2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO CARBON TETRACHLORIDE IN THE UNITED STATES

Carbon tetrachloride is a solvent that has been used in the past as a cleaning fluid or degreasing agent, as a grain fumigant, and industrially in the synthesis of refrigeration fluid and propellants for aerosol cans. Although most of these uses have been discontinued, the possibility still exists for carbon tetrachloride to be released to the environment, primarily through industrial processes or old bottles of cleaning agents containing carbon tetrachloride that may still be in the home. Degradation of carbon tetrachloride occurs slowly in the environment, which contributes to the accumulation of the chemical in the atmosphere as well as the groundwater. Carbon tetrachloride is widely dispersed and persistent in the environment, but is not detected frequently in foods.

The general population is not likely to be exposed to large amounts of carbon tetrachloride. Populations living within or very near waste sites, or areas of heavy carbon tetrachloride use would have an increased risk of exposure from contaminated media (air, water, or soil). Those likely to receive the highest levels of exposure are those who are involved in the production, formulation, handling, and application of carbon tetrachloride. Inhalation appears to be the major route of exposure for workers and also for the general population, which may be exposed to carbon tetrachloride in ambient air and from volatilization of contaminated water during showering or bathing. Ingestion via contaminated drinking water is an important route of exposure for the general population not living in areas where carbon tetrachloride is extensively used. Dermal contact from showering or bathing has not been shown to be a significant route of exposure to carbon tetrachloride.

Most carbon tetrachloride released to the environment is expected to volatilize rapidly due to its high vapor pressure. Outdoor measurements in several areas of the United States have reported average concentrations of carbon tetrachloride in air between 0.6 and 1.0 $\mu g/m^3$ (0.1–0.16 ppb). Typical indoor concentrations in homes in several U.S. cities were about 1.0 $\mu g/m^3$ (0.16 ppb), with some values up to 9 $\mu g/m^3$ (1.4 ppb). Indoor concentrations in indoor air were thought to be higher than in outdoor air because of the presence of carbon tetrachloride in building materials or household products. The majority of domestic water supplies contain carbon tetrachloride at concentrations below 0.5 µg/L. Children are expected to be exposed to carbon tetrachloride by the same routes that affect adults. Since carbon

tetrachloride has a low affinity for adsorption onto soil and dust particles, the risk of exposure for small children from ingesting soil or dust is likely to be low. The average daily intake of carbon tetrachloride for the general population is estimated as 0.1 µg/kg/day from inhalation exposure and 0.01 µg/kg/day from ingesting drinking water containing typical low concentrations of the chemical.

See Chapter 6 for more detailed information regarding concentrations of carbon tetrachloride in environmental media.

2.2 SUMMARY OF HEALTH EFFECTS

As a volatile halogenated alkane, carbon tetrachloride has depressant effects on the central nervous system that are most significant at high exposure levels. Carbon tetrachloride also produces irritant effects on the gastrointestinal tract. Most other toxic effects of absorbed carbon tetrachloride are related to its metabolism by mixed function cytochrome P-450 oxygenases (in humans, primarily CYP2E1, but also CYP3A). The liver is the most sensitive target in exposed humans and animals, independent of the route of administration, because of the abundance of CYP2E1 and other cytochromes. The kidneys are also sensitive targets in humans and animals. There is no conclusive evidence from epidemiological studies of workers or the general population that carbon tetrachloride is carcinogenic in humans. Carbon tetrachloride has been shown to be carcinogenic in animals following chronic inhalation or oral exposure. Alcohol consumption is an important risk factor for the development of serious toxicological effects following exposure to carbon tetrachloride, since alcohol induces CYP2E1, leading to increased production of reactive metabolites. Several case reports demonstrated that when groups of individuals were accidentally exposed to carbon tetrachloride in the workplace, the individuals who were heavy consumers of alcohol developed the most serious adverse effects.

