This document is a final draft. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency position on this chemical. It is being circulated for review of its technical accuracy and science policy implications.

0020
Carbon tetrachloride; CASRN 56-23-5; 00/00/0000

Human health assessment information on a chemical substance is included in IRIS only after a comprehensive review of toxicity data by U.S. EPA health scientists from several program offices, regional offices, and the Office of Research and Development. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the positions that were reached during the review process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website at http://www.epa.gov/iris/backgr-d.htm.

STATUS OF DATA FOR Carbon tetrachloride

File First On-Line 01/31/1987

Category (section)	Status	Last Revised
Chronic Oral RfD Assessment (I.A.)	on-line	00/00/0000
Chronic Inhalation RfC Assessment (I.B.)	on-line	00/00/0000
Carcinogenicity Assessment (II.)	on-line	00/00/0000

_I. HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS

__I.A. REFERENCE DOSE (RfD) FOR CHRONIC ORAL EXPOSURE

Carbon tetrachloride
CASRN -- 56-23-5
Section I.A. Last Revised -- 00/00/0000

The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD is intended for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. It is expressed in units of mg/kg-day. Please refer to the guidance documents at http://www.epa.gov/iris/backgr-d.htm for an elaboration of these

concepts. Because RfDs can be derived for the noncarcinogenic health effects of substances that are also carcinogens, it is essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

The previous oral RfD for carbon tetrachloride (posted on the IRIS database in 1987) was 0.0007 mg/kg-day, based the same 12-week study (Bruckner et al., 1986) used to derive the current RfD. The no-observed-adverse-effect level (NOAEL) was identified as 1 mg/kg (daily dose of 0.7 mg/kg-day) and the lowest-observed-adverse-effect level (LOAEL) as 10 mg/kg (daily dose of 7 mg/kg-day) for liver lesions as evidenced by mild centrilobular vacuolation and significantly increased serum sorbitol dehydrogenase (SDH) activity in rats. The RfD of 0.0007 mg/kg-day was calculated by applying a UF of 1,000 (three factors of 10 to account for interspecies and interhuman variability and extrapolation from subchronic to chronic exposure) to the NOAEL of 0.7 mg/kg-day.

___I.A.1. CHRONIC ORAL RfD SUMMARY

Critical Effect	Point of Departure*	UF	Chronic RfD
Elevated serum SDH activity	$BMDL_{2X\text{-}ADJ}$: 3.9 mg/kg-day	1,000	0.004 mg/kg-day
Subchronic oral rat study			
Bruckner et al., 1986			

*Conversion Factors and Assumptions –Animals were dosed 5 days/week; therefore, $BMDL_{2X}$ was multiplied by a factor of 5/7 to derive the $BMDL_{2X\text{-}ADJ}$.

___I.A.2. PRINCIPAL AND SUPPORTING STUDIES

Bruckner et al. (1986) administered analytical-grade carbon tetrachloride to groups of 15–16 adult male Sprague-Dawley rats at doses of 0, 1, 10, or 33 mg/kg by gavage in corn oil 5 days/week for 12 weeks (time-weighted average doses of 0, 0.71, 7.1, or 23.6 mg/kg-day). Body weight was measured twice weekly. Blood samples were taken from five rats from each group at 2-week intervals (2, 4, 6, 8, 10, and 12 weeks, and 2 weeks post-treatment). After 12 weeks, 7–9 animals from each group were sacrificed. The remaining animals were maintained without carbon tetrachloride treatment for an additional 2 weeks and then sacrificed. Following sacrifice, a terminal blood sample was taken by cardiac puncture. The liver and kidneys were removed, weighed, and processed for histopathological examination. Blood samples were used for determination of serum alkaline phosphatase (ALT), ornithine carbamoyl transferase (OCT), and SDH, all of which are indicators of liver injury, and blood urea nitrogen (BUN), an indicator of kidney damage.

At the end of the exposure period, substantial toxicity was evident in rats exposed to 33 mg/kg-day. Body weight gain in this group was significantly reduced by about 6% after

30 days and 17% after 90 days. Liver toxicity in this group was manifested by significantly elevated ALT (up to 34 times the control level), SDH (up to 50 times the control level), and OCT (up to 8 times the control level) from week 2 through the end of exposure, significantly increased liver:body weight ratio, and extensive occurrence of degenerative lesions (including lipid vacuolization, nuclear and cellular polymorphism, bile duct hyperplasia, and periportal fibrosis). Only moderate liver effects were seen in animals exposed to 10 mg/kg-day, as shown by a significant (two- to threefold) elevation of SDH during the second half of the exposure period and the presence of mild centrilobular vacuolization in the liver. Body weight gain was similar to controls. During the 2-week recovery period, serum ALT and SDH levels returned towards control levels in both mid- and high-dose rats. Hepatic lesions were still present in both groups, but severity was reduced for lesions other than fibrosis and bile duct hyperplasia, the severity of which did not change. No effects were observed in rats exposed to 1 mg/kg-day. This study identified a NOAEL of 1 mg/kg-day and a LOAEL of 10 mg/kg-day for carbon tetrachloride-induced liver toxicity.

Methods of Analysis. Elevated serum SDH was identified as a specific and sensitive biomarker of liver toxicity and the most appropriate data set from the Bruckner et al. (1986) study for defining the point of departure (POD) for the RfD. Travlos et al. (1996) found SDH to be a reliable predictor of hepatotoxicity based on an examination of data from 13-week toxicity studies for 50 chemicals and three chemical mixtures. Of the enzymes monitored by Bruckner et al. (1986), only SDH showed a statistically and biologically significant increase in the 10 mg/kg dose group. The data for the 10- and 12-week blood draws were similar. Therefore, both 10- and 12-week data were used for dose-response modeling using BMD methods (U.S. EPA, 2000).

All of the models for continuous data in U.S. EPA's BMD software (BMDS) (version 1.4.1) were fit to the 10- and 12-week SDH data. An increase in SDH activity 2 times the control mean, representing an increase in serum enzyme level considered to be biologically significant, was used as the benchmark response (BMR). None of the models for continuous data in BMDS provided an adequate fit of the 12-week SDH data. The power model provided the best fit of the 10-week SDH data (based on goodness-of-fit *p*-value $\geq$0.1 and the lowest Akaike's Information Criterion [AIC] value) and was therefore selected as the basis for the POD. This model estimated a BMD_{2X} of 7.32 mg/kg-day and a $BMDL_{2X}$ of 5.46 mg/kg-day. To obtain a value representing an average daily dose, the $BMDL_{2X}$ of 5.46 mg/kg-day was multiplied by 5/7, to yield an adjusted $BMDL_{2X}$ of 3.9 mg/kg-day.

Other data sets were also considered as the basis for the carbon tetrachloride RfD, including 10- and 12-week serum OCT and ALT data from Bruckner et al. (1986), rat liver histopathology data from Bruckner et al. (1986), and mouse liver histopathology data from a subchronic oral toxicity study by Condie et al. (1986). These data sets were either less suitable for defining a POD or produced BMDL values higher than that from the 10-week SDH data.

___I.A.3. UNCERTAINTY FACTORS

UF = 1,000

A default 10-fold UF for intraspecies differences (UF_H) was selected to account for variability in susceptibility among members of the human population in the absence of

quantitative information on the variability of human response to carbon tetrachloride. Intrahuman variability in CYP450 levels that are responsible for metabolism of carbon tetrachloride to reactive metabolites has been documented. This variation in CYP450, which is likely influenced by age-related differences or other factors (e.g., exposure to other chemicals that induce or inhibit microsomal enzymes), could alter susceptibility to carbon tetrachloride toxicity. Individual variability in nutritional status, alcohol consumption, or the presence of underlying disease could also alter metabolism of carbon tetrachloride or antioxidant protection systems. To account for these uncertainties, a factor of 10 was included for individual variability.

A default 10-fold UF for interspecies extrapolation (UF_A) was selected to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans. Metabolism of carbon tetrachloride to reactive species is the initial key event in the development of carbon tetrachloride toxicity. Also critical to carbon tetrachloride toxicity are cellular antioxidant systems that function to quench the lipid peroxidation reaction, thereby preventing damage to cellular membranes. PBPK models available for carbon tetrachloride were found unsuitable for repeat-dose oral scenarios, and could not be used for interspecies extrapolation. In the absence of data to quantify specific interspecies differences or a suitable PBPK model, a UF of 10 is included.

