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Provisional Peer-Reviewed Toxicity Values for

1,2,4-Trichlorobenzene
(CASRN 120-82-1)

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COMMONLY USED ABBREVIATIONS

BMD	Benchmark Dose
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
RfC	inhalation reference concentration
RfD	oral reference dose
UF	uncertainty factor

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR 1,2,4-TRICHLOROBENZENE (CASRN 120-82-1)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (U.S. EPA) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. U.S. EPA's Integrated Risk Information System (IRIS).
2. Provisional Peer-Reviewed Toxicity Values (PPRTVs) used in U.S. EPA's Superfund Program.
3. Other (peer-reviewed) toxicity values, including:
   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in U.S. EPA's IRIS. PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the U.S. EPA IRIS Program. All provisional toxicity values receive internal review by two U.S. EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all U.S. EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a 5-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV documents conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and Resource Conservation and Recovery Act (RCRA) program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV document and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other U.S. EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

Trichlorobenzene, specifically 1,2,4-trichlorobenzene CASRN 120-82-1, is a high-production-volume (HPV) chemical listed in U.S. EPA's toxic release inventory (TRI) database. Existing toxicity reference values include a chronic RfD for 1,2,4-trichlorobenzene (verified in November 1996) which is available in IRIS (U.S. EPA, 2008). The RfD of 0.01 mg/kg-day is based on increased adrenal weights and vacuolization of the *zona fasciculata* in the adrenal cortex in a multigenerational rat reproductive toxicity study (Robinson et al., 1981). Rats were exposed from birth through three generations; a NOAEL of 14.8 mg/kg-day was identified (Robinson et al., 1981). Uncertainty factors (UF), of 10 each, for interspecies extrapolation, protection of sensitive humans, and lack of chronic studies were applied to the NOAEL to derive the RfD. The two source documents were (1) the U.S. EPA (1985, 1988) Health Assessment Document (HAD) for Chlorinated Benzenes and (2) the Drinking Water Criteria Document (DWCD) for Trichlorobenzenes. The Drinking Water Standards and Health Advisories list (U.S. EPA, 2006) includes the same chronic RfD of 0.01 mg/kg-day as reported on IRIS. The Health Effects Assessment Summary Tables (HEAST; U.S. EPA, 1997) reports a subchronic RfD of 0.01 mg/kg-day for 1,2,4-trichlorobenzene, adopting the chronic RfD from IRIS as the subchronic RfD.

IRIS (U.S. EPA, 2008) does not report an RfC for 1,2,4-trichlorobenzene. The HEAST (U.S. EPA, 1997) lists chronic and subchronic RfCs of 0.2 and 2.0 mg/m^3 (respectively). The RfCs were based on 6- and 26-week rat, rabbit, dog and monkey inhalation studies by Kociba et al. (1981) and Coate et al. (1977). A nominal NOAEL of 104 ppm (772 mg/m^3) and total uncertainty factors of 100 and 1000 were used to derive the subchronic and chronic RfCs (respectively). No U.S. EPA source document for this assessment was listed.

Relevant documents in the Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991a, 1994a) include a Health Effects Assessment (HEA) for 1,2,4-Trichlorobenzene (U.S. EPA, 1987) in addition to the previously mentioned HAD for Chlorinated Benzenes (U.S. EPA, 1985) and DWCD for Trichlorobenzenes (U.S. EPA, 1988). The HEA includes chronic and subchronic "inhalation RfDs" of 0.18 and 1.75 mg/day for 1,2,4-trichlorobenzene based on a NOAEL of 3 ppm (22 mg/m^3) in a rat study by Watanabe et al. (1978); a LOAEL of 10 ppm (74 mg/m^3) was identified for increased urinary porphyrin excretion in this study. However, this derivation is not consistent with current U.S. EPA (1994b) methodology for RfC derivation.

The American Conference of Governmental Industrial Hygienists (ACGIH, 2007) has adopted a Short-Term Exposure Limit (STEL) ceiling of 5 ppm (~40 mg/m^3) for 1,2,4-trichlorobenzene. This value is intended to minimize the potential for ocular and upper respiratory tract irritation in exposed workers (ACGIH, 2007). The National Institute for Occupational Safety and Health (NIOSH, 2008) also recommends a ceiling limit of 5 ppm (~40 mg/m^3) for 1,2,4-trichlorobenzene based on the same effects. The Occupational Safety and Health Administration (OSHA, 2008) has no permissible exposure limit (PEL) for 1,2,4-trichlorobenzene.

In the IRIS, 1,2,4-trichlorobenzene is assigned to Weight-of-Evidence (WOE) Group D (not classifiable as to human carcinogenicity), last revised in 1991, based on the U.S. EPA (1986) Guidelines for Carcinogen Risk Assessment. The same classification is shown in the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). A cancer assessment for 1,2,4-trichlorobenzene is not included in the HEAST (U.S. EPA, 1997). The Health Assessment Document for Chlorinated Benzenes (U.S. EPA, 1985) and Health Effects Assessment for 1,2,4-Trichlorobenzene (U.S. EPA, 1987) also assigned the chemical to Group D.

The National Toxicology Program (NTP, 2008) has not assessed the toxicity or carcinogenicity of 1,2,4-trichlorobenzene and this compound is not included in the 11th Report on Carcinogens (NTP, 2005). 1,2,4-Trichlorobenzene has not been the subject of a monograph by the International Agency for Research on Cancer (IARC, 2008) or a toxicological profile by the Agency for Toxic Substances Disease Registry (ATSDR, 2008). An Environmental Health Criteria document for chlorinated benzenes (WHO, 1991), a Priority Substances List Assessment Report on trichlorobenzenes (Health Canada, 1993), and a toxicity review on halogenated benzenes (Leber and Bus, 2001) were consulted for relevant information. To identify toxicological information pertinent to the derivation of provisional toxicity values for 1,2,4-trichlorobenzene, literature searches were conducted in December 2007 using the following databases: MEDLINE, TOXLINE, BIOSIS (August 2000−December 2007), TSCATS1/2, CCRIS, DART/ETIC, GENETOX, HSDB, RTECS, and Current Contents (first half of 2008). Except where noted, the literature searches were not limited by date.

REVIEW OF PERTINENT DATA

Human Studies

No data were located in the review documents (WHO, 1991; Health Canada, 1993; Leber and Bus, 2001) or the literature search (see above) regarding the effects of 1,2,4-trichlorobenzene in humans exposed by any route.

Animal Studies

Oral Exposure

Subchronic Studies—The Chemical Manufacturers Association (CMA) (1989a) conducted a subchronic dietary study of 1,2,4-trichlorobenzene (99% pure) in F344 rats. Groups of 10 rats/sex/dose were given diets containing nominal concentrations of 0, 200, 600, or, 1800 ppm for 94 days. Due to the volatility of the test compound, the investigators mixed diets containing 110% of the target concentrations; diets were stored frozen and changed twice each week. Laboratory analyses of the diet showed that the mean concentrations were ±4% of the target concentrations. Based on estimates of weekly compound intake provided by the authors, average intakes over the study duration were 0, 14.6, 45.6, and, 133.7 mg/kg-day in males and 0, 17.0, 52.5, and 150.6 mg/kg-day in females. Daily observations for clinical signs were made, and both body weight and food consumption were recorded weekly. All rats were subjected to ophthalmoscopic examination prior to termination. Blood was collected prior to sacrifice for analysis of hematology (Hgb, Hct), erythrocyte count and morphology, platelet count and total and differential leukocyte counts) and serum chemistry (alanine aminotransferase [ALT], aspartate aminotransferase [AST], blood urea nitrogen [BUN], glucose, total protein, albumin, creatinine, total bilirubin, electrolytes, calcium and phosphorus). All rats received complete necropsies and the adrenals, brain, heart, kidneys, liver, testes with epididymides (males) and ovaries (females) were weighed. Complete histopathology examinations (30 tissues) were performed on control and high-dose rats of both sexes, and the kidney and liver were examined microscopically in all dose groups.

There were no deaths during the study (CMA, 1989a). Treated rats exhibited a higher incidence of chromodacryorrhea (red tears) and excessive lacrimation than controls, but these effects did not exhibit a clear dose-response relationship. Body weights were not different from controls in the treated animals, although food consumption was increased in mid- and high-dose males. Ophthalmoscopy was not affected by treatment. Table 1 shows the hematology and clinical chemistry findings. A borderline anemia was evident in high-dose males, with a trend toward this effect in high-dose females as well. Mean hemoglobin and Hct values were significantly reduced in both sexes at the high dose, and mean erythrocyte count was also reduced in high-dose males. Platelet and total leukocyte counts were increased in high-dose males. Clinical chemistry parameters affected by treatment included increases in BUN, total protein, calcium and albumin in high-dose males; decreased AST levels in mid- and high-dose males; and increased BUN in high-dose females. The authors reported that individual BUN values were within the normal range of variability for this species.

Table 1. Significant Changes in Rats Treated with 1,2,4-Trichlorobenzene in the Diet for 13 Weeks[a]

Males				
	Control	**200 ppm (14.6 mg/kg-d)**	**600 ppm (45.6 mg/kg-d)**	**1800 ppm (133.7 mg/kg-d)**
Hematology				
Erythrocyte count (10^6/μL)	8.55 ± 0.16[b]	8.56 ± 0.17	8.51 ± 0.21	8.12 ± 0.22[d]
Hematocrit (%)	50 ± 1	50 ± 1	49 ± 1	47 ± 1[d]
Hemoglobin (g/dL)	16.9 ± 0.2	16.9 ± 0.2	16.7 ± 0.4	15.7 ± 0.4[d]
Platelets (10^5/μL)	6.72 ± 0.32	6.71 ± 0.27	6.96 ± 0.45	7.80 ± 0.27[d]
Total leukocyte count (10^3/μL)	7.5 ± 1.0	7.9 ± 1.1	8.3 ± 0.9	8.9 ± 0.9[c]
Clinical Chemistry				
AST (IU/L)	84 ± 19	77 ± 7	66 ± 9[c]	61 ± 5[d]
BUN (mg/dL)	17.3 ± 1.5	16.2 ± 1.7	16.3 ± 1.0	19.3 ± 1.5[c]
Protein (g/dL)	6.3 ± 0.2	6.3 ± 0.2	6.5 ± 0.1	7.3 ± 0.2[d]
Albumin (g/dL)	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.4 ± 0.1[d]
Calcium (mg/dL)	10.3 ± 0.2	10.3 ± 0.3	10.4 ± 0.2	11.1 ± 0.3[d]
Organ Weights				
Adrenal weight (g)	0.0425 ± 0.0063	0.0805 ± 0.1124	0.0406 ± 0.0070	0.0509 ± 0.0060[c]
Adrenal/body weight (× 10,000)	1.57 ± 0.19	3.03 ± 4.41	1.53 ± 0.25	1.84 ± 0.17[c]
Kidney weight (g)	1.932 ± 0.163	2.030 ± 0.181	2.113 ± 0.185	2.455 ± 0.199[d]
Kidney/body weight (× 1000)	7.13 ± 0.36	7.45 ± 0.31	7.92 ± 0.39[d]	8.85 ± 0.28[d]
Liver weight (g)	7.116 ± 0.596	7.936 ± 0.615[c]	8.615 ± 0.678[d]	11.876 ± 1.063[d]
Liver/body weight (× 100)	2.63 ± 0.13	2.91 ± 0.10[d]	3.23 ± 0.14[d]	4.28 ± 0.17[d]
Testes weight (g)	2.884 ± 0.072	2.989 ± 0.241	2.877 ± 0.129	3.059 ± 0.073[d]
Histopathology				
Centrilobular hepatocyte hypertrophy	0/10[e]	0/10	5/10[f]	10/10[g]
Renal dilated tubules	0/10	0/10	0/10	10/10[g]
Renal granular casts	0/10	0/10	0/10	10/10[g]
Renal hyaline droplets	0/10	0/10	10/10[g]	10/10[g]
Interstitial nephritis	0/10	1/10	3/10	9/10[g]

Table 1. Significant Changes in Rats Treated with 1,2,4-Trichlorobenzene in the Diet for 13 Weeks[a]

Females				
	Control	**200 ppm (17.0 mg/kg-d)**	**600 ppm (52.5 mg/kg-d)**	**1800 ppm (150.6 mg/kg-d)**
Hematology				
(%)Hematocrit	50 ± 1	50 ± 1	50 ± 1	48 ± 1[c]
Erythrocyte count(10^6/uL)	8.09 ± 0.23	7.98 ± 0.15	8.00 ± 0.22	7.89 ± 0.11
Hemoglobin (g/dL)	17 ± 0.4	16.8 ± 0.4	16.8 ± 0.3	16.4 ± 0.1[c]
Clinical Chemistry				
BUN (mg/dL)	16.2 ± 1.3	17.2 ± 1.8	18.2 ± 1.8	19.4 ± 2.1[d]
Organ Weights				
Kidney/body weight (×1000)	7.76 ± 0.31	7.83 ± 0.45	8.34 ± 0.39[c]	8.34 ± 0.59[c]
Liver weight (g)	4.139 ± 0.298	4.278 ± 0.101	4.863 ± 0.408[d]	5.381 ± 0.294[d]
Liver/body weight (×100)	2.63 ± 0.15	2.71 ± 0.10	3.14 ± 0.37[d]	3.54 ± 0.20[d]
Histopathology				
Centrilobular hepatocyte hypertrophy	0/10	0/10	0/10	10/10[g]
Renal tubular mineral deposition	9/10	10/10	10/10	10/10

[a]CMA, 1989a
[b]Mean ± standard deviation
[c]Significantly different from control at $p < 0.05$
[d]$p < 0.01$
[e]Number affected/number examined
[f]$p < 0.05$ by Fischer's exact test conducted for this review
[g]$p < 0.0001$ by Fischer's exact test conducted for this review

Significant changes in organ weights were observed in the adrenal glands, liver, kidneys, and testes of male rats and in the kidneys and liver of female rats (CMA, 1989a). Table 1 details the organ-weight changes in all treatment groups. As the table indicates, absolute and relative adrenal weights were increased over control values (20% and 17%, respectively) in the high-dose males but not in females. Absolute and relative liver weights were significantly increased in males at all doses (with >60% increases at the high dose) and in females exposed to 600 or 1800 ppm (~30−35% increase at the high dose). Absolute kidney weight was increased in high-dose males but not at any dose in females; relative kidney weight was increased in both sexes at doses ≥600 ppm. Testes weights were modestly increased in high-dose males. The histopathology findings were consistent with the liver and kidney weight changes (see Table 1). Centrilobular hepatocyte hypertrophy was observed in all rats of both sexes at the high dose and in some mid-dose males, and may underlie the changes in observed organ weights. The authors characterized this finding as "minimal" in females and mid-dose males and "mild" in high-dose males, but severity scores are not reported. Kidney findings were largely restricted to males, although there was a suggestion of increased severity (but not increased incidence) of kidney tubular mineral deposition in high-dose females. All high-dose males exhibited dilated renal tubules and granular casts. Hyaline droplets were observed in all mid- and high-dose males. Finally, a dose-related increase in the incidence of interstitial nephritis was reported in male rats.

The constellation of kidney changes occurring only in male rats (especially hyaline droplets, dilated tubules, granular casts) is suggestive of male rat-specific alpha 2μ-globulin nephropathy, an endpoint that is not considered relevant to human health (U.S. EPA, 1991b). The absence of kidney effects in mice treated subchronically (CMA, 1989b) or chronically (with similar dosing ranges) (CMA, 1994b) and the finding of related kidney histopathology in male rats treated via the diet for 104 weeks (renal pelvis mineralization, transitional cell hyperplasia, increased severity of chronic progressive nephropathy; CMA, 1994a) or via inhalation for 26 weeks ("hyaline degeneration"; Coate et al., 1977) provide support for a potential relationship with alpha 2μ-globulin nephropathy. In female rats in this study (CMA, 1989b), kidney weights were increased and there was a suggestion of increased severity of renal tubular mineralization. In female rats exposed chronically via the diet, a statistically significant increase in the incidence of renal pelvis mineralization was observed (CMA, 1994a). The biological significance of these slight changes is uncertain. In the absence of data demonstrating the accumulation of alpha 2μ-globulin, it is not possible to conclusively demonstrate this mechanism. However, the closely related compound, 1,4-dichlorobenzene, is considered a model inducer of alpha 2μ-globulin nephropathy (U.S. EPA, 1991b). As available data provide suggestive support for a finding that the male rat kidney effects may not be relevant to humans, these effects are not considered as the basis for the LOAEL.

The authors identified the low dose (200 ppm) as a NOAEL for female rats and reported that a NOAEL for male rats was not identified (CMA, 1989a). The effect occurring at the low dose was an 11% increase in liver weights only in male rats. In the absence of accompanying histopathology, this response may be considered an adaptive physiologic change. However, at the mid-dose (600 ppm) the incidence of hepatocellular hypertrophy was increased in males and liver weights were increased significantly (~20%) in both sexes. U.S. EPA (2002) considers these two endpoints to be adverse when there is a known mode of action (MOA) for toxicity. In the case of 1,2,4-trichlorobenzene, there is evidence to suggest that liver enlargement is related to induction of liver enzymes that may result in secondary toxicity. Specifically, 1,2,4-trichlorobenzene is a potent inducer of liver enzymes, including cytochrome P-450,

cytochrome c reductase, benzpyrene hydroxylase, azoreductase, aniline hydroxylase, aminopyrine demethylase, heme oxygenase, and others (Carlson and Tardiff, 1976; Black et al., 1988; Cote et al., 1988; Ariyoshi et al., 1981). Induction of liver enzymes may be associated with secondary toxic effects (U.S. EPA, 2002). Several studies have shown toxic effects of 1,2,4-trichlorobenzene that may be attributable to enzyme induction: increased excretion of porphyrins (Carlson, 1977; Kociba et al., 1981; Watanabe et al., 1977, 1978), hematologic effects (CMA, 1989a,b; Black et al., 1988), reduced circulating levels of thyroid hormones (den Besten et al., 1991a) and thyroid histopathology (Cote et al., 1988; Black et al., 1988). Porphyrinuria may be the result of the induction of liver enzymes and/or disruption of liver function and heme metabolism and, as such, may be related to observations of anemia. Alterations in hepatic thyroxine metabolism may contribute to the reduction in circulating thyroid hormone levels and induction of thyroid histopathology. For the purpose of this review, based on the suggestive evidence that induction of liver enzymes by 1,2,4-trichlorobenzene may be associated with secondary toxicity, the mid dose (45.6 mg/kg-day) is considered a LOAEL based on hepatocellular hypertrophy and increased liver weight in male rats; the low dose (14.6 mg/kg-day) is considered a NOAEL.