Studies in animals, combined with limited observations in humans, indicate that the principal adverse health effects associated with inhalation exposure to carbon tetrachloride are central nervous system depression, liver damage, and kidney damage. Case reports in humans and studies in animals indicate that the liver, kidney, and central nervous system are also the primary targets of toxicity following oral exposure to carbon tetrachloride; gastrointestinal irritation has been frequently noted following accidental ingestion of high doses in humans. Limited dermal data suggest that carbon tetrachloride absorbed through the skin can cause, in addition to skin irritation, gastrointestinal effects such as nausea and vomiting and neurological effects such as polyneuritis in humans, and liver damage in animals. Based on the no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL)

values identified in the animal studies, the liver appears to be the most sensitive target. Several types of liver effects have been observed in humans and laboratory animals. At lower adverse effect levels, hepatocytes accumulate lipids, resulting in cellular vacuolization and fatty degeneration. At higher exposure levels, hepatocellular necrosis (cell death), fibrosis, and cirrhosis are observed. Hepatic carcinogenicity has been observed in laboratory rodents following chronic-duration inhalation or oral exposure to carbon tetrachloride. In animal studies, kidney effects, such as tubular cell degeneration and fatty accumulation, are typically observed at higher oral doses than hepatic effects. However, in rats with chronic progressive nephropathy, hepatotoxicity and exacerbation of the severity of renal disease occurred at similar effect levels following chronic inhalation exposure. Human case reports indicate that high oral or inhalation exposures sufficient to cause renal failure (progressive uremia and electrolyte retention) may cause delayed secondary damage (edema) to the lungs. Central nervous system effects following inhalation or oral exposure include headache, weakness, lethargy, stupor, blurred vision, and coma; neurological effects are generally observed at exposure levels higher than the thresholds for hepatic or renal toxicity. High-level inhalation or oral exposure is associated with mild hematological effects, primarily anemia in humans and animals, and reduced platelet function (clotting efficiency) in animals. Suppression of immune function (reductions in IgM antibody-forming cell activity, T-cell activity, lymphocyte counts, or host resistance to bacteria) has been observed in animals exposed short-term to oral doses higher than those causing liver effects.

No studies were located regarding reproductive effects in humans after exposure to carbon tetrachloride and the available human data for developmental effects are limited to epidemiological studies of pregnancy outcomes in women exposed to carbon tetrachloride and other halogenated hydrocarbons in drinking water. These data are inadequate for establishing a causal relationship between carbon tetrachloride exposure and developmental toxicity in humans. In animals exposed by inhalation for intermediate durations, reproductive effects included decreased fertility and testicular atrophy. In developmental studies in animals exposed by inhalation or ingestion, no fetal toxicity was observed in the absence of maternal toxicity and morphological defects were not observed in offspring. However, oral doses that produced clear maternal toxicity increased fetal mortality, in some cases, complete litter loss. It is not known whether litter loss is the result of toxicity to the fetus or to the placenta.

The following sections discuss significant effects resulting from exposure to carbon tetrachloride in greater detail: hepatic, renal, neurological, and cancer.

Hepatic Effects. Hepatotoxicity is the major effect of exposure to carbon tetrachloride by any route in humans and animals and is the basis for all MRLs derived for that compound. Liver injury is detectable by clinical signs (jaundice, swollen and tender liver), biochemical alterations (elevated levels of hepatic enzymes in the blood, loss of enzymatic activities in the liver), or histological examination (fatty degeneration and necrosis of central hepatocytes, destruction of intracellular organelles, fibrosis, cirrhosis). Elevated levels of serum enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase, and gamma glutamyl transferase) may provide evidence of hepatocellular injury in the absence of clinical signs, as was observed in workers occupationally exposed at intermediate-to-chronic durations at levels between 1.1 and 12 ppm. Degeneration or necrosis of the liver was noted in humans following acute inhalation exposure at 250 ppm or acute oral exposure at ≥110 mg/kg. In humans, acute lethal inhalation or oral exposures were associated with massive liver necrosis and steatosis. In rats, centrilobular vacuolization was observed at an acute oral dose of 20 mg/kg/day, whereas necrosis was observed at 80 mg/kg/day. Hepatic necrosis was also observed in guinea pigs following acute dermal exposure at 513 mg/cm^2. In chronic studies, significant increases in fatty change, fibrosis, and cirrhosis were observed in rats at 25 ppm; in the same study, the major non-neoplastic hepatic lesion in mice was necrosis. These species differences in hepatic effects may be related to the differential involvement of tumor necrosis factor alpha, which may facilitate necrosis, or transforming growth factor beta, which is an initiator of fibrosis.

