III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

Carbon tetrachloride (CCl_4), also known as tetrachloromethane, is a clear, colorless liquid having a moderately strong ethereal odor similar to that of chloroform. The more important physical properties are listed in Table XI-1. [1,2]

Two methods of carbon tetrachloride manufacture are chlorination of methane and direct chlorination of carbon disulfide. [2] Manufacture of carbon tetrachloride by 7 companies in the United States was reported by the US Tariff Commission for 1972. [3]

In the past, large quantities of carbon tetrachloride were used for drycleaning, degreasing, cleaning electrical parts, laboratory analyses, as a fire extinguisher, and in miscellaneous small applications. [1,4] Fairhall [4] calculated that 56.5% of the total 1946 US production of carbon tetrachloride, or approximately 82 million pounds, was used for making other halocarbons. In 1973, it was estimated that approximately 80% of the carbon tetrachloride produced in 1972 (997 million pounds [3]) was used to make fluorocarbons and that about 200 workers were exposed by this use. (HL Smith, written communication, August, 1973). Grain fumigation, another major use of carbon tetrachloride, was estimated to consume approximately 16 million pounds in 1972, and it was estimated that about 25,000 workers were exposed. (S Calkins, written communication, September, 1973) Carbon tetrachloride production which was about 1 billion, 11 million pounds in 1970, [5] declined in the last 2 years for which data are

available to 997 million pounds in 1972. [3,6]

NIOSH estimates that 160,000 people are potentially exposed to carbon tetrachloride in their working environment.

Historical Reports

Early studies of the effects of carbon tetrachloride on humans were made in England in connection with its use as an inhalation anesthetic. [7,8] In 1867, Smith [7] reported the results of 52 cases in which carbon tetrachloride was the anesthetic agent. He concluded that it was "useful in removing pain...while producing no nausea or sickness following its use."

Another early use of carbon tetrachloride was as a waterless shampoo. [9] Recommendations were made in 1909 in England to label carbon tetrachloride as a poison after a death resulted from its use for this purpose. [9] In some European countries, however, it continued to be used as a hair shampoo, resulting in further reports of serious illness and death. [10,11]

Health hazards from industrial use of carbon tetrachloride began to be reported in the early years of the twentieth century. In Germany, Lehmann [12] reported that he had received information in 1903 confirming that mental confusion, anesthesia, and other unwelcome effects occurred when new cleaning agents such as benzol and carbon tetrachloride were brushed on by hand.

In the United States, Hamilton [13] reported in 1915 that 4 men using carbon tetrachloride in the manufacture of rubber goods experienced nausea, vomiting, loss of appetite, loss of weight, weakness, and eye,

nose, and throat irritation. She also noted that men working without gloves were likely to develop dermatitis of the hands and arms.

Carbon tetrachloride was reported by Hall, [14,15] in 1921, to be an efficacious anthelmintic. Its use as an oral medication for this purpose stimulated considerable research into the pharmacologic and physiologic effects of carbon tetrachloride on humans. [16-18]

Effects of oral doses of carbon tetrachloride as a human anthelmintic administered to condemned prisoners in Ceylon were reported in 1922 and 1923. [16,17] Three condemned prisoners received 4 ml of carbon tetrachloride, two received 5 ml, and one received 5 ml plus an additional 3 ml 2 weeks later. The prisoners were executed 3-15 days after the administration of carbon tetrachloride. The livers of some showed no major microscopic or macroscopic changes upon autopsy, whereas the livers of others showed marked fatty degeneration. Although a dose-response relationship would be difficult to determine, it is significant that no fatalities occurred among the subjects within the 96 hours following ingestion of doses ranging from 3 to 8 ml of carbon tetrachloride.

In 1924, Wells [18] injected 1.5-2.5 ml of carbon tetrachloride through a tube into his duodenum to avoid eructation and began breath collection 10 minutes later. At this time the odor of carbon tetrachloride on his breath was unmistakable and he felt some dizziness. His method of determining carbon tetrachloride was reported to recover 97.8-99.5% of carbon tetrachloride from air experimentally blown through charcoal at rates of 1.0-13.4 liters/minute.

From the data presented by Wells, [18] average concentrations in his exhaled air for various periods after ingestion were calculated, and are

presented in Table XI-2. These data show that a large amount of the ingested carbon tetrachloride appeared in the exhaled breath and that it was detectable up to 36 hours after oral doses of 1.5-3 ml.

Effects on Humans

(a) Central Nervous System Effects

Carbon tetrachloride is a central nervous system depressant and this feature led to its early use as an anesthetic. [7,8] In many cases of ingestion and of occupational or accidental inhalation exposures to carbon tetrachloride, dizziness, headache, vertigo, giddiness, fatigue, and occasionally narcosis developed. [19-22]

Stevens and Forster [19] observed 17 cases of carbon tetrachloride poisoning between 1948 and 1953. In 7 of the cases they considered that there was neurological involvement, and headache, vertigo, weakness, blurring of vision, lethargy, and coma appeared soon after exposure. There were 2 fatalities among the 7 cases, and the brains of these were examined microscopically. One of these patients cleaned a sofa with a fluid containing carbon tetrachloride. Following his death 2 days after admission to the hospital, the brain showed scattered areas of demyelination with necrosis judged by the authors to be not unlike the lesions of acute perivenous encephalomyelitis. The other fatality was an alcoholic who died 7 days after washing parts of machines with carbon tetrachloride. In this man's brain the molecular layer of the cerebellar cortex was strikingly disorganized, and the Purkinje cells were pyknotic and greatly reduced in number. [19]

Similar Purkinje cell damage was reported by Cohen [20] in 1957 in 2

fatal cases from carbon tetrachloride ingestion that he described. Cohen [20] also reported finding patchy necrosis in the tissue connecting the cerebrum, cerebellum, and medulla oblongata, with cerebellar hemorrhages and extravasation of blood in the cerebrum. Other pathologic findings reported were liver necrosis and degeneration of the renal tubules.

Six workers in a motion picture film coating operation in which a solution of 85% carbon tetrachloride and 15% ethyl alcohol was used were the subject of a 1941 report by Heimann and Ford. [21] Carbon tetrachloride concentrations found with a portable combustion apparatus [23] in 6 samples of the work-environment air averaged 79 ppm (33-124 ppm). All 6 of the workers complained of fatigue after a "few" hours of work even though the work was not physically demanding. The sense of fatigue continued each workday, but disappeared on weekends. All of the men were given complete medical examinations, and medical histories were taken. Five of the 6 workers complained of nausea, and 4 experienced frequent vomiting. The authors [21] considered the fatigue of the workers to be the result of mild narcosis.

A soldier who cleaned bomb sights with carbon tetrachloride was reported in 1944 by Farrell and Senseman [22] to have had numbness, and ankle, knee, thigh, and wrist weakness. Physical and routine laboratory examinations were essentially negative except for the neuromuscular system. A fine spray of carbon tetrachloride was used to saturate a cloth wrapped around his fingers. He worked at the job 8 hours/day. The total exposure time and inhalation exposure concentrations were not reported. The case was diagnosed by the authors [22] as polyneuritis caused by absorption of carbon tetrachloride through the exposed skin. Following discontinuation

of the exposure, recovery was complete.

Experimental human studies of nervous system responses to inhaled carbon tetrachloride were reported by Lehmann and Schmidt-Kehl [24] in 1936. They used fans to distribute carbon tetrachloride spray throughout a 10-cubic meter room. The concentrations in the room were determined by hydrolysis of carbon tetrachloride with alkali in alcohol. The exposure concentrations ranged from 140 ppm (0.89 mg/liter) to 14,000 ppm (89 mg/liter). Exposures were for periods ranging from 50 seconds to 30 minutes. Two subjects usually were exposed at the same time and a total of 26 experiments were reported.

Perception of a light, transient odor was the only effect reported by Lehmann and Schmidt-Kehl [24] at 240 ppm (1.5 mg/liter) for 20 minutes. Dizziness and vertigo occurred after 10 minutes of exposure at concentrations of 600 ppm (4 mg/liter). Exposure at more than 600 ppm carbon tetrachloride resulted in increasingly severe effects that included headache, tiredness, giddiness, and salivation. Loss of consciousness occurred with exposure at 14,000 ppm (89 mg/liter) for 50 seconds. [24]

Responses reported by Davis [25] in a similar experiment included: none, with 5 hours of exposure at 75 ppm carbon tetrachloride; slight nausea after 30 minutes of exposure at 160 ppm; and nausea, vomiting, and headache after 30 minutes of exposure at 320 ppm.

Detection of a sweetish, not unpleasant odor was the only nervous system response to experimental carbon tetrachloride inhalation by human subjects observed by Stewart et al [26] and reported in 1961. The 6 individuals were exposed to carbon tetrachloride at a time-weighted average (TWA) concentration of 49 ppm (13-87 ppm) for 70 minutes. Odor was not

reported when 6 individuals were exposed on another occasion at a TWA concentration of 10.9 ppm (10-14.2 ppm) for 180 minutes. The exposures were conducted in a room 11 x 12 x 7.5 feet. The source of carbon tetrachloride was a 90-mm crystallization dish covered with a folded towel. A fan circulated carbon tetrachloride vapor throughout the room. The concentrations reported were obtained by sampling the room air at head height and passing the vapor through a continuously recording halide meter. The subjects did not report nausea, lightheadedness, or irritation during the exposures at either concentration, and impairment of equilibrium was not found when measured by the Romberg test and the heel-to-toe test during exposure.

(b) Eye Effects

Bilateral peripheral constriction of the visual color fields was found in 5 men examined by Wirtschafter [27] and reported in 1933. The men were employed in drycleaning establishments, and were exposed to carbon tetrachloride vapor 8-10 hours daily for 4, 5, 6, 10, and 26 weeks preceding examination. In 3 of the 5 cases, a delay of a few days to 3 weeks elapsed between first exposure and the onset of nausea, vomiting, dizziness, headache, and weakness. Three of the men complained of subsequently developing visual disturbances, which prompted the study of visual color fields. In one case, the color fields gradually returned to normal within 5 weeks after removal from exposure.

Marked optic atrophy of the right eye and a receding choked disc of the left eye were reported in 1950 by Smith [28] in a man who had cleaned silver bars for 8 months with a rag soaked in carbon tetrachloride. His visual impairment was noticed one week after termination of the job, and

medically confirmed on hospitalization 4 months later. At this time, the physical examination, apart from the eyes, gave negative results. Abnormalities were not found by electroencephalograms, skull and sinus roentgenograms, or tests for syphilis, blood sugar, or urea nitrogen. His vision improved somewhat during the following 3 years while he was not exposed to carbon tetrachloride.

