1952 and 1985 at the obstetrical and gynecological hospital of Ho Chi Minh City, Republic of Vietnam. Chemosphere 18:805-810.

Ideo, G; Ballati, G; Bellobuno, A; et al. (1985) Urinary D-glucaric excretion in the Seveso area, polluted by tetrachlorodibenzo-p-dioxin (TCDD): five years of experience. Environ Health Perspect 60:151-157.

Ikezuki, Y; Tsutsumi, O; Takai, Y; et al. (1999) Breast-fed infants possibly exposed to dioxins in milk, compared to formula-fed infants, have unexpectedly lower incidence of endometriosis in later adult life. Organohalogen Compounds 44:393-395.

Institute of Medicine, Committee to Review the Evidence Regarding the Link Between Exposure to Agent Orange and Diabetes, Division of Health Promotion and Disease Prevention. (2000) Veterans and Agent Orange: Herbicide/Dioxin Exposure and Type 2 Diabetes. National Academy Press, Washington, DC.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (1997) Polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans/ (Lyon, France) Monograph 69.

Jacobson, JL; Jacobson, SW. (1996) Intellectual impairment in children exposed to polychlorinated biphenyls *in utero*. N Engl J Med 335(11):783-789.

James, WH. (1987) The human sex ratio, part 1: a review of the literature. Hum Biol 59:721-52.

James, WH. (1997a) Re: "Total serum testosterone and gonadotropins in workers exposed to dioxin" [letter; comment]. Am J Epidemiol 145:569.

James, WH. (1997b) Reproductive effects of male dioxin exposure. The use of offspring sex ratios to detect reproductive effects of male exposure to dioxins [letter; comment by Toppari J, Skakkebaek, NE.]. Environ Health Perspect 105:162-163.

James, WH. (1997) The sex ratio of offspring sired by men exposed to wood preservatives contaminated by dioxin [letter; comment] Scand J Work Environ Health 23:69. [retracted by James, WH. In: Scand J Work Environ Health 1998 Oct;24(5):416].

James, WH. (1998) Re: the use of offspring sex ratios in the search for endocrine disruptors [letter; comment]. Environ Health Perspect 106:A472-A473.

Jennings, AM; Wild, G; Ward, JD; et al. (1988) Immunological abnormalities 17 years after accidental exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Br J Ind Med 45:701-704.

Jensen, AA. (1987) Polychlorobiphenyls (PCBs), polychlorodibenoz-p-dioxins (PCDDs), and polychlorodibenzofurnas (PCDFs) in human milk, blood and adipose tissue. Sci Total Environ 64:259-293.

Jirasek, L; Kalensky, J; Kulec, K. (1973) Chloracne and porphyria cutanea tarda in association with the production of herbicide. Cesk Dermatol 48:306-317.

Jirasek, L; Kalensky, K; Kubec, K; et al. (1974) Chronic poisoning by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Cesk Dermatol 49:145-157.

Jones, RE; Chelsky, M. (1986) Further discussion concerning porphyria cutanea tarda and TCDD exposure. Arch Environ Health 41:100-103.

Jones, G; Greig, JB. (1975) Pathological changes in the liver of mice given 2,3,7,8-TCDD. Experientia 31:1315-1317.

Jones, KG; Cole, FM; Sweeney, GD. (1981) The role of iron in the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol Appl Pharmacol 61:74-88.

Jung, D; Berg, PA; Edler, L; et al. (1998) Immunologic findings in workers formerly exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin and its congeners. Environ Health Perspect 106 Suppl 2:689-695.

Kahn, PC; Gochfeld, M; Nygren, M; et al. (1988) Dioxins and dibenzofurans in blood and adipose tissue of Agent Orange-exposed Vietnam veterans and matched controls. JAMA 259:1661-1667.

Kang, HK; Watanabe, KK; Breen, J; et al. (1991) Dioxins and dibenzofurans in adipose tissue of U.S. Vietnam veterans and controls. Am J Publ Health 81:344-349.

Kashimoto, T; Miyata, H; Fukushima, S; et al. (1985) PCBs, PCQs and PCDFs in blood of Yusho and Yu-Cheng patients. Environ Health Perspect 59:73-78.

Kelling, CK; Menahan, LA; Peterson, RE. (1987) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin treatment on mechanical function of the rat heart. Toxicol Appl Pharmacol 91:497-501.

Kellokumpu-Lehtinen P, Pelliniemi LJ. (1984) Sex ratio of human conceptuses. Obstet & Gynecol 64:220-222.

Khera, KS; Ruddick, JA. (1973) Polychlorinated dibenzo-p-dioxins: perinatal effects and the dominant lethal test in Wistar rats. Adv Chem 120:70-84.

Khoury, M. (1989) Epidemiology of birth defects. Epidemiol Rev 11:244-248.

Kimbrough, RD; Carter, CD; Liddle, JA; et al. (1977) Epidemiology and pathology of a tetrachlorodibenzodioxin poisoning episode. Arch Environ Health 32:77-85.

Kimmig, J; Schulz, KH. (1957a) Chlorinated aromatic cyclic ethers as the cause of so-called chloracne. Naturwissenshaften 44:337-338.

Kimmig, J; Schulz, KH. (1957b) Occupational chloracne caused by aromatic cyclic ethers. Dermatologica 115:540-546.

Kleeman, JM; Moore, RW; Peterson, RE. (1990) Inhibition of testicular steroidogenesis in 2,3,7,8-tetrachlorodibenzo-p-dioxin-treated rats: evidence that the key lesion occurs prior to or during pregnenolone formation. Toxicol Appl Pharmacol 106:112-125.

