

CARBON TETRACHLORIDE

Carbon tetrachloride, CCl_4 , is a clear, colorless, non-flammable and highly volatile liquid. The complete chlorine substitution makes carbon tetrachloride less volatile but more fat soluble than the less chlorinated methanes methylene chloride and chloroform (Appendices 1 and 2). Carbon tetrachloride is one of the oldest of the halogenated aliphatic solvents and has been extensively used as an industrial solvent, extraction medium and degreasing agent. Carbon tetrachloride has also been used for dry cleaning and in chemical syntheses and as a fumigant, fire-extinguisher and medicinally as an anthelmintic (51, 299). Although carbon tetrachloride, due to its hepatotoxicity, has been replaced to a large extent by other chlorinated solvents, the world production capacity of carbon tetrachloride was estimated to be about 1 million tons per year in 1973 (267).

TOXICITY OF CARBON TETRACHLORIDE

The early literature on carbon tetrachloride toxicity has been reviewed by Browning (51) and von Oettingen (299). Death at acute exposure to high concentrations of carbon tetrachloride may occur as a result of cardiac arrhythmia since carbon tetrachloride sensitizes the myocardium to the actions of endogenous catecholamines (76, 170, 180, 299) but more likely in respiratory failure since carbon tetrachloride is a potent narcotic

agent with an even more depressant effect on the CNS than methylene chloride or chloroform (101, 301). Concentrations around 3000-4000 ppm produce initial narcotic symptoms in animals such as drowsiness and stupor. Loss of equilibrium and coordination occurs at 7000-9000 ppm and complete unconsciousness and narcosis at exposure to 12000-20000 ppm for 30 minutes or longer (4, 419, 299). The LC_{50} for mice has been estimated to be about 9000 ppm for 2-7 hours (299, 301). The minimal fatal concentration for rats was 19000-24000 ppm for 30 minutes-2 hours (4, 299) and for cats 14300 ppm for 70 minutes (299). Humans may experience the first signs of CNS depression at concentrations of only 150-300 ppm followed by headache, dizziness and drowsiness at exposure to about 2000 ppm for 5-10 minutes (299). Concentrations around 10000 ppm may produce paresthesia, weakness, slurred speech and even unconsciousness in a few minutes and concentrations of about 25000 ppm may be fatal in 30 minutes-1 hour (299, 419). Several cases of severe CNS depression due to acute overexposure to carbon tetrachloride have been described in the early literature (112, 170, 299, 420). Histopathological examination of the CNS in acute fatal human carbon tetrachloride poisoning has revealed perivascular hemorrhage and edema in the white matter of the cerebrum, cerebellum and pons (258) occasionally in combination with areas of demyelination and necrosis as well as degeneration of Purkinje cells (77, 299, 420). Chronic occupational human exposure to carbon tetrachloride has been reported to produce unspecific signs of CNS involvement such as mental dullness, depression, irritability, loss of memory, headache, vertigo and dizziness (28, 170, 216, 296, 299, 402, 430) but

also peripheral neuropathy (152). A variety of both central and peripheral neuropathological changes have also been described in experimental animals after acute (77, 240, 258) or chronic (402) exposure to carbon tetrachloride.

Carbon tetrachloride is a well documented hepatotoxic agent (334, 337) of outstanding potency among the halogenated hydrocarbon solvents (99, 103, 143, 222, 223, 224, 316, 343, 448, 473) as well as in comparison to other organic solvents (103, 343, 473). The effects and mechanisms of liver injury in acute experimental carbon tetrachloride poisoning have been extensively investigated. The earliest ultrastructural changes are seen in the endoplasmic reticulum of centrilobular hepatocytes which shows extensive degranulation, fragmentation and vacuolization with an increase in free ribosomes within 1 hour after administration of carbon tetrachloride (38, 42, 100, 298). The ribosomal dissociation results in an altered ultracentrifugal sedimentation pattern (346, 399, 400). Injury to the endoplasmic reticulum is associated with an early depression of hepatic protein synthesis (376, 400, 401), shown by micro-autoradiography to occur in centrilobular hepatocytes (75, 280), as well as with loss of microsomal enzymes such as glucose-6-phosphatase and cytochrome P450 (338, 339, 343, 361). There is also evidence of beginning fat infiltration visible as lipid droplets in the cytoplasm (21, 38, 42, 298, 473). Liver triglycerides increase rapidly during the first hours of carbon tetrachloride poisoning - within 1 hour and 3 hours after oral administration of 0.25 ml/100 g carbon tetrachloride to rats liver triglyceride content was elevated 33.6% and 195% respectively (372). The calcium content of mitochondria is