A UF of 3 ($10^{0.5}$) for subchronic to chronic extrapolation (UF_S) was selected based on the following: (1) Qualitative information demonstrating that the target of toxicity following chronic oral exposure is the liver. The NCI oral cancer bioassay in rats and mice (NTP, 2007; NCI, 1977, 1976a, b; Weisburger, 1977) did not include an adequate evaluation of low-dose exposures; in rats, there was marked hepatotoxicity at the lowest dose tested, and in mice, survival was low in dosed animals because of the high incidence of liver tumors. For these reasons, the bioassay was not suitable for dose-response analysis. Nevertheless, complete nonneoplastic incidence data available through an NTP (2007) database of neoplastic and nonneoplastic data did not identify carbon tetrachloride-related histopathological changes in any organ systems or tissues other than the liver. Therefore, the NCI bioassay clearly identified the liver as a target organ following chronic exposures, consistent with the findings from subchronic oral studies and subchronic and chronic inhalation studies.

(2) Knowledge of the relationship between effect levels in subchronic and chronic inhalation studies. The JBRC inhalation bioassay, which included 13-week and 2-year inhalation studies in rats and mice (Nagano et al., 2007a, b; JBRC, 1998), provides information on the relationship between NOAELs and LOAELs from subchronic and chronic exposure durations. In the 13-week study, liver toxicity (increased liver weight and fatty liver) was observed in rats and mice at the lowest exposure concentration tested (LOAEL = 2 ppm, duration adjusted). Following chronic exposure, the LOAEL based on liver and kidney effects was 4 ppm (duration adjusted) and the NOAEL was 0.9 ppm (duration adjusted); the LOAEL concentration in the chronic study was, in fact, twofold higher than the LOAEL from the subchronic study. Other subchronic inhalation studies in rats and mice support a NOAEL in the range of 0.9–4 ppm, which is similar to or within fourfold of the NOAEL from the JBRC chronic inhalation bioassay.

(3) Early onset of liver toxicity. Cytotoxicity occurs early in the sequence of events. For example, Bruckner et al. (1986) observed increases in liver enzymes and liver cell vacuolization

after 4 days of exposure in an 11-day oral toxicity study, and increases in liver enzymes at week 2 in a 12-week oral toxicity study.

Thus the data suggest that an increase in the duration of the exposure may not increase the incidence and/or severity of the liver toxicity.

A UF to account for extrapolation from a LOAEL to a NOAEL (UF_L) was not used because the current approach is to address this extrapolation as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR represented by an increase in SDH activity 2 times the control mean was selected under an assumption that it represents a minimal biologically significant change.

A UF to account for deficiencies in the database (UF_D) of 3 ($10^{0.5}$) was selected. The oral database for this chemical includes extensive testing for subchronic toxicity in animals, a number of tests of immunotoxic potential, limited chronic oral bioassays in both rats and mice, and limited human data. Developmental toxicity testing by the oral route has been conducted. Testing for developmental toxicity by two groups of investigators (Narotsky and Kavlock, 1995; Wilson, 1954) found full-litter resorption at doses accompanied by some degree of maternal toxicity, ranging from piloerection to mortality. Because both studies used relatively high doses, neither study identified a NOAEL. The low dose of carbon tetrachloride (25 mg/kg-day) used in Narotsky et al. (1997b) caused neither maternal nor developmental effects when administered in either aqueous or corn oil vehicles, albeit the group sizes (12–14 dams/dose level) were smaller than the group size used in the typical developmental toxicity study. Nevertheless, the NOAEL in this developmental study (25 mg/kg-day) exceeds the POD for the RfD based on liver effects by over 6-fold and the LOAEL (50 mg/kg-day) by 13-fold, and is consistent with developmental toxicity endpoints as less sensitive than measures of hepatotoxicity. Also, as noted in Section 4.8.1 (Possible Childhood Susceptibility), the available life stage information on microsomal enzyme activity, and in particular CYP2E1, suggests that the developing organism would be no more susceptible to free radical-induced liver injury from carbon tetrachloride than adults. The carbon tetrachloride database lacks an adequate multigeneration study of reproductive function by any route of exposure. A database UF_D of 3 was applied to account for the lack of a multigeneration reproductive toxicity study.

___I.A.4. ADDITIONAL STUDIES/COMMENTS

No long-term toxicity data are available for humans with quantified oral exposures to carbon tetrachloride, but case reports identify the liver and kidney as the primary target organs following acute exposures. Evidence of acute oral hepatotoxicity in humans comes from observations of liver enlargement, elevated serum enzyme (aspartate aminotransferase [AST] and/or ALT), bilirubin levels, or histopathology (hepatocyte degeneration) (Ruprah et al., 1985; Stewart et al., 1963; Docherty and Nicholls, 1923; Docherty and Burgess, 1922). Other acute oral effects in humans include renal toxicity, usually delayed relative to hepatic toxicity (New et al., 1962) and lung effects secondary to renal failure (Umiker and Pearce, 1953). The prominence of hepatic injury in acutely exposed humans suggests that hepatic toxicity observed in subchronic animal studies is an important and relevant consideration.

Studies in laboratory animals indicate that hepatic toxicity is the predominant noncancer

effect of subchronic or chronic oral exposure to carbon tetrachloride. In these studies, evidence of hepatic damage included liver histopathology (fatty degeneration, necrosis, fibrosis, cirrhosis, inflammation, and regenerative activity), along with increases in liver weight and serum markers for hepatotoxicity (ALT, AST, OCT, SDH, and bilirubin) (Koporec et al., 1995; Allis et al., 1990; Bruckner et al., 1986; Condie et al., 1986; Hayes et al., 1986; NCI, 1977, 1976a, b; Weisburger, 1977; Litchfield and Gartland, 1974; Della Porta et al., 1961; Eschenbrenner and Miller, 1946; Edwards and Dalton, 1942; Edwards et al., 1942; Edwards, 1941).

Subchronic oral studies that also examined nonhepatic endpoints (Bruckner et al., 1986; Hayes et al., 1986) did not observe effects in the kidneys or other organs. There was some evidence for impairment of T-cell-dependent immunity in mice treated with 40 mg/kg-day for 14 days but not in rats at hepatotoxic doses (160 mg/kg-day for 10 days) (Guo et al., 2000; Smialowicz et al., 1991; Kaminski et al., 1990).

There is no direct evidence for effects on reproduction or development in humans exposed orally to carbon tetrachloride. One epidemiological study (Bove et al., 1995, 1992a, b) suggested associations between maternal exposure to carbon tetrachloride in drinking water and adverse birth outcomes (the strongest relationship was for low term birth weight), but subjects were exposed to multiple chemicals and the study included only a limited characterization of exposure. Studies in animals have found that relatively high oral doses of carbon tetrachloride (50 mg/kg-day and above) given on days 6–15 of gestation produce significant prenatal loss by increasing the incidence of full-litter resorptions (Narotsky et al., 1997a, b, 1995; Narotsky and Kavlock, 1995; Wilson, 1954); some evidence exists that reproductive effects are a consequence of a maternally mediated response to alterations in hormonal levels (Narotsky et al., 1995, 1997a). The doses producing litter resorption also produced overt toxic effects in dams (piloerection, kyphosis [or rounded upper back], and marked weight loss) and are well above the LOAELs for liver toxicity with longer-term exposure. Mice treated with carbon tetrachloride early in gestation did not show these effects (Hamlin et al., 1993).

Adrenal adenoma and pheochromocytomas were observed in mice exposed to carbon tetrachloride by gavage in an NCI bioassay in which carbon tetrachloride was used as a positive control for liver tumors (Weisburger, 1977). These tumors may indicate a potential noncancer health risk. Benign pheochromocytomas are tumors that originate in chromaffin cells of the adrenal gland medulla and secrete excessive amounts of catecholamines, usually epinephrine and norepinephrine. Because pheochromocytomas are not innervated, catecholamine secretion is unregulated, producing sustained sympathetic nervous system hyperactivity leading to hypertension, tachycardia, and cardiac arrhythmias (Hansen, 1998). Health effects related to pheochromocytoma formation in mice were not assessed in the NCI (1977) cancer bioassay. Therefore, the potential for secondary effects of pheochromocytoma on the cardiovascular system can only be inferred. The lowest exposure level associated with benign pheochromocytomas in mice (LOAEL of 1250 mg/kg-day, 5 days/week [approximately 900 mg/kg-day]) is approximately 2 orders of magnitude higher than levels at which liver effects become apparent in experimental animals. Therefore, the available data do not identify the adrenal gland as a sensitive target organ for carbon tetrachloride by oral administration.