Kline, J; Stein, Z; Susser, M. (1989) In: Conception to birth: epidemiology of prenatal development. New York: Oxford University Press.

Kociba, RJ; Keeler, CN; Park, CN; et al. (1976) 2,3,7,8-Tetrachlorodibenzo-p-dioxin: results of a 13-week oral toxicity study in rats. Toxicol Appl Pharmacol 35:553-574.

Kociba, RJ; Keyes, DG; Beyer, JE; et al. (1978) Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Toxicol Appl Pharmacol 46:279-303.

Kociba, RJ; Keyes, DG; Beyer, JE; et al. (1979) Long-term toxicologic studies of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in laboratory animals. Ann NY Acad Sci 320:397-404.

Koopman-Esseboom, C; Huisman, M; Weisglas-Kuperus, N; et al. (1994a) Dioxin and PCB levels in blood and human milk in relation to living areas in The Netherlands. Chemosphere 29(9-11):2327-2338.

Koopman-Esseboom, C; Huisman, M; Weisglas-Kuperus, N; et al. (1994b) PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemoshpere 28:1721-1732.

Koopman-Esseboom, C; Morse, DC; Weisglas-Kuperus, N; et al. (1994c) Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatr Res 36(4):468-73.

Koopman-Esseboom, C; Huisman, M; Touwen, BCL; et al. (1995a) Effects of PCB/dioxin exposure and feeding type on the infant's visual recognition memory. Chapter 5 in dissertation entitled: Effects of perinatal exposure to PCBs and dioxins on early human development. Erasmus Universiteit Rotterdam, pp. 75-86.

Koopman-Esseboom, C; Weisglas-Kuperus, N; de Ridder, MAJ; et al. (1995b) Effects of PCB/dioxin exposure and feeding type on the infant's visual recognition memory. Chapter 7 in Dissertation entitled: Effects of perinatal exposure to PCBs and dioxins on early human development. Erasmus Universiteit Rotterdam, pp. 107-121.

Koopman-Esseboom, C; Weisglas-Kuperus, N; de Ridder, MAJ; et al. (1996) Effects of polychlorinated biphenyl/dioxin exposure and feeding type on the infant's mental and psychomotor development. Pediatrics 97:700-706.

Kunstadter, P. (1982) A study of herbicides and birth defects in the Republic of Vietnam. Honolulu, Hawaii: National Academy Press.

Kuratsune, M. (1972) An abstract of results of laboratory examinations of patients with Yusho and of animal experiments. Environ Health Perspect Experimental Issue 5:129-136.

Kuratsune, M. (1989) Yusho, with reference to Yu-Cheng. In: Halogenated biophenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Kimbrough, RD; Jensen, AA, eds. 2nd ed. New York: Elsevier Science Publishers; pp. 381-400.

Kuratsune, M; Yoshimura, T; Matsuzaka, J; et al. (1972) Epidemiologic study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. Environ Health Perspect 1:119-128.

Kuriowa, Y; Murai, Y; Santa, T. (1969) Neurological and nerve conduction velocity studies of 23 patients with chlorobiphenyls poisoning. Fukuoka Acta Med 60:462-463.

Lai, TJ; Chen, YC; Chou, WJ; et al. (1993) Cognitive development in Yu-Cheng children. Presented at: 13th International Symposium on Chlorinated Dioxins and Related Compounds (Dioxin '93); September 20-24, 1993; Vienna, Austria.

Lai TJ; Guo, YL; Yu, ML; Ko, HC; Hsu, CC. (1994) Cognitive development in Yucheng children. Chemosphere 29:2405-2411.

Lamb, JC; Moore, JA; Marks, TA. (1980) Evaluation of 2,4-D, 2,4,5-T, and 2,3,7,8-TCDD toxicity in C578BL/6 mice: reproduction and fertility in treated mice and congenital malformations in their offspring. National Toxicology Program. NTP 80-44.

Landi, MT; Needham, LL; Lucier, G; et al. (1997) Concentrations of dioxin 20 years after Seveso. The Lancet 349:1811.

Lathrop, GD; Wolfe, WH; Albanese, RA; et al. (1984) An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides. Baseline morbidity study results. Brooks Air Force Base, TX: U.S. Air Force School of Aerospace Medicine, Aerospace Medical Division (unpublished).

Lathrop, GD; Wolfe, WH; Michalek, JE; et al. (1987) An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides. First follow-up examination results, January 1985-September 1987. Brooks Air Force Base, TX: U.S. Air Force School of Aerospace Medicine, Aerospace Medical Division (unpublished).

Lee, IP; Dixon, RL. (1978) Factors influencing reproduction and genetic toxic effects on male gonads. Environ Health Perspect 24:117-127.

Longnecker, MP; Michalek, JE. (2000) Serum dioxin level in relation to diabetes mellitus among Air Force veterans with background levels of exposure. Epidemiology 11:44-48.

Lü, YC; Wong, PN. (1984) Dermatological, medical, and laboratory findings of patients in Taiwan and their treatments. Am J Ind Med 5:81-115.

Lü, YC; Wu, YC. (1985) Clinical findings and immunological abnormalities in Yu-Cheng patients. Environ Health Perspect 59:17-29.

Lundgren, K; Collman, GW; Wuu, SW; et al. (1988) Cytogenic and chemical detection of human exposure to polyhalogenated aromatic hydrocarbons. Environ Mol Mutagen 11:1-11.