CMA (1989b) conducted a parallel subchronic study in B6C3F1/CrlBR hybrid mice. Groups of 10 mice/sex/dose were given diets containing target concentrations of 0, 200, 3500, or 7000 ppm 1,2,4-trichlorobenzene (99.48% pure) for 13 weeks. Due to the volatility of the test compound, the investigators mixed diets containing 110% of the target concentrations; diets were stored frozen and changed twice each week. Tests for stability of the diet material showed a predictable rate of loss over time; using this loss constant, the authors estimated that a 10% increase in nominal concentration would result in average concentrations near that of the target levels. Analysis of the administered diets was not performed. Based on estimates of weekly compound intake provided by the authors, average intakes over the study duration were 0, 67, 851, and 1222 mg/kg-day in males and 0, 87, 1184 and 1346 mg/kg-day in females. Observations for clinical signs were made daily; body weight and food consumption were measured weekly. Ophthalmoscopic examinations were administered before the study and prior to termination. Blood was collected prior to sacrifice for analysis of hematology (Hgb, Hct, erythrocyte count, platelet count, total and differential leukocyte counts, mean cell volume [MCV], mean cell hemoglobin [MCH], and mean cell hemoglobin concentration [MCHC]) and serum chemistry (sorbitol dehydrogenase [SDH], ALT, BUN, glucose, total protein, albumin, globulin, gamma glutamyl transferase [GGT], electrolytes, calcium and phosphorus). Mice were given complete necropsies, and the adrenals, brain, kidneys, liver, spleen and testes with epididymides were weighed. Complete histopathology examinations (38 tissues) were performed on control and high-dose mice, and the lung, kidney, and liver (as well as any gross lesions) were examined microscopically in all dose groups.

Treatment did not affect survival, incidence of clinical signs or ophthalmoscopic findings (CMA, 1989b). Food consumption was consistently lower than control values in mid- and high-dose mice of both sexes throughout most of the study. The study reported that average cumulative body weight gain was significantly lower than controls throughout the study in males of all doses. However, examination of individual body weight data revealed an apparent error in

the initial body weight recorded for one male control mouse[1]; this error resulted in a falsely inflated estimate of cumulative body weight gain for male control mice and distorted the body weight gain comparisons with treated male mice (leading to inflated differences from control). Average cumulative body weight gain was significantly lower than controls in high-dose females (female body weight gain was not subject to this error) and, notwithstanding the error in baseline male control weights, may have also been reduced in high-dose males. Terminal body weight values (which were unaffected by the error) were significantly ($p < 0.05$) lower than controls in mid- and high-dose males (14% lower for both doses) and in high-dose females (8% lower). Weekly body weight measures were significantly ($p < 0.05$) lower than controls in low dose males during Weeks 11 and 12 (7% lower each time) but not at termination. Hematology findings were unremarkable except in high-dose females, which exhibited reduced Hct, Hgb, MCV, and MCH (see Table 2); these changes are similar to those observed in the subchronic rat study (CMA, 1989a). Serum chemistry changes, shown in Table 2, include marked increases in AST and SDH in mice of both sexes exposed to ≥3500 ppm, with more pronounced effects evident in males than in females.

Statistically significant organ-weight changes were observed in the liver, spleen, kidney, and brain of both sexes and in the adrenals and testes of males (CMA, 1989b). Absolute and relative liver weights were markedly increased (57−128% above control values), despite body weight reductions, in the mid- and high-dose mice of both sexes (see Table 2). Liver was also the site of the only significant histopathology findings, consisting of cytomegaly/karyomegaly multinucleation, atrophy, degeneration, and microcytosis, as well as coagulative necrosis.

Table 2 shows the incidence of these lesions. As shown in Table 2, the absolute and relative spleen weights were reduced in high-dose females, which may be correlated with the hematology changes in this group. Absolute—but not relative—spleen weights were also decreased in high-dose males. Weight changes in the kidney, adrenal, brain, and testes were small increases in relative weight and/or decreases in absolute weight consistent with, and probably secondary to, decreased body weight in the mid- and high-dose groups. The kidney, adrenal, brain, and testes weight changes were not associated with toxicological or histopathological correlates.

The authors identified the low dose as a NOAEL for females and reported that a NOAEL was not identified for males given the reductions in cumulative body weight gain observed at all doses. However, as noted above, the cumulative body weight gain comparisons were distorted by the inclusion of an erroneously low initial body weight in one control mouse. Weekly measures of body weight in males exposed at the low dose were reduced slightly (7%) during Weeks 11 and 12 but not at termination; no other statistically significant changes were observed at this dose. In contrast, both food consumption and body weights were reduced at the mid dose in males, and clear evidence of liver toxicity (including 3-fold increases in ALT and/or SDH, histopathology and liver weight increases) was observed in both males and females at the mid dose. Thus, for the purpose of this review, the mid dose serves as a LOAEL (851 and 1154 mg/kg-day in males and females, respectively), and the low dose serves as a NOAEL (67 and 87 mg/kg-day in males and females).

[1] The initial (Week 0) body weight measurement for male control mouse #31937 was reported as 12.9 g, while other male controls weighed from 19.6−22.7 g. The Week 1 body weight for mouse #31937 was reported as 21.5 g, resulting in a 1-week body weight gain of 8.6 g. Such a weight gain is highly implausible, as is the chance that a mouse with such an unusually low body weight would be included in the study.

Table 2. Selected Changes in Mice Treated with 1,2,4-Trichlorobenzene in the Diet for 13 Weeks[a]

Males				
	Control	**200 ppm (67 mg/kg-d)**	**3500 ppm (851 mg/kg-d)**	**7000 ppm (1222 mg/kg-d)**
Clinical Chemistry				
ALT (IU/L)	44 ± 8.8[b]	56 ± 13.5	150 ± 71.4[c]	146 ± 47[d]
SDH (IU/L)	33.3 ± 4.09	33.1 ± 5.10	130.0 ± 72.38[c]	152.2 ± 37.75[c]
Protein (g/dL)	5.9 ± 0.25	6.1 ± 0.29	6.7 ± 0.34[c]	7.4 ± 0.15[c]
Albumin (g/dL)	3.5 ± 0.09	3.7 ± 0.31	3.8 ± 0.29	4.5 ± 0.32[c]
Terminal Body Weight (g)	25.0 ± 1.9	23.3 ± 1.8	21.6 ± 1.4[c]	21.5 ± 1.4[c]
Organ Weights				
Liver weight (g)	1.249 ± 0.068	1.209 ± 0.069	1.957 ± 0.232[c]	2.338 ± 0.261[c]
Liver/body weight (× 100)	5.017 ± 0.368	5.201 ± 0.232	9.034 ± 0.678[c]	10.860 ± 0.638[c]
Histopathology of Liver				
Coagulative necrosis	0/10[d]	1/10	0/10	2/10
Cytomegaly/karyomegaly with multinucleation, atrophy, degeneration, microcytosis	0/10[e]	0/10	10/10[e]	10/10[e]
Females				
	Control	**200 ppm (87 mg/kg-d)**	**3500 ppm (1184 mg/kg-d)**	**7000 ppm (1346 mg/kg-d)**
Hematology				
Hematocrit (%)	54.1 ± 1.39	55.5 ± 3.58	53.8 ± 1.24	49.1 ± 2.33[c]
Hemoglobin (g/dL)	15.71 ± 0.41	15.8 ± 0.97	15.7 ± 0.67	14.5 ± 0.26[c]
MCV (FL)	51 ± 0.5	51 ± 0.9	50 ± 1.3	48 ± 1.5[c]
MCH (pg)	14.7 ± 0.46	14.4 ± 0.19	14.7 ± 0.22	14.0 ± 0.22[c]
Clinical Chemistry				
ALT (IU/L)	51 ± 10.3	51 ± 29.2	73 ± 18.3	199 ± 124.4[c]
SDH (IU/L)	26.9 ± 3.43	29.9 ± 10.54	74.8 ± 23.62[c]	86.5 ± 26.26[c]
Protein (g/dL)	6.1 ± 0.15	5.9 ± 0.13	6.3 ± 0.42	6.7 ± 0.17[c]
Terminal Body Weight (g)	21.2 ± 0.7	21.6 ± 1.9	20.2 ± 1.2	19.6 ± 0.6[c]
Organ Weights				
Spleen weight (g)	0.0772 ± 0.0098	0.0785 ± 0.0111	0.0700 ± 0.0106	0.0626 ± 0.0075[c]
Spleen/body weight (× 1000)	0.3629 ± 0.0424	0.3618 ± 0.0229	0.3454 ± 0.0398	0.3189 ± 0.0300[c]
Liver weight (g)	1.088 ± 0.066	1.178 ± 0.139	1.734 ± 0.116[c]	2.292 ± 0.184[c]
Liver/body weight (× 100)	5.119 ± 0.223	5.443 ± 0.317	8.597 ± 0.499[c]	11.714 ± 1.057[c]

Table 2. Selected Changes in Mice Treated with 1,2,4-Trichlorobenzene in the Diet for 13 Weeks[a]

Females				
	Control	**200 ppm (87 mg/kg-d)**	**3500 ppm (1184 mg/kg-d)**	**7000 ppm (1346 mg/kg-d)**
Histopathology of Liver				
Coagulative necrosis	0/9	0/10	0/10	2/9
Cytomegaly/karyomegaly with multinucleation, atrophy, degeneration, microcytosis	0/9	0/10	10/10[e]	9/9[e]

[a]CMA, 1989b
[b]Mean ± standard deviation
[c]Significantly different from control at $p < 0.05$
[d]Number affected/number examined
[e]$p < 0.0001$ by Fischer's exact test conducted for this review

Cote et al. (1988) administered 1,2,4-trichlorobenzene (>99% pure, in corn oil) in the diet to groups of Sprague-Dawley rats (10/sex/dose) for 13 weeks. Dietary concentrations of 0, 1, 10, 100, or 1000 ppm were used. Diets were freshly made and stored in airtight containers to prevent evaporative loss of the test material. Based on measured body weights and food consumption, the authors estimated doses of 0, 0.07, 0.78, 7.8, or, 82 mg/kg-day in males and 0, 0.11, 1.4, 15, or 101 mg/kg-day in females. Clinical observations were performed daily, body weights were recorded weekly, and food consumption was measured monthly. Urine was collected monthly for analysis of pH, protein, and nitrite. At sacrifice after 13 weeks of exposure, all animals were subjected to complete necropsy and blood was collected for hematology (Hgb, Hct, erythrocyte count, total and differential leukocyte count, platelet count, prothrombin time, MCH, and MCHC) and clinical chemistry (electrolytes, inorganic phosphate, total bilirubin, ALP, AST, total protein, calcium, cholesterol, glucose, uric acid and LDH) evaluations. Weights of the brain, heart, liver, kidney, and spleen were recorded and comprehensive histopathology evaluations were conducted. Hepatic mixed function oxidase (aniline hydroxylase [AH] and aminopyrine demethylase [APDM]) activities were measured and bone marrow cytology evaluated.

One high-dose female rat died during the fourth week on study; the authors did not identify a cause of death but did not consider it to be related to exposure to 1,2,4-trichlorobenzene (Cote et al., 1988). The authors reported that treatment did not otherwise affect survival, incidence of clinical signs, body weight gain, urinalysis parameters, serum chemistry, or hematology in either sex (only body weight data shown in the original report). At the highest dose, activities of AH and ADPM were increased in males and ADPM was increased in females. In males exposed to 1000 ppm, relative liver weight and absolute and relative kidney weights were significantly ($p < 0.05$) increased over control values (20%, 31%, and 36%, respectively). Organ weights of female rats were not affected by treatment. Gross necropsy revealed nephrosis in one male rat of the highest-dose group. Histopathology findings were reported generally for the three trichlorobenzene isomers tested, without incidence data or, in most cases, specific reference to the dose or isomer involved. Liver changes included a mild-to-moderate increase in cytoplasmic volume and anisokaryosis of hepatocytes in "most" treated groups. Rats exposed to 1000 ppm 1,2,4-trichlorobenzene were reported to have marked liver changes characterized by aggregated basophilia and fatty infiltration leading to widespread midzonal vacuolation. All treated groups were reported to exhibit thyroid changes (reduced follicular size, increased epithelial height, reduced colloid density) with increasing severity at the highest doses. For both liver and thyroid changes, the authors considered the effects to be biologically significant only at the highest dose. Based on the authors' conclusions regarding liver and thyroid histopathology, the highest dose (82 and 101 mg/kg-day in males and females, respectively) is considered a LOAEL and the NOAEL is 7.8 or 15 mg/kg-day (males or females, respectively).

Carlson and Tardiff (1976) evaluated selected parameters in adult male albino rats given 1,2,4-trichlorobenzene (reagent grade, purity not reported) orally (presumably via gavage), in corn oil at doses of 0, 10, 20, or 40 mg/kg-day every day for 90 days, with or without a 30-day recovery period, in groups of 6 rats. Body weight gain was measured in all groups. Liver enzyme levels were assessed and blood was collected for hemoglobin and hct determinations. Upon sacrifice, livers were weighed and examined microscopically. Body weights were not affected by treatment. Liver weights were significantly ($p < 0.05$) increased at the high dose—both in the group sacrificed immediately after dosing (10% higher than control) and in the

recovery group (14% higher). Several liver enzyme levels (including cytochrome c reductase, cytochrome P-450, benzpyrene hydroxylase, and azoreductase) were increased by treatment. No effects were observed on hematology parameters or liver histopathology. Effect levels were not determined from this study due to the limited parameters evaluated.

Carlson (1977) evaluated the ability of chlorinated benzenes to induce hepatic porphyria in female rats (strain not reported). Groups of five rats were given gavage doses of 0, 50, 100, or 200 mg/kg 1,2,4-trichlorobenzene (purity not specified) in corn oil daily for 30, 60, 90, or 120 days prior to sacrifice. Liver porphyrins (total); urinary excretion of porphyrins, delta aminolevulinic acid (ALA) and porphobilinogen; and liver weights were the only endpoints measured. Absolute liver weights were significantly ($p < 0.05$) increased at the high dose after 30 days (20% over controls) and at all doses after 60 and 90 days of exposure (17 to 42%); however, liver weights were not different from control at any dose after 120 days of exposure. Liver porphyrins were slightly, but statistically significantly, increased at 100 and 200 mg/kg after 30, 90, and 120 days of exposure, but not after 60 days. The maximum increase in liver porphyrins, occurring after 90 days of exposure to 200 mg/kg, was 86%. Urinary porphyrin excretion was increased at the highest dose after 30 and 90 days (59% and 2-fold higher, respectively) but not after 60 or 120 days; urinary porphyrins were increased at the mid dose after 120 days. The increases in urinary porphyrin levels were small relative to the 10- to 100-fold increases seen with hexachlorobenzene; the authors characterized the effects of 1,2,4-trichlorobenzene on this parameter as "minimal." Other parameters were not affected by treatment. Based on increases in both liver and urinary porphyrins, as well as increased liver weights, the mid-dose (100 mg/kg-day) is considered a LOAEL for the purpose of this review. At the low dose (50 mg/kg-day), increases in absolute liver weight (up to 32%) were reported without effects on porphyrins; this is considered a NOAEL.

The U.S. EPA conducted a 1-month study in rats (Cicmanec, 1991). The study was designed to confirm the effects of 1,2,4-trichlorobenzene on the adrenal glands seen in a multigeneration reproductive toxicity study (Robinson et al., 1981). Groups of five rats (sex, strain, and age not specified) were given doses of 0 or 53 mg/kg-day in corn oil by daily gavage for 30 days. Purity of the compound was not reported. Body weights were recorded at study initiation and termination. Urine samples were collected for porphyrin analysis and blood was analyzed for serum corticosterone levels as a measure of adrenal cortical function. At sacrifice, adrenal gland weights and histopathology were evaluated. The results of porphyrin analysis were not reported. When compared with controls, treated rats had decreased serum corticosterone levels (32% lower) and increased absolute (14.8% higher) and relative (13.8%) adrenal gland weights (see Table 3). No statistical analyses were performed by the authors; however, statistical analysis (t-test) performed for this review indicated that the decrease in corticosterone levels was significant, while the adrenal weight differences were not. Microscopic examination of the adrenals showed moderate vacuolization of the *zona fasciculata* in all treated animals with only slight vacuolization in controls. Increased adrenal weight of greater than 10%, with accompanying histopathology, does constitute an adverse effect. No other study details are available. This study identifies a LOAEL of 53 mg/kg-day for effects on the adrenal glands; no NOAEL can be identified.

Table 3. Adrenal Effects in Rats Exposed to 1,2,4-Trichlorobenzene via Gavage for 30 Days[a]

Dose (mg/kg-d)	Absolute Adrenal Weight (mg)	Adrenal Weight Relative to Body Weight (mg/100 g)	Serum Corticosterone Level (ng/mL)
0	5.4 ± 0.60[b]	18.8 ± 1.7	483.9 ± 62.0
53	6.2 ± 0.58	21.4 ± 3.1	330.2 ± 42.9[c]

[a]Cicmanec, 1991
[b]Mean ± standard deviation
[c]Significantly different from control (p = 0.002) by t-test conducted for this review

In a study reported only in abstract form, Smith et al. (1978) administered oral daily doses (presumably via gavage) ranging from 1 to 173.6 mg/kg 1,2,4-trichlorobenzene (purity not given) to rhesus monkeys (number and sex not reported) for unspecified periods of time (at least 20−30 days). While endpoints were not enumerated in the abstract, it appears that mortality, clinical signs, body weight, and serum chemistry (including BUN, electrolytes, calcium, phosphate, creatinine phosphokinase, AST, ALT, ALP, and LDH) were monitored. The authors reported that doses of 25 mg/kg-day were nontoxic, while doses of 90 mg/kg-day were toxic and doses of 173.6 mg/kg-day were lethal within 20−30 days. At the highest dose, monkeys exhibited fine tremors and severe weight loss, as well as serum chemistry changes, prior to death. This study does not provide enough information to identify effect levels other than a Frank Effect Level (FEL) of 173.6 mg/kg-day associated with mortality and emaciation.