It is widely agreed that the reason for the special sensitivity of the liver to carbon tetrachloride toxicity is the inherently high rate of metabolism of carbon tetrachloride by this tissue, presumed to be associated with the high abundance of CYP2E1, particularly concentrated in the centrilobular zone. This hypothesis was verified for mice in a study that administered 1,590 mg carbon tetrachloride/kg body weight by intraperitoneal injection to wild-type or CYP2E1 knockout mice *(cyp2e1$^{-/-}$)*. In wild-type mice expressing CYP2E1, hepatotoxicity characterized by elevated serum enzyme levels (ALT and AST) and histopathology (centrilobular parenchymal degeneration and perivenular vacuolation) was observed 24 hours after treatment with carbon tetrachloride. None of these hepatic lesions were observed in CYP2E1 knockout mice treated with the same dose. In humans, CYP2E1 is also the primary enzyme responsible for metabolizing carbon tetrachloride at environmentally relevant concentrations, but others, particularly CYP3A, are also involved at higher concentrations. The reactive metabolites (trichloromethyl free radicals) generated by the oxidation of carbon tetrachloride are believed to trigger a spectrum of hepatocellular damage. Mechanisms that appear to be involved include direct binding of reactive metabolites to cellular proteins, peroxidation of unsaturated membrane lipids, and alterations in intracellular calcium levels. Release of proteolytic enzymes from dying cells has been shown to extend

the necrosis of hepatic tissue beyond the initial site exposed to carbon tetrachloride. The outcome of any carbon tetrachloride-induced injury has been demonstrated to depend on several factors, including the induction of P-450 enzymes and the presence of antioxidants and interactions with other chemicals.

Renal Effects. Injury to the kidney is also observed in many reports of carbon tetrachloride toxicity in humans, often at the same exposure levels that cause hepatic injury. The principal clinical signs in severe cases are oliguria or anuria, with resultant azotemia and edema, leading in turn to hypertension and pulmonary edema. Cells of the proximal tubule are most clearly injured by carbon tetrachloride, probably because of high content of cytochrome P-450. Renal injury is observed in animal studies, but usually at higher doses with lesser severity than in humans. In oral exposure studies, the effect levels for kidney toxicity are generally higher than for hepatic toxicity. Some intermediate-duration inhalation bioassays in rats reported the adverse effect level for the kidney to be the same as or higher than that for the liver. In a 2-year inhalation study in F344 rats, exposure to carbon tetrachloride at hepatotoxic levels increased the severity of chronic progressive nephropathy compared to the control group.

There is some evidence that the susceptibility of the kidney to carbon tetrachloride may increase in elderly animals. Both the liver and the kidney exhibit age-related reductions in CYP-450, which would result in relatively lower production of reactive metabolites following exposure, but findings have been qualitatively different in the two organs. Reductions in CYP3A and CYP2E1 activity have been noted in the liver of elderly humans and rats. In the rat kidney, however, one isoform of CYP3A was upregulated by 50% in old (25–26-month) rats, resulting in a net 11% increase (not statistically significant) in total CYP3A in the kidney, possibly contributing to increased renal vulnerability to carbon tetrachloride. In addition to the possibility that the kidney in the elderly generates relatively more reactive metabolites following exposure, reductions in the organ content of antioxidants protecting against reactive metabolites may contribute to sensitivity. Both the liver and the kidney exhibit age-related reductions in glutathione. One study in F344 rats (the same strain used in the chronic inhalation assay) reported a significant decrease in glutathione peroxidase activity in the kidney, but not the liver of 24-month-old rats compared to 6-month-old rats, consistent with a greater sensitivity in the kidney. Although the findings outlined above are suggestive, no study has specifically demonstrated age-related changes in the renal metabolism of carbon tetrachloride.

Neurological Effects. The primary acute toxicological effect of unmetabolized carbon tetrachloride is depression of the central nervous system. Acute-duration inhalation or oral exposures in humans have resulted in neurological effects such as headache, dizziness, and weakness, and, at higher exposures,

tremor, blurred vision, drowsiness, seizures, and loss of consciousness. Immediate fatalities occur from suppression of respiratory centers. Following ingestion of single doses, autopsy findings have included neurohistopathology in the cerebellum (demyelination and Purkinje cell damage with widespread hemorrhagic infarcts), but survivors appear not to have lasting cerebellar deficits. Suppression of autonomic respiratory centers is also observed in animals exposed by inhalation. Degeneration of the optic nerve has sometimes been noted in humans and repeated inhalation exposure studies in animals, sometimes at levels lower than those causing overt signs of central nervous system depression. It is likely that the observed neurohistopathology may be related to the generation of reactive metabolites in the neural tissues of exposed animals.