Two other cases of visual disturbances were observed by Smith, [28] in which the exposures included other chemicals in addition to carbon tetrachloride. A man who dipped razor cutting heads in a solution containing carbon tetrachloride and gasoline and spread them on a cloth in front of him for inspection developed headaches and dizziness after about 4 months of this work. The illness progressed during 16 months to include weakness, irritability, nausea, muscle cramps, mental confusion, disorientation, and memory loss, and finally blurred vision. Laboratory and physical findings were normal, except for visual tests. The final diagnosis was bilateral optic neuritis. The second case was a woman who used a solution of 20% carbon tetrachloride and 80% naphtha as a spot remover in a dressmaking factory. When hospitalized for blurring vision, her eyes showed marked constriction of the visual fields with scotomata. Other physical and laboratory findings were normal. The diagnosis was retrobulbar neuritis. [28]

Bilateral diminished vision in a man was reported by Gray [29] to have developed after 11 years of mixing chemicals, especially carbon tetrachloride, in the manufacture of polishes. In this case, jaundice, nausea, vomiting, weakness, and weight loss developed 2 months after the onset of blurred vision. At this time a slight enlargement of the liver,

elevated icteric index, positive van den Bergh, and slight hypochromic anemia were found. The patient improved following removal from carbon tetrachloride exposure.

(c) Liver and Kidney Effects

Acute and chronic exposures to carbon tetrachloride have resulted in liver and kidney damage. [29-36] Mortality from carbon tetrachloride exposure was most often the result of pathologic effects on the liver and kidneys and subsequent organ failure. Moderate-to-heavy alcohol consumption was a concomitant variable in many cases of carbon tetrachloride poisoning with liver or kidney injury.

Two cases of industrial poisoning from carbon tetrachloride used as a cleaning agent were reported by Franco [30] in 1936. In one case, carbon tetrachloride was used to clean ink from stencils, and in the other, for cleaning electrical condensers on a boat. In one case, the diagnosis was necrotizing nephrosis and, in the other, acute nephritis. This was one of the first reports to recognize renal injury from carbon tetrachloride poisoning instead of, or in addition to, hepatic dysfunctions.

Between 1948 and 1957, Guild et al [31] examined 20 patients suffering from carbon tetrachloride poisoning (16 by inhalation and 4 by ingestion). Exposure times varied from 30 minutes to 11 hours; exposure concentrations were not reported. Following carbon tetrachloride exposure, dizziness, nausea, and vomiting commonly occurred within 1-6 hours. All 20 patients developed anuria 1-7 days (average 3 days) after exposure. Eighteen of the patients had no previous history of renal disease. Three of the 16 people exposed by inhalation and 2 of the 4 ingestion cases died. Autopsy findings in 4 of the 5 fatalities included acute renal tubular and

hepatic centrilobular necroses. The cause of death in the fifth case was clinically determined to be severe hepatic insufficiency. [31] Eleven of the 16 people exposed by inhalation and all 4 who had ingested carbon tetrachloride had clinical and laboratory evidence of moderate to severe hepatic dysfunction. Alcohol was consumed daily in large quantities by 16 of the 20 patients.

A history of chronic alcoholism was common to 3 cases of carbon tetrachloride intoxication with kidney and liver involvement observed in 1939 by Smetana. [32] Autopsy of one patient who died 10 days after ingestion of an unknown amount of carbon tetrachloride showed centrilobular liver necrosis, jaundice, ascites, and nephrosis. Autopsy findings in another man who died 9 days after an exposure of several hours to carbon tetrachloride at unknown concentrations while cleaning furniture and draperies included nephrosis, fatty degeneration of the liver, and centrilobular liver necrosis. A clinical diagnosis of toxic hepatitis, acute renal tubular damage, toxic myocarditis, and possible cirrhosis of the liver was made on the third patient who was hospitalized 3 days after cleaning dresses with carbon tetrachloride for 3 hours in a close, stuffy room.

Fatal uremia was reported in 1942 by Ashe and Sailer [33] as the cause of death of a man exposed to carbon tetrachloride while cleaning machinery in an elevator shaft. Death occurred 17 days after a 4-hour exposure. Degeneration of the tubular epithelium of the nephrons, centrilobular liver necrosis, and fatty degeneration of the ganglion cells of the globus pallidus were found at autopsy.

Carbon tetrachloride poisoning in a felt manufacturing plant was the subject of a report in 1932 by McGuire. [34] The 7 employees cleaned the felt by passing it through an open vat that contained a 33% mixture of carbon tetrachloride which was warmed. The other components of the solution were not reported. Extent of the skin exposure was not indicated. The men, all of whom were wine drinkers, complained of nausea and vomiting 1-3 days following institution of the soaking procedure. Other complaints included burning sensations of the eyes or mouth, headache, and diarrhea. Four of the 7 workers experienced kidney "irritation," diagnosed as acute nephritis in one. Two of the 7 men had definite liver enlargement with jaundice.

Liver function and enzyme activity were compared with microscopic findings obtained by liver biopsy in a 1960 report by Lachnit and Pietschmann [35] of 2 cases of acute carbon tetrachloride intoxication. One case involved a laborer who used carbon tetrachloride for 3 hours to clean a machine in a relatively large and well-ventilated room. He developed nausea, giddiness, stomach pains, fever, and 3 days after exposure he was hospitalized. His liver was slightly enlarged and sensitive to pressure. Serum glutamic-oxaloacetic transaminase (SGOT) was 6,000 units/ml and serum aldolase was 300 units (1 unit = 0.001 extinction decrement/minute/ml serum). The enzymes returned to normal values (10-33 units/ml) 10-15 days after intoxication. Large areas of centrilobular necrosis were found in a liver biopsy on the 8th day after intoxication. The surrounding liver cells appeared normal. The other man had assisted in the cleaning operation and had not been so directly exposed. He developed the same, but less severe, clinical symptoms and was hospitalized on the

12th day after exposure. At that time, SGOT of 80 units and serum aldolase of 21 units were found. Hepatic cells showing degenerative changes and nuclei of different sizes were found in a liver biopsy on the 22nd day after exposure. [35]

Three workers acutely exposed to carbon tetrachloride and hospitalized with jaundice, nausea, and dizziness were described by Barnes and Jones [36] in 1967. One of the cases was associated with the delivery process (concentrations of carbon tetrachloride were in excess of 30 ppm), another case occurred when an employee drained water from a carbon tetrachloride storage tank (carbon tetrachloride concentrations of 75-600 ppm with the "main" level at 210 ppm), and the third case occurred when an employee cleaned sludge from a carbon tetrachloride tank. Abnormal liver function was found in all 3. Findings in a liver biopsy from one of the workers taken 3 weeks after the episode were considered by the authors [36] to be consistent with toxic hepatic injury from carbon tetrachloride poisoning.

Enlarged livers and elevated icteric indices, but no indications of kidney injury, were found by Gray [29] in 4 workers who had been chronically exposed to carbon tetrachloride for from 2 months to 11 years. The worker with the longest exposure was a chemical mixer. The other 3 workers, including 2 with a history of heavy chronic alcohol consumption, used carbon tetrachloride to clean machinery. Both kidney and liver damage were observed by Gray [29] in 3 other cases of carbon tetrachloride poisoning where exposure had been for 1-2 days in electric motor cleaning operations. One of these acutely poisoned patients with a history of heavy chronic alcohol consumption died following the carbon tetrachloride exposure. [29]

A foreman and another man who occasionally worked with him on the maintenance of telephone relay equipment within the German railway system frequently experienced nausea and stomach distress as reported by Dellian and Wittgens in 1962. [37] Sixteen measurements of carbon tetrachloride concentrations ranged from 10 to 100 ppm in various locations. The foreman, 54 years of age, had worked 4 years in the maintenance section using carbon tetrachloride on the average of 3 days/week. During the 4th year, colic in his right upper abdomen led to his incapacity to work. Clinical studies excluded the possibility of gall bladder infection. Laparoscopy confirmed that both lobes of the liver were enlarged and yellow-red in color with indistinct markings. Coarse fatty degeneration, undeveloped borders, and moderate infiltration of the periportal areas were found on microscopic examination of liver tissue. The SGOT was elevated and sulfobromophthalein (BSP) retention was increased. Fourteen months after this examination, a greater degree of fatty liver degeneration was found on biopsy, but SGOT had returned to normal. Kidney findings were normal at all examination times. There was no history of alcohol abuse or predisposing disease known to affect the liver. The investigators concluded that the effects were related to chronic exposure to carbon tetrachloride. [37]

In 1961, Stewart et al [26] reported that exposures to carbon tetrachloride at 10 or 11 ppm for 180 minutes did not affect liver or kidney function based on normal urinary urobilinogen, SGOT, and 15-minute phenolsulfonphthalein. However, exposure at 49 ppm for 70 minutes may have had some effect on liver function shown by a reduction of serum iron 1 or 2 days later in 2 of 4 subjects in whom it was measured.

Primary hepatic epithelioma secondary to cirrhosis was diagnosed at autopsy of a military fireman who had been extensively exposed to carbon tetrachloride from fire extinguishers during an unspecified number of years. [38] One particular day, he and an assistant were ordered to use a carbon tetrachloride extinguisher to destroy a swarm of wasps that had located in an enclosed space. Both men became intoxicated, and the assistant was hospitalized with nephritis. The fireman who suffered some "indisposition" was not hospitalized, but he began to feel very weak and nauseated the next day. The following day these symptoms became more pronounced and somnolence and anorexia developed. On the 6th day after exposure he developed hematuria which lasted 4 days, at which time he felt better and returned to work. During the next 4 years, 10-day episodes of anorexia, fatigue, fever, hematuria, and conjunctival subicterus occurred at intervals of 4-6 months. Four years after the acute poisoning, the fireman was hospitalized with weakness, fever, epigastric pain, cramps, and diarrhea. A bright pink liver with small nodules resembling micronodular cirrhosis and an enlarged spleen were seen by laparoscopy. The diagnosis of hepatic cirrhosis with portal hypertension was confirmed by splenoportography and laparoscopy. There were no known etiological factors such as icteric antecedents or heavy alcohol usage. Microscopic examination of biopsy material showed cirrhosis and an early epithelioma of the liver. The epithelioma was glandiform with clear cells. Lymph vessels under the thickened Glisson capsule were increased in number and inflamed. Five months after the surgery the patient was thin, subicteric, and asthenic, with abdominal pain and a hard, enlarged liver. Two months later he died from a ruptured neoplastic lobe. Irregular cirrhosis with early

hepatic cancer on the cirrhotic areas of the entire right lobe was found at autopsy. Cancerous pylephlebitis was also observed.