In another study published only in abstract form, Cragg et al. (1978) reported a subchronic study in rhesus monkeys. Groups of four rhesus monkeys (sex not specified) were given oral (presumably gavage) doses of 1,2,4-trichlorobenzene (purity not given) from 1−25 mg/kg-day for 120 days. The authors reported that body weight, clinical observations, hematology, and clinical chemistry (parameters not specified) were not affected at doses up to 25 mg/kg-day, and that cytochrome P-450 and P-448 were not induced at these doses; no data were presented to support these findings. A dose of 125 mg/kg was reported to be lethal for 1/4 monkeys and to cause temporary weight loss and cytochrome P-450 induction in survivors. The information presented in the abstract is insufficient to identify effect levels other than a FEL of 125 mg/kg-day associated with mortality.

Chronic Studies—In a carcinogenicity bioassay in rats, groups of F-344 rats (50 per sex per group) were fed basal diets containing target concentrations of 0, 100, 350, or 1200 ppm of 1,2,4-trichlorobenzene (98.9% pure) for 104 weeks (CMA, 1994a). Due to the volatility of the test compound, the investigators mixed diets containing 110% of the target concentrations. Diets were prepared weekly and frozen until use; in addition, the diet was analyzed for actual concentrations weekly for the first month and then monthly thereafter. Measured concentrations averaged over the 7 days between diet changes were within 4% of the target concentrations. Based on food intake, body weight, and measured compound concentrations in the diet, the mean daily consumed doses were reported by the authors to be 0, 5.6, 19.4, and 66.5 mg/kg-day for males and 0, 6.9, 23.5, and 81.4 mg/kg-day for females. Rats were examined for mortality and moribundity twice daily, for clinical signs daily, and they were given a thorough physical examination weekly. Body weights and food consumption were measured weekly during

Weeks 1−16 and monthly thereafter. Blood samples for hematology were collected during Weeks 52 and 78 of treatment and at termination. Blood smears were used to determine cellular morphology and leukocyte differentials for the controls and high-dose animals. All animals were given a complete necropsy upon death or sacrifice. Organ weights were recorded for the brain, kidneys, liver, and testes of 10 rats/sex/group and 36 tissues were collected for histopathology. Microscopic examination was carried out on all tissues in control and high-dose rats surviving to 104 weeks and in rats dying prematurely. Gross lesions were examined in the low- and mid-dose groups and the liver and kidney in the mid-dose group.

Survival was significantly (p-value not reported) reduced in high-dose males after 83 weeks of exposure, but was unaffected in other treatment groups; survival at termination was 84, 80, 84, and 60% in males and 76, 78, 72, and 72% in females in the control, low-, mid- and high-dose groups, respectively. The authors did not indicate the cause of the reduced survival, but they reported that there was no evidence that treatment-related histopathology was the cause. During the first 24 weeks, body weight and body weight gain were significantly lower in high-dose rats compared to controls, but the differences were small and overtaken by a rebound effect during the last half of the study. Overall (Weeks 1−104) mean total body weight gain was not affected by treatment. Food consumption was consistently significantly ($p \leq 0.05$) lower in treated rats than in controls; mean total food consumption was 4−7% lower than controls across all treatment groups. There are no biologically significant hematology findings. The only compound-related organ weight changes were significant ($p \leq 0.05$) increases in absolute and relative liver weights in the high-dose rats of both sexes (19−21% higher than controls for both parameters in both sexes). There was histopathologic evidence for compound-associated toxicity in the liver (slight-to-moderate centrilobular hepatocellular hypertrophy and diffuse fatty change in both sexes and focal cystic degeneration in males) at the high dose (see Table 4). Similarly, renal toxicity (mineralization of the renal pelvis in both sexes, transitional cell hyperplasia of the pelvic urothelium in males, and increased severity of chronic rat nephropathy in males) was observed at the high dose (see Table 4). The incidence of renal pelvis mineralization was also significantly increased in mid-dose males. The adrenal glands were not weighed in this study, but there were no treatment-related histopathologic changes in these organs. The incidences of adrenal cortical vacuolization were 11/50 and 13/50 in control and high-dose males (respectively) and 15/50 and 20/50 in control and high-dose females. The authors identified the mid dose (350 ppm) as a NOAEL for systemic toxicity. Although the male rats exhibited an increased incidence of renal pelvis mineralization (without an increase in severity) at the mid dose, available evidence suggests this effect may be related to male rat-specific hyaline droplet nephropathy (see additional discussion of this in the discussion of CMA 1989a above). For the purpose of this review, a LOAEL of 1200 ppm (66.7 mg/kg-day in males and 81.4 mg/kg-day in females) is identified from this study based on reduced survival (males only), reduced body weight (first 24 weeks of the study), and liver histopathology. The NOAEL is 350 ppm (19.4 mg/kg-day in males and 23.5 mg/kg-day in females).

There was no statistically significant increase in the incidence of neoplasia in any tissue—including liver and kidney (CMA, 1994a). The incidence of hematopoietic neoplasia was slightly elevated in males (15/50 and 22/50 in control and high-dose males; 10/50 and 10/50 in control and high-dose females), but the difference is not statistically significant. This study is adequate to assess the carcinogenicity of 1,2,4-trichlorobenzene in rats. Although survival is reduced at the high dose, adequate numbers of animals survived to termination. In addition, the high dose appears to have been a Maximum Tolerated Dose (MTD).

Table 4. Incidence of Significant Liver and Kidney Effects in Rats Exposed to 1,2,4-Trichlorobenzene in the Diet for 104 Weeks[a]

Male			
	Control	**350 ppm (19.4 mg/kg-d)**	**1200 ppm (66.5 mg/kg-d)**
Liver			
Grossly enlarged liver	1/50[b]	5/50	7/50
Hepatocellular hypertrophy	2/50	5/50	30/49[c]
Diffuse fatty change	5/50	5/50	14/49[c]
Focal cystic degeneration	9/50	4/50	19/49[c]
Kidney			
Granular/ pitted appearance	11/50	11/50	34/50[c]
Renal pelvis mineralization	34/50 (0.7)[d]	44/50[c] (1.0)	49/50[c] (3.0)
Transitional cell hyperplasia	2/50 (0.1)	2/50 (0.0)	34/50[c] (1.0)
Moderate chronic progressive nephropathy	8/50 (2.7)	4/50 (2.6)	29/50[c] (3.6)
Female			
	Control	**350 ppm (23.5 mg/kg-d)**	**1200 ppm (81.4 mg/kg-d)**
Liver			
Grossly enlarged liver	0/50	0/50	0/50
Hepatocellular hypertrophy	6/50	5/50	37/50[c]
Diffuse fatty change	15/50	21/50	30/50[c]
Focal cystic degeneration	0/50	0/50	0/50
Kidney			
Renal pelvis mineralization	39/48 (0.8)	47/50 (1.0)	48/50[c] (1.3)

[a]CMA, 1994a
[b]Number affected/total number examined
[c]Significantly different from control incidence ($p < 0.05$) by Fisher's exact test conducted for this review
[d]Mean severity score is given in parentheses

CMA (1994b) also evaluated potential carcinogenicity in mice. Groups of 50 mice/sex/dose were given 1,2,4-trichlorobenzene (98.9% pure) in the diet at target concentrations of 0, 150, 700, or 3200 ppm for 104 weeks. Due to the volatility of the test compound, the investigators mixed diets containing 110% of the target concentrations and analyzed the diet for actual concentrations. Diets were prepared weekly and frozen until use; in addition, the diet was analyzed for actual concentrations weekly for the first month and then monthly thereafter. Measured concentrations averaged over the 7 days between diet changes were within 2% of the target concentrations. Based on measured food consumption and body weight, as well as analysis of the actual diet concentrations, the authors estimated mean doses of 0, 21.0, 100.6, or 519.9 mg/kg-day in males and 0, 26.3, 127.0, or 572.6 mg/kg-day in females. Animals were observed twice daily for mortality and moribundity and daily for clinical signs of toxicity. Food consumption and body weights were measured weekly through Week 16 and monthly thereafter. Blood was collected for limited hematology analysis (cellular morphology and differential leukocyte counts) at Weeks 52 and 78 and at termination. Clinical chemistry was not evaluated in this study. Upon sacrifice, all mice were necropsied and the brain, liver with gall bladder, kidneys, and testes with epididymides were weighed. Comprehensive histopathology examinations were performed on all control and high-dose mice, while the liver, adrenals, and testes with seminal vesicles from all dose groups were examined microscopically.

Reduced survival was observed in high-dose animals beginning after Week 48. Survival to termination was significantly ($p < 0.05$) reduced in high-dose males and females; survival to the beginning of Week 105 was 90, 88, 82, and 10% (control through high dose) in males and 78, 76, 84, and 0% in females. A high incidence of hepatocellular carcinoma in high dose animals of both sexes explains the mortality at this dose. High-dose animals also exhibited reduced body weight, reduced total body weight gain, and reduced food consumption. In low and mid-dose mice, body weight and body weight gain were either increased or unchanged from control values. Similarly, there were no consistent dose-related effects on food consumption at doses other than the high dose. Clinical signs were observed only in moribund animals prior to death. There were no significant hematology findings in the limited analyses performed; sporadic, statistically significant changes in differential leukocyte counts did not exhibit a treatment-related pattern. Animal observations revealed dose-related increases in the incidence of distended abdomen in all treatment groups; the authors attributed this observation to liver enlargement and liver masses, with associated accumulation of ascitic fluids. Organ weights were not evaluated in high-dose females due to premature death. Absolute and relative liver weights were increased in a dose-related fashion in both males and females of all remaining groups (except relative liver weight in low-dose males), with marked changes (2-fold or higher) at the mid- and high doses (see Table 5). Other statistically significant organ weight changes were unrelated to treatment or attributable to body weight differences. Nonneoplastic histopathology findings were restricted to hepatocyte hypertrophy, the incidence of which was increased in mid- and high-dose males. The incidence of hepatocellular carcinoma was significantly increased in males and females of the mid- and high-dose groups, while the incidence of hepatocellular adenoma was increased only at the mid-dose in both sexes (CMA, 1994b). The low incidence of adenoma at the high dose is attributable to the high incidence of carcinomas at this dose; nearly 100% of high dose animals of both sexes exhibited carcinomas.

Table 5. Liver Changes in Mice Treated with 1,2,4-Trichlorobenzene in the Diet for 104 Weeks[a]

Males				
	Control	**150 ppm (21.0 mg/kg-d)**	**700 ppm (100.6 mg/kg-d)**	**3200 ppm (519.9 mg/kg-d)**
Terminal Body Weight (g)	32.5 ± 4.1	35.3 ± 4.6[c]	32.1 ± 3.6	26.7 ± 2.0[c]
Organ Weights at Termination				
Liver weight (g)	1.59 ± 0.60[b]	1.77 ± 0.43[c]	3.05 ± 1.72[c]	7.37 ± 1.84[c]
Liver/body weight (%)	4.962 ± 2.268	5.022 ± 1.392	9.522 ± 5.508[c]	27.364 ± 5.740[c]
Nonneoplastic Lesions				
Hepatocyte hypertrophy	0/49[d]	0/50	27/50[e]	20/50[e]
Neoplastic Lesions				
Hepatocellular adenoma	4/49	7/50	16/50[e]	2/50
Hepatocellular carcinoma	8/49	5/50	27/50[e]	50/50[e]
Females				
	Control	**150 ppm (26.3 mg/kg-d)**	**700 ppm (127.0 mg/kg-d)**	**3200 ppm (572.6 mg/kg-d)**
Terminal Body Weight (g)	28.6 ± 3.4	31.6 ± 5.8[c]	30.7 ± 3.8[c]	No survivors
Organ Weights at Termination				
Liver weight (g)	1.42 ± 0.23	1.87 ± 0.43[c]	3.98 ± 2.32[c]	No survivors
Liver/body weight (%)	5.057 ± 0.504	5.994 ± 0.910[c]	12.734 ± 6.817[c]	No survivors
Nonneoplastic Lesions				
Hepatocyte hypertrophy	0/50	0/50	1/50	8/50[e]
Neoplastic Lesions				
Hepatocellular adenoma	3/50	4/50	16/50[e]	8/50
Hepatocellular carcinoma	1/50	1/50	28/50[e]	46/50[e]

[a]CMA, 1994b
[b]Mean ± standard deviation
[c]Significantly different from control at $p < 0.05$
[d]Number affected/number examined (including premature deaths)
[e]$p < 0.01$ by Fischer's exact test conducted for this review

The authors characterized the low dose (150 ppm) as a NOAEL for systemic toxicity, presumably due to liver weight changes and liver histopathology at the mid-dose. However, the incidence of neoplasia in the livers of both male and female mice was markedly increased at the mid-dose, so the interpretation of the nonneoplastic changes in this organ is complicated by the prevalence of tumors. As a consequence, effect levels were not defined for this study.

In a review of a paper published in German, WHO (1991) reported that liver weight was not affected and gross and histological lesions of the liver were not observed in 20 male ICR-JCL mice given 78 mg/kg-day 1,2,4-trichlorobenzene via dietary administration for 6 months (Goto et al., 1972, as cited in WHO, 1991). No other information was available in the secondary source.

Reproductive/Developmental Studies—Robinson et al. (1981) conducted a multigeneration reproductive toxicity study using rats (strain not specified). Beginning at birth of the F_0 generation and continuing through postnatal day (PND) 32 of the F_2 generation, rats (17−23 litters per group for all generations) were continuously exposed to concentrations of 0, 25, 100, or 400 ppm 1,2,4-trichlorobenzene (purity not specified, solubilized in Tween 20) in the drinking water. Both water and vehicle control groups were included. Body weights, food consumption, and water intake were recorded regularly. Locomotor activity was assessed at intervals up to 90 days of age. F_0 and F_1 rats were bred to same-group animals at 90 days of age. Fertility, litter size, and neonatal sex, weight, and postnatal viability were monitored. Vaginal opening was assessed in F_2 female rats. Interim sacrifices were performed on each generation; the F_0 and F_2 interim sacrifices occurred on days 27 and 95 of age and the F_1 interim sacrifice occurred only on day 95 of age. At each time, 10 rats/sex/group were sacrificed; blood samples were collected for chemistry determinations (i.e., glucose, BUN, creatinine, electrolytes, uric acid, Ca, P, cholesterol, triglyceride, bilirubin, ALP, ALT, AST, LDH, creatinine phosphokinase [CPK], total protein, globulin, and albumin) and selected organs (liver, kidney, uterus, adrenals, lungs, heart, and gonads) were weighed. Livers and kidneys from control and high-dose F_1 rats sacrificed at 95 days of age were examined microscopically.

The authors indicated that body weight was not changed by treatment at any dose or in any generation; data were not shown (Robinson et al., 1981). Food intake of the F_0 and F_1 generations was not adversely affected by treatment at any dose, although a transient increase in food intake was seen in F_0 high dose males at 29 days of age. Water intake was significantly ($p < 0.05$) reduced at the high dose in F_0 females at 35 days of age (data not reported) and at both sexes of the F_0 generation at 83 days of age (12% and 17% lower than controls in males and females, respectively). It was not clear from the report whether the control water consumption data reported were for water or vehicle controls. Water intake was not affected in the F_1 generation. Representative compound intake rates estimated by the authors based on the water consumption and body weight data (F_0 generation at 83 days of age) were 2.5, 8.9, and 33 mg/kg-day in males and 3.7, 14.8, and 53.6 mg/kg-day in females in the 25-, 100-, and 400-ppm groups. The authors reported that no treatment-related changes were observed in serum chemistry analyses in any generation (data not shown). Gestation rate, maternal weight (data not shown), litter size, postnatal viability, growth (data not shown), and locomotor activity were not affected by treatment in any generation. Vaginal opening of the F_2 females was unchanged by exposure; this endpoint was not assessed in other generations. The only organ weight that was significantly affected by treatment was that of the adrenal glands, which were significantly ($p < 0.05$) increased at the high dose in both sexes of both F_0 and F_1 generations when assessed

at 95 days of age. Adrenal weights were 9−11% higher than water controls in males and 8−22% higher in females (see Table 6). No histopathology was observed in the liver or kidneys of high dose F_1 animals; adrenal histopathology was not assessed. U.S. EPA (2008) identified the high dose as a LOAEL based on increased adrenal gland weights, noting that this effect had been confirmed in an acute toxicity study using *i.p*. administration (also reported in Robinson et al., 1981) as well as in an unpublished U.S. EPA study (Cicmanec, 1991, as cited in U.S. EPA, 2008). The latter study, a 1-month rat gavage study using a comparable dose (53 mg/kg-day), also observed adrenal histopathology (increased vacuolization of the *zona fasciculata*), as well as decreased serum corticosterone levels. Increased adrenal weights, were also observed in male rats given 1,2,4-trichlorobenzene in the diet for 13 weeks at an average dose of 133.7 mg/kg-day (CMA, 1994a). For the purpose of this review, the mid dose (8.9 or 14.8 mg/kg-day in males or females, respectively) is, thus, identified as a NOAEL and the high dose (33 or 53.6 mg/kg-day in males or females, respectively) is a LOAEL for adrenal effects.

Table 6. Adrenal Weight Changes (Left Adrenal Only) at 95 Days of Age in Rats Treated with 1,2,4-Trichlorobenzene in the Drinking Water for Three Generations[a]

	Control (water)	**Control (Tween 20)**	**25 ppm (2.5 mg/kg-d)**	**100 ppm (8.9 mg/kg-d)**	**400 ppm (33 mg/kg-d)**
F_0 Male Adrenal weight (mg)	28.7 ± 1.78[b]	28.6 ± 1.09	28.8 ± 1.59	28.2 ± 1.10	31.8 ± 2.43[c]
F_1 Male Adrenal weight (mg)	27.1 ± 1.71	28.0 ± 1.57	28.8 ± 1.46	26.6 ± 0.82	29.6 ± 1.66[c]
	Control (water)	**Control (Tween 20)**	**25 ppm (3.7 mg/kg-d)**	**100 ppm (14.8 mg/kg-d)**	**400 ppm (53.6 mg/kg-d)**
F_0 Female Adrenal weight (mg)	34.1 ± 1.87	36.8 ± 1.14	35.6 ± 1.70	36.6 ± 1.08	41.5 ± 1.99[c]
F_1 Female Adrenal weight (mg)	35.8 ± 1.89	37.0 ± 1.36	34.8 ± 1.77	35.3 ± 1.07	38.5 ± 1.89[c]

[a]Robinson et al., 1981
[b]Mean ± standard error
[c]Significantly different from control at $p < 0.05$

In a study of embryotoxicity, Kitchin and Ebron (1983; also U.S. EPA, 1982) administered gavage doses of 0, 36, 120, 360, or, 1200 mg/kg-day 1,2,4-trichlorobenzene (>99% pure) in corn oil to timed-pregnant Sprague-Dawley rats (at least six per dose) on gestation days (GDs) 9−13. Upon sacrifice by decapitation on GD 14, maternal livers were weighed, sectioned, and examined microscopically; liver homogenates were analyzed for enzyme content. Uteri of control and 360 mg/kg-day rats were examined for implantations and resorptions; rats from lower dose groups were not examined. Live embryos were examined grossly and the presence of a beating heart, somite number and embryo size were noted.