Cancer. There are a few reports of cancer in people who have been exposed to carbon tetrachloride, but these data alone are not sufficient to show that carbon tetrachloride causes cancer in humans. Suggestive data in humans comes from occupational case-control studies that found positive associations between exposure to carbon tetrachloride and mortality from several types of cancer (lymphosarcoma, lymphatic leukemia, non-Hodgkin's lymphoma, or multiple myeloma). There is convincing evidence that exposure to carbon tetrachloride leads to hepatic tumors in rodents exposed by inhalation or dosed orally. The lowest cancer effect levels were observed for mice: 25 ppm by inhalation and 20 mg/kg/day orally.

Two kinds of processes appear to contribute to the carcinogenicity of carbon tetrachloride. Genotoxicity, primarily covalent binding to DNA in the liver, results from the direct binding of reactive carbon tetrachloride metabolites or lipid peroxidation products in animals exposed orally or by intraperitoneal injection. High oral doses have resulted in DNA breakage, detectable by electrophoresis. It is likely that DNA breakage following acute exposures at high levels may be secondary to liver necrosis that is characterized by the release of nucleases and other enzymes from lysosomes of degenerating hepatocytes. There is some evidence that carbon tetrachloride may also cause cancer by a nongenotoxic mechanism involving cellular regeneration. Mild hepatic necrosis stimulates cell division processes; the resulting increase in cell proliferation could result in either the replication of unrepaired DNA damage or the induction of additional errors during the replication process, both of which can produce heritable mutations that may result in an initiated preneoplastic cell.

The U.S. Department of Health and Human Services has determined that carbon tetrachloride may reasonably be anticipated to be a carcinogen. IARC has classified carbon tetrachloride in Group 2B, possibly carcinogenic to humans. EPA has determined that carbon tetrachloride is a probable human

carcinogen and derived an oral slope factor of $1.3x10^{-1}$ per (mg/kg/day). EPA is currently revising the carcinogenicity assessment for carbon tetrachloride.

2.3 MINIMAL RISK LEVELS

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for carbon tetrachloride. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

The liver is the most sensitive target organ for carbon tetrachloride toxicity by the oral and inhalation routes. Adverse effects are also observed in the kidney, which is a significant target in humans acutely exposed at high levels and in animals following chronic inhalation exposure. However, all derived MRLs for carbon tetrachloride are based on nonneoplastic hepatic effects, which occurred at lower exposure levels than effects in other target tissues in studies that quantified exposure. All derived MRLs are based on rat studies since the observed nonneoplastic hepatic effects (fatty degeneration, necrosis, fibrosis, and cirrhosis) in this species are similar to the range of histopathology observed in exposed humans. Conversely, in exposed mice, the most significant nonneoplastic features of hepatic histopathology are fatty degeneration and necrosis, but not fibrosis or cirrhosis. Thus, studies in rats would appear to be preferred as a basis for human health risk assessment for carbon tetrachloride. The MRLs for carbon tetrachloride were based on the lowest available LOAELs or the associated NOAELs (if available) in well-designed studies.