___I.A.5. CONFIDENCE IN THE CHRONIC ORAL RfD

Study -- Medium
Data Base -- Medium
RfD -- Medium

The overall confidence in this RfD assessment is medium. Confidence in the principal study, Bruckner et al. (1986), is medium. The 12-week gavage study is a well-conducted, peer-reviewed study that used three dose groups plus a control and collected interim data at 2-week intervals. The study is limited by relatively small group sizes (five to nine rats/group) and investigation of only two target organs (liver and kidney). Confidence in the oral database is medium. Two chronic oral animal studies were designed as cancer bioassays, and one of the two included only limited investigation of noncancer endpoints. The second chronic bioassay by NCI provided complete nonneoplastic incidence data; however, because of the marked hepatotoxicity in dosed rats at the lowest dose tested and the low survival in dosed mice as a result of the high incidence of liver tumors, the bioassay was not suitable for dose-response analysis. The toxicity of carbon tetrachloride has been more thoroughly investigated in a number of oral toxicity studies of subchronic duration, and a number of tests of immunotoxic potential are available. The oral database contains information on developmental toxicity but lacks an adequate multigeneration study of reproductive function.

___I.A.6. EPA DOCUMENTATION AND REVIEW OF THE CHRONIC ORAL RfD

Source Document – U.S. EPA, 2009

This document has been reviewed by EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of Carbon Tetrachloride* (U.S. EPA, 2009).

Agency Completion Date -- __/__/__ *[note: leave this BLANK until completion is reached*]

___I.A.7. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

__I.B. REFERENCE CONCENTRATION (RfC) FOR CHRONIC INHALATION EXPOSURE

Carbon tetrachloride
CASRN -- 56-23-5
Section I.B. Last Revised -- 00/00/0000

The RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory effects). The inhalation RfC (generally expressed in units of mg/m^3) is analogous to the oral RfD and is similarly intended for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action.

Inhalation RfCs are derived according to *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). Because RfCs can also be derived for the noncarcinogenic health effects of substances that are carcinogens, it is essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

An inhalation assessment for carbon tetrachloride was not previously available on IRIS.

___I.B.1. CHRONIC INHALATION RfC SUMMARY

Critical Effect	Point of Departure*	UF	Chronic RfC
Fatty changes in the liver Chronic inhalation toxicity study in rats Nagano et al., 2007b; JBRC, 1998	$BMCL_{10[HEC]}$: 14.3 mg/m^3	100	0.1 mg/m^3

*Conversion Factors and Assumptions – The $BMCL_{10[HEC]}$ was estimated from incidence data for fatty changes in the liver of rats using PBPK modeling for interspecies extrapolation.

___I.B.2. PRINCIPAL AND SUPPORTING STUDIES

In a study by the JBRC (Nagano et al., 2007b; JBRC, 1998), groups of F344/DuCrj rats (50/sex/group) were exposed (whole-body) to 0, 5, 25, or 125 ppm (0, 31.5, 157, or 786 mg/m^3) of carbon tetrachloride (99.8% pure) vapor for 6 hours/day, 5 days/week for 104 weeks. Animals were observed daily for clinical signs, behavioral changes, and mortality. Body weights were measured once a week for the first 14 weeks and every 2 weeks thereafter. Urinalysis, hematology, and clinical chemistry tests were conducted at study termination. All organs and tissues were examined for gross lesions and histopathologic changes, and organ weights were recorded for the adrenal gland, testis, ovary, heart, lung, kidney, spleen, liver, and brain.

Survival was high in all groups through week 64. After week 64, survival declined precipitously in the 125-ppm males and females primarily due to liver tumors and chronic

nephropathy; only three males and one female from this group survived to 104 weeks. Survival in the other treated groups (19–28/50 in males and 39–43/50 in females) was similar to controls. Body weights were reduced throughout most of the study in 125-ppm males (reduced 22% at termination) and after week 84 in 25-ppm males (reduced approximately 10% at termination). In females, body weight was reduced during the second year of the study in both the 125-ppm (reduced 45% at termination) and 25-ppm (reduced approximately 10% at termination) groups. The body weight decreases in the 25-ppm males and females at termination were statistically significant.

Hematology analyses showed trends for decreased red blood cell count, hemoglobin, and hematocrit in males and females at 25 and 125 ppm, although only the decreases for hemoglobin and hematocrit in 25-ppm females were statistically significant. Serum chemistry changes included statistically significant increases in AST (males), ALT (males and females), LDH (females), and GPT (females) at 25 ppm; the increases over control in individual serum chemistry parameters at 25 ppm ranged from 1.2- to twofold. There were also significant increases in BUN in both males and females at 25 ppm (25–63% over controls). At 125 ppm, BUN, creatinine, and inorganic phosphate were increased by two- to threefold over the control. There was a significant increase in creatine phosphokinase (CPK) in 25-ppm females but not males. An increase was reported in the number of male and female rats with high levels of proteinuria in the 5- and 25-ppm groups (too few data to test in the 125-ppm group). As discussed more fully in Section 4.6.2 of the *Toxicological Review of Carbon Tetrachloride* (U.S. EPA, 2009), the interpretation of the observed proteinuria in the F344 rat, a strain with a high spontaneous incidence of renal lesions, is problematic. Therefore, this endpoint was not considered an appropriate basis for the RfC.

Increases in the incidence and severity of nonneoplastic liver lesions (fatty change, fibrosis, cirrhosis) were seen at 25 and 125 ppm in both males and females. Liver lesions (e.g., fatty liver, granulation) in the 5-ppm group were of similar type, incidence, and severity as controls. In the kidney, there was a dose-related increase in the severity of chronic nephropathy at 25 and 125 ppm in both males and females. Nephropathy was characterized as severe in most members of the 125-ppm group. Other dose-related histopathological changes were increased severity of eosinophilic change (eosinophilic globules in cytoplasm) in the nasal cavity at ≥25 ppm in males and ≥5 ppm in females and increased incidence and severity of granulation in the lymph nodes at 125 ppm in both sexes. The incidence of hepatocellular adenomas and carcinomas was markedly increased in male and female rats at 125 ppm.

Nagano et al. (2007b; JBRC, 1998) also conducted a 2-year study in Crj:BDF1 mice using the same exposure protocol as for the rat. In the mouse study, the 25-ppm concentration was a LOAEL for effects on the liver (increased weight, serum chemistry changes indicative of damage, and lesions), kidney (serum chemistry changes and lesions), and spleen (lesions); decreased growth; and reduced survival. The 5-ppm level was a NOAEL.

<u>Methods of Analysis</u>. Fatty change in the liver of rats was selected as the specific endpoint for dose-response analysis because this histopathologic lesion is indicative of cellular damage and was a more sensitive endpoint than other histopathologic changes that were also present in 25-ppm rats in the JBRC study (Nagano et al., 2007b; JBRC, 1998).

Exposure levels studied in the 2-year JBRC rat bioassay were converted to estimates of internal doses by application of a PBPK model. BMD modeling methodology was used to analyze the relationship between the estimated internal doses and response (i.e., fatty change of the liver). The resulting BMDL values were converted to estimates of equivalent human exposure concentrations (HECs) by applying a human PBPK model.

Estimation of internal doses corresponding to the exposure concentrations studied in the 2-year JBRC rat bioassay was accomplished using a PBPK model for the rat (Thrall et al., 2000; Benson and Springer, 1999; Paustenbach et al., 1988). Time-averaged rate of metabolism of carbon tetrachloride in the liver (MRAMKL, µmol/hour/kg liver) was selected as the primary dose metric for liver effects, based on evidence that metabolism of carbon tetrachloride via CYP2E1 to highly reactive free radical metabolites plays a crucial role in the hypothesized MOA in producing liver toxicity.

BMD modeling methodology (U.S. EPA, 2000) was used to analyze the data (with exposure expressed in terms of internal dose). All of the models for dichotomous data in U.S. EPA's BMDS (version 1.4.1) were fit to the incidence data for fatty liver in male and female rats. Predicted internal doses associated with a BMR of 10% extra risk were calculated. In the male rat, the log-logistic model provided the best fit of the data (based on χ^2 $p \geq 0.1$ and lowest AIC value). For female rats, no models provided an adequate fit to the data when all dose groups were included, as assessed by the χ^2 goodness-of-fit test. After dropping the highest dose, the multistage model provided the best fit of the data.