doubled within 2-4 hours after carbon tetrachloride administration and shows an almost 20-fold maximal elevation at 40 hours (344, 442). By 5-10 hours there is lysosomal rupture as evidenced by an increased activity of non-sedimentable hydrolytic enzymes in liver homogenates (21, 394, 454). Structural damage to mitochondria characterized by enspherulation and swelling is seen at 10-16 hours (100, 244, 298) accompanied by loss of mitochondrial function such as oxidative activity and phosphorylation first observed at 10-20 hours (60, 72, 335, 339, 344, 390). Scattered necrotic hepatocytes are visible already at 6-8 hours (38, 100, 473). By 24-48 hours centrilobular necrosis and fat infiltration are seen as the final histopathological result of acute carbon tetrachloride poisoning (38, 100, 182, 244, 298, 401, 473). The order of injury to the cell organelles is also reflected in the time-sequence of appearance of various hepatocellular enzymes in serum (100, 394, 478). The degree of liver injury is dependent on the dose of carbon tetrachloride administered as shown for fat accumulation (224, 335), rise of serum enzymes (478), inhibition of protein synthesis (400) and histopathological changes (38).

In spite of the wealth of information available on carbon tetrachloride hepatotoxicity its basic events and mechanisms are still subject to discussion. However, it is generally agreed that the accumulation of triglycerides in the liver is due to a disruption of the mechanisms for synthesis and secretion of lipoproteins occurring in the endoplasmic reticulum (334, 337). As for the liver necrosis, no single event can be made responsible but it may be considered a

result of several effects (477). It is also agreed that carbon tetrachloride exerts its hepatotoxic effects after biotransformation to reactive free radicals (337) as originally suggested by Butler (57) and Slater (393). In the membranes of the endoplasmic reticulum, the site of carbon tetrachloride biotransformation, and later also in the membranes of other cell organelles, the free radicals bind rapidly to proteins and lipids (332) as well as react with the methylene bridges of polyunsaturated phospholipids thereby initiating autocatalytic lipoperoxidation (331). Both processes produce alterations of membranal structure which probably result in alterations of membranal function including permeability changes and loss of normal intracellular integrity and activity (477). The relative importance of the two processes cannot be determined since both covalent binding of free radicals (69, 74, 94, 95, 97, 466) and radical induced lipid peroxidation (153, 334, 337, 338) are considered as crucial and initial events in the development of liver injury.

Acute experimental carbon tetrachloride poisoning is not only associated with liver injury but may also produce alterations in other tissues (73, 299). However, histopathological changes have been observed mainly in the lung and kidney. In the lungs of rats given 0.25 ml/100 g carbon tetrachloride orally perivascular edema was observed at 4 hours and areas of atelectasis and intraalveolar hemorrhages at 8-12 hours after administration (158). Ultrastructurally, there was cytoplasmic edema, dislocation of ribosomes from the endoplasmic reticulum and disruption of mitochondria between 4 and 8 hours after administration. Necrosis of pneumocytes