Inhalation MRLs

The database for acute-duration inhalation exposure to carbon tetrachloride includes a few studies in humans as well as studies in laboratory animals. No neurological effects were observed in six volunteers exposed to 50 ppm carbon tetrachloride for 1–3 hours, but these individuals exhibited a decrease in serum iron levels that was not observed at 10 ppm (Stewart et al. 1961); the significance of serum iron to hepatic toxicity is not clear. Single exposures of 200 ppm for ≤3 hours resulted in hepatic effects (increased serum bilirubin), renal effects (proteinuria), and gastrointestinal effects (nausea) (Barnes and Jones 1967; Norwood et al. 1950). Ethanol consumption increases the severity of carbon tetrachloride toxicity and confounds dose-response assessments from case reports. Whereas two workers experienced only mild neurological effects (headache and dizziness) after 4 hours exposure at 250 ppm, a co-worker who was a heavy consumer of alcohol died from renal insufficiency six days after a 15-minute exposure (Norwood et al. 1950); severe effects in the fatal case were noted in the liver (necrosis), kidney (oliguria, nephrosis), and lung (edema secondary to kidney failure). A NOAEL for hepatic effects was not observed in acute-duration inhalation studies in animals. Effects in rats included slight fatty degeneration of the liver at 10 ppm, increasing in extent and severity at ≥25 ppm, cirrhosis at ≥200 ppm, and parenchymatous degeneration of renal tubules in female rats treated at 400 ppm following exposure 7 hours/day for 13 days in a 17-day period (Adams et al. 1952). Mild liver effects (altered glycogen distribution, hepatocytic steatosis, hydropic degeneration, and necrosis, and elevated serum alanine aminotransferase) were observed in rats exposed at 50 ppm for 6 hours/day for 4 days (David et al. 1981). Hepatic effects (fatty degeneration and elevated serum sorbitol dehydrogenase) were also reported in rats exposed to 100 ppm 8 hours/day, 5 days/week for 2 weeks (Paustenbach et al. 1986a). Exposure for 15 minutes at 180 ppm resulted in increased alanine aminotransferase and relative liver weight in rats (Sakata et al. 1987). Effects in other organ systems following acute-duration inhalation exposure of rodents include hematological effects (increased coagulation time) at 325 ppm (Vazquez et al. 1990), developmental effects (decreased fetal body weight and crown-rump length) at 330 ppm (Schwetz et al. 1974), and neurological effects (coma, inhibition of response to electrical stimulus) at 180–1,370 ppm (Frantik et al. 1994; Sakata et al. 1987); dogs exposed to 15,000 ppm for 2–10 hours exhibited depression of the central nervous system (Von Oettingen et al. 1949). No renal effects were observed in rats exposed once or repeatedly to 100 ppm (Adams et al. 1952; Paustenbach et al. 1986b). Hepatotoxicity appears to be the critical effect of acute-duration inhalation exposure because it occurs at the lowest LOAELs in laboratory animals. The Adams et al. (1952) study identified the lowest LOAEL of 10 ppm and highest NOAEL of 5 ppm. In this study, male or female Wistar rats (2–30 of one sex/group) were exposed to carbon

tetrachloride vapor at concentrations of 0, 5, 10, 25, 50, 100, 200, or 400 ppm, 7 hours/day, 5 days/week for 173–205 days of exposures.

No MRL was established for acute-duration inhalation exposure to carbon tetrachloride because a derivation based on the most suitable data (the minimal LOAEL of 10 ppm in rats reported by Adams et al. 1952) would result in an acute-duration MRL lower than the intermediate-duration MRL. The intermediate-duration inhalation MRL of 0.03 ppm, based on a NOAEL of 5 ppm and a LOAEL of 10 ppm for liver effects (Adams et al. 1952), is expected to be protective for acute-duration inhalation exposure.

- An MRL of 0.03 ppm has been derived for intermediate-duration inhalation exposure to carbon tetrachloride.

Limited human data are available for intermediate-duration inhalation exposure to carbon tetrachloride. Effects in humans exposed intermittently included gastrointestinal effects (nausea, dyspepsia) at 20–50 ppm, depression at 40 ppm, and narcosis at 80 ppm (Elkins 1942; Heiman and Ford 1941; Kazantzis and Bomford 1960). An occupational study of hepatic effects in workers exposed for <1–>5 years indicated that serum levels of hepatic enzymes were significantly elevated only at exposures >1 ppm, but the actual durations of exposure were not reported (Tomenson et al. 1995). Interpretation of this study is also limited by the finding that the group estimated to have had the highest exposure did not show the highest levels of serum enzymes. The liver appears to be the most sensitive target in animals exposed for intermediate durations. Fatty degeneration, sometimes with increased liver weight, was observed at a LOAEL of 10 ppm in rats, mice, and guinea pigs treated 6–8 hours/day, 5 days/week for 12–36 weeks or continuously for 90 days (Adams et al. 1952; DOE 1999; Japan Bioassay Research Center 1998; Prendergast et al. 1967), and 50–100 ppm in monkeys (Adams et al. 1952; Smyth et al. 1936). Increased serum enzymes and necrosis were observed in mice at 20 ppm and hamsters at 100 ppm (DOE 1999). Exposure to higher concentrations resulted in cirrhosis in guinea pigs (25 ppm) and rats (50–270 ppm) (Adams et al. 1952; Japan Bioassay Research Center 1998; Prendergast et al. 1967; Smyth et al. 1936). In studies examining other organs, renal effects (tubular degeneration) were noted at 50–200 ppm in rats (Adams et al. 1952; Smyth et al. 1936), at 90 ppm in rats and mice (Japan Bioassay Research Center 1998), and at 200 ppm in monkeys (Smyth et al. 1936). A neurological effect (injury to sciatic and optical nerves) was noted in rats at 50 ppm (Smyth et al. 1936). Hematological effects (decreased erythrocytes, hemoglobin, hematocrit; hemolysis, increased spleen weight) were observed in rats and mice exposed to 90–270 ppm (Japan Bioassay Research Center 1998; Smyth et al. 1936). Reproductive toxicity (decreased litters, testicular atrophy) was noted at 200 ppm (Adams et al. 1952; Smyth et al.