Johnstone [39] reported in 1948 that a woman who had been exposed to carbon tetrachloride for 17 months developed cancer of the liver. The woman was an assistant to a metallurgist and several times a day she dipped a sponge into a small open dish of carbon tetrachloride to clean the metal. After 2 months, she became increasingly fatigued and exhibited periodic jaundice. After 13 months of employment, she sought medical aid and was hospitalized. A markedly enlarged liver with nodular cirrhosis was found in an exploratory operation. Following a short recuperation, she returned to work for 4 months at which time she was forced to stop work because of extreme weakness, marked jaundice, and increasing weight. She died 3 years after the beginning of her employment. A cancer of the liver was found at autopsy. The type of cancer was not specified and results of laboratory studies were not reported. Five years prior to her employment, she had suffered "biliary colic," pain at the angle of the right scapula, and she had been studied for "gall bladder disease." Whether these factors had etiological significance was not discussed by the author. [39]

Hepatocellular carcinoma was reported by Tracey and Sherlock [40] in 1968 as the diagnosed cause of death of a 59-year-old man 7 years after acute poisoning with carbon tetrachloride. When he returned to his apartment from a cocktail party he noted the odor of carbon tetrachloride which had been used earlier that day to clean the rug. He slept in the apartment and 5 days later developed nausea, vomiting, and diarrhea. He became jaundiced and anuric on the 10th day. At this time, the liver was found to be enlarged 3 fingerbreadths below the costal margin. There was

no history of previous jaundice, symptoms of hepatic decompensation, or portal hypertension, but the patient, who had been in good health all his life, admitted to having several alcoholic drinks daily.

He remained almost completely anuric for 4 days and remained in the hospital for 9 weeks. Four years later when he was seen again, he had been in good health and had abstained from alcohol since his hospitalization. Clinical tests at this time were normal. A smooth, enlarged, nontender liver palpable 3 fingerbreadths below the right costal margin was found. He was readmitted to the hospital 3 years later with a history of anorexia, weight loss, nausea, vomiting, and diarrhea. His liver was enlarged 4 fingerbreadths below the right costal margin and a 6 x 6 cm tender mass was felt in the right epigastrium. A hepatocellular carcinoma was found by liver biopsy. He died five months later. The liver was extensively involved with tumors, and only a small amount of normal tissue remained. There was a moderate amount of fibrosis, but the parenchymal cell structure was so disrupted by tumors that no classification of the cirrhosis could be made.

(d) Carbon Tetrachloride in Blood and Exhaled Air

In 1951, Hamburger et al [41] reported that a concentration of carbon tetrachloride in the blood of 32 mg/liter was found at autopsy of a woman who died 11 days after cleaning her hair with carbon tetrachloride. In 1958, Hamburger [42] reported carbon tetrachloride concentrations in the blood of 0.09-2.30 mg/liter in 9 individuals hospitalized 1-5 days after they had become anuric from carbon tetrachloride poisoning. Among 6 other individuals examined after being anuric from carbon tetrachloride poisoning for 6 or more days, carbon tetrachloride was found in the blood of only

one. In this individual, carbon tetrachloride in the blood was 1.4 mg/liter on the 10th day of anuria. The case histories were described in detail by Richet et al. [43] Carbon tetrachloride was determined by reaction with pyridine in alkali as described by Fabre et al. [44]

A man who had a history of chronic alcohol consumption became oliguric after cleaning tiles in his bathroom for about 30 minutes with carbon tetrachloride. In a breath sample obtained 10 days after exposure, Markham [45] reported a carbon tetrachloride concentration of 4 mg/liter. The analytical method was not given.

During 6 exposures of 30 minutes' duration to carbon tetrachloride at 650-1,600 ppm (4.1, 4.2, 6.7, 8.6, 9.6, and 10.2 mg/liter), Lehmann and Schmidt-Kehl [24] collected expired air with an aspiration device and determined residual carbon tetrachloride concentrations. The respective percentages absorbed (61.0, 64.3, 57.2, 61.1, and 57.0) were calculated from the concentrations of carbon tetrachloride in inhaled air and those found in exhaled air.

Carbon tetrachloride was measured by Stewart et al [26] in the exhaled air during 5 hours after the end of human exposures. The concentrations found at 25 minutes after a 70-minute exposure at 49 ppm were about 20 μg/liter and about 6 μg/liter when exposures had been at 10 or 11 ppm for 3 hours. Breath concentrations ranged between about 1.0 and 2.5 μg/liter at 5 hours after removal from exposure, regardless of exposure concentration.

A worker became dizzy, weak, nauseated, and finally unconscious during exposure to a mixture of vapors including carbon tetrachloride. Ten minutes later he was coherent, though uncoordinated, and nauseated.

Concentrations of carbon tetrachloride in his breath following the episode were reported by Stewart et al [46] in 1965. Thirty minutes after his collapse, a breath sample contained 9.5 ppm (60 μg/liter) carbon tetrachloride, determined by infrared analysis. Carbon tetrachloride was still detectable in the breath 15 days after the exposure. The data were not presented, but the authors [46] reported normal findings for urinalysis, complete blood cell counts, 24-hour urinary urobilinogen, and SGOT measured serially over 2 weeks.

Concentrations of carbon tetrachloride in exhaled breath of a woman who drank a "pint" of a solution containing 2 parts carbon tetrachloride and 1 part methanol were studied by Stewart et al [47] and reported in 1963. Gastric lavage was carried out soon after ingestion. The first expired air sample contained 2,000-2,500 μg/liter of carbon tetrachloride. A series of exhaled breath samples were analyzed for carbon tetrachloride over the following 19 days. The samples showed a progressive decrease in carbon tetrachloride concentration to about 1.2 μg/liter 19 days after the ingestion.

Studies of absorption of carbon tetrachloride through the skin of the thumb were reported by Stewart and Dodd [48] in 1964. Three subjects each immersed one thumb in carbon tetrachloride for 30 minutes, and the concentrations of carbon tetrachloride in the exhaled air of the subjects were determined at 10, 20, and 30 minutes of immersion. The concentration ranges in the exhaled air at these times were respectively 0.025-0.819, 0.25-3.27, and 0.69-5.23 μg/liter. Carbon tetrachloride was still measurable in exhaled air of these subjects 5 hours later.

(e) Clinical Observations with Alcohol and Carbon Tetrachloride

Among the case studies presented in previous sections of this chapter, alcohol consumption was frequently mentioned as a coexisting factor. The first serious effects of carbon tetrachloride as an anthelmintic for humans were observed when it was administered by Smillie and Pessoa [49] to 2 alcoholics in a group of 34 persons, 9-25 years of age; daily alcohol consumption was reported for 16 of the 20 cases of carbon tetrachloride poisoning discussed by Guild et al [31]; all of the 7 men studied by McGuire [34] were wine drinkers; the 3 cases reported by Smetana [32] were chronic alcoholics; and the 2 fatalities studied by Gray [29] were long-term alcohol addicts.

In addition to these cases, others have found alcohol to be a prominent factor in those hospitalized with carbon tetrachloride poisoning. Alcohol was considered in 1948 to be a predisposing factor in 8 of 10 cases studied by Abbott and Miller [50]; it was involved in 8 of 12 cases reported by Joron et al [51] in 1957, and in 17 of 19 cases discussed by New et al [52] in 1962. In the report by New et al, [52] the serious cases of renal failure all involved alcohol consumption, whereas alcohol was not a factor in the 4 cases with only liver or central nervous system involvement.

Thompson [53] mentioned the case of a known chronic alcoholic who frequently drank on his job of bottling carbon tetrachloride and who was seriously poisoned while his coworkers were not affected.

Epidemiologic Studies

Surveys of a number of industries that used carbon tetrachloride were reported by Elkins [54] in 1942. Carbon tetrachloride in the workroom air was collected in amyl acetate, decomposed to chloride by burning the solution, and the chloride was determined by silver nitrate titration. [55] Ranges of concentrations and their averages were reported, but the number of samples and sampling times were not. A survey of 5 drycleaning plants where carbon tetrachloride was used revealed the following effects and exposure concentrations:

(1) Nausea, vomiting, belching, drowsiness, headache, dizziness, and lassitude occurred among several women engaged in a spot cleaning operation after several days of exposure to carbon tetrachloride at an average of 35 ppm (10-80 ppm). In this plant, each woman used about one quart of solvent per day in a well-ventilated room.

(2) Workers in a drycleaning plant complained of feeling sick, but quantitative data about the type and frequency of symptoms or number of people involved were not reported. Carbon tetrachloride concentrations during operations that would produce the maximum amount of vapor averaged 65 ppm (35-115 ppm). The frequency and duration of the exposures at these concentrations were not reported. The symptoms were not completely alleviated when undescribed operational changes were made that reduced the average concentration to a reported 25 ppm.

(3) An unspecified number of workers in a drycleaning plant where carbon tetrachloride concentrations averaged 25 ppm (15-60 ppm) were reported to be seriously affected in some way.

(4) An operator of a drycleaning shop where measured carbon tetrachloride concentrations averaged 20 ppm (5-40 ppm) used a gas mask because he had been nauseated.

(5) Several women using small amounts of carbon tetrachloride for spot removal in a large, well-ventilated room were nauseated, and one woman's illness of several weeks was attributed to the exposure. No measurements of carbon tetrachloride in the air were made at the time the nausea and illness occurred, but Elkins [54] believed that only low concentrations could have occurred. After dilution of the carbon tetrachloride with an equal volume of naphtha and installation of a canopy hood over the work area, the concentration of carbon tetrachloride in the air was 5 ppm, and there were no further complaints.

In a survey of two plants where multigraph operators used carbon tetrachloride, Elkins [54] reported that:

(1) Nausea, loss of appetite, and loss of weight occurred in 4 persons in an office where ink was removed from multigraph machines using about 1 gallon of carbon tetrachloride daily. Carbon tetrachloride concentrations reported only for the worst conditions ranged from 40 to 375 ppm, with an average of 85 ppm. Concentrations of this magnitude were considered to occur only once in 2-3 weeks. The factors causing the worst environmental conditions were not explained, nor was the frequency of occurrence of the symptoms reported.

(2) Five multigraph operators who used slightly more than 1 quart of carbon tetrachloride daily in a relatively small area experienced nausea. No measurements of carbon tetrachloride concentrations were given for the conditions under which the nausea occurred. After installation of

general ventilation and apparent alleviation of symptoms, the concentrations of carbon tetrachloride were 10-25 ppm.