None of the rats treated with 1200 mg/kg-day survived beyond the 3rd day of treatment and 2/9 rats given 360 mg/kg-day died (Kitchin and Ebron, 1983, U.S. EPA, 1982). Body weight gain was markedly reduced in survivors given 360 mg/kg-day (17 g weight loss versus 37 g gain in controls). Liver weight and hepatic microsomal protein content were not affected by treatment; however, hepatic enzyme induction was observed at 120 and 360 mg/kg-day. Microscopic examination of the livers revealed slight hepatocellular hypertrophy in 1/9 rats exposed to 120 mg/kg and moderate hypertrophy in 7/8 rats (presumably including one rat that

died prematurely) exposed to 360 mg/kg-day. Treatment at 360 mg/kg-day resulted in fewer implantations, an increase in the incidence of dead embryos (this increase was not significant on a litter basis), reduced head and crown-rump lengths, decreased number of somites, and decreased embryonic protein content (see Table 7) when compared with controls. The decrease in implantations was not likely to be caused by the treatment, since implantation occurs on GD 6, and the dosing started on GD 9. Uterine and embryonic parameters were not evaluated in lower-dose groups, precluding the clear identification of embryotoxic effect levels from this study.

Table 7. Significant Changes in Uterine and Embryonic Parameters in Rats Exposed to 1,2,4-Trichlorobenzene from GDs 9−13[a]

	Uterine parameters (12 litters examined)			Embyronic parameters (24 treated and 26 control embryos examined)			
Dose (mg/kg-d)	**Implantations**	**Dead Embryos/ Total Number of Embryos**	**Litters With Dead Embryos/ Total Number of Litters**	**Embryonic Head Length (mm)**	**Embryonic Crown-Rump Length (mm)**	**Somites**	**Protein (µg)**
0	14.16 ± 0.31[b]	0/161	0/12	4.72 ± 0.06	8.17 ± 0.09	49.6 ± 0.6	2670 ± 108
360	12.5 ± 0.79[c]	25/138[d]	3/12	4.43 ± 0.10[c]	7.69 ± 0.17[c]	47.7 ± 0.6[c]	2053 ± 121[e]

[a]Kitchin and Ebron, 1983
[b]Mean ± standard error of mean
[c]Significantly different from control at $p < 0.05$
[d]$p < 0.001$
[e]$p < 0.01$

Black et al. (1988; Ruddick et al., 1983) exposed timed-pregnant Sprague-Dawley rats (about 14/group) to gavage doses of 0, 75, 150, or 300 mg/kg-day 1,2,4-trichlorobenzene (99.5% pure, in corn oil) on GD 6−15. The dams were sacrificed on GD 22, whereupon the uterus with ovaries was removed for examination and liver, kidney, spleen, heart, and brain were weighed. Body weights were recorded before and after uterine removal. Maternal blood collected at sacrifice was analyzed for hematology (Hgb, Hct, erythrocyte count, total and differential leukocyte count, MCV, MCH, and MCHC) and serum chemistry (electrolytes, inorganic phosphorus, total bilirubin, ALP, AST, total protein, calcium, cholesterol, glucose, uric acid and LDH). Liver samples were homogenized for analysis of liver enzymes. Finally, 24 maternal tissues were subjected to histopathology evaluation. Uterine contents were examined for resorptions and live or dead pups. Pups were weighed and examined grossly for abnormalities; live fetuses were prepared for skeletal or visceral examination and histopathology.

There were no treatment-related deaths or clinical signs during the study (Black et al., 1988; Ruddick et al., 1983). Body weights of treated dams were not statistically distinguishable from control values, although they were lower in high-dose animals (data not provided in publication). Absolute and relative liver weights were significantly ($p < 0.05$) increased in the high-dose dams (6% increase in relative weight; absolute weight increase not

reported). No other organ weights were affected by treatment. Maternal mixed function oxidase activity was increased at 150 and 300 mg/kg-day. Maternal hemoglobin concentration (7% and 6% below control, at 150 and 300 mg/kg-day, respectively) and Hct (31% at both doses) were reduced at 150 and 300 mg/kg-day, but reticulocytes were not increased. Mild histopathology changes consisting of follicle size reduction were observed in the thyroids of dams treated at the high dose. The authors reported mild hepatic lesions (increased periportal cytoplasmic eosinophilia and mild anisokaryosis of hepatocellular nuclei) at the mid and high doses. Increased splenic hematopoiesis was reported to occur in some animals, but details were not provided. Incidences of histopathology changes were not reported. Treatment with 1,2,4-trichlorobenzene did not affect pregnancy rate, resorptions, incidence of dead fetuses, litter size, fetal weight or the incidences of gross, skeletal, or visceral abnormalities. In the absence of any fetotoxic or teratogenic effects, the high dose (300 mg/kg-day) is considered a freestanding NOAEL for developmental toxicity. Based on mild anemia (reduced Hgb and Hct) and mild hepatic lesions in dams, the mid dose (150 mg/kg-day) is considered a systemic LOAEL; the NOAEL is 75 mg/kg-day.

In a series of papers reporting the development of a teratology-screening assay (Chernoff and Kavlock, 1983; Gray et al., 1983; Gray and Kavlock, 1984; Gray et al., 1986), investigators administered gavage doses of 0 or 130 mg/kg-day 1,2,4-trichlorobenzene to groups of 25 pregnant CD-1 mice on GD8−12, after which dams were allowed to deliver. Maternal survival, body weight change and pregnancy rate were recorded, as well as the postnatal viability and body weight of offspring on PND 1 and 3. Dams that had not delivered 3 days after expected parturition date were sacrificed and uteri were examined for implantation sites. Dead pups were necropsied and evaluated for gross abnormalities. Groups of three male and three female pups were randomly assigned to same-treated dams for fostering on PND 6. At 30 days of age, offspring number and weights were recorded, as was the number of females with patent vaginas. Locomotor activity in the pups was assessed at 21, 58, and 210 days of age. Female offspring that became pregnant were removed for delivery and their litter size, weight, and sex ratio were evaluated. At about 250 days of age, the male mice were sacrificed and necropsied; body, liver, kidney, testes, and seminal vesicle weights were recorded. Tabular data presented in the studies showed that treatment did not result in differences from control for any parameter assessed. This series of studies identified a NOAEL of 130 mg/kg-day for postnatal effects of gestational exposure to 1,2,4-trichlorobenzene in mice.

Inhalation Exposure

Subchronic Studies—Kociba et al. (1981) exposed groups of 20 male Sprague-Dawley rats, four male New Zealand rabbits and two male beagle dogs by inhalation to 0, 30, or, 100 ppm (0, 223, or, 742 mg/m^3) of 1,2,4-trichlorobenzene (99.41% pure) vapor for 7 hours/day, 5 days/week, for a total of 30 exposures in 44 days. The following endpoints were monitored: body weight, clinical signs, hematology (total erythrocytes, differential leukocytes, packed cell volume [PCV] and Hgb), clinical chemistry parameters (BUN, ALT, and ALP), organ weights (liver, kidneys, spleen, adrenals [in dogs and rabbits], heart, brain, thymus [rats] and testes), and gross and microscopic examination of most tissues and organs in all dogs and rabbits as well as five control and high-dose rats. Urine samples, taken from control and high-concentration rats only, were analyzed for coproporphyrin and uroporphyrin as indicators of hepatotoxicity. At the end of the experiment, these levels were observed to be elevated, so a second, confirmatory

experiment was undertaken with 10 male rats per exposure at 0, 30, and 100 ppm; urine samples were collected after 15 and 30 days of exposure and analyzed for coproporphyrin and uroporphyrin.

Analysis of chamber concentrations indicated values close to the nominal concentrations (30 ± 4 ppm and 104 ± 14 ppm) (233 ± 31 mg/m^3 and 771 ± 104 mg/m^3) (Kociba et al., 1981). Exposure to 1,2,4-trichlorobenzene did not result in clinical signs or statistically significant effects on body weight gain in any of the species tested (statistical analysis not provided). Results from hematologic examinations and clinical chemistry tests revealed no compound-related effects in any of the three species. In rats, changes in organ weights included statistically significant ($p < 0.05$) increases in absolute and relative liver weight (each 11% higher than control) and increases in relative kidney weight (9%) at the 100-ppm level. Urinary uroporphyrin and coproporphyrin levels were significantly ($p < 0.05$) elevated in rats in the original experiment. Furthermore, these levels were significantly elevated at both time points and at both exposure levels in the confirmatory experiment (see Table 8). In rabbits, relative liver weight was significantly decreased at the 30- and 100-ppm levels (each 83% of control) and absolute and relative testes weights were increased (30% and 43% higher than control, respectively) at the 100-ppm level. While there was a statistically significant decrease in BUN in rabbits, the authors considered the measure within the range of normal variability. In dogs, absolute and relative liver weights were nominally increased over control values at the 100-ppm level, but the small number of animals precluded statistical comparison. Exposure to 1,2,4-trichlorobenzene did not result in gross or microscopic alterations in any tissues of any species tested.

Table 8. Significant Changes at Urinary Porphyrins in Rats Inhaling 1,2,4-Trichlorobenzene for 15 or 30 Days[a]

Exposure (ppm)	15 Exposure Days		30 Exposure Days	
	Coporphyrin (µg/24 h)	Uroporphyrin (µg/24 h)	Coporphyrin (µg/24 h)	Uroporphyrin (µg/24 h)
First Experiment				
0	Not measured	Not measured	4.2 ± 1.4	0.4 ± 0.2
100	Not measured	Not measured	12.4 ± 2.8 [a]	11.5 ± 0.4[b]
Second Experiment				
0	3.6 ± 0.9	4.6 ± 1.0	6.2 ± 2.1	6.3 ± 3.9
30	16.2 ± 2.7[b]	11.6 ± 1.3[b]	15.8 ± 3.0[b]	12.8 ± 1.3[b]
100	33.8 ± 2.9[b]	10.8 ± 4.6[b]	15.2 ± 2.7[b]	13.5 ± 2.3[b]

[a]Kociba et al., 1981
[b]Significantly different from control, $p < 0.05$

The authors considered the testes weight increases in rabbits to be unrelated to treatment (Kociba et al., 1981), but they provided no rationale for this conclusion. While the investigators considered the increased urinary excretion of porphyrins to be a physiological effect of 1,2,4-trichlorobenzene inducing hepatic microsomal enzymes (cytochrome P-450), rather than a

toxic effect on destruction of heme-containing cytochromes or inhibition of heme synthesis, no data were provided to support this hypothesis. For the purpose of this review, the exposure level of 30 ppm of 1,2,4-trichlorobenzene (223 mg/m^3) is defined as a LOAEL for rats in this study based on increased excretion of porphyrins; no NOAEL can be defined for rats from these data. Due to the small numbers of rabbits and dogs used in this study, effect levels for these species cannot be reliably defined.

In a subsequent (albeit published earlier) study by the same group of investigators (Watanabe et al., 1977, 1978), the effects of inhaled 1,2,4-trichlorobenzene (99.6% pure) on both liver and urinary excretion of porphyrins were evaluated in groups of 10 male and 26 female Sprague-Dawley rats. The rats were exposed to 0, 2.8, or 10.2 ppm (0, 21, or 76 mg/m^3) 6 hours/day, 5 days/week, for 3 months. Animals were observed for clinical signs daily and body weights were recorded weekly. Between four and five females/group were sacrificed after 2 weeks, 1 month, or 2 months of exposure and 2 or 4 months post-exposure for assessment of total liver porphyrins. Urine was collected at these same intervals from the rats maintained for the entire experiment; these samples were analyzed for porphyrin, coporphyrin, and creatinine. All animals received gross necropsy and weights of brain, kidney, liver, and lung were recorded. Histopathological examinations were not performed because the Kociba et al. study (1981), conducted earlier, publication delayed, had reported no histopathological findings at higher exposure levels.

Measured chamber concentrations of 1,2,4-trichlorobenzene averaged 2.8 and 10.2 ppm over the duration of the experiment (Watanabe et al., 1977, 1978). Only one female control and one female of the 3-ppm group died; no treatment-related effect on mortality was observed. Likewise, treatment did not affect clinical signs, body weight, organ weights, gross necropsy findings, or total liver porphyrins. Statistically significant increases (over control levels) in urinary porphyrin excretion were observed sporadically over the 3-month period in rats exposed to 10 ppm (see Table 9 for results at 3 months of exposure); levels were not different from controls 2 or 4 months after exposure ceased. While the authors maintained that the increased urinary porphyrin excretion was of minor toxicological significance, they suggested that this effect might directly precede toxic manifestations of exposure to 1,2,4-trichlorobenzene noted by other investigators (Rimington and Ziegler, 1963). As noted previously with the oral dosing studies, the scenario of hepatic enzyme induction, tissue damage, possibly mediated by quinine metabolites, hepatic hypertrophy, and increased liver weight, appears to be the progression (Carlson and Tardiff, 1976; Carlson, 1977; Watanabe et al., 1977, 1978; Ariyoshi et al., 1981; Kociba et al., 1981; Black et al., 1988; Cote et al., 1988; CMA, 1989a,b). Consequently, the authors considered the exposure concentration of 2.8 ppm (21 mg/m^3) to be the NOAEL for rats, as this level did not increase urinary porphyrin excretion. For the purpose of this review, a minimal LOAEL of 10.2 ppm (76 mg/m^3) is identified based on increased porphyrin excretion; the NOAEL is 2.8 ppm (21 mg/m^3).

Table 9. Significant Changes at Urinary Porphyrins in Rats Inhaling 1,2,4-Trichlorobenzene (Results after 3 Months of Exposure)[a]

	Male		Female	
24-h Excretion				
Exposure (ppm)	**Coproporphyrin (µg/24 h)**	**Uroporphyrin (µg/24 h)**	**Coproporphyrin (µg/24 h)**	**Uroporphyrin (µg/24 h)**
0	6.1 ± 3.4	1.3 ± 1.0	5.3 ± 9.4	0.6 ± 0.3
3	8.5 ± 5.4	2.2 ± 1.7	2.4 ± 0.5	0.7 ± 0.1
10	11.4 ± 5.9[b]	4.1 ± 2.4[b]	3.1 ± 1.0	1.0 ± 0.2[b]
Excretion Adjusted for Creatinine				
Exposure (ppm)	**Coproporphyrin (µg/mg creatinine)**	**Uroporphyrin (µg/mg creatinine)**	**Coproporphyrin (µg/mg creatinine)**	**Uroporphyrin (µg/mg creatinine)**
0	0.58 ± 0.33	0.11 ± 0.05	0.67 ± 1.23	0.07 ± 0.04
3	0.78 ± 0.42	0.17 ± 0.09	0.35 ± 0.14	0.10 ± 0.04
10	1.0 ± 0.5	0.31 ± 0.12[b]	0.32 ± 0.11	0.10 ± 0.02

[a]Watanabe et al., 1977
[b]Significantly different from control, $p < 0.05$

Chronic Studies—Coate et al. (1977) exposed groups of 30 male Sprague-Dawley rats, 16 male New Zealand rabbits and 9 male cynomolgus monkeys *(Macaca fascicularis)* to nominal concentrations of 0, 25, 50, or 100 ppm (0, 186, 371, or 742 mg/m^3) of 1,2,4-trichlorobenzene vapor (99.07% pure) 7 hours/day, 5 days/week, for 26 weeks. Average measured concentrations of 1,2,4-trichlorobenzene were 0, 25.3, 49.2, and 92.8 ppm (0, 188, 365, and 689 mg/m^3) for rats and rabbits and 0, 24.8, 49.2, and 94.5 ppm (0, 184, 365, and 701 mg/m^3) for monkeys. Daily observations were performed and body weights were measured weekly for the first 4 weeks, biweekly for the next 8 weeks, and monthly for the remainder of the study. Hematology (complete blood count) and clinical chemistry parameters (BUN, total bilirubin, ALT, AST, ALP, and LDH) were monitored in rabbits and monkeys throughout the study and in rats at sacrifice. The following tests were conducted before and during the exposure period: ophthalmoscopy, pulmonary function, and operant behavior (in monkeys) and ophthalmoscopy (in rabbits). Selected rats (five/group) were sacrificed after 1, 3, and 6 months of exposure. All animals that died during the study and/or were sacrificed were subjected to a complete necropsy with gross pathological examination followed by histopathological examination of selected organs (brain, lungs, heart, liver, kidneys, spleen, eyes, spinal cord, bone marrow, and abdominal skin).

Coate et al. (1977) reported that no compound-related effects were observed on body weights, survival, hematology, or clinical chemistry tests, ophthalmic tests in rabbits and monkeys, or histologic examinations in rabbits and monkeys (data not shown). Tabular data on pulmonary function and operant behavior tests in monkeys also indicated no effect of exposure. Several rabbits (16/64 across all groups including control) and monkeys (4/36, also distributed across all groups) died during the study from pneumonia, but the lack of a concentration-response relationship suggests that this is not a treatment-related effect.