1936). Hepatotoxicity is identified as the critical effect of intermediate-duration inhalation exposure to carbon tetrachloride since it was noted at the lowest LOAELs.

The intermediate-duration inhalation MRL for carbon tetrachloride is based on a NOAEL of 5 ppm and a LOAEL of 10 ppm for liver effects in rats identified in a study by Adams et al. 1952. In this study, Wistar rats (15/sex/group) were exposed to carbon tetrachloride vapor at concentrations of 0, 5, 10, 25, 50, 100, 200, or 400 ppm, 7 hours/day, 5 days/week for periods between 173 and 205 days. Fatty degeneration and increased liver weights were evident at concentrations of ≥10 ppm, cirrhosis occurred at ≥50 ppm, and necrosis at ≥200 ppm. Renal effects (cloudy swelling of the tubular epithelium) were first evident at ≥50 ppm, with increased kidney weights and degeneration of renal tubular epithelium evident at ≥200 ppm. Testicular atrophy was observed at ≥200 ppm. A human equivalent concentration of the identified rat NOAEL of 5 ppm ($NOAEL_{HEC}$) was calculated by multiplying the duration-adjusted rat NOAEL ($NOAEL_{ADJ}$) by the ratio of the rat and human blood:gas partition coefficients. The $NOAEL_{ADJ}$ is 0.9 ppm (5 ppm x 7 hours/24 hours x 5 days/7 days) and the blood:gas partition coefficient ratio is 1.7 (4.52/2.64). Because the ratio was greater than 1, a default value of 1 was applied, resulting in a $NOAEL_{HEC}$ of 0.9 ppm. An uncertainty factor of 30 was applied to the $NOAEL_{HEC}$ of 0.9 ppm (3 for extrapolation from animals to humans using a dosimetric adjustment and 10 for human variability).

- An MRL of 0.03 ppm has been derived for chronic-duration inhalation exposure to carbon tetrachloride.

The chronic-duration inhalation database for carbon tetrachloride includes the occupational study by Tomenson et al. (1995) and 2-year bioassays in rats and mice (Japan Bioassay Research Center 1998; Nagano et al. 1998). As discussed under the intermediate-duration MRL, elevated hepatic serum enzymes were observed in workers who had been exposed to concentrations >1 ppm for <1–5 years, but the actual durations of exposure were not reported (Tomenson et al. 1995). Interpretation of this study is also limited by the finding that the group estimated to have had the highest exposure did not show the highest levels of serum enzymes.

In the 2-year bioassays, groups of F344/DuCrj rats and BDF_1 mice (50/sex) were treated at 0, 5, 25 or 125 ppm, 6 hours/day, 5 days/week for 104 weeks (Japan Bioassay Research Center 1998; Nagano et al. 1998). Male rats exhibited increased hemosiderin deposition in the spleen at 5 ppm and above, but this effect was the result of anemia that was observed at 13 weeks, but not 104 weeks. For most observed effects, the lowest concentration of 5 ppm was a NOAEL and 25 ppm was a LOAEL: hematological

(decreased hemoglobin, hematocrit in female rats and increased extramedullary splenic hematopoeisis in mice), body weight (reduced body weight gain), renal (altered clinical chemistry values in both species, chronic progressive glomerulonephrosis in rats, protein casts in mice), and hepatic (increased liver weight and serum enzymes in both species; fibrosis, cirrhosis, severe fatty change and granulation in rats; thrombus, necrosis, and, degeneration in mice). Mice showed increased mortality from hepatic cancer at 25 ppm, rats at 125 ppm. The severity of proteinuria, but not renal histopathology, was elevated in male and female rats treated at ≥5 ppm compared to controls; this lesion was not used as the basis for MRL derivation because the severity in control rats was so high (>90% with scores of 3+ or 4+). Hepatotoxicity is selected as the critical effect of chronic-duration inhalation exposure because the severity of effects at 25 ppm was greater compared to other end points. Furthermore, selection of hepatotoxicity as the critical effect of chronic exposure is consistent with the database for intermediate-duration inhalation exposure.