was observed at 12-24 hours. Guinea pigs given 0.25 ml/100 g carbon tetrachloride intraperitoneally showed an increasing dilatation of the endoplasmic reticulum and fatty infiltration in granular pneumocytes from 3 hours up to 48 hours after administration (458). The lungs of rabbits after 2 subcutaneous injections of 1 ml/kg or up to 20 injections of 0.5 ml/kg carbon tetrachloride showed dystelectasis, hemorrhages and edema at light microscopic examination (240). In mice and dogs carbon tetrachloride is a weak nephrotoxic agent with slight proteinuria and swelling of proximal convoluted tubules observed only after administration of near lethal doses (222, 223, 317). A single dose of 0.25 ml/100 g carbon tetrachloride to rats produced cellular edema and swelling and enspherulation of mitochondria in the proximal tubules paralleled by decreased succinic dehydrogenase activity, which was associated with impairment of energy-requiring sodium reabsorption with consequent abnormal urine output (433). Repeated small intraperitoneal injections of carbon tetrachloride produced an increased urinary excretion of serum-like proteins in rats, which was considered a possible result of kidney injury (313). High intraperitoneal doses (0.3-1.0 ml/100 g) carbon tetrachloride to rats caused tubular necrosis within 2-6 hours (199, 231). Thus, microscopically there are similarities between the effects of carbon tetrachloride on the liver, lung and kidney. Little is known about the biochemical alterations and the mechanisms of carbon tetrachloride induced lung and kidney injury. In the rat lung rapid destruction of microsomal mixed function oxidase activity was noted after oral or inhalational administration of carbon tetrachloride (71) whereas glucose-6-

phosphatase activity showed no significant alterations in rat kidney after oral administration (74, 433). Uncoupling of oxidative phosphorylation after carbon tetrachloride administration to rats was shown to occur not only in the liver but also in the kidney (98). Lipid peroxidation has not been observed in rat lung (73, 466) or rat kidney (73, 74, 336, 417, 466) as a consequence of carbon tetrachloride administration but irreversible binding of radioactivity from ^{14}C -carbon tetrachloride to macromolecules takes place in both organs as well as in several other tissues (73, 466).

Although carbon tetrachloride is an excellent experimental hepatotoxic agent acute carbon tetrachloride induced hepatic injury in humans had dwindled to a problem of minor clinical importance. However, there are numerous early reports of acute liver as well as kidney and lung injuries in humans after ingestion or inhalation of large amounts of carbon tetrachloride (33, 77, 112, 164, 170, 258, 288, 296, 395, 456). The clinical picture of acute carbon tetrachloride poisoning is characterized by an initial more or less severe CNS depression followed shortly or within a few days by symptoms of liver injury such as nausea, vomiting and abdominal pain sometimes accompanied by jaundice. Later, acute renal insufficiency and lung and heart failure may develop. The outcome in severe cases of acute carbon tetrachloride poisoning is usually fatal. Microscopic examination reveals centrilobular liver necrosis with fat infiltration, degeneration of kidney tubules and signs of pulmonary injury such as edema, congestion, hemorrhage and bronchopneumonia. Cases of less severe acute hepato-renal failure generally show complete recovery. However, Tracey and

Sherlock (446) suggested a link between acute carbon tetrachloride induced liver injury and the development of hepatoma 7 years later.

The deleterious effects of carbon tetrachloride, particularly on the liver, are also obvious at chronic exposure, although reports on chronic carbon tetrachloride poisoning are few in comparison to the number of reports on acute carbon tetrachloride hepatotoxicity. Several authors describe subjective signs of gastro-intestinal disorders such as nausea, vomiting, anorexia and abdominal cramps and pain in workers chronically exposed to carbon tetrachloride (216, 296, 430, 464). Objective signs of liver injury are rare (33, 216, 296, 402, 430), which has been taken as evidence against any specific direct chronic effect of carbon tetrachloride on the liver and led to the suggestion that carbon tetrachloride may exert an indirect effect on the gastro-intestinal tract via the CNS (430). However, chronic occupational exposure has been found to cause liver cirrhosis (170, 268, 321, 464). Cirrhosis of the liver may also develop in experimental animals subject to long-term inhalation of carbon tetrachloride vapors. Smyth *et al.* (402) exposed rats, guinea pigs and monkeys to 50-400 ppm carbon tetrachloride 8 h/day, 5 days/week for up to 226 exposures. The guinea pigs developed cirrhosis at all exposure levels and the rats at 100, 200 or 400 ppm after 173, 115 and 54 exposures respectively whereas the monkeys only showed slight fatty degeneration of the liver after 188 exposures to 50 or 200 ppm carbon tetrachloride. Kidney damage was also observed in some animals evidenced by swelling of the tubular epithelium followed by degeneration. Adams *et al.* (4) exposed rats,