Histopathologic findings were restricted to the liver and kidney of rats; the incidences of these effects are not reported. Exposed rats are reported to have enlarged hepatocytes, a finding that was more apparent at 4 weeks than at 13 weeks and greater at 50 and 100 ppm than at 25 ppm (no additional details were provided). The authors reported a slight increase in the degree of vacuolization of hepatocytes at 4 and 13 weeks, a slight increase in the incidence and degree of granuloma of the liver at 4 weeks, and an increase in the degree of biliary hyperplasia at 4 and 13 weeks. The authors did not indicate the levels at which the latter findings were observed; however, according to the report, these effects do not exhibit a concentration-response relationship. All groups of rats (presumably including controls) exhibited "hyaline degeneration" in kidney sections. The authors indicated that the severity of this lesion was "slightly increased"—although not in a concentration-related manner—in all exposed rats at 4 weeks of exposure but only in the 100-ppm group at 13 weeks. Neither liver nor kidney changes were present in rats sacrificed after 26 weeks of exposure.

Details that would enable a determination of whether the renal lesion constituted hyaline droplet (alpha 2μ-globulin) nephropathy were not provided (Coate et al., 1977), but its description as hyaline degeneration, transient nature and occurrence in male rats are consistent with the description of this lesion (U.S. EPA, 1991b); further, evidence for hyaline droplet nephropathy has also been observed in other rat studies (CMA, 1989a, 1994a). The absence of information on the incidence and/or severity of the hepatic lesions observed in the rats, coupled with the reported lack of concentration-response relationship and the absence of effects in the rats treated longest, preclude the identification of a reliable LOAEL value from the rat data. For rabbits and monkeys, the highest exposure levels of 92.8 ppm (689 mg/m^3) and 94.5 ppm (701 mg/m^3), respectively, represent NOAELs.

Summary of Oral and Inhalation Studies

Table 10 provides a summary of the cited studies in the Derivations Section.

Other Studies

Other Routes

den Besten et al. (1991a) compared the liver, kidney, and thyroid toxicity of several chlorinated benzenes after a single intraperitoneal exposure of male Wistar rats to 1, 2, or 4 mmol/kg of each compound (181, 362, or 543 mg/kg of trichlorobenzene). Of the compounds tested, 1,2,4-trichlorobenzene induced the greatest hepatotoxicity as measured by increase in serum ALT and evidence of liver histopathology 72 hours after dosing. In addition, only 1,2,4-trichlorobenzene exposure resulted in severe degenerative damage to the kidney. Serum thyroxine (T4) levels plunged rapidly after exposure to 2 or 4 mmol/kg 1,2,4-trichlorobenzene; levels were significantly below controls after only 5 hours and reached a nadir 24 hours after dosing. Serum triiodothyronine levels (T3) were not affected by treatment. Among the compounds tested, the decline in T4 levels was correlated with the relative binding affinity of the phenolic metabolites to the plasma transport protein for thyroxine (transthyretin). The authors suggested that alterations in hepatic thyroxine metabolism may also have contributed to the reduction in T4 levels.

In a study published in Japanese with an English abstract and tables, Yamamoto et al. (1982) applied 1,2,4-trichlorobenzene in acetone to the dorsal skin of Slc:ddy mice twice weekly for 2 years. The solution of 1,2,4-trichlorobenzene was 60% for the high dose and 30% for the low dose; the volume applied was 0.03 mL/application. Each treated group

contained 75 animals of each sex and there were 50 vehicle control animals of each sex. Mortality, growth rate, urinalysis, hematology, clinical chemistry, organ weights, and histopathology were evaluated. Growth rates in treated and control mice were comparable through 83 weeks. Mean survival days were significantly ($p < 0.05$) reduced in the 60% 1,2,4-trichlorobenzene groups of males and females and, also, in the 30% treatment group of females. Spleen weights were increased in males of both treatment groups and adrenal weights were increased in high-dose females. Dermal effects (e.g., keratinization, edema, cell infiltration, fibrosis, etc.) were apparent in all treated groups with evidence of dose-related changes. Males treated at the highest concentration exhibited increases in AST, ALT, and BUN; other serum chemistry and hematology findings were unremarkable. The incidences of several nonneoplastic changes were reportedly increased in treated rats, including lung inflammation; liver degeneration, inflammation and amyloidoisis; kidney inflammation and amyloidosis; adrenal amyloidosis and spleen amyloidosis. Further information on the nature of these lesions is not available. Treatment did not increase the incidence of any single tumor type.

Toxicokinetics

WHO (1991) summarized the toxicokinetics of chlorobenzenes, reporting that these compounds are readily absorbed across both the respiratory and gastrointestinal tracts. WHO (1991) noted that data on hexachlorobenzene suggest that oral absorption of chlorobenzenes is likely affected by exogenous factors including the presence of bile and/or lipids in the gastrointestinal tract. Koss and Koransky (1975) reported greatly enhanced absorption of orally administered hexachlorobenzene when administered to rats in olive oil when compared with administration in an aqueous solution (80% absorption from olive oil vs. 6% in aqueous solution). Based on this information, it is possible that, in toxicity studies of 1,2,4-trichlorobenzene administered in corn oil, absorption was enhanced by the vehicle.

Carlson and Tardiff (1976) have shown that 1,2,4-trichlorobenzene is a potent inducer of liver enzymes including P-450; it has also been demonstrated to induce delta-aminolevulinic acid synthetase, the rate-limiting enzyme in heme synthesis and heme oxygenase, which is the rate-limiting enzyme in heme degradation (Ariyoshi et al., 1981).

Only two studies have examined the importance of quinone metabolites in the hepatotoxicity of 1,2,4-trichlorobenzene. den Besten et al. (1991b) demonstrated that secondary metabolism to hydroquinones (after initial epoxide formation) was strongly correlated with covalent binding to protein in rat liver microsomes *in vitro*. The authors observed complete inhibition of protein binding with the addition of the reducing agent ascorbic acid, providing support for quinones as the sole reactive species formed. Mizutani and Miyamoto (1999) examined the role of quinone metabolites *in vivo* using male ddY mice exposed to a single i.p. dose of 1,2,4-trichlorobenzene. The mice were pretreated with either butylated hydroxyanisole (BHA, an inducer of DT-diaphorase, the enzyme that detoxifies quinone intermediates) or dicoumarol (an inhibitor of DT-diaphorase). Pretreatment with BHA markedly suppressed hepatotoxicity (as measured by serum ALT levels), while dicoumarol enhanced hepatotoxicity in mice treated with 1,2,4-trichlorobenzene. This study provided evidence for the importance of quinone metabolites in the liver toxicity of 1,2,4-trichlorobenzene.

Mutagenicity

Mutagenicity and *in vitro* clastogenicity studies of 1,2,4-trichlorobenzene have yielded uniformly negative results, while studies of *in vivo* clastogenicity have been positive. With or without metabolic activation, the chemical did not induce reverse mutation in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 or mitotic recombination in *Saccharomyces cerevisiae* strain D3 (Ethyl Corp., 1975; Schoeny et al., 1979; Lawlor et al., 1979). In a hepatocyte primary culture DNA repair assay, 1,2,4-trichlorobenzene gave negative results (CMA, 1984). With or without metabolic activation, 1,2,4-trichlorobenzene did not increase the frequency of chromosomal aberrations in cultured Chinese hamster ovary cells (Bioassay Systems Corp., 1982). Cell transformation was induced by 1,2,4-trichlorobenzene treatment of adult rat liver epithelial cells (CMA, 1984). In a study published in Italian (Parrini et al., 1990; English abstract reviewed), micronuclei were reportedly induced by intraperitoneal administration of 1,2,4-trichlorobenzene in male Swiss CD-1 mice; no other details are available in the English abstract. In another test of clastogenicity, exposure to doses of 210 to 840 mg/kg 1,2,4-trichlorobenzene via intraperitoneal injection also increased the frequency of micronucleated polychromatic erythrocytes in the bone marrow of male NMRI mice (Mohtashamipur et al., 1987).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR 1,2,4-TRICHLOROBENZENE

Subchronic p-RfD

The toxicological database for 1,2,4-trichlorobenzene includes several oral studies that are potentially relevant to the derivation of a subchronic p-RfD for this chemical. These include subchronic (CMA, 1989a; Cote et al., 1988; Carlson, 1977) studies and a 30-day (Cicmanec, 1991) study in rats; a multigeneration reproductive toxicity study in rats (Robinson et al., 1981); and one adequate developmental toxicity study in rats (Black et al., 1988). In addition, there is a subchronic study in mice (CMA, 1989b). Other developmental toxicity studies were performed; however, effect levels could not be identified for one (Kitchin and Ebron, 1983), and the remaining studies were teratology screening assays that failed to identify a LOAEL for 1,2,4-trichlorobenzene (Chernoff and Kavlock, 1983; Gray et al., 1983; Gray and Kavlock, 1984; Gray et al., 1986). Carlson and Tardiff (1976) conducted a subchronic study in rats, but few endpoints are examined and the effect levels are not defined. In addition, Smith et al. (1978) and Cragg et al. (1978) studied the effects of subchronic exposure in monkeys; however, these studies are reported only in abstract form without enough information to define effect levels. Table 10 provides a summary of the effect levels and critical effects in the studies that were considered for derivation of a subchronic p-RfD. A review of these studies suggests that 1,2,4-trichlorobenzene affects the liver in mice, and results in liver, thyroid, adrenal and hematologic effects in rats. Among the available studies, LOAELs for liver and adrenal effects in rats were of the same order of magnitude, including a LOAEL for liver and thyroid histopathology in rats (82−101 mg/kg-day from Cote et al., 1988); a LOAEL for liver effects in male rats (45.6 mg/kg-day from CMA, 1989a); a freestanding LOAEL for adrenal effects in rats (53 mg/kg-day from Cicmanec, 1991); and a LOAEL for adrenal effects in rats(33 or 54 mg/kg-day for males and females, respectively, from Robinson et al., 1981). LOAELs for the other studies (CMA, 1989b and Black et al., 1988) were higher (150−1184 mg/kg-day); thus, these studies were not considered for use in deriving the subchronic p-RfD.

Table 10. Summary of Oral Noncancer Dose-Response Information Suitable for Subchronic p-RfD Derivation

Species, Sex, Number	Dose (mg/kg-d)	Exposure Regimen	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Adjusted[a] LOAEL (mg/kg-d)	Responses at the LOAEL	Reference
Subchronic Studies							
Rat M/F 10/sex/dose	0, 14.6. 45.6, or 133.7 (M) 0, 17.0, 52.5, or 150.6 (F)	Diet for 13 wk	14.6 (M)	45.6 (M)	45.6 (M)	Increased liver weight and increased incidence of hepatocyte hypertrophy in males only	CMA, 1989a
Rat M/F 10/sex/dose	0, 0.07, 0.78, 7.8, or 82 (M) 0, 0.11, 1.4, 15, or 101 (F)	Diet for 13 wk	7.8 (M) 15 (F)	82 (M) 101 (F)	82 (M) 101 (F)	Liver and thyroid histopathology	Cote et al., 1988
Rat F 5/dose	0, 50, 100, or 200	Daily gavage for 30, 60, 90, or 120 d	50	100	100	Increased liver weight with transiently increased liver and urinary porphyrins	Carlson, 1977
Rat Sex not specified 5/dose	0 or 53	Daily gavage for 30 d	NA	53	53	Decreased serum corticosterone levels, vacuolization of *zona fasciculata* and nonsignificant increase in adrenal weights	Cicmanec, 1991
Mouse M/F 10/sex/dose	0, 67, 851, or 1222 (M) 0, 87, 1184, or 1346 (F)	Diet for 13 wk	67 (M) 87 (F)	851 (M) 1184 (F)	851 (M) 1184 (F)	Liver toxicity in both males and females	CMA, 1989b
Reproductive/Developmental Toxicity Studies							
Rat M/F 17−23 litters/ group	0, 2.5, 8.9, or 33.0 (M) 0, 3.7, 14.8, or 53.6 (F)	Drinking water for 3 generations	8.9 (M) 14.8 (F)	33.0 (M) 53.6 (F)	33.0 (M) 53.6 (F)	Increased adrenal weights in both sexes of F_0 and F_1 generations at 95 days of age	Robinson et al., 1981
Rat F 14/dose	0, 75, 150, or 300	Daily gavage on GD 6−15	75 (maternal) 300 (fetal)	150 (maternal) NA (fetal)	150 (maternal) NA (fetal)	Mild anemia and mild hepatic lesions in dams	Black et al., 1988; Ruddick et al., 1983

Table 10. Summary of Oral Noncancer Dose-Response Information Suitable for Subchronic p-RfD Derivation

Species, Sex, Number	Dose (mg/kg-d)	Exposure Regimen	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Adjusted[a] LOAEL (mg/kg-d)	Responses at the LOAEL	Reference
Reproductive/Developmental Toxicity Studies							
Mice F 25/dose	0 or 130	Daily gavage on GD 8−12	130	NA	NA	None	Chernoff and Kavlock, 1983; Gray et al., 1983, 1986; Gray and Kavlock, 1984

[a]Adjusted for continuous exposure
NA = Not applicable

In order to select a point-of-departure (POD) for the subchronic p-RfD, each of the selected studies (CMA, 1989a; Cote et al., 1988; Cicmanec, 1991; Robinson et al., 1981) was evaluated to determine whether the data for the critical endpoint(s) were amenable to benchmark dose (BMD) modeling. Cote et al. (1988) did not report incidences or severity of liver and thyroid histologic findings, so these endpoints could not be modeled. Because Cicmanec (1991) used only one dose, the data on adrenal weight changes in this study could not be modeled. CMA (1989a) reported dose-related changes in liver weights and hepatocellular hypertrophy incidences (see Table 1), so these endpoints were modeled. Finally, adrenal weight changes in male and female F_0 rats (see Table 6) reported by Robinson et al. (1981) were modeled. Adrenal weight changes were also reported in F_1 rats; however, dose estimates were provided only for F_0 rats. Appendix A provides details of the modeling efforts and selection of best fitting models. The best fit is defined as those with the best goodness of fit and Akaike's information criterion (AIC) scores. The benchmark response level is defined as 1 standard deviation (SD) above the control mean. Table 11 shows the BMD and BMDL predictions from the best-fitting models for each of these data sets.

Table 11. Comparison of BMD and BMDL Predictions for Available Data sets

Reference	Endpoint Modeled	Best-fitting Model	BMD (mg/kg-d)	BMDL (mg/kg-d)
CMA, 1989a	Male Rat Centrilobular Hepatocyte Hypertophy Incidence	Gamma	33.09	14.35
CMA, 1989a	Male Rat Absolute Liver Weight	Linear (constant variance)	21.28	17.49
CMA, 1989a	Male Rat Relative Liver Weight	Linear (constant variance)	11.27	9.41
Robinson et al., 1981	Male Rat Absolute Adrenal Weight	Power (modeled variance)	31.27	16.63
Robinson et al., 1981	Female Rat Absolute Adrenal Weight	Polynomial (constant variance)	28.60	25.51

Among the available data sets, the data for increased relative liver weight in male rats (CMA, 1989a) gave the lowest BMDL (9.41 mg/kg-day). While the hepatocellular hypertrophy that probably underlies this change in liver weight might be a better endpoint, the analysis of the BMD of this endpoint yields a slightly higher value. Being conservative, this value for increased liver weights is selected as the POD for derivation of the subchronic p-RfD. A composite uncertainty factor (UF) of 100 is applied to the BMDL to calculate a **subchronic p-RfD of 0.09 mg/kg-day** as follows:

Subchronic p-RfD = BMDL ÷ UF
= 9.41 mg/kg-day ÷ 100
= **0.09 mg/kg-day or 9×10^{-2} mg/kg-day**

The composite UF of 100 is composed of the following:

- A full UF of 10 is applied for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans. Data to define these parameters for 1,2,4-Trichlorobenzene are unavailable.

- A full UF of 10 for intraspecies differences is used to account for potentially susceptible individuals in the absence of information on the variability of response in humans. Data to define these parameters for 1,2,4-Trichlorobenzene are unavailable.

- No database UF is applied. The toxicological database for oral exposure to 1,2,4-trichlorobenzene includes high-quality chronic and subchronic bioassays in two species and adequate developmental toxicity and multigeneration reproduction studies. Although there are multigeneration reproductive toxicity data in only one species, available information suggests that systemic maternal toxicity occurs at lower doses than reproductive or developmental effects. Available studies have not identified neurotoxicity at high doses, indicating that the lack of a neurotoxicity study is not a significant concern.

Confidence in the principal study (CMA, 1989a) is medium. This subchronic toxicity study used an acceptable number of animals and an appropriate range of dose levels. A variety of endpoints were measured and the study identified both a NOAEL (one sex) and LOAEL (both sexes). Confidence in the database, which includes high-quality chronic and subchronic bioassays in two species, developmental toxicity studies, and a multigeneration reproduction study, is medium. Confidence in the subchronic p-RfD is, therefore, medium.

Chronic p-RfD

Because a chronic RfD of 0.01 mg/kg-day (1E-2 mg/kg-day) exists on IRIS (U.S. EPA, 2008), a chronic p-RfD is not derived in this assessment.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR 1,2,4-TRICHLOROBENZENE

The animal studies provide sufficient information for derivation of p-RfCs for 1,2,4-trichlorobenzene. Table 12 summarizes the noncancer dose-response data from available inhalation studies. In the study by Kociba et al. (1981), the low concentration of 223 mg/m^3 serves as a LOAEL for the increased urinary excretion of porphyrins in rats, while the study by Watanabe et al. (1978) defines a LOAEL of 74 mg/m^3 for increased urinary excretion of porphyrins in rats; a NOAEL of 21 mg/m^3 is established, as well. In the Coate et al. (1977) study, the highest exposure concentration (689−701 mg/m^3) is a freestanding NOAEL for rabbits and monkeys exposed 7 hours/day, 5 days/week, for 4 or 13 weeks. Due to the lack of information on the incidence and/or severity of hepatic lesions reported in rats, the reported lack of concentration-response relationship, and the absence of these effects in rats treated for 26 weeks in the same study, effect levels for this species could not be identified.