The chronic-duration inhalation bioassay in rats is selected as the principal study because it provided a NOAEL of 5 ppm and a LOAEL of 25 ppm for hepatic effects (increased serum enzyme levels, liver weight, and liver histopathology) without increased mortality (Japan Bioassay Research Center 1998; Nagano et al. 1998). The duration-adjusted rat NOAEL ($NOAEL_{ADJ}$) for hepatic effects was multiplied by the ratio of the rat and human blood:gas partition coefficients to derive a human equivalent concentration of the identified chronic rat NOAEL of 5 ppm. The $NOAEL_{ADJ}$ is 0.9 ppm (5 ppm x 6 hours/24 hours x 5 days/7 days) and the blood:gas partition coefficient ratio is 1.7 (4.52/2.64). Using standard procedures, a default value of 1 was applied because the ratio was greater than 1, resulting in a chronic-duration $NOAEL_{HEC}$ of 0.9 ppm. An uncertainty factor of 30 was applied to the $NOAEL_{HEC}$ of 0.9 ppm (3 for extrapolation from animals to humans using dosimetric adjustment and 10 for human variability), resulting in a chronic-duration inhalation MRL of 0.03 ppm.

Oral MRLs

- An MRL of 0.02 mg/kg/day has been derived for acute-duration oral exposure to carbon tetrachloride.

Limited data in humans and several studies in laboratory animals are available for acute-duration oral exposure to carbon tetrachloride. In humans, hepatic toxicity (fatty accumulation, necrosis) has been noted following ingestion of single doses of carbon tetrachloride in the range of 80–180 mg/kg (Docherty and Burgess 1922; Docherty and Nicholls 1923; Phelps and Hu 1924). Single doses of 70 mg/kg had no overt neurological effect, but various neurological symptoms indicative of depression of the central

nervous system have been reported at doses between 114 and 10,800 mg/kg (Cohen 1957; Hall 1921; Leach 1922; Stevens and Forster 1953; Stewart et al. 1963). Gastrointestinal effects in humans following ingestion of single doses include nausea at ≥100 mg/kg (Ruprah et al. 1985) and vomiting and abdominal pain at 680–910 mg/kg (Hardin 1954; New et al. 1962; Smetana 1939; Umiker and Pearce 1953; von Oettingen 1964). In laboratory animals, mild hepatic effects (cytoplasmic vacuolization and increased serum enzymes) have been reported to occur following treatment with single doses of 40–80 mg/kg or repeated dosing at 5–20 mg/kg/day (Bruckner et al. 1986; Kim et al. 1990b; Korsrud et al. 1972; Smialowicz et al. 1991). No renal effects or positive results in special tests for immunological function were observed in rats following repeated administration at 5–160 mg/kg/day (Bruckner et al. 1986; Smialowicz et al. 1991). Renal effects (fatty degeneration, swelling of convoluted tubules) were observed in dogs given single doses of 3,200–6,400 mg/kg (Chandler and Chopra 1926; Gardner et al. 1925). Hepatic toxicity is selected as the critical effect of acute-duration oral exposure to carbon tetrachloride because effects were observed at the lowest effect level.

The study of Smialowicz et al. (1991) is selected as the principal study because it provides the lowest LOAEL for hepatotoxicity, the critical effect. In this study, groups of 5–6 male Fischer rats were dosed by oral gavage with 0, 5, 10, 20, or 40 mg/kg/day for 10 consecutive days and evaluated for hepatic and renal toxicity (organ weight and serum parameters and histology) as well as some immunological end points. Another set of animals was exposed at 40, 80, or 160 mg/kg/day and evaluated for additional immunological parameters. Liver toxicity was the most sensitive effect observed in this study and was dose related in severity. Centrilobular vacuolar degeneration was barely detectable in all six animals of the 5 mg/kg/day group, whereas no liver effects were observed in any of the six controls. Hepatocellular necrosis was first evident at 10 mg/kg/day. At higher doses, serum levels of ALT and AST became significantly elevated ($p<0.01–0.05$) (20 and 40 mg/kg/day), as did relative liver weight (40 mg/kg/day). No renal effects were observed at the highest dose of 40 mg/kg/day and no immunological effects were observed at doses as high as 160 mg/kg/day. The LOAEL of 5 mg/kg/day is considered to be minimal because the type and degree of hepatic histopathology was relatively mild. A total uncertainty factor of 300 was applied to the minimal LOAEL of 5 mg/kg/day (3 for the use of a minimal LOAEL, 10 for extrapolation between animals to humans, and 10 for human variability).