Smyth et al [56] and Smyth and Smyth [57] reported 8-hour time-weighted average exposure concentrations which they calculated from carbon tetrachloride determinations and job analyses for 73 workers employed in carbon tetrachloride and fire extinguisher manufacture, 10 workers in drycleaning plants, and 2 workers involved with other uses of carbon tetrachloride. The exposure concentrations were determined using a portable 50-cm interference refractometer. Summaries of the environmental data are shown in Tables XI-3, XI-4, and XI-5.

Carbon tetrachloride TWA exposures of the workers studied in these various plants (see Table XI-5) ranged from 5 to 117 ppm with peak exposures at 22-1,680 ppm. Hematology, liver and kidney function, and fields of vision were studied in 77 workers using carbon tetrachloride in 10 plants. The distribution of significant findings for visual fields, determined with a "simple" portable perimeter, and icteric indices are shown in Table XI-5. Nine of the men had definitely restricted visual fields. Four of these 9 workers were engaged in container-filling operations. An additional 26 men had slightly restricted visual fields, 40 had normal visual fields and the measurement was not made on 2 men. Thirteen of 67 men tested had elevated icteric indices. Hematology, kidney function studies, and other measures of liver function did not show significant results of exposure.

Ophthalmological studies of 108 people who were chronically exposed to carbon tetrachloride were conducted by Moeller. [58] A group of 46 people who were mostly locksmiths from various factories had exposures to

carbon tetrachloride varying from 1 hour daily to 1 hour weekly, for an average of 7.7 years. Another group of 62 people worked in a single, large locksmith shop where cleaning operations were performed at least 1-3 hours daily, without protective clothing, by dipping mops into buckets of carbon tetrachloride. Air analyses in this shop gave carbon tetrachloride concentrations of 6.4-9.5 ppm (40-60 mg/cu m).

Vision, ocular foreground (probably lens) and eye ground (fundi oculi), intraocular pressure, and corneal sensitivity were studied in the group of 46 workers. In this investigation, he found reduced corneal sensitivity in 28 of the workers, and temporal atrophy of the optic nerve in a 63-year-old subject who had been exposed to carbon tetrachloride for 27 years and to carbon monoxide for 33 years.

In the group of 62 workers and in a control group of 82 persons, Moeller [58] studied outer limits of the field of vision and color fields of vision on a spherical perimeter, instant adaptation on the recording nyctometer, dark adaptation on a recording adaptometer, sense of color with charts and an anomaloscope, near point measurement on a proximeter, and blind spots with a scotometer.

Among these workers, 43 had reduced corneal sensitivity, 4 had restricted outer limits of the visual fields for white, 4 had subnormal or questionable dark adaption curves, 15 had color limits of the visual field which clearly deviated from normal, and in 7 there were instrument detectable changes in color perception.

Kazantzis and Bomford, [59] in a 1960 report, described a factory in which quartz crystals and various pieces of equipment were cleaned with carbon tetrachloride. The carbon tetrachloride could be smelled in the

vicinity of the equipment as well as near another bench where plates were cleaned with a cloth dipped in beakers of carbon tetrachloride. The 3,000 sq ft room was divided by a partial partition. Concentrations of carbon tetrachloride were measured on both sides of the partition, both before and after the beakers were covered. Before they were covered, 67, 70, and 97 ppm were found on one side of the partition, and 45, 45, and 47 ppm on the other side. After the beakers were covered, the concentrations found were 0, and 8, and 9 ppm on the one side and less than 8 ppm on the other side.

Five days after corrective action, 17 of 18 employees were interviewed and examined by Kazantzis and Bomford. [59] Fifteen of the 17 interviewees had experienced for up to 2 years one or more of the following: nausea, 15; anorexia, 12; vomiting, 7; flatulence, 7; gastric discomfort or distention, 10; and depressive symptoms of headache or giddiness, 10. Nausea had usually developed on Tuesday or Wednesday of each week and vomiting on Thursday or Friday. One affected individual had previously been studied in the hospital because of the severity of his symptoms. The only physical or clinical abnormality found was an SGOT measurement of 76 units/ml. After his return to work and 5 days after the corrective measures were taken, SGOT measured in this individual and in 7 coworkers was normal. At the same time, the fundi and visual fields were normal in the 17 individuals studied; no abnormalities were found in the urine; and no other abnormal physical signs were discovered except for abdominal tenderness without guarding in 3 men. All workers were symptom-free within one week and there were no recurrences up to 6 months later.

In 1967, Barnes and Jones [36] reported a comparative study of exposed and unexposed workers in a plant where carbon tetrachloride was

used in the manufacture of polyfluorohydrocarbons. Sixteen of the plant's employees who handled carbon tetrachloride and 11 others who worked in sections of the plant remote from carbon tetrachloride were studied. The process was automated and enclosed, but intermittent exposure occurred when carbon tetrachloride was delivered by road tanker and discharged into receiving tanks and when the pipes and tanks were periodically cleaned and repaired.

Urinary urobilinogen was found in 6 of those handling carbon tetrachloride, and there was protein in the urine of 3 others of this group. Both the average zinc turbidity and average thymol turbidity of the exposed group were elevated compared to those of the control group. Serum bilirubin averaged 1.36 mg% (0.45-4.0) compared to the control group average of 0.46 mg% (0.20-0.60). The SGOT averaged 37.3 (25-48) Sigma Frankel (SF) units in the exposed group and 32.7 (27-38) SF units in the control group. [36]

An epidemic of carbon tetrachloride poisoning in a parachute plant in which 137 people became ill with sudden onset of abdominal cramping, nausea, and vomiting over a 10-day period in early December 1943 was described by Doyle and Baker. [60] Health records showed that a great many employees had frequent or even constant headaches and that complaints of gastric distress had been especially prevalent for several weeks. All 675 or more employees in the plant had a waxy, "anemic appearance," and the great majority complained of constant headaches, "sour stomach," weakness, and colds. Food, water, and infectious diseases were carefully ruled out as causative factors.

The investigators [60] found that carbon tetrachloride, labeled 99.005%, used for cleaning the parachutes, was constantly present in open

containers on each of 30-40 tables covering half of the 120 by 300 feet of floor space. Gauze pads were soaked in carbon tetrachloride and then rubbed against soiled areas of parachutes. Of the 137 persons who became acutely ill, 135 were engaged in this task. To remedy the situation, the number of open containers of carbon tetrachloride was reduced to 9, windows and doors were opened as much as possible, the building was thoroughly aired between shifts, and the carbon tetrachloride containers were kept covered when not in use. No measurements of carbon tetrachloride in the air had been made before these corrective measures were taken. One afternoon, one week later, 2 room air samples taken between 2 work tables, rather than in the breathing zone of workers, contained 163.6 and 193.5 ppm of carbon tetrachloride. A halogenated hydrocarbon detector was used for the determinations.

Rabes [61] reported in 1972 on a study of 51 workers exposed to carbon tetrachloride for 3-27 years in an electrical plant where turbines and generators were overhauled and cleaned. The process occurred 2-3 times/year and lasted about 14 days each time. Carbon tetrachloride was the only solvent ever used in these cleaning operations. During each 8-hour cleaning shift, a tank containing 20-30 liters of carbon tetrachloride was uncovered and the stripped-down components of the electrical equipment were cleaned in and around it. Special ventilation was not used. The author [61] noted that it was difficult to determine the carbon tetrachloride exposure but that, as an indication, in a 19-month period the maintenance section used 18,100 kg of carbon tetrachloride. Carbon tetrachloride concentrations of 6.3 ppm (40 mg/cu m, or 40 μg/liter) were found at the beginning of a work shift, and of 9.5 ppm (60 mg/cu m) after 5

minutes of work in an unspecified location in the plant. Work clothes sprinkled with carbon tetrachloride were seldom changed.

Fifty-one exposed workers and a comparison group of 27 unexposed workers were given clinical examinations, and laboratory analyses of blood and urine were made. [61] All test subjects (exposed and comparison) were evaluated for previous diseases affecting the liver and for occupational contact with other hepatotoxic substances. The 51 subjects were classified according to years of exposure to carbon tetrachloride, ie, 1-5, 6-10, 11-15, 16-20, or greater than 20. Table XI-6 shows the calculated average biochemical measurements used to evaluate liver function. Serum iron and glutamic dehydrogenase (GDH) were increased in the carbon tetrachloride-exposed group compared to the control group. Both indicators of liver changes were increased by a statistically significant amount after 5 years of exposure to carbon tetrachloride--23% increase for serum iron and 92% increase for GDH. In order to exclude distortion of the data due to previous liver damage, the test subjects were classified into 2 additional groups, those with and those without previous liver damage. Analysis of the data in this way excluded previous liver damage as a significant factor in the results.

Animal Toxicity

(a) Effects of Liquid Carbon Tetrachloride

Centrilobular liver necrosis was found in dogs after ingestion, inhalation, subcutaneous and intraperitoneal injection, and rectal administration of carbon tetrachloride by Gardner et al [62] and reported in 1924. A single 0.5 ml/kg oral dose of carbon tetrachloride produced frank,

fresh central necrosis in the livers of 2 dogs killed 48 hours after administration. Pathologic changes were not found in the kidneys of these 2 dogs. With carbon tetrachloride doses of 0.05, 0.1, and 0.25 ml/kg, indefinite microscopic signs of pathologic changes including swelling and paleness of parenchymal cells were seen in the liver. No gross or microscopic abnormalities were seen in 2 dogs killed 48 hours after a single dose of 0.01 ml/kg. Centrilobular necrosis of the liver was found in rabbits administered oral doses ranging between 0.1 and 6 ml/kg.

Monkeys given 1-2 ml/kg of carbon tetrachloride by stomach tube on 3 days/week showed no evidence of incipient fibrosis after 176 days, [62] but marked accumulation of fat in the center of the liver lobules, and vacuolation and swelling of liver cells were found by microscopic examination. Of 15 liver function tests, only 30-minute BSP retention reflected liver damage. The normal range of BSP retention was given as 0.35-1.5%. BSP retention of 8.2-8.5% was found in 4 monkeys after 15 doses; 14.0-27.0% was found after 31 or 32 doses; and 18.0% was found in 1 monkey after 52 doses.

The intensity and character of damage and repair occurring simultaneously in kidneys and liver were studied by Lundh. [63] Long-Evans strain rats were injected subcutaneously with carbon tetrachloride as follows: the first group received 1 injection of 0.4 ml/kg; the second group received 1 injection of 4 ml/kg; and the third group received 0.4 ml/kg every 3 days over periods of 72 hours-12 weeks. Animals in the single injection groups were autopsied serially at 2, 4, 8, 16, 20, 24, 30, 36, 42, 48, 72, 96, 120, and 168 hours. Animals in the multiple injection group were autopsied serially at 72 hours and at 2, 3, 4, 5, 6, 7, 8, 10, and 12 weeks.