Manz, A; Berger, J; Dwyer, JH; et al. (1991) Cancer mortality among workers in chemical plant contaminated with dioxin. Lancet 338:959-964.

Martin, JV. (1984) Lipid abnormalities in workers exposed to dioxin. Br J Ind Med 41:254-256.

Mastroiacovo, P; Spagnolo, A; Marni, E; et al. (1988) Birth defects in the Seveso area after TCDD contamination. JAMA 259:1668-1672.

Masuda, Y; Kuroki, H; Haraguchi, K; et al. (1985) PCB and PCDF congeners in the blood and tissues of Yusho and Yu-Cheng patients. Environ Health Perspect 59:53-58.

May, G. (1973) Chloracne from the accidental production of tetrachlorodibenzo-dioxin. Br J Ind Med 30:276-283.

May, G. (1982) Tetrachlorodibenzodioxin: a survey of subjects ten years after exposure. Br J Ind Med 39:128-135.

Mayani, A; Barel, S; Soback, S; et al. (1997) Dioxin concentrations in women with endometriosis. Hum Reprod 12:373-375.

McConnell, EE; Moore, JA; Dalgard, DW. (1978a) Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rhesus monkey (Macaca mulatta) following a single oral dose. Toxicol Appl Pharmacol 43:175-187.

McConnell, EE; Moore, JA; Haseman, JK; et al. (1978b) The comparative toxicity of chlorinated dibenzo-p-dioxins in mice and guinea pigs. Toxicol Appl Pharmacol 44:335-356.

McMichael, AJ. (1976) Standardized mortality ratios and the "healthy worker effect": scratching beneath the surface. J Occup Med 18:128-131.

McNulty, WP. (1977) Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin for rhesus monkeys: brief report. Bull Environ Contam Toxicol 18:108-109.

Mebus, CA; Reddy, VR; Piper, WN. (1987) Depression of rat testicular 17-hydroxylase and 17,20-lyase after administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Biochem Pharmacol 36(5):1727-1731.

Michalek, JE. (1989) The value of epidemiology. Applied Industrial Hygiene, Special Issue 68-72.
Michalek, JE; Wolfe, WH; Miner, JC. (1990) Health status of Air Force veterans occupationally exposed to herbicides in Vietnam II. Mortality. JAMA 264:1832-1836.

Michalek , JE; Pirkle, JL; Caudill, SP; et al. (1996) Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 10-year follow-up. J Toxicol Environ Health 47:209-220.

Michalek JE; Ketchum NS; Akhtar FZ. (1998) Postservice mortality of US Air Force veterans occupationally exposed to herbicides in Vietnam: 15-year follow-up. Am J Epidemiol 148:786-792.

Michalek JE; Rahe AJ; Boyle CA. (1998) Paternal dioxin, preterm birth, intrauterine growth retardation, and infant death. Epidemiology 9:161-167.

Michalek JE; Rahe AJ; Boyle CA. (1998) Paternal dioxin and the sex of children fathered by veterans of Operation Ranch Hand [letter]. Epidemiology 9:474-475.

Michalek JE; Ketchum NS; Check IJ. (1999) Serum dioxin and immunologic response in veterans of Operation Ranch Hand. Am J Epidemiol 149:1038-1046.

Miller, RW; Blot, WJ. (1972) Small head size after *in utero* exposure to atomic radiation. Lancet ii:784-787.

Miller, LV; Stokers, JD; Silpipat, C. (1978) Diabetes mellitus and autonomic dysfunction after Vacor rodenticide ingestion. Diabetes Care 1:73.

Missouri Dioxin Health Studies Progress Report. (1983) Missouri Division of Health, Centers for Disease Control, St. Joseph's Hospital of Kirkwood, St. Louis University Hospital.

Mocarelli, P; Marocchi, A; Brambilla, P; et al. (1986) Clinical laboratory manifestations of exposure to dioxin in children. A six year study of the effects of an environmental disaster near Seveso, Italy. JAMA 256:2687-2695.

Mocarelli, P; Pocchiari, F; Nelson, N. (1988) Preliminary report: 2,3,7,8-tetrachlorodibenzo-p-dioxin. Exposure to humans--Seveso, Italy. MMWR 37:733-736.

Mocarelli, P; Needham, LL; Marocchi, A; et al. (1991) Serum concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin and test results from selected residents of Seveso, Italy. J Toxicol Environ Health 32:357-366.

Mocarelli P; Brambilla P; Gerthoux PM; et al. (1996) Change in sex ratio with exposure to dioxin [letter]. Lancet 348:409.

Mocarelli, P; Gerthoux, PM; Ferrari, E; et al. (2000) Paternal concentrations of dioxin and sex ratio of offspring. Lancet 355:1858-1863.

Moore, RW; Peterson, RE. (1988) Androgen catabolism and excretion in 2,3,7,8-tetrachlorodibenzo-p-dioxin-treated rats. Biochem Pharmacol 37:560-562.

Moore, RW; Potter, CL; Theobald, HM; et al. (1985) Androgenic deficiency in male rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 79:99-111.

Moore, RW; Bookstaff, RC; Mably, RA; et al. (1991) Differential effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on responsiveness of male rats to androgens, 17B-estradiol, luteinizing hormone, gonadotropin releasing hormone, and progesterone. Presented at: Dioxin '91, 11th international symposium on chlorinated dioxins and related compounds; Research Triangle Park, NC.

Morrow, AF; Baker, G; Burger, HG. (1986) Different testosterone and LH relationships in infertile men. J Androl 7:310-315.