Table 12. Summary of Inhalation Noncancer Dose-Response Information

Species, Sex, Number	Exposure Concentration (ppm)	Exposure	NOAEL	LOAEL	Responses	Reference
Subchronic Studies						
Rat M 20/conc.	0, 30 or 100	7 h/d, 5 d/wk for 44 d	NA	30 ppm (223 mg/m^3)	Increased urinary excretion of porphyrins.	Kociba et al., 1981
Rat M/F 10 M and 26 F/conc.	0, 2.8 or 10.2	6 h/d, 5 d/wk for 3 mo	2.8 ppm (21 mg/m^3)	10.2 ppm (76 mg/m^3)	Increased urinary excretion of porphyrins; minimal LOAEL.	Watanabe et al., 1977, 1978
Chronic Studies						
Rat M 30/conc.	0, 25.3, 49.2, or 92.8	7 h/d, 5 d/wk for 4, 13 or 26 wk	Cannot be determined	Cannot be determined	Hepatic lesions were reported to occur in treated rats at 13 wk; however, there was not a clear concentration-response, neither incidence nor severity was reported, and no effects were observed at 26 wk.	Coate et al., 1977
Rabbit M 16/conc.	0, 25.3, 49.2, or 92.8	7 h/d, 5 d/wk for 26 wk	92.8 ppm (689 mg/m^3)	NA	None	Coate et al., 1977
Monkey M 9/conc.	0, 24.8, 49.2, or 94.5	7 h/d, 5 d/wk for 26 wk	94.5 ppm (701 mg/m^3)	NA	None	Coate et al., 1977

The NOAELs and LOAELs from Watanabe et al. (1977, 1978) were first adjusted to an equivalent continuous exposure concentration, then converted to human equivalent concentrations ($NOAEL_{[HEC]}$ and $LOAEL_{[HEC]}$) based on the guidance provided in U.S. EPA (1994b). The human equivalent concentration was then calculated using the appropriate dosimetric adjustment (U.S. EPA, 1994b). As increased urinary excretion of porphyrins is an extrarespiratory effect, 1,2,4-trichlorobenzene was treated as a Category 3 gas and the blood:gas partition coefficients were used to make the dosimetric adjustment. In the absence of blood:gas partition coefficients for 1,2,4-trichlorobenzene, the default ratio of 1.0 was used U.S. EPA (1994b). Table 13 shows a summary of the NOAEL and LOAEL values and the corresponding HEC values.

Table 13. Calculation of Human Equivalent Concentrations

Study	Species	Effect	Effect Level (mg/m^3)	Adjusted Effect Level[a] (mg/m^3)	Dosimetric Adjustment[b]	Human Equivalent Concentration (mg/m^3)
Watanabe et al., 1977, 1978	Rat	Increased urinary excretion of porphyrins	NOAEL = 21 LOAEL = 76 BMCL = 26	$NOAEL_{[ADJ]}$ = 3.8 $LOAEL_{[ADJ]}$ = 14 $BMCL_{[ADJ]}$ = 4.6	1.0	$NOAEL_{[HEC]}$ = 3.8 $LOAEL_{[HEC]}$ = 14 $BMCL_{[HEC]}$ = 4.6

[a]Exposure concentration adjusted to equivalent continuous concentration based on exposure regimen (number of hours/day and days/week; see Table 12)
[b]Ratio of blood:gas partition coefficients

In order to identify a POD for both the subchronic and chronic p-RfCs, BMD modeling was performed on the significant changes in urinary coproporphyrin and uroporphyrin excretion in male and female rats exposed for 3 months (see Table 9) using the nominal exposure concentrations. Appendix B provides details of the modeling and results. The recommended Benchmark Response (BMR) of 1 SD from the control mean (U.S. EPA, 2000) was used in the absence of a biologically-based benchmark response level. No model fit was achieved with female uroporphyrin data, the male coproporphyrin data, or the male uroporphyrin results when reported in µg/mg creatinine. However, adequate fit was achieved with the male uroporphyrin data when reported in µg/24 hours. For this data set, the test for homogenous variance indicated adequate fit to the variance data and the linear model provided adequate fit to the means. The benchmark concentration (BMC_{1sd}) and the 95% lower confidence limit ($BMCL_{1sd}$) resulting from this model were 5.76 and 3.47 ppm (43 and 26 mg/m^3), respectively. The $BMCL_{1sd[HEC]}$ calculated as shown in Table 13 was 4.6 mg/m^3. This value was used as the POD for both the subchronic and chronic p-RfCs for 1,2,4-trichlorobenzene.

Subchronic p-RfC

For the subchronic p-RfC derivation, the $BMCL_{1sd\ [HEC]}$ was divided by a composite UF of 300, as shown below:

Subchronic p-RfC = $BMCL_{1sd[HEC]} \div UF$
= $4.6\ mg/m^3 \div 300$
= **$0.02\ mg/m^3$** or **$2 \times 10^{-2}\ mg/m^3$**

The composite UF of 300 is composed of the following:

- A UF of 3 ($10^{0.5}$) is used to account for the extrapolation from rats to humans using dosimetric adjustments. The interspecies UF includes a factor of 1 for species differences in pharmacokinetic considerations (as a dosimetric adjustment was used) and 3 for pharmacodynamic considerations in accordance with U.S. EPA (1994b).

- A full UF of 10 is used for the protection of sensitive individuals in the absence of information to determine potentially susceptible populations.

- A UF of 10 is used to account for database deficiencies; the toxicological database for inhaled 1,2,4-trichorobenzene contains a limited chronic study in three species and two subchronic studies, but it does not contain any reproductive or developmental toxicity studies by the inhalation route of exposure. Available oral studies do not indicate that the fetus is especially sensitive to the effects of 1,2,4-trichlorobenzene; in the one adequate developmental toxicity study, maternal toxicity was observed at a dose below that affecting the fetus (Black et al., 1988). Likewise, a multigeneration oral reproductive toxicity study did not indicate effects on reproductive endpoints.

Confidence in the key study is medium. The study was well designed, thoroughly documented, and carefully conducted. It identifies both a NOAEL and LOAEL. However, only two exposure concentrations are included and only a limited number of toxicological endpoints are evaluated. Confidence in the database is medium. Though it includes two subchronic studies, a limited chronic study in three species, and oral data on both reproductive and developmental toxicity, the database lacks inhalation data on reproductive and developmental toxicity, as well as a well documented chronic inhalation toxicity study in rats, a sensitive species. Available studies have not identified neurotoxicity at high concentrations, indicating that the lack of a neurotoxicity study is not a significant concern. Medium confidence in the subchronic p-RfC follows.

Chronic p-RfC

For the chronic p-RfC derivation, the $BMCL_{[HEC]}$ was divided by a UF of 3000, including 3 for extrapolation from rats-to-humans using dosimetric adjustments, 10 for protection of sensitive individuals and 10 for database deficiencies (each discussed above under Subchronic p-RfC Derivation), as well as an additional UF of 10 for use of a subchronic study. The absence of a well documented chronic study in a sensitive species (such as rat) is accounted for by the use of a full 10-fold UF for extrapolation from subchronic-to-chronic effects. The chronic p-RfC is calculated below:

Chronic p-RfC = $BMCL_{[HEC]} \div UF$
= $4.6\ mg/m^3 \div 3000$
= **$0.002\ mg/m^3$** or **$2 \times 10^{-3}\ mg/m^3$**

Confidence in the key study is medium. The study was well designed, thoroughly documented, and carefully conducted. It identifies both a NOAEL and LOAEL. However, only two exposure concentrations are included and only a limited number of toxicological endpoints are evaluated. Confidence in the database is medium. Though it includes two subchronic studies, a limited chronic study in three species, and oral data on both reproductive and developmental toxicity, the database lacks inhalation data on reproductive and developmental toxicity as well as a well documented chronic toxicity study in rats. Medium confidence in the chronic p-RfC follows.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR 1,2,4-TRICHLOROBENZENE

Weight-of-Evidence Classification

Under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), 1,2,4-trichlorobenzene is considered *"Likely to Be Carcinogenic to Humans"* by the oral route of exposure based on a finding of increased tumor incidence in more than one sex of mouse. Chronic (2-year) dietary administration of 1,2,4-trichlorobenzene at a concentration of ≥700 ppm produced statistically significant increased incidences of hepatocellular carcinomas and adenomas in male and female B6C3F1 mice (CMA, 1994b). The incidence of carcinoma approached 100% in both sexes treated at 3200 ppm (see Table 5) and led to marked reductions in survival at this dose. Holsapple et al. (2006) reviewed the relevance of mouse liver tumors. They conclude that rodent liver tumors induced by porphyrogenic compounds may be relevant as a predictor of human toxicity, particularly if the metabolism of the compound is similar in rodents and humans. However, they also conclude that the MOA for the induction of tumors by such agents is probably cytotoxicity, not mutagenicity. Short-term tests in bacteria and yeast have given negative evidence for mutagenicity both with and without metabolic activation. A test for DNA repair in mammalian cells was negative, while *in vivo* tests for clastogenicity (micronucleus formation) were positive.

Mode of Action Discussion

The U.S. EPA (2005) Guidelines for Carcinogen Risk Assessment defines MOA as "A sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes and resulting in cancer formation. Toxicokinetic processes leading to the formation or distribution of the active agent (i.e., parent material or metabolite) to the target tissue are not part of the mode of action." Examples of possible carcinogenic MOAs include mutagenic, mitogenic, anti-apoptotic (inhibition of programmed cell death), cytotoxic (with reparative cell proliferation), and immunologic suppression.

Very little information is available on the potential mode by which 1,2,4-trichlorobenzene increases the incidence of liver tumors in mice. Only two studies have been conducted (both in mice): a subchronic toxicity study (CMA, 1989b) and the chronic bioassay (CMA, 1994b) that reported an increased incidence of hepatocellular adenomas and carcinomas in male and female B6C3F1 mice exposed to 1,2,4-trichlorobenzene for 2 years. The subchronic study reports increased absolute and relative liver weights, increased serum ALT,

and/or SDH and liver histopathology (including cytomegaly, karyomegaly, multinucleation, atrophy, degeneration, microcystis) at dietary concentrations of 3500 ppm and higher (CMA, 1989b). The chronic study (CMA, 1994b) reports increased absolute and relative liver weight at ≥150 ppm and hepatocellular hypertrophy, adenomas and carcinomas at ≥700 ppm; liver enzymes were not analyzed. No mechanistic studies examining cancer endpoints were located. Quinone metabolites have been implicated in the hepatotoxicity of 1,2,4-trichlorobenzene in mice (den Besten et al., 1991b; Mizutani and Miyamoto, 1999) and may play a role in liver carcinogenesis as well. Quinones alkylate cellular nucleophiles (e.g., glutathione or tissue macromolecules) and can cause oxidative stress via redox cycling between the semiquinone anion radical and the corresponding hydroquinone or benzoquinone (den Besten et al., 1994). These limited data are inadequate for outlining potential key events in the MOA for 1,2,4-trichlorobenzene-induced hepatocellular tumors.

Quantitative Estimates of Carcinogenic Risk

Oral Exposure

Oral data are sufficient to derive a quantitative estimate of cancer risk from 1,2,4-trichlorobenzene; this derivation is shown below. Male and female B6C3F1 mice both exhibited increased incidences of hepatocellular carcinomas and adenomas in a chronic bioassay (CMA, 1994b). The incidence of combined adenomas and carcinomas was not reported, nor could the incidences be combined. Although the incidence of adenomas was increased at the mid dose in both sexes, there was no increase in adenoma incidence at the high dose due to the high incidence of carcinomas at this dose; thus, only the carcinoma incidence was used in quantitative estimates of cancer risk. The MOA for liver tumors produced by 1,2,4-trichlorobenzene has not been fully elucidated. Existing data do not confirm a mutagenic MOA for 1,2,4-trichlorobenzene, however, the contribution of a linear MOA to induction of liver tumors cannot be ruled out based on available data; thus, a linear assessment was conducted.

The dose-response data used in the quantitative cancer assessment are shown in Table 5 and also below in Table 14. Animal doses in the CMA (1994b) mouse study were first converted to human equivalent doses (HEDs) by adjusting for differences in body weight between humans and mice. In accordance with U.S. EPA (2005) guidelines for carcinogen risk assessment, a factor of $BW^{3/4}$ was used for cross-species scaling. Using this scaling factor, the straight dose (mg) in humans is obtained by multiplying the straight animal dose (mg) by the ratio of human:animal body weight raised to the 3/4 power. For doses expressed per unit body weight (mg/kg or mg/kg-day), the relationship is reciprocal and the human dose (mg/kg) is obtained by multiplying the animal dose (mg/kg) by the ratio of animal:human body weight raised to the 1/4 power. Because the test article was administered in the diet *ad libitum* for 2 years, no adjustment for discontinuous exposure or less-than-lifetime administration is necessary. The equation used to calculate the HEDs is shown below and the HEDs are presented in Table 14.

$$HED = Dose \times (W/70\ kg)^{1/4}$$

where:

Dose = average daily animal dose (mg/kg-day)
W = average mouse body weight during the study (kg)
70 kg = reference human body weight (U.S. EPA, 1988)

Table 14. Dose-Response Data for Liver Tumors in Male and Female Mice[a]

Male				Female			
Animal Dose (mg/kg-d)	**Average Body Weight[b] (kg)**	**Human Equivalent Dose (mg/kg-d)**	**Incidence of Hepatocellular Carcinoma**	**Animal Dose (mg/kg-d)**	**Average Body Weight[b] (kg)**	**Human Equivalent Dose (mg/kg-d)**	**Incidence of Hepatocellular Carcinoma**
0	NA	0	8/49	0	NA	0	1/50
21	0.0373	3.19	5/50	26.3	0.0327	3.87	1/50
100.6	0.0365	15.2	27/50	127.0	0.0322	18.6	28/50
519.9	0.0302	74.9	50/50	572.6	0.0286	81.4	46/50

[a]CMA, 1994b
[b]Calculated from body weight measurements made during the study

Dose-response modeling of the data in Table 14 was performed to obtain a POD for a quantitative assessment of cancer risk. The POD is an estimated dose (expressed in human-equivalent terms) near the lower end of the observed range that marks the starting point for extrapolation to lower doses. Appendix C provides details of the modeling effort. Table 15 shows the $BMD_{10[HED]}$ and $BMDL_{10[HED]}$ predicted by the multistage model for the liver tumor data in males and females.

Table 15. Summary of Human Equivalent BMDs and BMDLs Based on Hepatocellular Carcinoma Incidence Data in Male and Female Mice

	$BMD_{10[HED]}$ (mg/kg-d)	**$BMDL_{10[HED]}$ (mg/kg-d)**
Male	6.23	3.50
Female	6.84	5.00

As the table shows, the BMD estimates for both sexes are very similar. The $BMDL_{10[HED]}$ for liver tumors in male mice (3.50 mg/kg-day) is slightly lower than that for females (5.00 mg/kg-day) and is selected as the POD for the provisional oral slope factor (p-OSF). A **provisional oral slope factor of 0.029 or 2.9×10^{-2} $(mg/kg\text{-}day)^{-1}$** was calculated by dividing 0.1 (10%) by the $BMDL_{10[HED]}$ of 3.50 mg/kg-day. The p-OSF for 1,2,4-trichlorobenzene should not be used with exposures exceeding the POD ($BMDL_{10[HED]}$ = 3.50 mg/kg-day), because below this level the fitted dose-response model better characterizes what is known about the carcinogenicity of 1,2,4-trichlorobenzene. Table 16 shows the doses associated with specific levels of cancer risk based on the provisional oral slope factor estimated herein.

Table 16. Doses of 1,2,4-Trichlorobenzene Associated With Specific Levels of Cancer Risk	
Risk Level	**Dose (mg/kg-d)**
10^{-4}	0.003
10^{-5}	0.0003
10^{-6}	0.00003

Inhalation Exposure

Inhalation studies of 1,2,4-trichlorobenzene are inadequate for the purpose of estimating an inhalation unit risk for this compound. Only one chronic inhalation study is identified (Coate et al., 1977) and, in the study, the neoplastic changes are not reported.

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APPENDIX A. DETAILS OF BENCHMARK DOSE MODELING FOR SUBCHRONIC ORAL p-RfD

Model-Fitting Procedure for Continuous Data:

The model fitting procedure for continuous data is as follows. The simplest model (linear) is first applied to the data while assuming constant variance. If the data are consistent with the assumption of constant variance ($p \geq 0.1$), then the fit of the linear model to the means is evaluated and the polynomial, power and Hill models are fit to the data while assuming constant variance. Among the models providing adequate fit to the means ($p \geq 0.1$), the one with the lowest AIC for the fitted model is selected for BMD derivation. If the test for constant variance is negative, the linear model is run again while applying the power model integrated into the BMDS to account for nonhomogenous variance. If the nonhomogenous variance model provides an adequate fit ($p \geq 0.1$) to the variance data, then the fit of the linear model to the means is evaluated and the polynomial, power and Hill models are fit to the data and evaluated while the variance model is applied. Among those providing adequate fit to the means ($p \geq 0.1$), the one with the lowest AIC for the fitted model is selected for BMD derivation. If the test for constant variance is negative and the nonhomogenous variance model does not provide an adequate fit to the variance data, then the data set is considered unsuitable for modeling. If after these attempts, no model provides an adequate fit to the data, the highest dose is dropped, if appropriate, and the entire procedure is repeated. If no fit is obtained after dropping the highest dose, the next highest dose is dropped, if appropriate and the procedure is repeated. Dose-dropping continues until: (1) adequate fit is obtained; (2) there are only controls and two dose groups remaining. If no fit is obtained following application of this procedure, then the data set is not considered to be amenable to BMD modeling.

Model-Fitting Results for Male Rat Absolute and Relative Liver Weights (CMA, 1989a)

Data on male rat absolute and relative liver weights were modeled according to the procedure outlined above using BMDS version 1.4.1 with default parameter restrictions. In the absence of data regarding a biologically meaningful change in liver weight, the BMR was chosen to be 1 SD from the control mean, as recommended by U.S. EPA (2000). The linear model with constant variance provided adequate fit to both data sets. Table A-1 shows the modeling results for each data set. A BMD_{1sd} and $BMDL_{1sd}$ of 21.28 and 17.49 mg/kg-day, respectively, were predicted using the absolute liver weight data. A BMD_{1sd} and $BMDL_{1sd}$ of 11.27 and 9.41 mg/kg-day, respectively, were predicted using the relative liver weight data. Figures A-1 and A-2 show the fit of the linear model with constant variance to these two data sets.