A human PBPK model (Thrall et al., 2000; Benson and Springer, 1999; Paustenbach et al., 1988) was used to estimate continuous human equivalent concentrations (HECs, in mg/m^3) that would result in values for the internal dose metric (MRAMKL) equal to the $BMDL_{10}$ values for fatty changes of the liver. The HEC calculated from male rat data (14.3 mg/m^3) was used as the POD for RfC derivation.

___I.B.3. UNCERTAINTY FACTORS

UF = 100

A default 10-fold UF for intraspecies differences (UF_H) was selected to account for variability in susceptibility among members of the human population in the absence of quantitative information on the variability of human response to carbon tetrachloride. Intrahuman variability in CYP450 levels that are responsible for metabolism of carbon tetrachloride to reactive metabolites has been documented. This variation in CYP450, which is likely influenced by age-related differences or other factors (e.g., exposure to other chemicals that induce or inhibit microsomal enzymes), could alter susceptibility to carbon tetrachloride toxicity. Individual variability in nutritional status, alcohol consumption, or the presence of underlying disease could also alter metabolism of carbon tetrachloride or antioxidant protection systems. To account for these uncertainties, a factor of 10 was applied for individual variability.

A UF of 3 ($10^{0.5}$) was selected for interspecies extrapolation (UF_A) to account for potential pharmacodynamic differences between rats and humans. As pharmacokinetic and

pharmacodynamic components are assumed to contribute equally to the uncertainty in interspecies extrapolation and the product of the two components is assumed by default to be 10, a numeric value of $10^{0.5}$ (3.2, expressed as the numeral 3 after rounding) is assigned to each component. Cellular antioxidant systems function to quench the lipid peroxidation reaction and prevent damage to cellular membranes. The pharmacokinetic model was used to adjust for pharmacokinetic differences across species; therefore, an additional UF was not included for pharmacokinetic differences between species. In the absence of data to quantify specific interspecies differences for cellular protective mechanisms, a UF of 3 is applied to account for species differences in pharmacodynamics.

A UF to account for extrapolation from a LOAEL to a NOAEL (UF_L) was not used because the current approach is to address this extrapolation as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 10% change in fatty changes of the liver was selected under an assumption that it represents a minimal biologically significant change.

A UF to extrapolate from a subchronic to a chronic exposure duration was not necessary because the RfC was derived from a study using a chronic exposure protocol.

A UF to account for database deficiencies (UF_D) of 3 ($10^{0.5}$) was selected. The inhalation database for this chemical includes extensive testing for subchronic toxicity in animals, 2-year chronic inhalation bioassays in rats and mice, one study of immunotoxic potential, and human epidemiology data. Testing for developmental toxicity was limited to one inhalation study in the rat that found effects only at high, maternally toxic exposure concentrations. This study did not use an exposure concentration low enough to identify a NOAEL for either maternal or fetal toxicity. Nevertheless, the developmental effects at the LOAEL were modest, and were limited to decreased fetal body weight (7%) and decreased crown-rump length (3.5%). The LOAEL for developmental effects (in the presence of maternal toxicity) in this study (334 ppm) was 66-fold higher than the NOAEL from the principal study (5 ppm). Developmental toxicity has been tested more extensively by the oral route, although all adequate studies were conducted in the same species (rat); the oral NOAEL for developmental toxicity exceeded both the oral NOAEL and LOAEL for liver toxicity. Microsomal enzymes that are responsible for metabolizing carbon tetrachloride, particularly CYP2E1, are lower in the developing organism than the adult, and do not achieve adult levels in humans until sometime between 1 and 10 years. Thus, life stage information on microsomal enzyme activity suggests that the developing organism would be no more susceptible to free radical-induced liver injury from carbon tetrachloride than adults. On balance, the available information suggests that further developmental toxicity testing would not likely result in a POD smaller than that based on liver toxicity. The database lacks an adequate multigeneration study of reproductive function by any route of exposure; therefore a threefold UF was applied.

___I.B.4. ADDITIONAL STUDIES/COMMENTS

Case reports of acute high-level exposure to carbon tetrachloride vapor or long-term occupational exposure provide evidence of hepatotoxic and nephrotoxic effects of carbon tetrachloride in humans. Observations indicative of an effect on the liver in these cases include jaundice, increased serum enzyme levels, and, in fatal cases, necrosis of the liver (Stewart et al.,

1965; New et al., 1962; Kazantzis and Bomford, 1960; Norwood et al., 1950). Delayed effects on the kidney have also been reported in acute overexposure cases. Other effects associated with carbon tetrachloride exposure in humans are gastrointestinal symptoms (nausea and vomiting, diarrhea, and abdominal pain) and neurological effects indicative of central nervous system depression (headache, dizziness, and weakness). Tomenson et al. (1995) conducted a cross-sectional epidemiology study of hepatic function in workers exposed to carbon tetrachloride. They found suggestive evidence of an effect of occupational carbon tetrachloride exposure on serum enzymes indicative of hepatic effects at workplace concentrations in the range of 1–4 ppm.

The liver and kidney are the most prominent targets of carbon tetrachloride in subchronic and chronic inhalation studies of laboratory animals. Hepatic toxicity in these studies was demonstrated by histopathology (centrilobular fatty degeneration, necrosis, fibrosis, cirrhosis, hepatitis, and regenerative activity) as well as increases in liver weight and serum markers for liver damage (Nagano et al., 2007a, b; Benson and Springer, 1999; JBRC, 1998; Prendergast et al., 1967; Adams et al., 1952; Smyth et al., 1936). Hepatic effects were observed in animals exposed to carbon tetrachloride concentrations as low as 2 ppm (adjusted to continuous exposure). Renal damage was reported less frequently in these animal studies and generally at higher concentrations than those causing liver damage.

In the subchronic studies, effects on the kidneys were generally observed at concentrations higher than the LOAEL for liver effects. With chronic exposure, the sensitivity of the kidney and liver as target organs were comparable in the rodent. The JBRC chronic rat study (Nagano et al., 2007b; JBRC, 1998) reported liver toxicity (serum enzyme changes, fatty liver, fibrosis, cirrhosis) and kidney toxicity (increases in BUN, creatinine, inorganic phosphorus, and severity of chronic progressive nephropathy) at exposure concentrations of 25 ppm (≥4 ppm, duration adjusted). An increase in the severity of proteinuria was reported in male and female rats at the lowest tested concentration of 5 ppm (0.9 ppm, duration adjusted). While the increased severity of proteinuria could be related to the nephropathy observed at ≥25 ppm, the biological significance of the finding of proteinuria at 5 ppm is unclear.

___I.B.5. CONFIDENCE IN THE CHRONIC INHALATION RfC

Study -- High
Data Base -- Medium
RfC -- Medium

The overall confidence in this RfC assessment is medium. Confidence in the JBRC bioassay (Nagano et al., 2007b; JBRC, 1998), the principal study, is high. This chronic study was well conducted, using two species and 50 animals/sex/group. The JBRC chronic study was preceded by a 13-week subchronic study, and an extensive set of endpoints was examined in both studies. Confidence in the database, which includes the JBRC 2-year chronic inhalation bioassays in rats and mice, subchronic toxicity studies, and one study of immunotoxic potential, is medium. Testing for developmental toxicity by inhalation exposure found effects only at high, maternally toxic exposure concentrations but was limited to a single inhalation study in a single species that did not test an exposure concentration low enough to identify a NOAEL for maternal or fetal toxicity. The database lacks an adequate inhalation multigeneration study of reproductive function.

___I.B.6. EPA DOCUMENTATION AND REVIEW OF THE CHRONIC INHALATION RfC

Source Document – U.S. EPA, 2009

This document has been reviewed by EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of Carbon Tetrachloride* (U.S. EPA, 2009).

Agency Completion Date -- __/__/__ *[note: Leave this BLANK until completion is reached]*

___I.B.7. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

_II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Carbon tetrachloride
CASRN -- 56-23-5
Section II. Last Revised -- 00/00/0000

This section provides information on three aspects of the carcinogenic assessment for the substance in question: the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral and inhalation exposure. Users are referred to Section I of this file for information on long-term toxic effects other than carcinogenicity.

The rationale and methods used to develop the carcinogenicity information in IRIS are described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) and the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). The quantitative risk estimates are derived from the application of a low-dose extrapolation procedure, and are presented in two ways to better facilitate their use. First, route-specific risk values are presented. The "oral slope factor" is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a "unit risk" is a plausible upper bound on the estimate of risk per unit of concentration, either per μg/L drinking water (see Section II.B.1.) or per $\mu g/m^3$ air breathed (see Section II.C.1.). Second, the estimated concentration of the chemical substance in drinking water or air when associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000 is also provided.