Moses, M; Prioleau, PG. (1985) Cutaneous histologic findings in chemical workers with and without chloracne with past exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Am Acad Dermatol 12:497-506.

Moses, M; Lilis, R; Crow, KD; et al. (1984) Health status of workers with past exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin in the manufacture of 2,4,5-trichlorophenoxyacetic acid. Comparison of findings with and without chloracne. Am J Ind Med 5:161-182.

Murai, K; Okamura, K; Tsuji, H; et al. (1987) Thyroid function in "Yusho" patients exposed to polychlorinated biphenyls (PCB). Environ Res 44:179-187.

Murray, FJ; Smith, FA; Nitschke, KD; et al. (1979) Three generation reproduction study of rats given 2,3,7,8-tetrachlorodibenzodioxin in diets. Toxicol Appl Pharmacol 50:241-252.

Muzi, G; Gorski, JR; Rozman, K. (1989) Mode of metabolism is altered in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-treated rats. Toxicol Lett 47:77-86.

Nakanishi, Y; Shigematsu, N; Kurita, Y; et al. (1985) Respiratory involvement and immune status in Yusho patients. Environ Health Perspect 59:31-36.

Nakayama, J; Hori, Y; Toshitani, S. (1993) Dermatological findings in the annual examination of the patients with Yucho in 1991-1992. Fukuoka Igaku Zasahi 84:294-299.[English Abstract].

Nakayama, J; Hori, Y; Toshitani, S. (1995) Dermatological findings in the annual examination of the patients with Yucho in 1993-1994. Fukuoka Igaku Zasahi 86:277-281.[English Abstract].

National Diabetes Data Group. (1979) Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039-1057.

National Toxicology Program (NTP). (1982a) Carcinogenesis bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Osborne-Mendel rats and B6C3F_1 mice (gavage study). Technical Report Series No. 201. Washington, DC: DHEW Publication No. (NIH) 82-1765.

National Toxicology Program (NTP). (1982b) Carcinogenesis bioassay 1 of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Swiss-Webster mice (dermal study). Technical Report Series No. 201. Washington, DC; DHEW Publication No. (NIH) 82-1757.

Neal, RA; Beatty, PW; Gasiewicz, TA. (1979) Studies of the mechanism of toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Endocrinology 101:292-296.

Needham, LL; Patterson, DG; Houk, VN. (1991) Levels of TCDD in selected human populations and their relevance to human risk assessment. In: Banbury Report 35. Biological basis for risk assessment of dioxins and related compounds. Gallo, Scheuplein, Heijden, eds. Cold Spring Harbor Laboratory Press: pp. 229-257.

Needham LL; Gerthoux PM; Patterson DG, Jr.; et al. (1997). Serum dioxin levels in Seveso, Italy, population in 1976. Teratog Carcinog Mutagen 17:225-240.

Neuberger, M; Landvoigt, W; Demt, F. (1991) Blood levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin in chemical workers after chloracne and in comparison groups. Int Arch Occup Environ Health 63:325-327.

Neubert, D; Dillman, I. (1972) Embryotoxic effects inmice treated with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Naunyn-Schmeideberg's Arch Pharmacol 272:243-264.

Neubert, R; Maskow, L; Webb, J; et al. (1993) Chlorinated dibenzo-p-dioxins and dibenzofurans and the human immune system. 1. Blood cell receptors in volunteers with moderately increased body burdens. Life Sci 53:1995-2006.

Neubert, R; Maskow, L; Delgado, I; et al. (1995) Chlorinated dibenzo-p-dioxins and dibenzofurans and the human immune system. 2. In vitro proliferation of lymphocytes from workers with quantified moderately-increased body burdens. Life Sci 56:421-436.

Norback, DH; Allen, JR. (1973) Biological responses of the nonhuman primate, chicken, and rat to chlorinated dibenzo-dioxin ingestion. Environ Health Perspect 6:233-240.

Okey AB; Giannone JV; Smart W; et al. (1997) Binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin to AH receptor in placentas from normal versus abnormal pregnancy outcomes. Chemosphere 34:1535-1547.

Oliver, RM. (1975) Toxic effects of 2,3,7,8-tetrachlorodibenzo 1,4 dioxin in laboratory workers. Br J Ind Med 32:49-53.

Olshan, AF; Mattison, DR. (1994) Male-mediated developmental toxicity. New York: Plenum Press.

Olson, JR; Holscher, MA; Neal, RA. (1980) Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the Golden Syrian hamster. Toxicol Appl Pharmacol 55:67-78.

Ott, MG; Olson, RA; Cook, RR; et al. (1987) Cohort mortality study of chemical workers with potential exposure to the higher chlorinated dioxins. J Occup Med 29:422-429.

Ott, MG; Messerer, P; Zober, A. (1993) Assessment of past occupational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin using blood lipid analyses. Int Arch Occup Environ Health 65:1-8.

Ott, MG; Zober, A; Germann, C. (1994) Laboratory results for selected target organs in 138 individuals occupationally exposed to TCDD. Chemosphere 29:2423-2437.

Ott, MG; Zober A. (1996a) Cause specific mortality and cancer incidence among employees exposed to 2,3,7,8-TCDD after a 1953 reactor accident. Occup Environ Med 53:606-612.

Ott, MG; Zober A. (1996b) Morbidity study of extruder personnel with potential exposure to brominated dioxins and furans. II. Results of clinical laboratory studies. Occup Environ Med 53:844-846.