Marked swelling of the renal tubular epithelium and collapse of Bowman's space occurred 2 hours after injection of 4 ml/kg of carbon tetrachloride. In the livers, disappearance of small arterioles and medial necrosis of larger arteries were seen at 4 hours after injection. Severe renal damage was still seen at 48 hours and a normal state had not been completely attained at 120 hours after injection of carbon tetrachloride. [63] Severe destructive changes were still present at 120 hours in the liver parenchyma.

Degeneration of the cytoplasm of the proximal tubular cells of the kidneys was found 4 hours after the single 0.4 ml/kg injection of carbon tetrachloride. At this time, liver cells around the central vein were eosinophylic. The degenerative changes in the kidney became maximal 16-24 hours after the injection, and at 48 hours, the kidneys had regained normal microscopic appearance. Liver damage progressed slowly from the central cells to the rest of the liver and from eosinophilic and granular changes to marked vacuolation and eventually cellular death. Maximum liver damage was observed 36-48 hours after injection of carbon tetrachloride and cellular regeneration was complete at 120 hours.

Until 2 weeks after the start of injections, the nephrotoxic and regenerative patterns found in the group of animals given carbon tetrachloride doses of 0.4 ml/kg at 72-hour intervals were similar to those found in the group given a single dose. At 2 weeks, the regenerative processes were not complete between injections, and dilation of renal tubules with flattening and atrophy of proximal tubular cells was seen. The liver cells did not recover between any of the injections and fibrotic and cirrhotic changes began at about 2 weeks.

Ornithine carbamoyl transferase (OCT) activity was found to vary in relation to the severity of liver damage in a study reported by Musser and Spooner [64] in 1968. Wistar rats were given carbon tetrachloride at a dose of 0.125 mg/g by intraperitoneal injection and killed by decapitation at various intervals 4-120 hours after injection. Autopsies were performed immediately after death and serum and tissue OCT determinations were made. Liver cell damage determined by microscopic examination was graded on a 1-4 scale, ranging from scattered individual cell necrosis to confluent areas of necrosis. The index of liver cell damage increased with time after injection to a maximum at 48 hours, then decreased to 0, ie, normal, at 120 hours. Serum OCT was significantly elevated at 8 hours after injection of carbon tetrachloride, maximum at 48 hours, and still abnormally high at 96 hours. The lowest level of tissue OCT was at 24 hours and the highest at 46 hours. After 48 hours, the tissue OCT decreased toward normal levels but rose significantly at 120 hours. Serum OCT levels at various times after carbon tetrachloride administration are shown in Table XI-7.

The effects on serum OCT activity of 33 solvents, including carbon tetrachloride, were reported by DiVincenzo and Krasavage [65] in 1974. Carbon tetrachloride was dissolved in corn oil and administered by intraperitoneal (IP) injection to mature male guinea pigs in doses of 5, 25, 50, 75, and 150 mg/kg. Twenty-four hours after the injections, 2 ml of blood was withdrawn by heart puncture from anesthetized animals, and serum OCT activity was determined spectrophotometrically. Each animal was killed after blood sampling by an overdose of diethyl ether and the liver was removed and examined. Control serum OCT values were 2.02 (SD of 1.61) International Units (IU) with a reported range of 0 to 8.9 IU. The value

of 8.9 IU suggested to the authors some incipient liver damage in an unexposed, presumably healthy animal. Carbon tetrachloride doses of 5, 25, 50, 75, and 150 mg/kg gave average serum OCT activity of 3.8, 37.1, 63.5, 53.5, and 64.4 IU, respectively. Liver damage and lipid accumulation were found after all doses except 5 mg/kg. [65]

Absorption of liquid carbon tetrachloride through the skin was studied by Lapidus [66] and McCord. [67] Lapidus [66] studied carbon tetrachloride in the blood, liver, and brain of 4 rabbits after immersion of one ear of each rabbit in carbon tetrachloride. Ear immersion times were 5, 6, 8, and 9 hours, and precautions were taken to avoid inhalation of carbon tetrachloride vapor. Substantial amounts of carbon tetrachloride were found in the blood (12-13 mg/100 g), liver (0-90 mg/g), and fat (0-300 mg/g). A trace of carbon tetrachloride was found in the brain of the rabbit whose ear was immersed for 9 hours, but none was found in the brains of the other rabbits. The analytical method required at least 5 mg of carbon tetrachloride in a sample.

McCord [67] administered carbon tetrachloride under a leak-proof bandage placed on the clipped abdominal skin of 3 animals (species not stated). Three times daily for 7 or 8 days, he injected either 7.5, 1.6, or 1.2 ml/kg of carbon tetrachloride under the bandages. The first animal (7.5 ml/kg/injection) died on the 7th day, the second (1.6 ml/kg/injection) died on the 8th day, and the third (1.2 ml/kg/injection) was killed after the 7th day. Autopsies were limited to macroscopic observations. Subcutaneous necrosis was evident in the areas where the carbon tetrachloride had been applied, the livers showed a dark purplish mottling, and inflammation was seen throughout the body of each animal.

(b) Chronic Inhalation Effects

An experiment was reported by Prendergast et al [68] in which 15 rats, 15 guinea pigs, 3 rabbits, 2 dogs, and 3 monkeys were exposed to carbon tetrachloride at 82 ppm for 8 hours/day, 5 days/week for a total of 30 exposures. The report also included the results of exposing similar groups of animals to carbon tetrachloride at 10 ppm or 1.0 ppm for 90 days or a total of 2,160 hours of continuous exposure. Weight losses or decreased growth occurred with all species under each exposure condition except for rats at 1 ppm.

The lungs of all animals repeatedly exposed at 82 ppm showed interstitial inflammation or pneumonitis and a high percentage of the livers of all species except dogs had a mottled appearance. Fatty changes were most severe in the livers of guinea pigs, then rats, then rabbits, then dogs, and least severe in monkeys. Livers of guinea pigs exposed at 82 ppm showed fatty infiltration, fibrosis, bile duct proliferation, hepatic cell degeneration and regeneration, focal inflammatory cell infiltration, alteration of lobular structure, and early portal cirrhosis. Three guinea pigs died during the experiment, 1 each after 20, 22, and 30 exposures, and 1 monkey died after seven exposures. [68]

Exposures at 10 ppm for 90 days of continuous exposure resulted in a "high incidence" (not explained) of enlarged and discolored livers in all species except dogs. [68] Liver changes were found in all species by microscopic examination. The changes included fibroblastic proliferation, collagen deposition, hepatic cell degeneration and regeration, and alteration of the structure of the liver lobule. Liver fat content of exposed guinea pigs averaged 35.4% (SD of 10.7%) compared to the control

average of 11.0% (SD of 3.6%). [68] Liver enzymatic activities of rats and guinea pigs, the most affected species, were normal except for a moderate reduction in succinic dehydrogenase. The other enzymes studied were reduced nicotinamide adenine dinucleotide (NADH), reduced nicotinamide adenine dinucleotide phosphate (NADPH), lactic dehydrogenase (LDH), and glucose-6-phosphate dehydrogenase (G6PD).

Specific pathological changes attributable to the continuous exposure at 1.0 ppm were not observed. The fat content of the livers of the guinea pigs averaged 9.7% (SD 2.4%) compared to 11.0% (SD 3.6%) in controls. [68]

Smyth et al [56] and Smyth and Smyth [57] reported on monkeys, rats, and guinea pigs exposed to carbon tetrachloride for 8 hours/day, 4-6 days/week for periods of time up to 321 days. Carbon tetrachloride atmospheres were obtained by metered dilution of air saturated with carbon tetrachloride at different temperatures. Monkeys were exposed at 50 and 200 ppm, and rats were exposed at 50, 100, 200, and 400 ppm. Guinea pigs were first exposed at 50, 100, 200, and 400 ppm. Since all guinea pigs exposed at 100, 200, or 400 ppm died by 94 days of age (Table XI-8), the diet was changed and other guinea pigs were exposed at 25, 50, 100, and 200 ppm. The death rate of guinea pigs fed the new diet continued to be high (Table XI-8). Optic nerve degeneration was found in 1 or 2 guinea pigs exposed at each concentration, and fatty degeneration was found in the ocular muscle of 3 to 6 guinea pigs at each concentration. Mortality was not a significant outcome of exposure of rats and monkeys to the various concentrations of carbon tetrachloride.

Rats were killed periodically for study. Initially the 5 groups of rats each contained 24 animals. Evidence of regeneration of liver cells and of interstitial cell proliferation was found after 126 exposures at 50 ppm and developing liver cirrhosis was evident after 189 exposures. Splenocytes were increased in rats after 121 exposures. Some damage to the myelin sheath of the sciatic nerve was seen in a rat after 96 exposures, and in another after 121 exposures. Degenerative changes were seen in the eye muscles of 3 rats. There was some evidence of kidney damage in rats exposed at 50 ppm, but it was not marked in any animal. At 100 ppm, liver, kidney, and nerve damage similar to that observed at 50 ppm became apparent after 20 exposures but did not develop to a greater extent than in rats exposed at 50 ppm. Splenocytes were increased after 49 exposures and increased pigment, not seen in rats exposed at 50 ppm, became noticeable in rats after 107 exposures at 100 ppm. Some necrosis was seen in rat livers by these investigators [56,57] after 20 exposures at 200 ppm. Cellular regeneration was apparent after 115 exposures, and cirrhosis after 150 exposures. Other pathological developments that occurred at lower exposure concentrations also occurred in rats at 400 ppm but they were more severe with the increased exposure. Two rats were found with optic nerve degeneration.

The exposure history of the monkeys is shown in Table XI-9. Fatty changes without evidence of degeneration were found in the livers after 62 exposures at 50 ppm. After 188 exposures at this concentration, there was evidence of adaptation and the livers appeared less abnormal than had been observed earlier, and after 225 exposures and 28 days' rest, the liver appeared practically normal. The kidneys appeared normal at 188 days, but

cloudy swelling was seen at 225 days; this improved with 28 days of rest. Spleen and nerve tissue appeared normal in all the monkeys exposed at 50 ppm.