The previous cancer assessment for carbon tetrachloride was posted on the IRIS database

in 1987. At that time, carbon tetrachloride was classified as a B2 carcinogen (probable human carcinogen), based on the finding of treatment-related hepatocellular carcinomas in rats, mice, and hamsters. An oral slope factor (SF) of 1.3×10^{-1} $(mg/kg\text{-}day)^{-1}$ was derived using linear extrapolation procedures and liver tumor data sets from the hamster (Della Porta et al., 1961), mouse (NCI, 1977, 1976a, b; Edwards et al., 1942), and rat (NCI, 1977, 1976a, b). An inhalation unit risk (IUR) of 1.5×10^{-5} $(\mu g/m^3)^{-1}$ was derived from the oral SF by route-to-route extrapolation (assuming an air intake of 20 m^3/day, body weight of 70 kg, and 40% absorption rate by humans).

__II.A. EVIDENCE FOR HUMAN CARCINOGENICITY

___II.A.1. WEIGHT-OF-EVIDENCE CHARACTERIZATION

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), carbon tetrachloride is characterized as *likely to be carcinogenic to humans* by all routes of exposure. This cancer weight of evidence determination is based on: (1) inadequate evidence of carcinogenicity in humans and (2) sufficient evidence in animals (i.e., hepatic tumors in multiple species [rat, mouse, and hamster] by oral and inhalation routes of exposure in response to carbon tetrachloride and evidence of pheochromocytomas in mice by oral and inhalation routes of exposure).

Carbon tetrachloride has been shown to be a liver carcinogen in rats, mice, and hamsters in eight bioassays of various experimental designs by oral and inhalation exposure. A general correspondence has been observed between hepatocellular cytotoxicity and regenerative hyperplasia and the induction of liver tumors. At lower exposure levels, this correspondence is less consistent. In particular, in the JBRC 2-year inhalation cancer bioassay in the mouse (Nagano et al., 2007b, JBRC, 1998), the lowest exposure concentration tested (5 ppm) was not hepatotoxic, whereas the incidence of liver adenomas in female mice at that concentration was statistically significantly increased compared to concurrent and historical controls.

A hypothesized carcinogenic MOA for carbon tetrachloride-induced liver tumors has been proposed and includes the following key events: (1) metabolism to the trichloromethyl radical by CYP2E1 and subsequent formation of the trichloromethyl peroxy radical, (2) radical-induced mechanisms leading to hepatocellular cytotoxicity, and (3) sustained regenerative and proliferative changes in the liver in response to hepatotoxicity. A substantial amount of data exists that supports these hypothesized key events in the cancer MOA for carbon tetrachloride. Data to characterize these key events at low-exposure levels, however, are limited. This is of particular concern for liver tumor MOA considerations in light of: (1) the finding that liver tumors in female mice occurred at non-cytotoxic doses (Nagano et al., 2007b; JBRC, 1998) and (2) the fundamental reactivity of direct and indirect products of carbon tetrachloride metabolism. Therefore, the MOA of carbon tetrachloride at low exposure levels can be hypothesized, but is unknown at this time. Hypothesized MOAs are discussed further in the *Toxicological Review of Carbon Tetrachloride* (U.S. EPA, 2009), Sections 4.7.3 and 4.7.4.

___II.A.2. HUMAN CARCINOGENICITY DATA

Studies in humans are inadequate to show an association between exposure to carbon

tetrachloride and carcinogenicity. There is some evidence for certain types of cancer in occupational populations thought to have had some exposure to carbon tetrachloride, including non-Hodgkin's lymphoma (NHL) (Blair et al., 1998; Spirtas et al., 1991), lymphosarcoma and lymphatic leukemia (Checkoway et al., 1984; Wilcosky et al., 1984), esophageal and cervical cancer (Blair et al., 1990, 1979), breast cancer (Cantor et al., 1995), astrocytic brain cancer (Heineman et al., 1994), and rectal cancer (Dumas et al., 2000). In these cases, exposure to carbon tetrachloride was poorly characterized and confounded by simultaneous exposures to other chemicals. Additionally, these studies were designed to evaluate tetrachloroethylene and trichloroethylene and had only limited ability to examine other chemical exposures such as carbon tetrachloride. None of the human epidemiology studies reported associations with cancer of the liver, which is the main site of carcinogenicity in animal studies, but this may be because of a lack of power to detect a relatively rare human tumor.

___II.A.3. ANIMAL CARCINOGENICITY DATA

Carbon tetrachloride has been shown to induce hepatocellular carcinomas in rodents by oral, inhalation, and parenteral exposure. Researchers at the NCI conducted a series of gavage studies in mice of various strains and found large increases in the incidence of liver tumors in treated mice (Andervont, 1958; Edwards and Dalton, 1942; Edwards et al., 1942; Edwards, 1941). A similar result was obtained in hamsters (Della Porta et al., 1961). These animal studies were generally conducted using a single high dose of carbon tetrachloride, but one early study was conducted with multiple dose levels in order to investigate dose-response relationships for induction of liver tumors (Eschenbrenner and Miller, 1946). Eschenbrenner and Miller (1946) found liver tumors (hepatomas) in strain A male and female mice that received carbon tetrachloride by gavage (in olive oil) daily or every 4 days for 4 months.

Oral bioassays of carbon tetrachloride using groups of 50 animals/sex were conducted in mice and rats by NCI (1977, 1976a, b) as a positive control for bioassays of chloroform, trichloroethylene, and 1,1,1-trichloroethane. The bioassay in mice employed very high doses (1,250 or 2,500 mg/kg, 5 days/week for 78 weeks) that produced close to 100% incidence of hepatocellular carcinoma. The incidence of adrenal adenoma and pheochromocytoma was also significantly increased in both dose groups in male and female mice. The bioassay in rats (47 or 94 mg/kg for males and 80 or 159 mg/kg for females, 5 days/week for 78 weeks) produced only a low incidence of liver tumors, but high early mortality, particularly in the high-dose group, may have affected the power of this study to detect a carcinogenic effect. Even so, the increase in carcinomas was statistically significant in low-dose females (4/49) in relation to pooled controls (1/99).

Carbon tetrachloride produced evidence of carcinogenicity in inhalation bioassays in rats and mice (Nagano et al., 2007b; JBRC, 1998). In rats, intermittent exposure (6 hours/day, 5 days/week) to 125 ppm for 2 years produced marked significant increases in the incidence of hepatocellular carcinomas and adenomas in both males and females. The incidence of tumors was not increased in rats exposed to 5 or 25 ppm by the same protocol although the incidence of liver carcinoma (3/50) in 25-ppm females exceeded the range of historical control incidence from JBRC 2-year bioassays. In mice, marked significant increases in hepatocellular carcinomas and (to a lesser extent) adenomas occurred at both 25 and 125 ppm in both sexes. Also, a statistically significant increase in the incidence of liver adenomas in female mice at 5 ppm was observed

compared to the concurrent control and exceeded the historical control range for hepatocellular adenomas from JBRC 2-year bioassays. The assays in mice also found significant increases in the incidence of benign adrenal pheochromocytomas in males at 25 or 125 ppm and females at 125 ppm, exposure levels at or above those associated with liver hepatocellular carcinoma and adenoma. Only one pheochromocytoma in a high-dose male mouse was classified as malignant.

Subcutaneous injections of carbon tetrachloride at an average dose of 0.29 mg/kg-day for 33–47 weeks induced hepatocellular carcinomas in Osborne-Mendel, Japanese, and Wistar rats but not in Sprague-Dawley or black rats (Reuber and Glover, 1970, 1967a, b). Intraperitoneal injections at an average of 86 mg/kg-day induced hepatomas in C3H mice (Kiplinger and Kensler, 1963).

___II.A.4. SUPPORTING DATA FOR CARCINOGENICITY

Carbon tetrachloride has been extensively studied for its genotoxic and mutagenic effects. Overall, results are largely negative. There is little direct evidence that carbon tetrachloride induces intragenic or point mutations in mammalian systems. The mutagenicity studies that have been performed using transgenic mice have yielded negative results, as have the vast majority of the mutagenicity studies that have been conducted in bacterial systems. However, since oxidative DNA adducts can be converted into mutations, the inability to detect mutations in the transgenic mouse assays may be an indication of efficient repair of oxidative lesions, a preferential formation of large chromosomal mutations that are inefficiently detected in the transgenic models, or a reflection of the limitations and sensitivity of the specific assays that were performed with carbon tetrachloride. The two positive mutation/DNA damage studies conducted in *Escherichia coli* were seen in strains that are particularly sensitive to oxidative damage. Moreover, the intrachromosomal recombination induced by carbon tetrachloride in *Saccharomyces cerevisiae* is believed to result from double-stranded DNA breaks leading to deletion mutations. These results are consistent with DNA breakage originating from oxidative stress or lipid peroxidation products that occur concurrently with cytotoxicity.