- An MRL of 0.007 mg/kg/day has been derived for intermediate-duration oral exposure to carbon tetrachloride.

The intermediate-duration oral toxicity database for carbon tetrachloride is somewhat limited in that no human data are available and many studies in laboratory animals restricted analysis to the liver. The

incidence and severity of hepatic effects were dose-related in animal studies. A NOAEL of 1 mg/kg and a LOAEL of 10 mg/kg was identified for significantly elevated sorbitol dehydrogenase (SDH) and mild centrilobular vacuolization in rats exposed 5 days/week for 12 weeks (Bruckner et al. 1986); at 33 mg/kg, ALT and ornithine carbamyl transferase activities were increased and cirrhosis was observed. In mice ingesting carbon tetrachloride 5 days/week for 12–13 weeks, no hepatic effects were detected at a dose of 1.2 mg/kg (Condie et al. 1986). Significant elevation in some serum enzymes (ALT, aspartate aminotransferase [AST], lactate dehydrogenase [LDH]), and mild necrosis were seen in mice at doses of 12 mg/kg and higher (Condie et al. 1986; Hayes et al. 1986). More extensive hepatic lesions (fatty accumulation, fibrosis, cirrhosis, necrosis) were noted in rats at doses of 20–25 mg/kg and higher (Allis et al. 1990; Koporec et al. 1995). At 100 mg/kg/day, hepatic effects in rats also included cytomegaly and various types of hyperplasia, which were perhaps adaptive responses to necrosis (Koporec et al. 1995). Effects in other organ systems include reduced body weight gain at doses between 33 and 100 mg/kg/day (Bruckner et al. 1986; Koporec et al. 1995) and neurological effects (increased serotonin synthesis) at 290 mg/kg/day (Bengtsson et al. 1987). No renal effects were observed in rats exposed at 33 mg/kg/day (Bruckner et al. 1986) or mice exposed at 1,200 mg/kg/day (Hayes et al. 1986). Increased mortality was observed in rats exposed at 25 mg/kg/day (Koporec et al. 1995). Cancer (hepatoma) was observed in mice treated with 20 mg/kg/day for 120 days and hamsters treated once weekly with 120 mg/kg/day for 30 weeks (Eschenbrenner and Miller 1946; Della Porta et al. 1961). Hepatotoxicity was selected as the critical effect of intermediate-duration oral exposure to carbon tetrachloride because it occurred at the lowest effect level.

The rat study of Bruckner was selected as the principal study because it provided the lowest LOAEL for the critical effect. In this study, male Sprague-Dawley rats (15–16 per group) were administered 0, 1, 10, or 33 mg/kg carbon tetrachloride by gavage in corn oil 5 days/week for 12 weeks. Blood samples were taken at biweekly intervals throughout the study for analysis of serum parameters related to liver and kidney toxicity and both organs were examined for histopathology at termination. Slightly elevated blood levels of sorbitol dehydrogenase and mild centrilobular vacuolation of the liver were observed at 10 mg/kg, but not at 1 mg/kg. Cirrhosis, extensive degenerative hepatic lesions, and significantly elevated serum enzyme levels (ornithine carbamyl transferase and alanine aminotransferase) were observed at the high dose of 33 mg/kg. No renal effects were observed at any dose. The NOAEL of 1 mg/kg was adjusted for intermittent exposure (5 days/7 days) and divided by a total uncertainty factor of 100 (10 for extrapolation between animals to humans and 10 for human variability).

No data were located on the effects of chronic-duration oral exposure in humans. Carbon tetrachloride was employed as a positive control for hepatic cancer in several chronic oral gavage bioassays in rats and mice exposed 5 days/week for 78 weeks (NCI 1976a, 1976b, 1977); serious effects were observed at the lowest tested doses. Exposure at ≥47 mg/kg reduced survival by 46% in rats because of severe hepatotoxicity. Portal cirrhosis, bile duct proliferation, and fatty accumulation were observed in more than 55% of treated rats. In the same study, survival in mice treated at doses of ≥1,250 mg/kg was reduced by ≥80% compared to controls on account of hepatic carcinogenicity. Since a no-effect level was not identified and ATSDR does not base MRLs on doses at which serious effects occur, a chronic-duration oral MRL was not derived for carbon tetrachloride.