At 200 ppm, evidence of liver damage with marked increase in interstitial cells and cellular infiltration into septa was observed after 62 exposures. After 225 exposures, this degeneration had not progressed, no cirrhosis was found, and there was no evidence of regeneration. The liver of a monkey examined 28 days after the last of 225 exposures appeared normal. In the kidneys of monkeys, light granular swelling without definite tubular degeneration was seen after 62 exposures at 200 ppm. There was increased secretion in the tubules, but little or no cell detritus and no desquamated cells. The animal examined 28 days after the last of 225 exposures had normal kidneys except for increased tubular secretion. There were some slight fatty changes in the ocular muscle and in the sciatic nerve. Other organs appeared normal. [56, 57]

In a chronic exposure study reported by Adams et al, [69] guinea pigs and rats were exposed at 5, 10, 25, 100, 200, and 400 ppm for 7 hours/day, 5 days/week for up to 184 exposures over a period of 258 days. The number of animals involved in each exposure and the number of animals surviving each exposure were not always reported. Eight or 9 guinea pigs of each sex were apparently used at most concentrations. Fifteen rats of each sex were used at concentrations of 25 ppm or more, 20 of each sex at 10 ppm, and 23 females and 26 males were exposed at 5 ppm. Numerous biochemical and organ weight measurements and microscopic findings were reported but it was not possible to determine from the report which measurements were omitted at the different concentrations. Consistent

responses in guinea pigs that were reported were increased liver weights at all exposure concentrations, a moderate amount of fatty degeneration at 10 ppm and above, and a moderate amount of liver cirrhosis at 25 ppm or more. Increased liver weights of rats occurred at all exposure concentrations of 10 ppm or more, accompanied by fatty degeneration. Liver cirrhosis was not detected in rats at exposures below 50 ppm.

Chronic exposures of rabbits were also studied by Adams et al. [69] One or 2 rabbits of each sex were reported to have survived exposures at 10, 25, 50, and 100 ppm. Liver weights were not increased by exposure at 10 ppm, but were increased by exposures at 25 ppm or more. Moderate fatty degeneration of the liver with cirrhosis developed after 178 exposures in 248 days at 25 ppm. Effects observed at lower concentrations were also observed at 50 and 100 ppm. In addition, decreased growth and increased kidney weights were observed and blood clotting time, indicative of liver injury, increased 1.5-2 times by exposures to carbon tetrachloride at 50 and 100 ppm. [69]

Some indications of microscopic liver changes were seen in 2 monkeys exposed to carbon tetrachloride at 100 ppm by Adams et al. [69] Body weight loss was the only abnormal finding reported in monkeys exposed to carbon tetrachloride at 50 ppm and no abnormal findings were reported in 2 others exposed at 25 ppm. The monkeys were exposed 148-198 times for 7 hours each time.

(c) Carcinogenesis

Induction of hepatomas in mice of the C3H and A strains was reported by Edwards [70] in 1941. The mice were fed 0.1 ml of a 40% solution of carbon tetrachloride in olive oil by stomach tube 2 or 3 times/week for a

total of 23-58 feedings and autopsied 2-147 days after the last feeding. (Body weights were not given, but if the mice weighed about 25 grams, the doses would have been about 1.5 ml/kg body weight.) This dose caused liver necrosis, and eventually cirrhosis. Hepatomas were found in 126 of 143 C3H strain mice and in all 54 A strain mice. The normal incidences of hepatomas in mice of comparable age (11-12 months) were 10% in the C3H strain and 0.5% in the A strain. Administration of olive oil to control animals did not increase the incidence of hepatomas above normal.

Subsequent reports of the experiment in 1942 by Edwards and Dalton [71] and Edwards et al [72] included more data from these strains and also data on 3 additional strains (Y, C, and L) that had normal hepatoma incidences of less than 2% at 12-16 months of age. Hepatoma incidences of 60, 82, and 47% in Y, C, and L strains, respectively, were reported after carbon tetrachloride treatment. Aliquots of mice were killed periodically after one or more doses of carbon tetrachloride, and all were killed before one year of age. Regenerative processes were observed throughout the experiment and atypical mitotic forms such as triple mitoses were frequent. Large cells with a peculiar, faintly basophilic reticulated cytoplasm and small nuclei were seen often in the livers of mice treated for one month or more. These cells were concentrated along strands of fibrous tissue. The hepatic tumors, described in detail by the investigators, [71] were usually multiple, as many as 10 in each liver. Neither invasion of blood vessels by hepatoma nor metastases were seen. Neither adenocarcinomas nor hemangioendotheliomas were present in any of the livers. The authors did not find evidence that tumors were induced in any other organ.

Rudali and Mariani [73] conducted an experiment in which 30 XVII Ivry strain mice were fed by stomach tube 0.1 ml of a 40% solution of carbon tetrachloride in oil, 2-3 times/week. Spontaneous liver tumors had not been observed up to this time in this strain of mice which was used by the Institut de Radium. Twenty-one animals were used to study the progress of the lesions for up to 66 days. Nine animals were given 48 carbon tetrachloride doses over 118 days. These 9 animals were autopsied after 132, 143, 232, 234, and 310 days. Microscopic adenomas were found in the first 4 mice. In each of the 5 mice killed after 310 days, macroscopic tumors were found and 4 of these had signs of malignancy. No metastases were found.

Effects of size and spacing of multiple doses of carbon tetrachloride on induction of hepatomas were reported by Eschenbrenner [74] in 1944. Doses of carbon tetrachloride administered were 1.6, 0.8, 0.4, 0.2, and 0.1 ml/kg body weight. Each dose was dissolved in olive oil and 0.005 ml/kg of the solution was administered by stomach tube. At each dose level, 5 groups of strain A mice were used to vary the intervals between doses by 1, 2, 3, 4, or 5 days. Each mouse was given 30 doses. Each of the doses had been found previously to cause central necrosis of the liver lobules. All animals were examined for hepatomas 150 days after the first dose. One mouse with hepatoma was found among 60 mice dosed daily. Eighteen mice with hepatomas were found among 60 mice dosed every other day, and with this dosing interval, there was an increased incidence of hepatomas as the magnitude of the individual doses of carbon tetrachloride increased. With intervals of 3 days between doses, 37 mice with hepatomas were found among 60 treated mice, and there was no apparent relationship to

the magnitude of the individual doses. With intervals of 4 days between doses, 55 mice with hepatomas were found among 75 treated mice, and again there was no relationship to the magnitude of the individual doses. Twenty-five mice with hepatomas were found among 44 mice treated at 5-day intervals, and there appeared to be a relationship to the magnitude of the individual doses. No hepatomas were found among 28 control mice that received olive oil without carbon tetrachloride. Three spontaneous hepatomas which differed from the induced tumors in color and microscopic characteristics were found, one in a control animal and 2 in experimental animals.

Eschenbrenner and Miller [75] reported in 1946 that single oral doses of carbon tetrachloride of 12.5 μl/kg or more caused liver cell necrosis in both male and female strain A mice. Doses of 6.25 μl/kg did not result in any observations of liver cell necrosis. Doses of 12.5 μl/kg resulted in observable liver cell necrosis in 1 of 8 mice examined. All higher doses caused liver cell necrosis in all mice examined. The investigators then administered 120 daily doses of 6.25, 12.5, 25, or 50 μl/kg. Each dose of carbon tetrachloride was dissolved in olive oil and 5 ml/kg of the solution was administered by stomach tube. The mice were examined one month after the last dose when they were 9 months old. No hepatomas were found in the mice given the nonnecrotizing doses of 6.25 μl/kg. All mice given the higher, necrotizing doses had hepatomas. Other groups of mice were given 30 necrotizing carbon tetrachloride doses of 100, 50, or 25 μl/kg at 4-day intervals. Very small hepatomas were found by microscopic examination in 2 of 10 mice given doses of 25 μl/kg. Larger

hepatomas were found macroscopically in two-thirds of the mice given the higher doses at 4-day intervals.

Transplantation of tumors induced by carbon tetrachloride has succeeded in some cases. One hepatoma from an A strain mouse was successfully transplanted and the subcutaneous transplants were invasive. [71] This tumor, which was first transplanted 6 months after the donor animal had received the last of 36 doses of carbon tetrachloride, was carried through at least 4 transplant generations. Transplantation of 7 other tumors, 3 from strain A and 4 from C3H mice, was unsuccessful in this experiment. The number of carbon tetrachloride doses and the examination times relative to the last dose were not reported for other mice of strain A and mice of strain C3H from which 7 hepatoma transplants were unsuccessfully attempted. [71]

Andervont and Dunn [76] reported in 1952 that there were no successes with 1-4 transplantations of each of 8 hepatomas that were induced in I strain mice by 22 weekly 0.25-ml oral doses of 4% carbon tetrachloride in olive oil (approximately 0.4 ml/kg). The mice were 3-9 months old when the experiment started. The ages of the donor mice at the time the transplantations were made were not reported.

In a later experiment with C3H mice, reported in 1955 by Andervont and Dunn, [77] and in 1958 by Andervont, [78] 28 of 30 tumors from carbon tetrachloride treated mice grew when transplanted. The mice had received, one week apart, two 0.2-ml oral doses of a 2% solution of carbon tetrachloride in olive oil (approximately 0.16 ml/kg), and subsequently they received, at weekly intervals, 17 doses of a 3% solution of carbon tetrachloride. The ages of the mice at the beginning of the experiment

were 3.5-7.5 months. [78] The time intervening between the last dose of carbon tetrachloride and the transplantation of the tumors was not given. However, for the tumors described in detail, the ages of the donor mice were given as 11, 12, 12, and 14 months indicating that as much as 2.5-5 months could have elapsed between the end of dosing and the tumor transplantation. The C3H strain had a substantial incidence of spontaneous hepatomas, 10% at 11-12 months of age, [70] and up to 50% at older ages, [70,78] and the spontaneous hepatomas of this strain had been successfully transplanted. [76]

Successful transplantation of hepatomas induced by carbon tetrachloride in a strain of mice in which spontaneous hepatomas had not been observed was reported by Leduc and Wilson [79] in 1959. Carbon tetrachloride was administered to BUB strain mice by stomach tube in doses of 0.1 ml of a 40% solution in olive oil, 3 times/week for a total of 45-66 doses. About 1/3 of the mice were given iv 0.2 ml/day thorotrast, a thorium containing x-ray contrast medium, on 3 days before carbon tetrachloride administration was started and X-ray pictures were taken at 2-4 week intervals for detection of tumors. No hepatomas were found in 20 control mice examined 192-514 days after injection of thorotrast only, but hepatomas were found in mice treated only with carbon tetrachloride. Two transplantations were attempted from mice that had not been given thorotrast but had been treated for 50-52 weeks with carbon tetrachloride. These transplants which were made 12 days and 11 weeks after the last carbon tetrachloride dose were not successful. Successful transplantations were made of 5 hepatomas from a thorotrast-treated mouse that had been given 45 doses of carbon tetrachloride and killed 8 months after the last

dose. The authors commented on the ability of thorotrast to cause vascular tumors in the liver and other organs, but concluded from their data that thorotrast did not have an important role in development and transplantability of these hepatomas.