Päpke, O; Ball, M; Lis, ZA. (1992) Various PCDD/PCDF patterns in human blood resulting from different occupational exposures. Chemosphere 25:1101-1108.

Pareschi, PL; Tomasi, F. (1989) Epidemiology of diabetes mellitus. In: Epidemiology and screening of diabetes. Morsiani, M, ed. Boca Raton, FL: CRC; pp. 77-101.

Patandin, S; Koopman-Esseboom, C; de Ridder, MA; et al. (1998) Effects of environmental exposure to polychlorinated biphenyls and dioxins on birth size and growth in Dutch children. Pediatr Res 44:538-545.

Patandin, S; Lanting, CI; Mulder, PGH; et al. (1999) Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J Pediatr 134:33-41.

Patterson, DG; Holler, JS; Lapeza, CR; et al. (1986a) High-resolution gas chromatography/high-resolution mass spectrometric analysis of human adipose tissue for 2,3,7,8-tetrachlorodibenzo-p-dioxin. Anal Chem 58:705-713.

Patterson, DG; Hoffman, RE; Needham, LL; et al. (1986b) 2,3,7,8-tetrachlorodibenzo-p-dioxin levels in adipose tissue of exposed and control persons in Missouri. JAMA 256:2683-2686.

Patterson, DG, Jr.; Fingerhut, MA; Roberts, DW; et al. (1989) Levels of polychlorinated dibenzo-p-dioxins and dibenzofurans in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Am J Ind Med 16:135-146.

Pauwels, A; Cenijn, P; Covaci, A; et al. (1999) Analysis of PCB congeners by (GC-ECD) and dioxin-like toxic equivalence (by CALUX assay) in females with endometriosis and other fertility problems. Organohalogen Compounds 44:407-410.

Pazdernik, TL; Kozman, KK. (1985) Effect of thyroidectomy and thyroxine on 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced immunotoxicity. Life Sci 36:695-703.

Pazderova-Vejlupkova, J; Nemcova, M; Pickova, J; et al. (1981) The development and prognosis of chronic intoxication by tetrachlorodibenzo-p-dioxin in man. Arch Environ Health 36:5-11.

Pearn, JH. (1983) Teratogens and the male. Med J Aust 2:16-20.

Pesatori, AC; Zocchetti, C; Guercilena, S; et al. (1998) Dioxin exposure and non-malignant health effects: a mortality study. Occup Environ Med 55:126-131.

Phuong, NTN; Thuy, TT; Phuong, PK. (1989a) An estimate of differences among women giving birth to deformed babies and among those with hydatidiform mole seen at the OB-GYN hospital of Ho Chi Minh City in the south of Vietnam. Chemosphere 18:801-803.

Phuong, NTN; Hung, BS; Vu, DQ; et al. (1989b) Dioxin levels in adipose tissue of hospitalized women living in the south of Vietnam 1984-85 with a brief review of their clinical histories. Chemosphere 19:933-936.

Piacitelli, LA; Marlow, DA. (1997) NIOSH 2,3,7,8-tetrachlorodibenzo-p-dioxin matrix. 33:510-514.

Piacitelli, LA; Sweeney, M.H; Patterson, DG; et al. (1992) Serum levels of 2,3,7,8-substituted PCDDs and PCDFs among workers exposed to 2,3,7,8-TCDD contaminated chemicals. Chemosphere 25:251-254.

Pirkle, JL; Wolfe, WH; Patterson, DG, Jr.; et al. (1989) Estimates of the half-life of 2,3,7,8-TCDD Vietnam veterans of Operation Ranch Hand. J Toxicol Environ Health 27:165-171.

Pluim, HJ; Koppe, JG; Olie, K; et al. (1992) Effects of dioxins on thyroid function in newborn babies. Letter to the Editor. Lancet 339:1303.

Pluim, HJ; de Vijlder, JJM; Olie, K; et al. (1993) Effects of pre- and postnatal exposure to chlorinated dioxins and furans on human neonatal thyroid hormone concentrations. Environ Health Perspect 101(6):504-508.

Pluim, HJ; Koppe, JG; Olie, K; et al. (1994) Clinical laboratory manifestations of exposure to background levels of dioxins in the perinatal period. Acta Paediatr 83(6):583-587.

Pluim, HJ; van der Goot, M; Olie K; et al. (1996) Missing effects of background dioxin exposure on development of breast- fed infants during the first half year of life. Chemosphere 33:1307-1315.

Pocchiari, F; Silvano, V; Zampieri, A; et al. (1979) Human health effects from accidental release of tetrachlorodibenzo-p-dioxin (TCDD) at Seveso, Italy. Ann NY Acad Sci 77:311-320.

Pocchiari, F. (1980) Accidental TCDD contamination in Seveso (Italy): epidemiological aspects. FIFRA Docket No. 415, Exhibit 1469.

Pocchiari, F; Silano, V; Zampieri, A. (1980) Human health effects from accidental release of TCDD at Seveso (Italy). FIFRA Docket No. 415, Exhibit 1470.

Poland, A; Glover, E. (1980) 2,3,7,8-tetrachlorodibenzo-p-dioxin: segregation of toxicity with the Ah locus. Mol. Pharmacol. 17:86-94.

Poland, AP; Smith, D; Metter, G; et al. (1971) A health survey of workers in a 2,4-D and 2,4,5-T plant. Arch Environ Health 22:316-327.

Poli, A; Francheschini, L; Puglist, L; et al. (1980) Increased total and high-density lipoprotein cholesterol with apoprotein changes resembling streptozotoxin diabetes in tetrachlorodibenzodioxin (TCDD)-treated rats. Biochem Pharmacol 29:835-838.