An evaluation based on the weight of evidence suggests that carbon tetrachloride is more likely an indirect than a direct mutagenic agent. In general, genotoxic effects have been observed in a consistent and close relationship with cytotoxicity, lipid peroxidation, and/or oxidative DNA damage. Mutagenic effects, if they occur, are likely to be generated through indirect mechanisms resulting from oxidative stress or lipid peroxidation products. Under highly cytotoxic conditions, bioactivated carbon tetrachloride can exert genotoxic effects. These tend to be modest in magnitude and are manifested primarily as DNA breakage and related sequelae. Chromosome loss leading to aneuploidy may also occur to a limited extent.

Challenges in evaluating the carbon tetrachloride genotoxicity database must be acknowledged. Although the cellular effects of carbon tetrachloride are described adequately at doses at or above those that induce cytotoxicity, there is a paucity of data describing DNA damaging events at doses below those that are cytotoxic. Additionally, there exists some level of uncertainty as to whether assays used to assess the genotoxicity of carbon tetrachloride were of sufficient quality to assess genotoxicity at doses that do not induce cytotoxicity.

__II.B. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

___II.B.1. SUMMARY OF RISK ESTIMATES

____II.B.1.1. Oral Slope Factor – 7×10^{-2} per mg/kg-day

The oral slope factor is derived from the LED_{10}, the 95% lower bound on the exposure associated with an 10% extra cancer risk, by dividing the risk (as a fraction) by the LED_{10}, and represents an upper bound, continuous lifetime exposure risk estimate:

LED_{10}, lower 95% bound on exposure at 10% extra risk – 1.54 mg/kg-day
ED_{10}, central estimate of exposure at 10% extra risk – 2.27 mg/kg-day

The slope of the linear extrapolation from the central estimate is
0.1/(2.27 mg/kg-day) = 4×10^{-2} per mg/kg-day.

The slope factor for carbon tetrachloride should not be used with exposures exceeding the POD (1.54 mg/kg-day), because above this level, the fitted dose-response model better characterizes what is known about the carcinogenicity of carbon tetrachloride.

____II.B.1.2. Drinking Water Unit Risk* - 2×10^{-6} per µg/L

Drinking Water Concentrations at Specified Risk Levels

Risk Level	Lower Bound on Concentration Estimate*
E-4 (1 in 10,000)	50 µg/L
E-5 (1 in 100,000)	5 µg/L
E-6 (1 in 1,000,000)	0.5 µg/L

* The unit risk and concentration estimates assume water consumption of 2 L/day by a 70 kg human.

____II.B.1.3. Extrapolation Method

Multistage model with linear extrapolation from the POD (LED_{10}).

___II.B.2. DOSE-RESPONSE DATA

Tumor Type – hepatocellular adenoma and carcinoma
Test Species – female BDF1 mouse
Route – inhalation (route-to-route extrapolation performed using PBPK modeling)
References – Nagano et al., 2007b; JBRC, 1998

Incidence of liver tumors in female BDF1 mice exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)

Tumor	Female mouse			
	0 ppm	5 ppm	25 ppm	125 ppm
Hepatocellular adenoma or carcinoma	4/50[a]	9/49	44/50[b]	48/49[b]

[a]Statistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.01$).
[b]Tumor incidence significantly elevated compared with that in controls by Fisher Exact test ($p \leq 0.01$).

Sources: Nagano et al. (2007b); JBRC (1998).

Oral SF using linear low-dose extrapolation approach and route-to-route extrapolation

Tumor	Dose groups modeled	Model parameters	HED (mg/kg-d)	Average HED (mg/kg-d)[a]	Oral SF $(mg/kg\text{-}d)^{-1}$
Female mouse hepatocellular adenoma and carcinoma	0, 5, 25 ppm (125 ppm group dropped from analysis)	MRAMKL; Fisher model[b] BMR = 10%	1.40	1.54	6.5×10^{-2}
		MRAMKL; Thrall model[b] BMR = 10%	1.69		

[a]The average represents an arithmetic mean of the two HEC values based the Fisher and Thall models.
[b]BMD modeling was performed using internal doses based on the application of two mouse PBPK models. The dose metric used was time-averaged rate of metabolism per kg liver (MRAMKL), in units of μmol/hour/kg liver.

___II.B.3. ADDITIONAL COMMENTS

Studies of carbon tetrachloride carcinogenicity by the oral exposure route are not sufficient to derive a quantitative estimate of cancer risk using low-dose linear approaches. No epidemiological investigations of the possible carcinogenicity of carbon tetrachloride associated with oral exposure have been performed. The cancer studies by Edwards et al. (1942) in the mouse and Della Porta et al. (1961) in the hamster included a control and only one dose group, and animals were dosed for less than a lifetime (2 months and 30 weeks, respectively). Neither study provided body weight information, so that doses could not be estimated with certainty. Despite the relatively short dosing periods and the fact that animals were kept on study for less than a lifetime (approximately 6.5 months in the case of Edwards et al. [1942], and approximately 1 year in the case of Della Porta et al. [1961]), liver tumor incidence was very high (71% in the case of Edwards et al. [1942], and 100% of the hamsters that died or were sacrificed between weeks 43 and 55 in the case of Della Porta et al. [1961]). In the NCI bioassays (1977, 1976a, b), liver tumor incidence in the mouse was virtually 100% in both dose groups. In the rat, liver tumor incidence was low and failed to show a dose-response relationship (in the female rat, tumor incidence was higher in the low-dose group [4/46] than in the high-dose group [1/30], presumably because early mortality in the high-dose group precluded tumor formation). Thus, none of the available oral studies of carbon tetrachloride carcinogenicity

provided data sets amenable to dose-response modeling.

Therefore, PBPK modeling was applied to extrapolate inhalation tumor data to the oral route. Because liver tumors and pheochromocytomas have been observed in experimental animals following both inhalation and oral exposures, the data sets evaluated as the basis for the IUR were considered appropriate for estimation of an oral SF. Data for female mouse liver tumors yielded the highest estimate of the SF of those data sets modeled [i.e., 7×10^{-2} (mg/kg-day)$^{-1}$].

___II.B.4. DISCUSSION OF CONFIDENCE

Relevance to humans. As noted in EPA's 2005 *Guidelines for Carcinogen Risk Assessment*, "… agents observed to produce tumors in both humans and animals have produced tumors either at the same site (e.g., vinyl chloride) or different sites (e.g., benzene) (NRC, 1994). Hence, site concordance is not always assumed between animals and humans." Thus, it is not clear whether the tumors observed in rodent bioassays would be predictive of human tumors of the same or different sites.

The MOA for liver tumor induction has not been established, but the hypothesized MOAs that have been investigated are assumed to be relevant to humans. There is no available evidence in humans for hepatic cancer associated with carbon tetrachloride exposure. The experimental animal literature, however, shows carbon tetrachloride to consistently induce liver tumors across species and routes of exposure. Further, there are similarities between experimental animals and humans in terms of carbon tetrachloride metabolism, antioxidant systems, and evidence for the liver as a sensitive target organ. Together, this supports a conclusion that experimental evidence for liver cancer is relevant to humans.

Pheochromocytomas, on the other hand, were observed in only one species (the mouse). In humans, pheochromocytomas are rare catecholamine-producing neuroendocrine tumors that are usually benign, but may also present as or develop into a malignancy (Eisenhofer et al., 2004; Salmenkivi et al., 2004; Tischler et al., 1996). In humans, hereditary factors have been identified as important in the development of pheochromocytomas (Eisenhofer et al., 2004). In the mouse, few chemicals have been reported to cause adrenal medullary tumors (Hill et al., 2003), and the MOA for this tumor in mice is unknown. The relevance of mouse pheochromocytomas to humans is similarly unknown, although parallels between this tumor in the mouse and human led investigators to concluded that the mouse might be an appropriate model for human adrenal medullary tumors (Tischler et al., 1996). Like the human, pheochromocytomas in the mouse are relatively rare, as are metastases. Both the morphological variability of the mouse pheochromocytomas and the morphology of the predominant cells are comparable to those of human pheochromocytomas. An important characteristic of mouse pheochromocytomas is expression of immunoreactive phenylethanolamine-N-methyltransferase (PNMT); human pheochromocytomas are also usually PNMT-positive (Tischler et al., 1996). Overall, this supports a conclusion that experimental evidence for pheochromocytomas is potentially relevant to humans.