guinea pigs and monkeys to 5-400 ppm carbon tetrachloride 7 h/day, 5 days/week for up to 198 exposures. The rats showed centrilobular fatty degeneration of the liver with cirrhosis and swelling and degeneration of the kidney tubular epithelium after 127-146 exposures to 50-400 ppm. At 10 or 25 ppm there was fatty degeneration of the liver but no cirrhosis or kidney damage. The guinea pigs showed liver and kidney damage at all exposure levels $\geq$ 10 ppm and the rabbits at all exposure levels $\geq$ 25 ppm. The monkeys did not develop liver cirrhosis even after 163 exposures to 100 ppm but only slight swelling and fatty degeneration of centrilobular hepatocytes. A more recent study by Prendergast *et al.* (329) reports severe liver changes including fatty infiltration, fibrosis, bile duct proliferation and cirrhosis in rats, guinea pigs, monkeys, rabbits and dogs exposed to 80 ppm carbon tetrachloride 8 h/day, 5 days/week for 6 weeks. Liver changes were also noted after continuous exposure to 10 ppm for 90 days in all species whereas exposure to 1 ppm for 90 days was harmless. Animal experimentation has also shown that carbon tetrachloride is carcinogenic. Mice developed cirrhosis and hepatoma and hamsters cirrhosis and hepatocellular carcinoma when fed with repeated small doses of carbon tetrachloride (19, 91, 113, 114, 115). Rats injected subcutaneously with repeated small doses developed liver cirrhosis but also hyperplastic liver nodules and hepatocellular carcinoma (341, 342).

UPTAKE, DISTRIBUTION, BIOTRANSFORMATION AND ELIMINATION OF CARBON TETRACHLORIDE

Carbon tetrachloride vapors are readily absorbed at inhalation and liquid carbon tetrachloride is well absorbed from the gastro-intestinal tract (51, 299). Lapidus (241) noted CNS depressant effects and found carbon tetrachloride in the tissues after local application to rabbit skin. According to Stewart and Dodd (422) liquid carbon tetrachloride is likely to be absorbed in toxic amounts through intact human skin. Carbon tetrachloride vapors have been shown to be absorbed to a small extent through monkey skin (266). The respiratory uptake of carbon tetrachloride vapors has only rarely been determined. Monkeys breathing a concentration of 46 ppm carbon tetrachloride for 139-344 minutes retained 30.4% of the inhaled amount (266) whereas rabbits retained 22.4% when exposed to 50 mg/l carbon tetrachloride for 1 hour (246). It was also noted that the rabbits exhaled 78.5% of the absorbed amount within 6 hours after exposure. One monkey inhaling 46 ppm ^{14}C -carbon tetrachloride for 344 minutes exhaled 40% and 11% of the calculated absorbed amount as unchanged ^{14}C -carbon tetrachloride and $^{14}\text{CO}_2$ respectively during 1800 hours after exposure (266). The expiration of carbon tetrachloride during a long time after acute exposure is also illustrated by a case of human oral poisoning where carbon tetrachloride could be identified in the exhaled air still 400 hours later (421). Robbins (348) stated that approximately all of a dose of carbon tetrachloride injected into the intestine of the dog was exhaled unchanged. Rats injected intraduodenally with 1 ml/kg carbon tetrachloride exhaled about 75% of the dose as ^{14}C -

carbon tetrachloride and about 1% of the dose as $^{14}\text{CO}_2$ during 18 hours after administration, most of which was recovered during the first 6 hours (312). Rats given a higher dose (2.5 ml/kg) of carbon tetrachloride orally exhaled about 80% of the dose as unchanged carbon tetrachloride and about 0.4% of the dose as $^{14}\text{CO}_2$ in 24 hours (377). Soucek (409) could not identify carbon tetrachloride in the exhaled air of rats injected subcutaneously with 0.4 g/kg for more than 98 hours after injection when 58-100% of the dose had been recovered in this way, most of it during the first 8 hours. He also noted an exponential decrease of the carbon tetrachloride concentration in the exhaled air with a half-time of 9.4 hours. The carbon tetrachloride breath concentration decreased exponentially also after human oral poisoning (421) and after inhalation of carbon tetrachloride by monkeys (266).