Potter, CL; Sipes, GI; Russel, HD. (1983) Hypothyroxinemia and hypothermia in rats in response to 2,3,7,8-tetrachlorodibenzo-p-dioxin administration. Toxicol Appl Pharmacol 69:89-95.

Potter, CL; Moore, RW; Inhorn, SL; et al. (1986) Thyroid status and thermogenesis in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 84:45-55.

Reggiani, G. (1978) Medical problems raised by the TCDD contamination in Seveso, Italy. Arch Toxicol 40:161-188.

Reggiani, G. (1980a) Acute human exposure to TCDD in Seveso, Italy. J Toxicol Environ Health 6:27-43.

Reggiani, G. (1980b) Direct testimony before the US EPA. FIFRA Docket No. 415, Exhibit 861.

Rehder, H; Sanchioni, L; Cefis, F; et al. (1978) Pathological-embryological investigations in cases of abortion related to the Seveso accident. J Swiss Med 108(42):1617-1625.

Report to the Minister for Veterans' Affairs. (1983) Case-control study of congenital anomalies and Vietnam service. Canberra: Australian Government Printing Service.

Rier, SE; Martin, D; Bowman, RE; et al. (1993) Endometriosis in rhesus monkeys (Macaca mulatta)following chronic exposure to 2,3,7,8 tetrachlorodibenzo-p-dioxin. Fundam Appl Toxicol 21:433-441.

Roegner, RH; Grubbs, WD; Lustik, MB; et al. (1991) Air Force Health Study: an epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides. Serum dioxin analysis of 1987 examination results. NTIS# AD A-237-516 through AD A-237-524.

Rogan, WJ. (1982) PCBs and cola-colored babies: Japan 1968 and Taiwan 1979. Teratology 26:259-261.

Rogan, W. (1989) Yu-Cheng. In: Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Kimbrough, RD; Jensen, AA, eds. 2nd ed. New York: Elsevier Pub.; pp. 401-415.

Rogan, WJ; Gladen, BC; Guo ,YL; et al. (1999) Sex ratio after exposure to dioxin-like chemicals in Taiwan [letter]. Lancet 353:206-207.

Rogan, WJ; Gladen, BC; Hung, K-L; et al. (1988) Congenital poisoning by polychlorinated biphenyls and thair contaminants in Taiwan. Science 241:334-6.

Romkes, N; Safe, S. (1988) Comparative activities of 2,3,7,8-tetrachlorodibenzo-p-dioxin and progesterone as antiestrogens in the female rat uterus. Toxicol Appl Pharmacol 92:368-380.

Romkes, N; Piskorska-Pliszynska, J; Safe, S. (1987) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on hepatic and uterine estrogen receptor levels in rats. Toxicol Appl Pharmacol 87:306-314.

Roth, W; Voorman, R; Aust, SD. (1988) Activity of thyroid hormone-inducible enzymes following treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 92:65-74.

Rozman, K; Rozman, T; Greim, H. (1984) Effect of thyroidectomy and thyroxine on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced toxicity. Toxicol Appl Pharmacol 72:372-376.

Rozman, K; Rozman, T; Scheufler, E; et al. (1985) Thyroid hormones modulate the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). J Toxicol Environ Health 16:481-491.

Ruangwies, S; Bestervelt, LL; Piper, DW; et al. (1991) Human chorionic gonadotropin treatment prevents depressed 17-α-hydroxylase/C17-20 lyase activities and serum testosterone concentrations in 2,3,7,8-tetrachlorodibenzo-p-dioxin-treated rats. Biol Reprod 45:143-150.

Schaum, J; Winters, D; Phillips, L; Lorber, M. (1999) TEQ Doses for CDD/Fs and PCBs General Population Exposure to Dioxin-Like Compounds in the United States During the 1990s. Organohalogen Compounds 44:181-184.

Schecter, A; Constable, JD; Bangerf, JV; et al. (1989) Elevated body burdens of 2,3,7,8-tetrachlorodibenzo-p-dioxin in adipose tissue of U.S. Vietnam veterans. Chemosphere 18:431-438.

Schiller, CM; King, MW; Walden, R. (1986) Alterations in lipid parameters associated with changes in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced mortality in rats. In: Chlorinated dioxins and dibenzofurans in perspective. Rappe, C; Choudhary, G; Keith, LH, eds. Chelsea, MI: Lewis Pub.; pp. 285-302.

Schnorr TM, Lawson CC, Whelan EA, Dankovic DA, Deddens JA, Piacitelli LA, Reefhuis J, Sweeney MH, Connally LB, Fingerhut MA. (`) Spontaneous abortion, sex ratio and paternal occupational exposure to 2,3,7,8 tetrachlorodibenzo-p-dioxin. In press, Environmental Health Perspectives.

Singer, R; Moses, M; Valciukas, J; et al. (1982) Nerve conduction velocity studies of workers employed in the manufacture of phenoxy herbicides. Environ Res 29:297-311.

Smith, AG; Francis, JE; Kay, SJE; et al. (1981) Hepatic toxicity and uroporphyrinogen decarboxylase activity following a single dose of 2,3,7,8-tetrachloro-dibenzo-p-dioxin to mice. Biochem Pharmacol 30:2825-2830.

Smith, AH; Fisher, DO; Pearce, N; et al. (1982) Congenital defects and miscarriages among New Zealand 2,4,5-T sprayers. Arch Environ Health 37:197-200.