Choice of low-dose extrapolation approach. The MOA is a key determinant of which approach to apply for estimating low-dose cancer risk. The MOA of carbon tetrachloride liver

carcinogenicity has been investigated extensively; however, much of this research has been conducted at relatively high exposure levels. The MOA(s) at low exposure levels is not known.

For liver tumors, a nonlinear extrapolation approach was explored in the *Toxicological Review of Carbon Tetrachloride* (U.S. EPA, 2009), Section 5.4.5 as an alternative to the linear low-dose extrapolation approach for cancer risk estimation. Such an approach would be supported by a conclusion that the hypothesized cytotoxicity-proliferative MOA is operative at all doses; however, bioassay results inconsistent with the nonlinear approach include evidence of female mouse hepatocarcinongenicity at non-cytotoxic doses, potential for genotoxicity at low doses, and the absence of MOA information regarding the observed pheochromocytomas in mice.

The linear extrapolation approach assumes that some cancer risk exists at all non-zero exposures, and that this risk increases linearly with exposure. While consistent with the recognized biological reactivity of carbon tetrachloride, uncertainties in this low-dose extrapolation approach are associated with the lack of MOA information at low exposures. Additional MOA information in the low-dose region to establish whether a linear or nonlinear approach applies to carbon tetrachloride liver tumors would significantly reduce the uncertainty associated with estimating the magnitude of liver tumor risk.

The effect on risk estimates derived using a linear extrapolation approach of using only data on carbon tetrachloride liver tumor response at levels below those associated with increased cell replication was examined. The risk calculations did not prove particularly sensitive to the limitation of data points to those below which increased cell replication was reported. This consistency in cancer risk estimates provided some confidence that the IUR and SF estimates based on liver tumor data are not driven by high doses associated with significant hepatotoxicity.

In data sets where early mortality is observed, methods that can reflect the influence of competing risks and intercurrent mortality on site-specific tumor incidence rates are preferred. Survival curves for female rats and mice from the JBRC bioassay show early mortality in some treated groups. Because liver tumors were the primary cause of early deaths in these groups, failure to apply a time-to-tumor analysis is not likely to significantly influence the IUR for liver tumors. The impact on the IUR from pheochromocytomas is unknown.

Under the linear low-dose extrapolation approach, cancer risk estimates were calculated by straight line extrapolation from the POD to zero, with the multistage model used to derive the POD. (The one exception is the male mouse pheochromocytoma data set, where the log-probit model was used.) It is unknown how well this extrapolation procedure predicts low-dose risks for carbon tetrachloride. The multistage model does not represent all possible models one might fit, and other models could conceivably be selected to yield different results consistent with the observed data, both higher and lower than those included in this assessment.

For pheochromocytomas, only a linear low-dose extrapolation approach was used to estimate human carcinogenic risk in the absence of any information on the MOA for this tumor. MOA information to inform the approach to low-dose extrapolation for carbon tetrachloride-induced pheochromocytomas would significantly reduce the uncertainty associated with the magnitude of risk from exposure to this tumor type.

Cancer risk estimates for liver tumors and pheochromocytomas developed using a linear low-dose extrapolation approach were not combined because different dose metrics were used in the dose-response/PBPK analysis of these two tumor types. Deriving the IUR or oral SF for data on one tumor site, however, may underestimate the carcinogenic potential of carbon tetrachloride. For the IUR based on male mouse pheochromocytomas, because of the poor resolution of the dose-response relationship for male rodent liver tumors, the magnitude of the potential risk underestimation cannot be characterized. Because the SF based on female mouse liver tumors was an order of magnitude greater than that for female mouse pheochromocytomas, any underestimation of the SF is expected to be small.

Interspecies extrapolation. Extrapolating dose-response data from animals to humans was accomplished using PBPK models in the rat, mouse, and human. Availability of a PBPK model generally reduces the pharmacokinetic component of uncertainty associated with animal to human extrapolation; however, any PBPK model has its own associated uncertainties. Specific uncertainties in the PBPK modeling for carbon tetrachloride are discussed the *Toxicological Review of Carbon Tetrachloride* (U.S. EPA, 2009), Section 5.3.

Route-to-route extrapolation for the oral SF. Studies of carbon tetrachloride carcinogenicity by the oral route were determined to be insufficient to derive a quantitative estimate of cancer risk. Therefore, a human PBPK model was used to extrapolate inhalation data to the oral route. A simple approximation method was used that assumed continuous infusion of carbon tetrachloride from the human GI tract to the liver and that absorption of carbon tetrachloride from the GI tract is essentially complete. Doses extrapolated from inhalation to oral exposures in this analysis were approximations because they did not account for oral bioavailability or absorption kinetics, information that is not available for carbon tetrachloride. To the extent that GI absorption is less than 100%, the current estimation method for route-to-route extrapolation would tend to overestimate the SF.

Statistical uncertainty at the POD. Parameter uncertainty can be assessed through confidence intervals. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. For the log-probit model applied to the male mouse pheochromocytoma data, there is a reasonably small degree of uncertainty at the 10% excess incidence level (the POD for linear low-dose extrapolation); the lower bound on the BMD (i.e., the $BMDL_{10}$) is 1.8-fold lower than the BMD. For the multistage model applied to the female mouse liver tumor data, there is similarly a reasonably small degree of uncertainty at the 10% excess incidence level; the lower bound on the BMD (i.e., the $BMDL_{10}$) is approximately 1.5-fold lower than the BMD.

Bioassay selection. The study by Nagano et al. (2007b; also reported as JBRC, 1998) was used for development of the IUR. A full report of the bioassay findings was published in 2007, although the study itself was conducted in the mid-1980s. Although not a recently conducted study, this bioassay was well-designed, using two species (rats and mice), four dose groups, including an appropriate untreated control, and 50 animals/sex/group. Examination of toxicological endpoints in both sexes of rats and mice was appropriate. No issues were identified with this bioassay that might have contributed to uncertainty in the cancer assessment.

Alternative bioassays for developing an IUR were unavailable.

Choice of species/gender. For liver tumors, modeling was performed using JBRC inhalation bioassay data for the female mouse and female rat. The male rat liver tumor data were not modeled because these data sets lacked the resolution desired for dose-response modeling; the male mouse liver data were modeled, but provided similarly poor dose-response curve resolution. Tumor frequencies increased from control levels to close to maximal responses without any intervening dose levels having submaximal responses. In the female mice and rats, lower but biologically significant levels of tumor response were seen at intermediate dose levels. Also, notably, increased levels of hepatocellular proliferation were not reported for rodents at these intermediate levels, so that dose-response modeling based on these data may be more applicable to an evaluation of cancer risk at noncytotoxic exposures. There is no indication that male rodents are more sensitive to carbon tetrachloride liver tumor induction and that use of female data only underestimated potential risk. For pheochromocytomas, JBRC inhalation data sets for both male and female mice were amenable to modeling, and the data set yielding the highest estimate of cancer risk could be selected.

Human population variability. Neither the extent of interindividual variability in carbon tetrachloride metabolism nor human variability in response to carbon tetrachloride has been fully characterized. Factors that could contribute to a range of human response to carbon tetrachloride include variations in CYP450 levels because of age-related differences or other factors (e.g., exposure to other chemicals that induce or inhibit microsomal enzymes), genetic polymorphisms in drug metabolism enzymes, transporters, and receptors (all of which can markedly affect susceptibility to a toxic chemical), nutritional status, alcohol consumption, or the presence of underlying disease that could alter metabolism of carbon tetrachloride or antioxidant protection systems. Incomplete understanding of the potential differences in metabolism and susceptibility across exposed human populations represents a source of uncertainty.

__II.C. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

___II.C.1. SUMMARY OF RISK ESTIMATES

____II.C.1.1. Inhalation Unit Risk – 6×10^{-6} per µg/m^3

The IUR is derived from the LEC_{10}, the 95% lower bound on the exposure associated with a 10% extra cancer risk, by dividing the risk (as a fraction) by the LEC_{10}, and represents an upper bound, continuous lifetime exposure risk estimate.