Hepatocellular carcinomas, less than 5 mm in size, were found and reported in 1967 by Reuber and Glover [80] in Buffalo strain rats given carbon tetrachloride subcutaneously twice weekly for 12 weeks at a dose of 0.65 ml/kg. In another study reported by Reuber and Glover [81] in 1970, male rats of the Japanese, Osborne-Mendel, Wistar, Sprague-Dawley, and Black strains were injected subcutaneously twice weekly until death with carbon tetrachloride at a dose of 1.3 ml/kg. The carbon tetrachloride was in corn oil solution. The Osborne-Mendel rats survived up to 105 weeks, Japanese rats up to 78 weeks, Wistar rats up to 68 weeks, Black rats up to 18 weeks, and Sprague-Dawley rats up to 16 weeks. Carcinomas were not observed in the Black and Sprague-Dawley rats, all of which died with severe liver cirrhosis at an early age. Some of the rats of the 3 longer-lived strains developed liver cell carcinomas; the frequency of carcinoma was 12 of 15 in Japanese rats, 8 of 13 in Osborne-Mendel, and 4 of 12 in Wistar rats. Incidences of carcinomas greater than 5 mm in these groups, were 8, 4, and 1, respectively.

Development of liver cell carcinomas in male and female Syrian golden hamsters after 30 weekly doses of a 5% solution of carbon tetrachloride in corn oil administered by stomach tube was reported in 1961 by Della Porta et al. [82] Ten hamsters of each sex were used in the experiment. They were initially 12 weeks old. During the first 7 weeks, the carbon tetrachloride dose was 0.0125 ml, and during the remaining 21

weeks of administration it was 0.00625 ml. Since the animals weighed about 100 grams, the doses were about 0.125 and 0.0625 ml/kg. One female died at the 10th week of administration, and 3 females and 5 males died or were killed between the 17th and 28th weeks. After all the doses were administered, the survivors were kept under observation for 25 additional weeks. During this time, 3 females died between weeks 41 and 54, and the rest were killed at week 55. Necrotic changes and hemorrhages involving the regenerated parenchyma were found in livers of animals that died within 48 hours of treatment. All animals that died during the time the doses were being given showed changes consistent with postnecrotic cirrhosis. One or more liver-cell carcinomas were found in each of the 5 males and 5 females examined 43-55 weeks after the first carbon tetrachloride dose.

Costa et al [83] exposed albino rats for up to 7 months at an unspecified inhalation concentration of carbon tetrachloride. The rats were killed serially 2-10 months after beginning of exposure. Thirty rats completed the experiment. Twelve of the rats were found to have adenocirrhosis and 10 had liver nodules measuring up to 1 cm which were microscopically diagnosed as incipient or established liver carcinomas. Established carcinomas were found in 5 livers and incipient carcinomas were found in 5 others.

(d) Teratogenesis and Mutagenesis

Smyth et al [56] and Smyth and Smyth, [57] in their chronic exposure study, observed 3 generations of rats exposed at 50, 100, 200, or 400 ppm carbon tetrachloride. The numbers of animals in the second and third generations were few, and there was evidence of reduced fertility at 200 and 400 ppm. There was no report of incidences of embryonic or fetal abnormalities.

Schwetz et al [84] reported the effects of repeated exposures to carbon tetrachloride on rat embryo and fetal development. Groups of pregnant Sprague-Dawley rats were exposed at 300 and 1,000 ppm for 7 hours a day on days 6 through 15 of gestation. Day 0 of pregnancy was considered to be the day on which sperm was seen in vaginal smears. Concentrations of carbon tetrachloride in the exposure chambers were continuously monitored by combustion analysis. In addition, an infrared spectrophotometer with a multipath gas cell was used 3 times daily to analyze the chamber air and substantiate the concentration calculations.

A significant decrease in fetal body weights and crown-rump lengths as compared with controls was found when pregnant rats were exposed at 300 and 1,000 ppm on days 6 through 15 of gestation. Fetuses showed no anatomical abnormalities by gross examination but the incidence of microscopic sternebral anomalies was significantly increased by exposure at 1,000 ppm. Subcutaneous edema was found in 33% of the control litters, 59% of the litters from dams exposed at 300 ppm, and 50% of the litters from dams exposed at 1,000 ppm. The authors concluded that carbon tetrachloride was not teratogenic.

The conception rates, numbers of implantations, and litter sizes were normal, even though maternal body weights were significantly less than control body weights. [84]

(e) Metabolism and Mechanism of Action

When carbon tetrachloride is administered to mammals, it is metabolized to a small extent. The metabolites include chloroform, hexachlorethane, and carbon dioxide. [85-87] Some of the carbon tetrachloride metabolic products also are incorporated into fatty acids by

the liver and into liver microsomal proteins and lipids. [88,89] A consequence of carbon tetrachloride breakdown is lipoperoxidation in the liver. [90-92] The initial pathological injury occurs in the endoplasmic reticulum. [93] Within the endoplasmic reticulum cytochrome P-450, [94] protein synthesis, [95] and glucose-6-phosphatase [96] are markedly depressed within the first 6 hours.

The acute liver necrotic action of carbon tetrachloride is dependent upon its metabolic breakdown as evidenced by (1) little effect of carbon tetrachloride in chickens and ducks, neither of which metabolize it [97]; (2) the increased carbon tetrachloride effects from alcohols, chlorinated hydrocarbons, and barbiturates which increase its metabolism [98-101]; and (3) decreased activity of glucose-6-phosphatase and aminopyrine demethylase in the whole animal after carbon tetrachloride administration but insensitivity of these enzymes to the physical presence of carbon tetrachloride in vitro. [102]

McCollister et al [85] found that about 1/2 of the carbon-14 from labeled carbon tetrachloride inhaled by monkeys at 46 ppm for 139-300 minutes was later excreted in the expired air, mostly as carbon tetrachloride, and less than 5% as carbon dioxide. Only small amounts of labeled carbon appeared in the urine, not as carbon tetrachloride but either as urea or as an unidentified nonvolatile substance.

Butler [86] found that small amounts of chloroform were excreted in exhaled air of dogs after inhalation of carbon tetrachloride, and that the conversion could take place in tissue homogenates. Fowler [87] showed that both chloroform and hexachlorethane were metabolites of carbon tetrachloride administered to rabbits. Gordis [88] found

trichloromethylated fatty acids present in labeled liver lipid after feeding a mixture of chlorine-36 and carbon-14 labeled carbon tetrachloride to rats, and Rao and Recknagel [89] found a small amount of labeling of rat microsomal proteins and lipids after administering carbon-14 labeled carbon tetrachloride.

The metabolism of carbon tetrachloride is dependent upon microsomal hydroxylating enzyme systems such as cytochrome P-450. Reduction of cytochrome P-450 and probably of other microsomal enzymes by feeding low protein diets to rats decreased metabolism to carbon dioxide, [103] and decreased toxicity of carbon tetrachloride (the LD50 of orally administered carbon tetrachloride increased from 6.4 to 14.7 ml/kg). [104] Pretreatment with phenobarbital to stimulate cytochrome P-450 increased metabolism of orally administered carbon tetrachloride to carbon dioxide, markedly increased fat in the liver, and increased plasma concentrations of bilirubin compared to rats given only similar doses of carbon tetrachloride. [100,101]

Preexposure to DDT also has been shown to increase both the P-450 content of rat liver cells and the toxicity of carbon tetrachloride. [100, 104] Ethanol administration increased the activity of the liver hydroxylating enzyme system. Ethanol pretreatment increased the necrotic liver injury from carbon tetrachloride, but had less effect on the liver fat accumulation caused by carbon tetrachloride. [98,99,105,106]

Dogs chronically treated with phenobarbital for 12 months then given a single dose of carbon tetrachloride were similarly much more affected than dogs given only carbon tetrachloride as indicated by increased liver triglyceride content, increased diene conjugates, and increased SGOT. [90]

Carbon tetrachloride was found to promote lipid peroxidation in the liver of rats at oral doses of 0.3-1.0 ml/kg, but not at doses of 0.1 ml/kg. [91] Hashimoto et al [92] compared the lipid peroxidation in the liver of a woman who died with massive liver necrosis after drinking carbon tetrachloride with that of the liver from a victim who died from a traffic accident. They concluded on the basis of liver lipid conjugated dienes that extensive peroxidative degeneration had occurred as a result of the carbon tetrachloride poisoning.

(f) Effects of Alcohol with Carbon Tetrachloride

Robbins [107] conducted a series of experiments in which he measured the concentration of carbon tetrachloride in the exhaled air of dogs absorbing carbon tetrachloride from the alimentary canal under various conditions. Carbon tetrachloride in the exhaled air was determined by the thermal conductivity method (which was said to be accurate within 100-200 ppm). He found that when 10 or 50 ml of carbon tetrachloride was administered simultaneously with 40 ml of alcohol, the carbon tetrachloride concentrations in the exhaled air were considerably greater than when the carbon tetrachloride was administered alone. The concentration of the administered alcohol was not given. The author found that absorption of carbon tetrachloride was increased when given with alcohol but he did not consider that the increased toxicity was due to the increased absorption.

A higher mortality and more extensive liver necrosis in dogs was found when Gardner et al [62] administered 4 ml of 95% ethyl alcohol orally with 4 ml carbon tetrachloride than when carbon tetrachloride was given alone. The enhancing effect of alcohol did not occur when the carbon tetrachloride administered was 0.05 or 0.10 ml/kg.

Cornish and Adefuin [98] conducted a series of experiments to determine the effects of ethyl alcohol ingestion on carbon tetrachloride toxicity using serum enzymes (SGOT, SGPT, and isocitric dehydrogenase) and liver morphology as toxicity determinants. In all experiments, 2 groups of 6 rats each were used. In each experiment 5 ml/kg of ethyl alcohol was administered orally to the 6 rats of one group; the 6 rats of the other group did not receive any alcohol. Except for the alcohol treatment, the 2 groups of rats in each experiment were treated alike.

Exposures to carbon tetrachloride for 2 hours at 10,000 ppm caused marked degenerative fatty infiltration of the livers of all exposed animals, whether or not they were given ethanol. With exposure to carbon tetrachloride for 2 hours at 1,000 ppm, the livers showed degenerative fatty infiltration, but necrosis was not observed, and there were no increases in serum enzyme activities. Effects similar to these were observed when ethanol was administered 2 hours before exposure to carbon tetrachloride at this level. However, when ethanol was administered 16-18 hours before the carbon tetrachloride exposure, more severe effects were found. In addition to degenerative fatty infiltration, there was liver cell necrosis, and the activities of all 3 serum enzymes were elevated at 48 hours after exposure.