Smith, AH; Patterson, DG, Jr.; Warner, ML; et al. (1992) Serum 2,3,7,8-tetrachlorodibenzo-p-dioxin levels of New Zealand pesticide applicators and their implications for cancer hypotheses. J Natl Cancer Inst 84:104-108.

Steenland, K; Piacitelli, L; Deddens, J; et al. (1999) Cancer, heart disease, and diabetes in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin [see comments]. J Natl Cancer Inst 91:779-786.

Stehr, PA; Stein, G; Falk, H; et al. (1986) A pilot epidemiologic study of possible health effects associated with 2,3,7,8-tetrachlorodibenzo-p-dioxin contaminations in Missouri. Arch Environ Health 41:16-22.

Stellman, SD; Stellman, JM; Sommer, JF. (1988) Health and reproductive outcomes among American Legionnaires in relation to combat and herbicide exposure in Vietnam. Environ Res 2:150-174.

Stockbauer, JW; Hoffman, RE; Schramm, WF; et al. (1988) Reproductive outcomes of mothers with potential exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Am J Epidemiol 128:410-419.

Strik, JJTWA. (1979) The occurrence of chronic-hepatic porphyria in man caused by halogenated hydrocarbons. In: Chemical porphyria in man. Strik, JJTWA; Koeman, JH, eds New York: Elsevier/North-Holland; pp. 3-9.

Suskind, RR. (1985) Chloracne, "the hallmark of dioxin intoxication." Scand J Work Environ Health 11:165-171.

Suskind, RR; Hertzberg, VS. (1984) Human health effects of 2,4,5-T and its toxic contaminants. JAMA 251:2372-2380.

Suskind, R; Cholak, J; Schater, LJ; et al. (1953) Reports on clinical and environmental surveys at Monsanto Chemical Co., Nitro, West Virginia, 1953. Cincinnati, OH: Department of Environmental Health, University of Cincinnati (unpublished).

Sweeney, GD. (1986) Porphyria cutanea tarda, or the uroporphyrinogen decarboxylase deficiency disease. Clin Biochem 19:3-15.

Sweeney, AM; Meyer, MR; Aarons, JH; et al. (1988) Evaluation of methods for the prospective identification of early fetal losses in environmental epidemiology. Am J Epidemiol 127:843-850.

Sweeney, MH; Fingerhut, MA; Connally, LB; et al. (1989) Progress of the NIOSH cross-sectional medical study of workers occupationally exposed to chemicals contaminated with 2,3,7,8-TCDD. Chemosphere 19:973-977.

Sweeney, MH; Fingerhut, MA; Patterson, DG; et al. (1990) Comparison of serum levels of 2,3,7,8-TCDD in TCP production workers and in an unexposed comparison group. Chemosphere 20:993-1000.

Sweeney, MH; Hornung, RW; Wall, DK; et al. (1992) Prevalence of diabetes and increased fasting serum glucose in workers with long-term exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Presented at: 12th International Symposium on Dioxins and Related Compounds; August 24-28; Tampere, Finland.

Sweeney, MH; Fingerhut, MA; Arezzo, JC; et al. (1993) Peripheral neuropathy after occupational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Am J Ind Med 23:845-858.

Swift, LL; Gasiewicz, TA; Dewey Dunn, G; et al. (1981) Characterization of the hyperlipemia in guinea pigs induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 59:489-499.

Tanaka, K; Tsukazaki, N; Yoshida, H; et al. (1995) Polychlorinated biphenyls (PCBs) and polychlorinated quaterphenyls (PCQs) concentrations in skin surface lipids and blood of patients with Yusho. Fukuoka Igaku Zasahi 86:202-206.[English Abstract].

Taylor, JS. (1979) Environmental chloracne: update and overview. Ann NY Acad Sci 320:295-407.

Tenchini, ML; Cimaudo, C; Pucchetti, G; et al. (1983) A comparative cytogenetic study on cases of induced abortions in TCDD-exposed and nonexposed women. Environ Mutagen 5:73-85.

Theobold, HM; Peterson, RE (1994) Developmental and reproductive toxicity of dioxins and ah-receptor agonists. In: Dioxins and health. Schecter, A; Constable, JD; Bangers, JV; et al., eds. New York: Plenum Press; pp. 309-346.

Thomas, WF; Grubbs, WD; Karrison TG; et al. (1990) The Air Force Health Study. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. 1987 Followup Examination Results. NTIS (AD A 222 304, AD A 222 573): Springfield VA.

Tonn, T; Esser, C; Schneider, EM; et al. (1996) Persistence of decreased T-helper cell function in industrial workers 20 years after exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ Health Perspect 104:422-426.

Townsend, JC; Bodner, KM; van Peenen, PFD; et al. (1982) Survey of reproductive events of wives of employees to chlorinated dioxins. Am J Epidemiol 115:695-713.

Tsuji, H; Ikeda, K; Suzuki, N; et al. (1995) Laboratory findings in patients with Yusho: 26 year follow-up study. Fukuoka Igaku Zasahi 86:273-276.[English Abstract].

Tuchmann-Duplessis, H. (1977) Embryo problems posed by the Seveso accident. Le Concours Medical No. 44, November 26.

Tuchmann-Duplessis, H. (1980a) Direct testimony before the US EPA. FIFRA Docket No. 415, Exhibit 864.

Tuchmann-Duplessis, H. (1980b) Tables in direct testimony before the US EPA. FIFRA Docket No. 415, Exhibit 864a.