Table A-1. Model Predictions for Liver Weight Changes in Male Rats[a]				
Model	**Variance *p*-value[b]**	**Means *p*-value[b]**	**BMD_{1sd} (mg/kg-d)**	**$BMDL_{1sd}$ (mg/kg-d)**
Absolute Liver Weight				
Linear (constant variance)[c]	0.1802	0.4346	21.28	17.49
Relative Liver Weight				
Linear (constant variance)[c]	0.4288	0.2090	11.27	9.41

[a]CMA, 1989a
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Betas restricted to ≥0

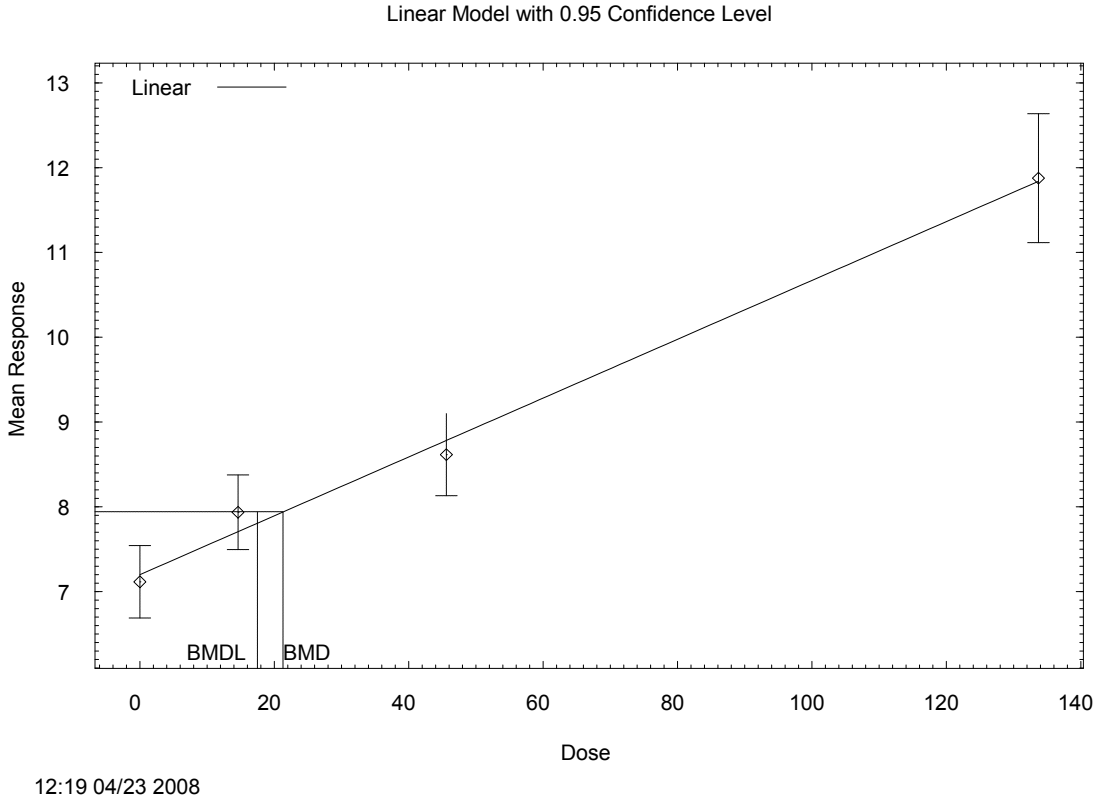

Figure A-1. Fit of Linear Model (Homogenous Variance) to Data on Male Rat Absolute Liver Weight (CMA, 1989a)

BMDs and BMDLs indicated are associated with a change of 1 SD from the control and are in units of mg/kg-day.

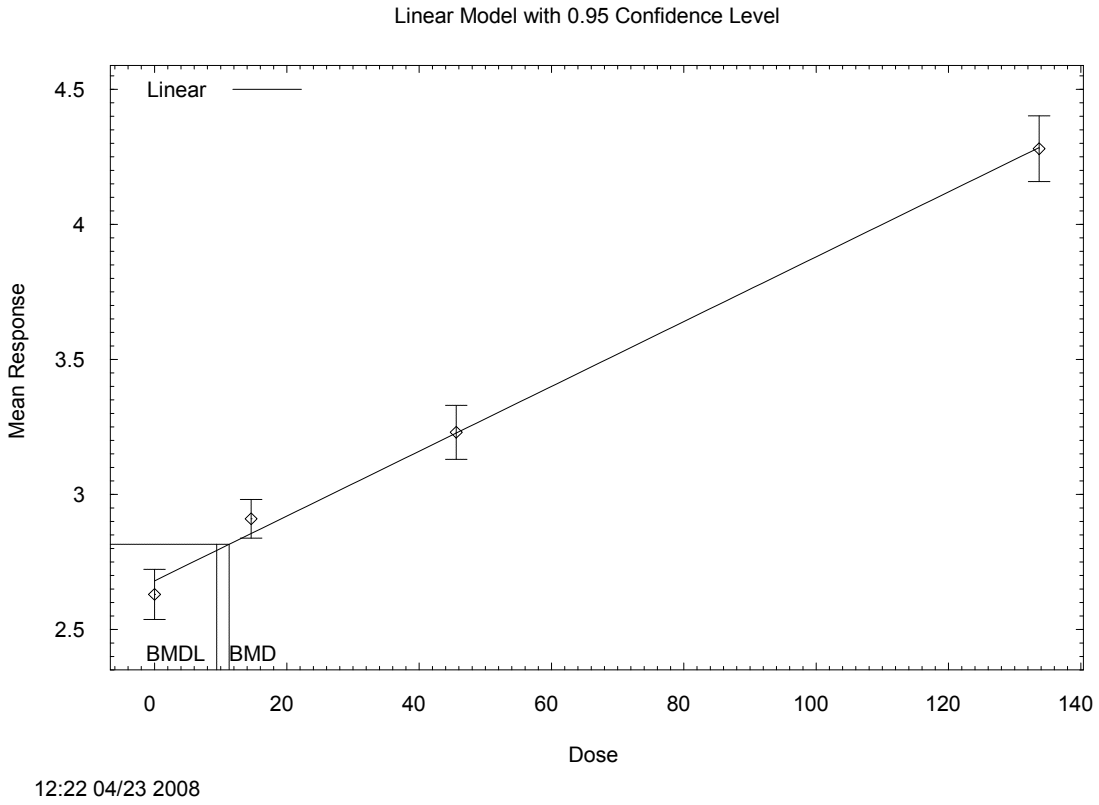

Figure A-2. Fit of Linear Model (Homogenous Variance) to Data on Male Rat Relative Liver Weight (CMA, 1989a)

BMDs and BMDLs indicated are associated with a change of 1 SD from the control and are in units of mg/kg-day.

Model-Fitting Results for Male and Female Rat Absolute Adrenal Weights (Robinson et al., 1981)

Data on male and female rat absolute adrenal weights were modeled according to the procedure outlined above using BMDS version 1.4.1 with default parameter restrictions. In the absence of data regarding a biologically meaningful change in adrenal weight, the BMR was chosen to be 1 SD deviation from the control mean, as recommended by U.S. EPA (2000). Table A-2 shows the modeling results for both data sets. Using the male adrenal weight data, the test for constant variance was negative, indicating that the variance should be modeled; the variance model in the software provided adequate fit. The linear model did not provide adequate fit to the means, so the polynomial and power models were fit to the data (there were not enough data points to fit the Hill model). Both of these models provided adequate fit to the means in the male adrenal weight data, but the power model provided better fit based on lower AIC. The BMD_{1sd} and $BMDL_{1sd}$ predicted by the power model were 20.57 and 16.84 mg/kg-day, respectively. Figure A-3 shows the fit of the power model (modeled variance) to the male adrenal weight data. Using the female adrenal weight data, the test for constant variance was positive, but the linear model did not provide adequate fit to the means. The polynomial and power models were applied, but only the polynomial model provided adequate fit to the means. This model predicted a BMD_{1sd} and $BMDL_{1sd}$ of 28.60 and 25.51 mg/kg-day, respectively, for the female adrenal weight data. Figure A-4 shows the fit of the polynomial model with constant variance to the female rat adrenal weight data.

Table A-2. Model Predictions for Adrenal Weight Changes in Male and Female Rats[a]

Model	Variance *p*-value[b]	Means *p*-value[b]	AIC	BMD_{1sd} (mg/kg-d)	$BMDL_{1sd}$ (mg/kg-d)
Male adrenal weight					
Linear (constant variance)[c]	0.02696	0.1062	86.12	16.25	11.83
Linear (modeled variance)[c]	0.593	0.0272	82.70	14.12	9.26
Polynomial (modeled variance)[c,d]	0.593	0.1753	78.98	20.57	16.84
Power (modeled variance)[e,f]	0.593	0.3385	77.66	31.27	16.63
Hill (modeled variance)[e]	NA[e]	NA	NA	NA	NA
Female adrenal weight					
Linear (constant variance)[c]	0.1522	0.01715	83.73	15.64	12.34
Polynomial (constant variance)[c,d,f]	0.1522	0.1800	79.03	28.60	25.51
Power (constant variance)[e]	0.1522	0.0652	81.00	30.31	17.62
Hill (constant variance)[e]	NA	NA	NA	NA	NA

[a]Robinson et al., 1981
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Betas restricted to ≥0
[d]Polydegree = 2 (lowest degree with adequate fit)
[e]Power restricted to ≥1
[f]Best fitting model
[g]NA = not applicable (insufficient degrees of freedom available to fit this model)

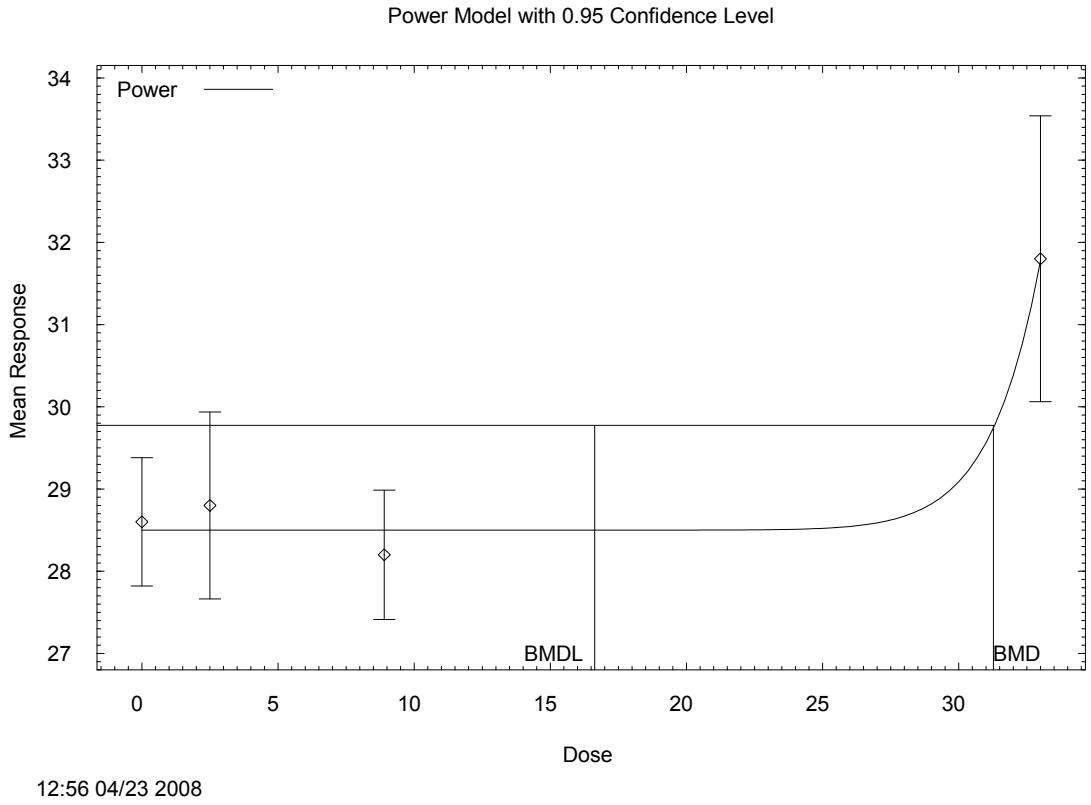

Figure A-3. Fit of Power Model (Modeled Variance) to Data on Male Rat Absolute Adrenal Weight (Robinson et al., 1981)

BMDs and BMDLs indicated are associated with a change of 1 SD from the control and are in units of mg/kg-day.

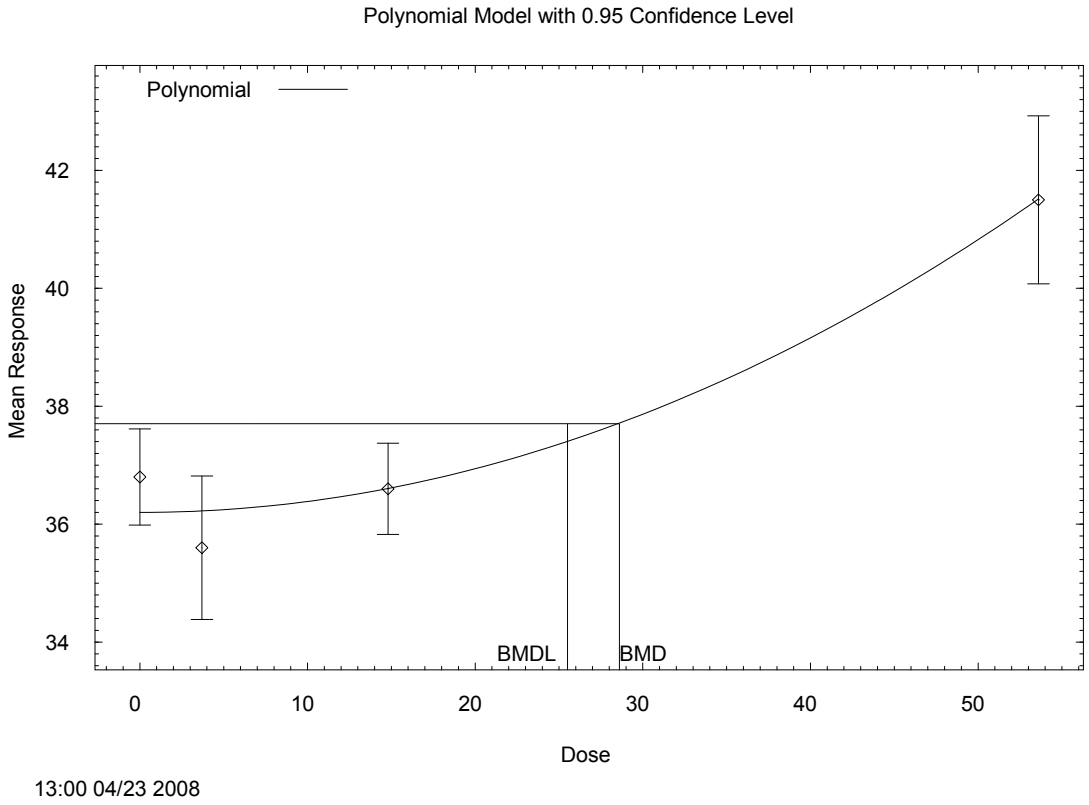

Figure A-4. Fit of Polynomial Model (Homogenous Variance) to Data on Female Rat Absolute Adrenal Weight (Robinson et al., 1981)

BMDs and BMDLs indicated are associated with a change of 1 SD from the control and are in units of mg/kg-day.

Model-Fitting Procedure for Quantal Data:

The model fitting procedure for dichotomous data is as follows. All available dichotomous models in the U.S. EPA BMDS are fit to the incidence data using the extra risk option. The multistage model is run for all polynomial degrees up to n - 1 (where n is the number of dose groups including control); the lowest degree polynomial providing adequate fit is used for comparison with the other models, per U.S. EPA (2000) guidance. Goodness of fit is assessed by the χ^2 test. When several models provide adequate fit to the data (χ^2 $p \geq 0.1$), models are compared using the AIC. The model with the lowest AIC is considered to provide the best fit to the data. When several models have the same AIC, the model resulting in the lowest BMDL is selected. In accordance with U.S. EPA (2000) guidance, BMDs and lower bounds on the BMD (BMDLs) associated with an extra risk of 10% are calculated for all models.

Model-Fitting Results for Male Rat Centrilobular Hepatocyte Hypertrophy (CMA, 1989a)

Data on the incidence of centrilobular hepatocyte hypertrophy in male rats (see Table 1) were modeled according to the procedure outlined above using BMDS version 1.4.1 with default parameter restrictions. Table A-3 shows the modeling results. With the exception of the quantal linear model, which the software indicated was an invalid model choice, all of the models in the software provided adequate fit to the data ($p > 0.1$). The gamma model provided the best fit as assessed by AIC. The BMD_{10} and $BMDL_{10}$ predicted by this model for the data on incidence of centrilobular hepatocyte hypertrophy in male rats are 33.09 and 14.35 mg/kg-day, respectively.

Table A-3. Model Predictions for Incidence of Male Rat Centrilobular Hepatocyte Hypertrophy[a]

Model	Degrees of Freedom	χ^2	χ^2 Goodness-of-Fit *p*-Value[b]	AIC	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)
Gamma (power ≥1)[c]	3	0.00	1.0000	15.8635	33.09	14.35
Log-logistic (slope ≥1)	2	0.00	1.0000	17.8629	40.29	16.74
Logistic	2	0.00	1.0000	17.8629	41.94	18.95
Multistage (degree = 1)[d]	3	3.99	0.2626	23.0471	6.43	4.04
Log-probit (slope ≥1)	2	0.00	1.0000	17.8629	35.74	16.04
Probit	2	0.00	1.0000	17.8629	38.66	17.28
Quantal Linear	Invalid model choice per BMDS software					
Weibull (power ≥1)	2	0.00	0.9999	17.8631	37.60	13.40

[a]CMA, 1989a
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Best-fitting model
[d]Degree of polynomial initially set to (n - 1) where n = number of dose groups including control; model selected is lowest-degree model providing adequate fit. Betas restricted to ≥0.