LEC_{10}, lower 95% bound on exposure at 10% extra risk – 1.78×10^4 µg/m^3
EC_{10}, central estimate of exposure at 10% extra risk – 3.13×10^4 µg/m^3

The slope of the linear extrapolation from the central estimate EC_{10} is $0.1/(3.13 \times 10^4$ µg/m$^3) = 3 \times 10^{-6}$ per µg/m^3.

The unit risk for carbon tetrachloride should not be used with exposures exceeding the POD (LEC_{10}) or 1.8×10^4 µg/m^3, because above this level the fitted dose-response model better characterizes what is know about the carcinogenicity of carbon tetrachloride.

Air Concentrations at Specified Risk Levels:

Risk Level	Lower Bound on Concentration Estimate
E-4 (1 in 10,000)	17 µg/m^3
E-5 (1 in 100,000)	1.7 µg/m^3
E-6 (1 in 1,000,000)	0.17 µg/m^3

____II.C.1.2. Extrapolation Method

Log-probit model with linear extrapolation from the POD (LEC_{10}).

___II.C.2. DOSE-RESPONSE DATA

Tumor Type – pheochromocytoma
Test Species – male BDF1 mouse
Route – inhalation
Reference – Nagano et al. 2007b; JBRC 1998

Incidence of pheochromocytoma in male BDF1 mice exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)

Tumor	Male mouse			
	0 ppm	**5 ppm**	**25 ppm**	**125 ppm**
Pheochromocytoma	0/50[a]	0/50	16/50[b]	32/50[b]

[a]Statistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.01$).
[b]Tumor incidence significantly elevated compared with that in controls by Fisher Exact test ($p \leq 0.01$).

Sources: Nagano et al. (2007b); JBRC (1998).

IUR using linear low-dose extrapolation approach

Tumor	Dose groups modeled	Model parameters	HEC (mg/m^3)	Average HEC[a] (mg/m^3)	IUR $(\mu g/m^3)^{-1}$
Male mouse pheochromocytoma	0, 5, 25, 125 ppm	MCA; Fisher model[b] BMR = 10%	12.00	17.78	5.6×10^{-6}
		MRAMKL; Thrall model[b] BMR = 10%	23.56		

[a]The average represents an arithmetic mean of the two HEC values based the Fisher and Thall models.
[b]BMD modeling was performed using internal doses based on the application of two mouse PBPK models. The dose metric used was time-averaged arterial blood concentration (MCA), in units of µmol/L.

___II.C.3. ADDITIONAL COMMENTS

The 104-week inhalation bioassay in rats and mice conducted by JBRC (Nagano et al., 2007b; JBRC, 1998) provided data adequate for dose-response modeling. In this bioassay, F344 rats and BDF1 mice were exposed to 0, 5, 25, or 125 ppm carbon tetrachloride, 6 hours/day, 5 days/week, for 2 years.

Dose-response modeling was conducted for five tumor data sets from the JBRC inhalation bioassay: adenoma and carcinoma of the liver in female rats, adenoma and carcinoma of the liver in male and female mice, and pheochromocytomas in male and female mice. The male rat data for liver adenomas and carcinomas were not modeled because these data sets lacked the resolution desired for dose-response modeling. Tumor frequencies increased from control levels to close to maximal responses without any intervening dose levels having submaximal responses.

Exposure levels studied in the 2-year JBRC rat and mouse bioassay were converted to estimates of internal dose metrics by application of PBPK modeling. BMD modeling methodology (U.S. EPA, 2000) was used to analyze the relationship between the estimated internal doses and response (i.e., liver tumors in rats and mice and pheochromocytomas in mice). The resulting BMCL values were converted to estimates of equivalent HECs by applying a human PBPK model. Data for male mouse pheochromocytomas yielded the highest estimate of the IUR of those data sets modeled [i.e., 6×10^{-6} $(\mu g/m^3)^{-1}$].

It is noted that whereas the male mouse pheochromocytoma data set yielded the highest estimate of the IUR for carbon tetrachloride based on analysis of tumor data from the JBRC bioassay (Nagano et al., 2007b) and application of PBPK modeling for interspecies extrapolation, female mouse liver tumor data yielded the highest estimate of the oral SF based on the data from the same bioassay and route-to-route extrapolation using PBPK modeling. While it may appear counterintuitive that the use of data from a single inhalation bioassay (Nagano et al., 2007b) could result in the use of different data sets for estimating cancer potency by the oral and inhalation routes, the situation arises because of the use in PBPK modeling of different dose metrics for the liver and adrenal gland that could result in different relationships between environmental exposure and internal dose within a species (i.e., rat in the current bioassay) and

across species (i.e., rats and humans).

___II.C.4. DISCUSSION OF CONFIDENCE (INHALATION EXPOSURE)

See discussion in Section II.B.4.

__II.D. EPA DOCUMENTATION, REVIEW, AND CONTACTS (CARCINOGENICITY ASSESSMENT)

___II.D.1. EPA DOCUMENTATION

Source Document – U.S. EPA, 2009

This document has been reviewed by EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of Carbon Tetrachloride* (U.S. EPA, 2009).

___II.D.2. EPA REVIEW

Agency Completion Date -- __/__/__ *[note: Leave BLANK until completion is reached]*

___II.D.3. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

_III. [reserved]
_IV. [reserved]
_V. [reserved]

_VI. BIBLIOGRAPHY

Carbon tetrachloride
CASRN -- 56-23-5
Section VI. Last Revised -- 00/00/0000

__VI.A. ORAL RfD REFERENCES

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_VII. REVISION HISTORY

Carbon tetrachloride
CASRN -- 56-23-5
File First On-Line 01/31/1987

Date	Section	Description
03/01/1988	I.A.1.	Dose conversion clarified
03/01/1988	I.A.2.	Principal study corrected
03/01/1988	I.A.4.	Text added
03/01/1988	I.A.4.	Text revised
03/01/1988	I.A.6.	Verification and meeting dates changed
03/01/1988	II.A.2.	Text corrected
03/01/1988	II.B.4.	Confidence statement revised
03/01/1988	II.C.4.	Confidence statement revised
03/01/1988	III.A.	Health Advisory added
06/30/1988	I.A.7.	Primary contact changed
12/01/1989	I.A.4.	Corrected citation year for Condie et al.
12/01/1989	VI.	Bibliography on-line
06/01/1990	IV.A.1.	Area code for EPA contact corrected
06/01/1990	IV.F.1.	EPA contact changed
08/01/1990	III.A.2.	Uncertainty factor text corrected
08/01/1990	III.A.10.	Primary contact changed
01/01/1991	II.	Text edited
01/01/1991	II.C.1.	Inhalation slope factor removed (global change)
03/01/1991	I.A.7.	Primary contact changed
06/01/1991	I.A.	Text edited
06/01/1991	II.	Text edited
06/01/1991	IV.B.1.	EPA contact changed
06/01/1991	IV.B.2.	EPA contact changed
08/01/1991	VI.C.	Blair et al., 1979 and Milham, 1976 references added
01/01/1992	IV.	Regulatory actions updated
04/01/1992	IV.A.1.	CAA regulatory action withdrawn
10/01/1992	VI.C.	Missing reference added
04/01/1997	III., IV., V.	Drinking Water Health Advisories, EPA Regulatory Actions, and Supplementary Data were removed from IRIS on or before April 1997. IRIS users were directed to the appropriate EPA Program Offices for this information.
01/12/2000	I., II.	This chemical is being reassessed under the IRIS Program.
00/00/0000	I., II., VI.	RfD and cancer assessment sections updated; RfC assessment added.

_VIII. SYNONYMS

Carbon tetrachloride
CASRN -- 56-23-5
Section VIII. Last Revised -- 00/00/0000

56-23-5
Acritet
Benzinoform
Carbona
Carbon chloride
Carbon tet
Carbon tetrachloride
Carbo tetrachloride
Czterochlorek wegla
ENT 4,705
Fasciolin
Flukoids
Freon 10
Halon 104
Mecatorina
Methane tetrachloride
Methane, tetrachloro-
Necatorina
Necatorine
Perchloromethane
R 10
Tetrachloorkoolstof
Tetrachloormetaan
Tetrachlorkohlenstoff, tetra
Tetrachlormethan
Tetrachlorocarbon
Tetrachloromethane
Tetrachlorure de carbone
Tetrachorkohlenstoff uvasol
Tetraclorometano
Tetracloruro di carbonio
Tetrafinol
Tetraform
Tetrasol
Univerm
Ventox
Vermoestricid
WLN: GXGGG