Carbon tetrachloride exposures for 2 hours at concentrations of 250 ppm or less caused no changes from controls in either serum enzyme activities or liver morphology. When ethanol was administered 16-18 hours before the carbon tetrachloride exposures at 100 and 250 ppm, a slight increase in liver lipid was found by microscopic examination. With exposures to carbon tetrachloride at 25 or 50 ppm for 2 or 8 hours, no

changes from controls were found, with or without ethanol pretreatment.

Ethanol treatments without carbon tetrachloride exposure caused no changes from controls in the serum enzymes or liver structure.

Correlation of Exposure and Effect

Symptoms referable to gastrointestinal and central nervous disturbances have been found to occur early in exposure to carbon tetrachloride and at relatively low levels of exposure. [24,31,33,54] Nausea, vomiting, belching, drowsiness, and headaches were found by Elkins [54] in surveys of workers exposed at 10-25 ppm in one factory and at 35 ppm (10-80) in another. Similar effects were found by Kazantzis and Bomford [59] in workers exposed to carbon tetrachloride between 45 and 97 ppm. These effects subsided when concentrations were reduced to 9 ppm or less.

Eye irritation and dysfunction were reported in some cases of occupational exposures to carbon tetrachloride. [27-29,56-58] Smyth et al [56] and Smyth and Smyth [57] found restricted visual fields in 4 workers whose 8-hour TWA exposures were estimated at 7, 10, 10, and 24 ppm with peak exposures of 66, 26, 173, and 232 ppm, respectively.

Various eye abnormalities were found by Moeller [58] in 62 workers in a locksmith shop where carbon tetrachloride concentrations of 6.4-9.5 ppm were found. Details of the sampling and analysis were not reported. Among the 62 workers, 70% had reduced visual fields and 15 individuals had visual color fields deviating from normal. Similar eye abnormalities were reported in other occupational cases where the air concentrations of carbon tetrachloride were not measured. [27-29]

Impaired function and kidney and liver tissue changes have been

reported in cases of both acute and chronic exposure to carbon tetrachloride. [20,26,30-40,53,61] Renal tubular necrosis and severe centrilobular liver necrosis were found in autopsy examinations of 4 fatalities among 20 cases of carbon tetrachloride exposures studied by Guild et al. [31] In all 20 cases, anuria developed 1-7 days following exposure.

Elevated serum transaminases and an enlarged discolored liver with fatty degeneration were observed in a 54-year-old man who had been using carbon tetrachloride in his work for 4 years. [37] Air samples at 16 locations in the plant contained carbon tetrachloride at concentrations of 10-100 ppm. No predisposing diseases or other etiological factors were found in a review of the patient's medical history.

Elevated serum aldolase and SGOT were compared with tissue changes in 2 men exposed to an unspecified amount of carbon tetrachloride. [35] In both cases, serum enzymes were elevated when the men were hospitalized with gastrointestinal and central nervous system symptoms resulting from carbon tetrachloride exposure. Large areas of centrilobular necrosis were found in one man and degenerative cellular changes were found in the other man in liver biopsies.

Increased serum iron and GDH were found in 51 workers with 5 or more years of periodic exposure to carbon tetrachloride. [61] Air samples in the work area contained carbon tetrachloride concentrations of 6.3 ppm at the beginning of a workshift and 9.5 ppm, 5 minutes after the beginning of the work period. The data were not sufficient to determine whether the environmental concentrations were always near the reported range. Serum iron and GDH were the only findings in clinical examinations and a series

of biochemical studies of the 51 exposed workers which varied from findings in a comparison group of 27 persons. The serum iron was elevated 23% and the GDH was elevated 92%. [61]

Serum iron was depressed in 2 of 4 subjects experimentally exposed by Stewart et al [26] at 49 ppm carbon tetrachloride for 70 minutes, but was not depressed in any subjects exposed at 10 or 11 ppm carbon tetrachloride for 180 minutes.

Zinc and thymol turbidity, serum bilirubin, and SGOT were elevated in a group of workers in a polyfluorocarbon manufacturing plant who handled carbon tetrachloride, compared to workers who did not handle carbon tetrachloride. [36] Urobilinogen was found in the urine of 6 of 16 carbon tetrachloride handlers and in none of the nonhandlers of carbon tetrachloride. No estimate of the exposure of the 16 workers was reported.

Smyth et al [56] and Smyth and Smyth [57] noted elevated icteric indices in 11 workers with estimated 8-hour TWA exposures of 26-39 ppm. Icteric indices were normal in 11 other workers with estimated 8-hour TWA exposures of 5-24 ppm.

Three human cases of hepatic carcinoma, 2 after repeated exposure, and 1 after a single exposure, have been reported. [38-40] In each case, the diagnosis was confirmed at autopsy. The extent of the exposure to carbon tetrachloride in these cases was only reported qualitatively and no definitive causal relationship between carbon tetrachloride and the resultant carcinomas was established, but data from animal studies show that carcinomas have resulted in a variety of experimental animals with various routes of intake. [70-83]

Reuber and Glover [81] found carcinomas greater than 5 mm occurring in 3 strains of rats injected 1-2 times a week with 1.3 ml/kg of carbon tetrachloride subcutaneously until the animals died. Japanese, Osborne-Mendel and Wistar strain rats had carcinoma frequencies of 80%, 60%, and 33%, respectively; the incidences of carcinomas greater than 5 mm were 53%, 30%, and 8%, respectively.

Established and incipient carcinomas were found by Costa et al [83] in albino rats exposed by inhalation to carbon tetrachloride for up to 7 months. The concentrations of carbon tetrachloride were not reported. Twelve of 30 rats surviving the experiments had adenocirrhosis and liver nodules measuring as large as 1 cm. Incipient or established carcinomas were found in 10 of these.

Liver damage within the first 120 hours after a single 0.125 ml injection of carbon tetrachloride was directly related to serum OCT activity in rats. [64] In guinea pigs serum OCT activity reflected liver damage but there was no dose-response relationship with doses of 5, 25, 50, 75 and 150 mg/kg. [65]

Intermittent carbon tetrachloride exposures (7 or 8 hours/day, 5 days/week) had adverse effects on guinea pigs and rats at concentrations of 10 ppm and guinea pigs at 5 ppm. [69] Female guinea pigs exposed to carbon tetrachloride at 5 ppm had increased liver weights relative to body weights after 143 exposures.

Both guinea pigs and rats exposed at 10 ppm for 7 hours/day, 5 days/week, 136 times, had increased liver weights and liver lipid contents compared to controls. [69] Fatty degeneration of the livers was also observed. These effects were more pronounced at 25 and 50 ppm. Exposures

at 50 ppm caused depression of growth, increased liver and kidney weights, and increased total liver lipid content. Fatty degeneration and cirrhosis of the liver, and swelling of the renal tubules were also observed. [69]

Intermittent exposures by Smyth et al [56] and Smyth and Smyth [57] of rats and guinea pigs to carbon tetrachloride at concentrations of 50 and 100 ppm affected liver weight, liver lipid content, and kidney weights. Fatty degeneration and cirrhosis of the liver, evidence of regeneration of liver cells, and some swelling of the kidneys were also observed.

Four monkeys exposed 5 hours/day, 4-6 days/week to carbon tetrachloride at 50 ppm had slight fatty liver changes at 62 days, evidence of adaptation at 188 days, and practically normal livers 28 days after the last of 225 exposures.

Continuous exposure of guinea pigs, monkeys, rats, and dogs to carbon tetrachloride at 10 ppm for 90 days caused depressed growth in all species. [68] Monkeys had an emaciated appearance and suffered hair loss. All animals, except the dogs, had enlarged or discolored livers, but the tissue structure was not well described or compared to controls. The enzymatic activities of the livers of the rats and guinea pigs, the most affected species, were normal; however, the fat content of the livers of exposed guinea pigs was 35% compared to 11% in the controls.

In a similar experiment, where the same species were exposed continuously for 90 days at 1 ppm, there were no signs of toxicity during testing. [68]

Absorption of liquid carbon tetrachloride through the skin of humans and animals has been demonstrated experimentally. [48,66,67] Lapidus [66] found carbon tetrachloride in the blood, liver, and fat of 4 rabbits after

each had had 1 ear immersed in carbon tetrachloride. The immersion times for the 4 rabbits were 5, 6, 8, and 9 hours.

Human subjects who each immersed one thumb in liquid carbon tetrachloride for 30 minutes had measurable amounts of carbon tetrachloride in exhaled air 5 hours later. [48] The investigators concluded that the amount of carbon tetrachloride that could penetrate the skin depended on the type of skin, the area exposed, and the duration of exposure. Using the data from the experimental exposure of 1 thumb, they estimated that the amount of carbon tetrachloride absorbed during topical exposure of both hands for 30 minutes would be equivalent to a vapor exposure of about 10 ppm for 3 hours.

Absorption of liquid carbon tetrachloride through the skin could have been a contributing factor in many occupational cases. [22,45,54,56,57,59,60] However in most reports it was not considered by the investigators, although its occurrence was evident because of the nature of the work being performed.

Alcohol was a concomitant factor in many of the human cases of carbon tetrachloride poisoning, especially in cases where severe liver and kidney damage occurred. [31,32,34,50-53] That ingestion of ethyl alcohol can increase the hepatoxicity of carbon tetrachloride has been demonstrated by animal experiments. Marked degenerative fatty infiltration and early centrilobular liver necrosis were found in rats given ethyl alcohol (5 ml/kg) orally 16-18 hours before exposure to carbon tetrachloride at 1,000 ppm for 2 hours. These changes did not occur in controls exposed only to carbon tetrachloride. [98] With 2-hour exposures to carbon tetrachloride at 100 ppm, SGOT activity was increased in rats given alcohol before

exposure, but not in those which were not given alcohol. Serum enzymes or liver tissue were not altered in rats pretreated with alcohol and exposed at 25 or 50 ppm carbon tetrachloride for 2 or 8 hours.

Similar potentiating or augmenting effects including increased liver enzyme activity, increased liver triglyceride content, and increased carbon tetrachloride metabolism were found when carbon tetrachloride exposure occurred together or in temporal proximity with exposure to barbiturates or chlorinated hydrocarbons such as DDT. [90,94,103,104]

A summary of concentration response data for inhalation exposures to carbon tetrachloride is presented in Table XI-10.