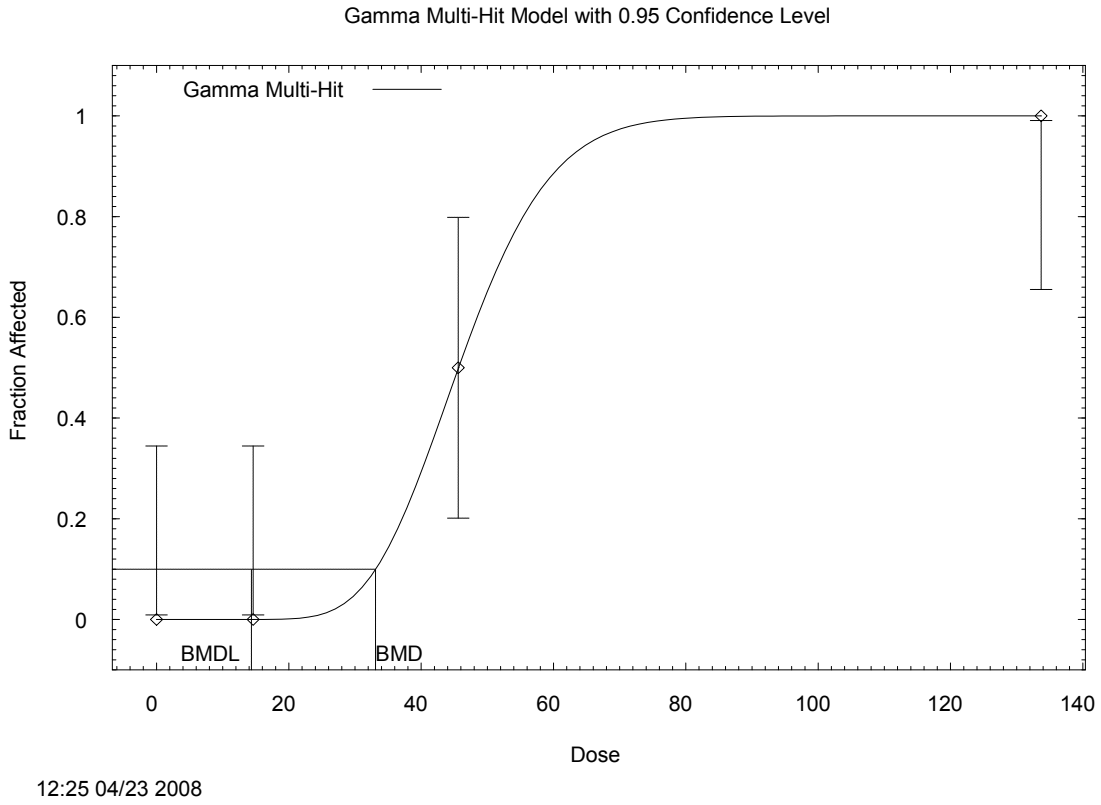

Figure A-5. Fit of Gamma Model to Data on Incidence of Male Rat Centrilobular Hepatocyte Hypertrophy (CMA, 1989a)

BMDs and BMDLs indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

APPENDIX B. DETAILS OF BENCHMARK DOSE MODELING FOR SUBCHRONIC AND CHRONIC INHALATION p-RfCs

Model-Fitting Procedure for Continuous Data:

The model fitting procedure for continuous data is as follows. The simplest model (linear) is first applied to the data while assuming constant variance. If the data are consistent with the assumption of constant variance ($p \geq 0.1$), then the fit of the linear model to the means is evaluated and the polynomial, power and Hill models are fit to the data while assuming constant variance. Among the models providing adequate fit to the means ($p \geq 0.1$), the one with the lowest AIC for the fitted model is selected for BMD derivation. If the test for constant variance is negative, the linear model is run again while applying the power model integrated into the BMDS to account for nonhomogenous variance. If the nonhomogenous variance model provides an adequate fit ($p \geq 0.1$) to the variance data, then the fit of the linear model to the means is evaluated and the polynomial, power and Hill models are fit to the data and evaluated while the variance model is applied. Among those providing adequate fit to the means ($p \geq 0.1$), the one with the lowest AIC for the fitted model is selected for BMD derivation. If the test for constant variance is negative and the nonhomogenous variance model does not provide an adequate fit to the variance data, then the data set is considered unsuitable for modeling. If after these attempts, no model provides an adequate fit to the data, the highest dose is dropped, if appropriate, and the entire procedure is repeated. If no fit is obtained after dropping the highest dose, the next highest dose is dropped, if appropriate and the procedure is repeated. Dose-dropping continues until: (1) adequate fit is obtained; (2) there are only controls and two dose groups remaining. If no fit is obtained following application of this procedure, then the data set is not considered to be amenable to BMD modeling.

Model-Fitting Results for Urinary Porphyrin Levels in Rats (Watanabe et al., 1977, 1978):

Data on male coproporphyrin levels (reported in µg/24 hours) and uroporphyrin levels (reported in µg/24 hours as well as in µg/mg creatinine), as well as data on female uroporphyrin levels (reported in µg/24 hours), were modeled according to the procedure outlined above using BMDS version 1.4.1 with default parameter restrictions. In the absence of data regarding a biologically meaningful change in urinary porphyrin levels, the BMR was chosen to be 1 SD from the control mean, as recommended by U.S. EPA (2000). Although modeling of the male coproporphyrin levels indicated adequate fit to both the variance and means using the linear model with constant variance, the initial test for a difference among the responses was not significant ($p > 0.05$), indicating that modeling of the data set would not be appropriate. The linear model with constant variance provided adequate fit to the male data on uroporphyrin levels when reported in µg/24 hours. Using the data on male uroporphyrin levels reported in µg/mg creatinine, adequate fit of the variance data was achieved with the homogenous variance model, but the linear model did not provide adequate fit to the means data. Because only three dose groups were available for modeling, other model forms could not be used for this data set. Using the data on female uroporphyrin levels reported in µg/24 hours, the test for homogenous variance was negative and the nonhomogenous variance model in the BMDS did not provide adequate fit to the variance data either, indicating that this data set is not suitable for BMD modeling. Table B-1 shows the modeling results for each data set.

Table B-1. Model Predictions for Urinary Porphyrins in Male and Female Rats[a]

Model	Variance *p*-value[b]	Means *p*-value[b]	BMC_{1sd} (ppm)	$BMCL_{1sd}$ (ppm)
Male coproporphyrin in µg/24 h[c]				
Linear (constant variance)[d]	0.4636	0.7486	8.84	4.54
Male uroporphyrin in µg/24 h				
Linear (constant variance)[d]	0.1769	0.947	5.76	3.47
Male uroporphyrin in µg/mg creatinine				
Linear (constant variance)[d]	0.1802	<0.0001	4.08	2.69
Polynomial (constant variance)[d]	NA [e]	NA	NA	NA
Power (constant variance)[e]	NA	NA	NA	NA
Hill (constant variance)[e]	NA	NA	NA	NA
Female uroporphyrin in µg/24 h				
Linear (constant variance)[d]	0.07468	0.8540	4.78	3.03
Linear (modeled variance)[d]	0.0347	0.8213	5.33	3.20

[a]Watanabe et al., 1977, 1978
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Test for a difference among the responses was not significant ($p = 0.32$)
[d]Betas restricted to ≥0
[e]Power restricted to ≥1
[f]NA = not applicable (insufficient degrees of freedom available to fit this model)

In summary, BMD modeling was successful only for the male uroporphyrin data reported in µg/24 hours. A BMC_{1sd} and $BMCL_{1sd}$ of 5.76 and 3.47 ppm, respectively, were predicted using these data. Figure B-1 shows the fit of the linear model with constant variance to these data.

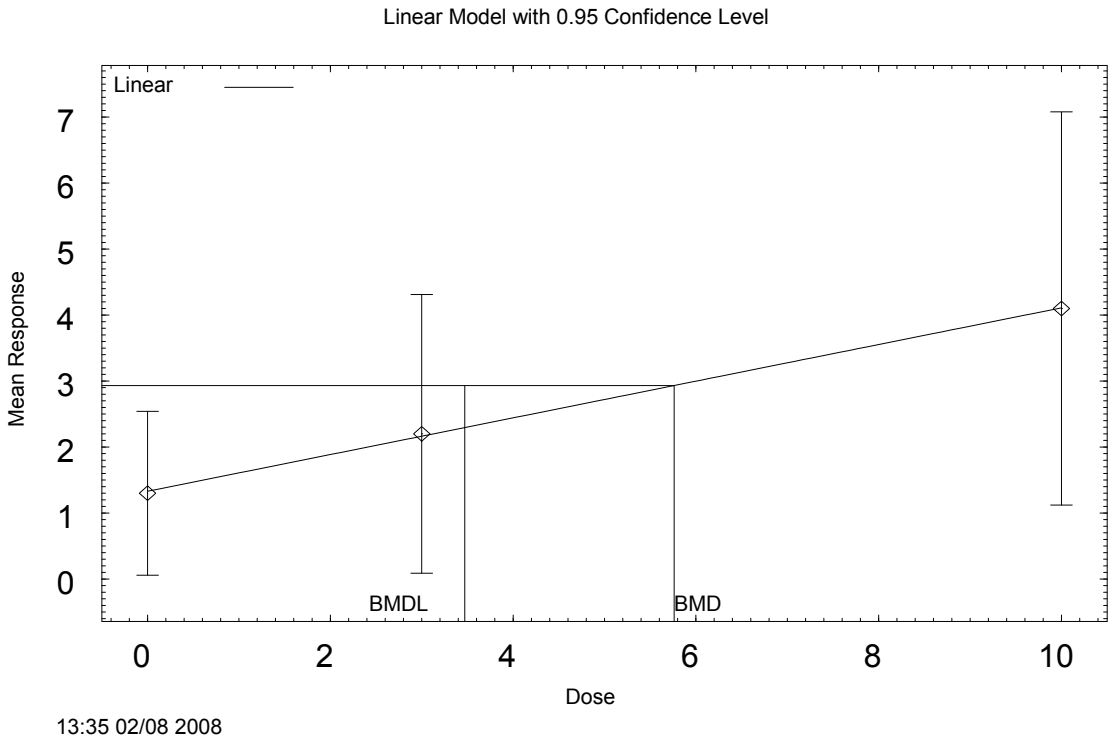

Figure B-1. Fit of Linear Model (Homogenous Variance) to Data on Male Uroporphyrin Levels (measured in µg/24 hours)

BMCs and BMCLs indicated are associated with a change of 1 SD from the control and are in units of ppm.

APPENDIX C. DETAILS OF BENCHMARK DOSE MODELING FOR ORAL SLOPE FACTOR

Model-Fitting Procedure for Cancer Incidence Data:

The model-fitting procedure for dichotomous cancer incidence data is as follows. The multistage-cancer model in the U.S. EPA BMDS is fit to the incidence data using the extra risk option. The multistage model is run for all polynomial degrees up to n - 1 (where n is the number of dose groups including control); the lowest degree polynomial providing adequate fit is selected, per U.S. EPA (2000) guidance. Goodness of fit is assessed by the χ^2 test; adequate fit is indicated by a *p*-value greater than 0.1. In accordance with U.S. EPA (2000) guidance, benchmark doses (BMDs) and lower bounds on the BMD (BMDLs) associated with an extra risk of 10% are calculated.

Model-Fitting Results for Hepatocellular Carcinomas in Mice (CMA, 1994b):

Table 14 shows the dose-response data on liver carcinoma incidence in male and female mice (CMA, 1994b). The incidence and human equivalent dose data were modeled according the procedure outlined above using BMDS version 1.4.1 with default parameter restrictions. As assessed by the χ^2 goodness-of-fit test, the multistage model with 2-degree polynomial was the lowest degree polynomial providing adequate fit to the male tumor data (χ^2 $p \geq 0.1$) (see Table C-1). The multistage model did not provide adequate fit to the female hepatocellular carcinoma data using the full data set. After exclusion of the high-dose group, adequate fit was achieved with the 2-degree multistage model. The liver tumor data in males generated the lower of the two $BMDLs_{10[HED]}$. Figure C-1 shows the fit of the multistage model (2-degree) to the data set for males, while Figure C-2 shows the fit of the multistage model to the reduced data set for females.

Table C-1. Model Predictions for Hepatocellular Carcinomas in Male and Female Mice[a]

Model	Degrees of Freedom	χ^2	χ^2 Goodness-of-Fit *p*-Value[b]	AIC	$BMD_{10[HED]}$ (mg/kg-d)	$BMDL_{10[HED]}$ (mg/kg-day)	Multistage Cancer Slope Factor $(mg/kg\text{-}d)^{-1}$
Male Mice: All Doses							
Multistage (degree = 2)[c]	2	1.62	0.4456	150.778	6.2274	3.5040	0.029
Female Mice: All Doses							
Multistage (degree = 3)[c]	2	7.62	0.0221	130.175	3.2527	2.6036	0.038
Female Mice: High Dose Excluded							
Multistage (degree = 2)[c]	1	0.98	0.3213	93.3518	6.8444	5.0006	0.020

[a]CMA, 1994b
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Degree of polynomial initially set to (n - 1) where n = number of dose groups including control; model selected is lowest degree model providing adequate fit. Betas restricted to ≥0.

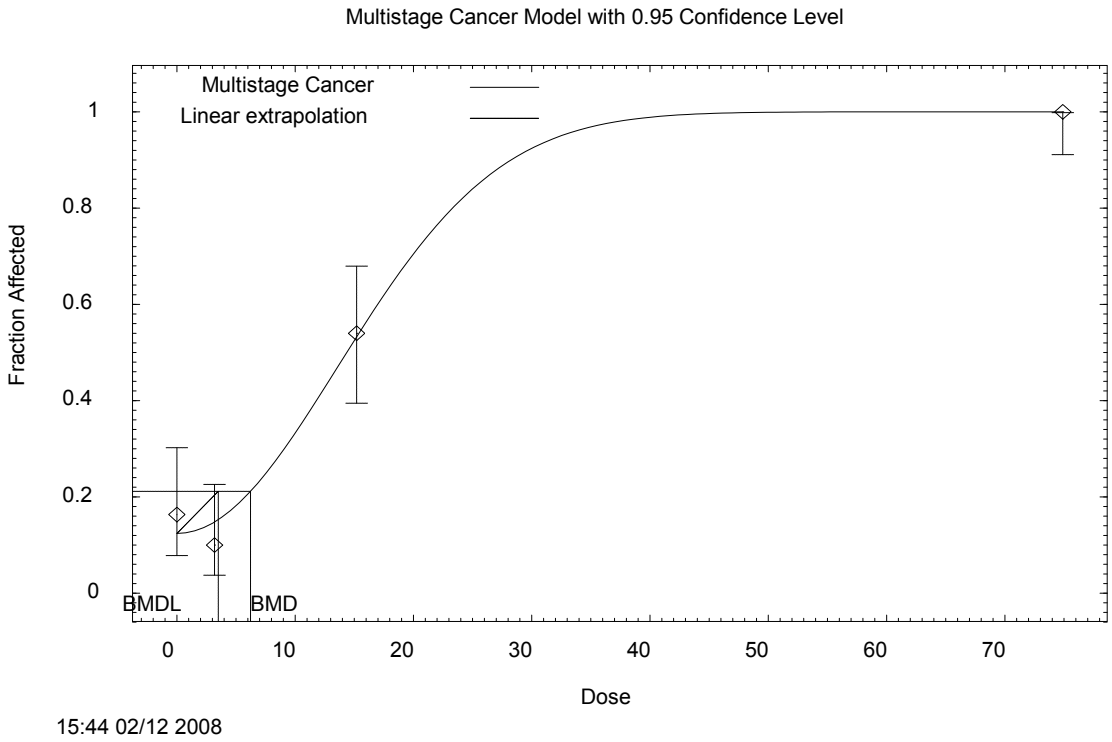

Figure C-1. Fit of Multistage (2-degree) Model to Data on Hepatocellular Carcinomas in Male Mice (CMA, 1994b)

BMDs and BMDLs indicated are human equivalent doses associated with an extra risk of 10% and are in units of mg/kg-day.

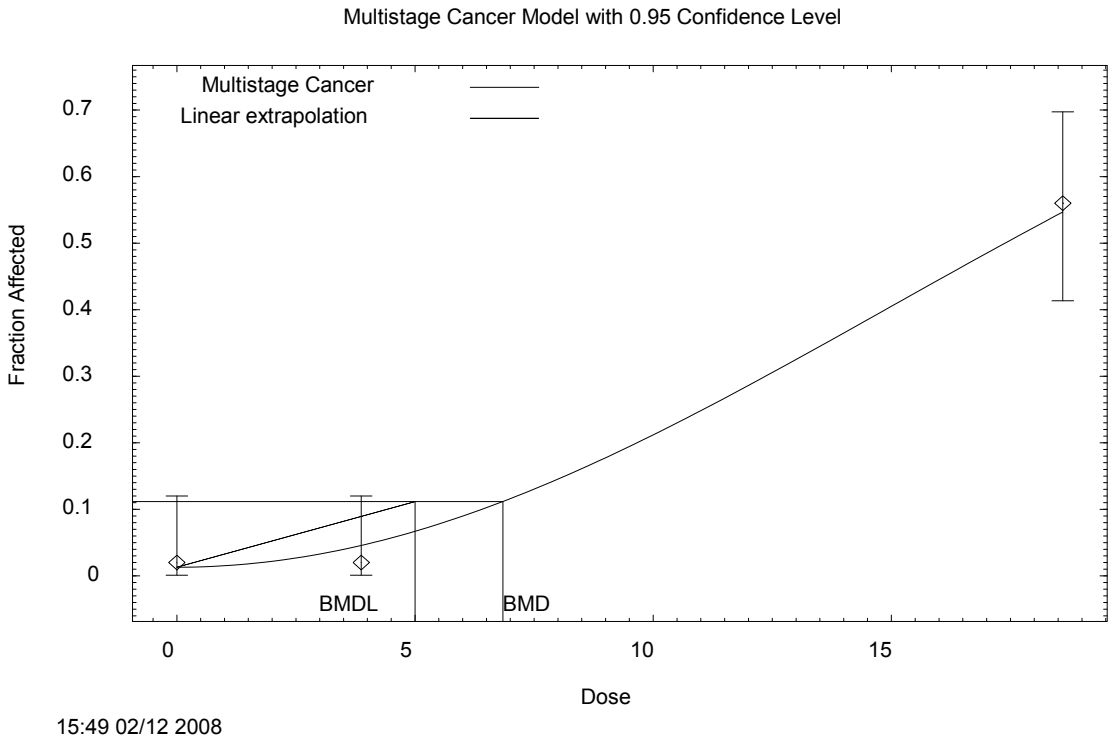

Figure C-2. Fit of Multistage (2-degree) Model to Data (excluding high dose) on Hepatocellular Carcinomas in Female Mice (CMA, 1994b)

BMDs and BMDLs indicated are human equivalent doses associated with an extra risk of 10% and are in units of mg/kg-day.