Few distribution studies of carbon tetrachloride may be found in the literature. In an early study Robbins (348) administered large doses (100 ml) orally to dogs and found the highest concentration of carbon tetrachloride 24 hours later in the bone marrow followed by the brain, blood, kidney, liver, spleen and pancreas. McCollister *et al.* (266) analyzing the tissues of a monkey immediately after inhalation of 46 ppm ^{14}C -carbon tetrachloride for 300 minutes found an eight times higher amount of radioactivity in the body fat than in the blood. The liver and bone marrow radioactivity levels were also higher than that of the blood whereas the brain, kidney, heart, spleen, muscle, lung and bone levels were lower. Twenty-four hours after subcutaneous injection of 1.45-3.65 g/kg carbon tetrachloride to rats a twenty times higher

concentration was registered in adipose tissue compared to blood whilst the liver, lung and brain concentrations were equal to that of the blood (409). The highest concentration of carbon tetrachloride (787 $\mu\text{g/g}$) was found in body fat 6 hours after oral administration of 1 ml/kg to rabbits (133). The liver concentration was 96 $\mu\text{g/g}$ and the kidney and muscle concentrations about 20 $\mu\text{g/g}$. All tissue levels declined rapidly during the subsequent 42 hours.

The formation of CO_2 from carbon tetrachloride administered to animals (266, 312, 377) represents its complete oxidative dechlorination and has been shown to take place in liver microsomes supplemented with O_2 and the soluble fraction of the liver homogenate or a NADPH-generating system (354, 377). The reaction is accompanied by an increased urinary output of chlorions (215). The biotransformation of carbon tetrachloride to CO_2 also explains the presence of small amounts of radioactive urea and carbonate in the urine of monkeys administered ^{14}C -carbon tetrachloride by inhalation (266). Hathway (174) suggests that the formation of CO_2 from carbon tetrachloride is due to the oxidation of chloroform, another recognized carbon tetrachloride metabolite (57, 133, 312), since the biotransformation of carbon tetrachloride to CO_2 has a less sharply defined pH-optimum (354) and occurs at a much slower rate both *in vitro* (354) and *in vivo* (312) than the biotransformation of chloroform to CO_2 . The formation of CO_2 from carbon tetrachloride would thus follow the pathways outlined above for the oxidative dechlorination of chloroform. The formation of $\text{CCl}_3\cdot$ radicals as the initial step in carbon tetrachloride biotransformation, which subsequently bind

irreversibly to cellular macromolecules and initiate lipid peroxidation, is supported indirectly by the appearance of small amounts chloroform, a hydrogen abstraction product of the reaction of the $\text{CCl}_3\cdot$ radical with e.g. unsaturated fatty acid side-chains, in the exhaled air of dogs (57) and rats (312) and in the tissues of rabbits (133) administered carbon tetrachloride as well as by the presence of hexachloroethane, a dimerization product of $\text{CCl}_3\cdot$ radicals, in rabbit tissues after administration of carbon tetrachloride (133). Chloroform is also produced by tissue slices, especially from liver, incubated with carbon tetrachloride (57, 312). Recently, direct evidence for the formation of the $\text{CCl}_3\cdot$ radical has been obtained using electron spin resonance spectroscopy (42, 59, 328). The cleavage of the $\text{CCl}_3\text{-Cl}$ bond may occur either by enzyme catalyzed homolysis, in which case both $\text{CCl}_3\cdot$ and $\text{Cl}\cdot$ radicals would be formed, or by enzyme catalyzed electron capture with formation of the $\text{CCl}_3\cdot$ radical and chlorions, the energy requirements of both reactions which are compatible with the energy level of a living system (45, 153, 174). Recent results of investigations of carbon tetrachloride biotransformation *in vitro*, using irreversible binding of radioactivity from radiolabeled carbon tetrachloride to proteins and lipids as evidence of carbon tetrachloride activation to free radicals, indicate that the cleavage of the $\text{CCl}_3\text{-Cl}$ bond proceeds via microsomal cytochrome P450 and NADPH dependent reductive pathways (233, 392, 453, 467) suggested by Castro and co-workers (67, 68, 96, 467) to occur specifically during the reduction of the carbon tetrachloride-cytochrome P450 complex by NADPH in the catalytic presence of cytochrome P450 reductase.