Urabe, H; Asahi, M. (1985) Past and current dermatological status of Yusho patients. Environ Health Perspect 59:11-15.

van Miller, JP; Lalich, JJ; Allen, JR. (1977) Increased incidence of neoplasms in rats exposed to low levels of 2,3,7,8-tetrachlorodibenzodioxin. Chemosphere 10:625-632.

Vartiainen, T; Jaakkola, JJ; Saarikoski, S; et al. (1998) Birth weight and sex of children and the correlation to the body burden of PCDDs/PCDFs and PCBs of the mother. Environ Health Perspect 106:61-66.

Vena, J; Boffetta, P; Becher, H; et al. (1998) Exposure to dioxin and nonneoplastic mortality in the expanded IARC international cohort study of phenoxy herbicide and chlorophenol production workers and sprayers. Environ Health Perspect 106 Suppl 2:645-653.

Vos, JG; Moore, JA; Zinkl, JG. (1974) Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in C57B1/6 mice. Toxicol Appl Pharmacol 29:229-241.

Walker, AE; Martin, JV. (1979) Lipid profiles in dioxin-exposed workers [letter]. Lancet i:446-447.

Webb, KB; Evans, RG; Knudsen, DP; et al. (1989) Medical evaluation of subjects with known body levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Toxicol Environ Health 28:183-193.

Weisglas-Kuperus, N; Sas, TCJ; Koopman-Esseboom, C; et al. (1995) Immunologic effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in Dutch infants. Pediatr Res 38:404-410.

Weisglas-Kuperus N; Patandin S; Berbers GAM; Sas TCJ: Mulder PGH; Sauer PJJ; Hooijkaas H. (2000) Immunologic Effects of Background Exposure to Polychlorinated Biphenyls and Dioxins in Dutch Preschool Children. Environmental Health Perspectives 108:1203-1207.

Wilcox, AJ; Weinberg, CR; O'Connor, JF; et al. (1988) Incidence of early loss of pregnancy. New Engl J Med 319:189-194.

Wilson, GL; LeDoux, SP. (1989) The role of chemicals in the etiology of diabetes mellitus. Toxicol Pathol 17:357-363.

Wolfe, WH; Michalek, JE; Miner, JC; et al. (1988) Serum 2,3,7,8-tetrachlorodibenzo-p-dioxin levels in Air Force Health Study personnel. MMWR 37:309-311.

Wolfe, WH; Michalek, JE; Miner, JC. (1992a) Diabetes versus dioxin body burden in veterans of Operation Ranch Hand. Presented at: 12th International Symposium on Chlorinated Dioxins; August 24-28; Tampere, Finland.

Wolfe, WH; Michalek, JE; Miner, JC; et al. (1992b) Air Force Health Study. An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides. Reproductive outcomes. Brooks Air Force Base, TX: Epidemiology Research Division, Armstrong Laboratory, Human Systems Division (AFSC).

Wolfe, WH; Michalek, JE; Miner, JC; et al. (1995) Paternal serum dioxin and reproductive outcomes among veterans of Operation Ranch Hand. Epidemiology 6:17-22.

Yamashita, F; Hayashi, M. (1985) Fetal PCB syndrome: clinical features, intrauterine growth retardation and possible alteration in calcium metabolism. Environ Health Perspect 59:41-45.

Yen, YY; Lan, SJ; Ko, YC; et al. (1989) Follow-up study of reproductive hazards of multiparous women consuming PCBS-contaminated rice oil in Taiwan. Bull Environ Contam Toxicol 43:647-655.

Yoon, JW; Kim, CJ; Pak, CY; et al. (1987) Effects of environmental factors on the development of insulin-dependent diabetes mellitus. Clin Invest Med 10:457-469.

Yu, ML; Hsu, CC; Gladen, BC; et al. (1991) *In utero* PCB/PCDF exposure: relation of developmental delay to dysmorphology and dose. Neurotoxicol Teratol 13:195-202.

Yu, ML; Hsu, CC; Guo, YL; Lai, TJ; Chen, SJ; Luo, JM. (1994) Disordered behavior in the early-born Taiwan Yucheng children. Chemosphere 29:2413-2422.

Yu et al. (1997) Increased mortality from chronic liver disease and cirrhosis. AJIM 31(2):172-175.

Yu, ML; Hsin, JW; Hsu, CC; Chan, WC; Guo, YL. (1998) The immunologic evaluation of the Yucheng children. Chemosphere 37:1855-1865.

Yu, ML, Gui YL, Hsu, CC, Rogan WJ. (2000) Menstruation and reproduction in women with polychlorinated biphenyl (PCB) poisoning: long term follow-up interviews of the women from the Taiwan Yucheng cohort. International Journal of Epidemiology. 29:672-677.

Zack, JA; Suskind, RR. (1980) The mortality experience of workers exposed to tetrachlorodibenzodioxin in a trichlorophenol process accident. J Occup Med 22(1):11-14.

Zinkl, JG; Vos, JG; Moore, JA; et al. (1973) Hematologic and clinical chemistry effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environ Health Perspect 5:111-118.

Zober, A; Messerer, P; Huber, P. (1990) Thirty-four-year mortality follow-up of BASF employees exposed to 2,3,7,8-TCDD after the 1953 accident. Int Arch Occup Environ Health 62:139-157.

Zober, MA; Ott, MG; Päpke, O; et al. (1992) Morbidity study of extruder personnel with potential exposure to brominated dioxins and furans. I. Results of blood monitoring and immunological tests. Br J Ind Med 49:532-544.