III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

Methylene chloride, (CH2C1 2), also known as methylene dichloride and dichloromethane, is a colorless liquid at room temperature (25 C) with a pleasant odor. [1] Its odor threshold has been determined to be about 200 ppm. [2,3] Some of its physical properties are listed in Table XII-1. [1,4-7]

Commercial production of methylene chloride is by chlorination of methane or methyl chloride which yields a mixture of chloromethanes. Methylene chloride is fractionated from the mixture after washing and alkali scrubbing. Reduction of higher chlorinated methanes, another method of possible manufacture, has no industrial importance. [1,8]

The United States production of methylene chloride increased from 73,963,000 pounds in 1955 to 471,276,000 pounds in 1972. [9-26]

In 1972, manufacture of methylene chloride was reported by 6 companies in the United States. [26] In addition to potential exposures associated with its manufacture, employees of at least 50 companies were potentially exposed to methylene chloride in the manufacture for the retail market of paint and varnish removers, insecticides and fumigants, solvents, cleaners, pressurized spray products, fire extinguishers, and Christmas tree bubble lights. [27]

Persons exposed to methylene chloride in their work could be subjected to additional exposure by home use of commercially available products containing methylene chloride. [27]

Exposure to methylene chloride could occur with each of its many

industrial uses. It is used widely in industry for paint stripping, manufacture of photographic film, and in aerosol propellants. It is used as a solvent in degreasing, in the diphasic treatment of metal surfaces, in the textile and plastic industries, as the carrier in rapid-dry paints, and for extracting heat-sensitive edible fats and essential oils. [1,7,8]

Because of its use as an extractant, it is allowed as a food additive in amounts up to 30 ppm in spice oleoresins, and up to 10 ppm in roasted and instant decaffeinated coffee (21 CFR 121.1039).

NIOSH estimates 70,000 people in the US are potentially exposed to methylene chloride in their working environment.

Historical Reports

In 1867, in England, Richardson [28] reported on human and animal experiments with what he thought was methylene chloride. However, his description of its properties included the observation that the material burned readily, indicating that he was not using methylene chloride.

Junker [29] in 1883 summarized 10 deaths in England from "methylene" anesthesia and pointed out that what was used as methylene chloride was in fact a mixture of chloroform and methyl alcohol. Therefore effects of methylene chloride described prior to this report are of doubtful validity.

The following year Regnauld and Villejean [30] reported that the material obtained from England and sold in France as methylene chloride was also a mixture of chloroform and methyl alcohol. Using purified methylene chloride, they found that if anesthesia was prolonged, anesthetized dogs consistently developed clonic movements and epileptiform attacks. In 1922, similar effects were reported by Hellwig [31] to occur in humans, and were

confirmed in 1923 by Bourne and Stehle [32] in dogs and humans. These investigators [31,32] reported a stage of methylene chloride anesthesia, not characteristic of other agents, in which analgesia and unconsciousness could be obtained without loss of muscle tone. With careful administration of methylene chloride, these states could be maintained for hours.

Grasset and Gauthier [33] reported that because of its analgesic properties methylene chloride could be used during labor without concurrent loss of consciousness or muscle tone. All 44 of the women in the study experienced retrograde amnesia.

In 1933, Nuckolls [34] exposed guinea pigs to methylene chloride vapor in concentrations of approximately 10,000, 20,000, and 50,000 ppm for 2 hours. Each group of animals was observed after 5 minutes, 30 minutes, 1 hour, and 2 hours of exposure. At all concentrations, the guinea pigs developed tremors fairly soon after the onset of exposure. Other muscular activity was noticed, for example, twitchings, noticeable movements of the diaphragm, trembling, and loss of coordination. After 1 hour of exposure at 10,000 ppm or 5 minutes of exposure at either 20,000 or 50,000 ppm, most animals became partly anesthetized, and, as the exposure continued, a few were completely anesthetized. Convulsive movements were noted in all animals, even in those that were anesthetized. Autopsy revealed congested and edematous lungs in some animals.

Collier's [35] 1936 report was the first in the English language of adverse effects of occupational exposure to methylene chloride. In this report he quoted a manufacturer of lacquers who discontinued the use of methylene chloride because of its effects on the workers: "...it dopes them, makes them stupid, they suffer from headache, are unreliable at their

work, are awfully apt to stumble around and hurt themselves, are irritable, unhappy and require constant supervision if they are to be kept from making silly mistakes."

The first report of adverse effects in the American literature appeared in 1947. [36] In this report, 4 men had been overcome by methylene chloride exposure in a hops extraction process, and one died. Concentrations were not reported.

Effects on Humans

(a) Central Nervous System Function During Experimental Exposures

Central nervous system (CNS) function was reported to be impaired during methylene chloride exposure by Fodor and Winneke, [37] Winneke, [38] and Stewart et al. [39]

Fodor and Winneke [37] and Winneke [38] used two indicators of behavioral performance: (1) the visual critical flicker frequency (CFF), and (2) an auditory vigilance task.

The CFF is a measure of the frequency of flickering light at which perception of the flickering changes to nonperception, or vice versa. It was determined experimentally by starting with a rapidly flickering light and gradually decreasing the flickering rate until the subject perceived the flickering. The brightness of the flicker light and the on-off ratio were held constant throughout the experiment. During every exposure of each subject, 8 CFF determinations were made at each of the following times: 30, 80, 130, 180, and 230 minutes.

The auditory vigilance task involved detection of faint and frequently occurring auditory signals. The subject listened through

earphones to a series of pulses of white noise, 0.3 seconds in duration and 2 seconds apart. The noise was 30 decibels above the threshold of sound and at random intervals with a probability of 0.033, the intensity was decreased by 4.8 decibels. The subject was required to detect this difference and report it. Each session lasted 3 hours and consisted of 4 observation periods of 45 minutes each. The measurements of performance were the percentages of signals missed within each of the 12 successive 15-minute observation periods.

In the study reported by Fodor and Winneke, [37] female volunteers, 20-30 years of age, were each exposed to methylene chloride at 300 and 800 ppm. For each subject, exposure at the different concentrations occurred 1 week apart. The subjects were tested individually during 4-hour exposure periods. The 18 x 10 x 9-ft exposure chamber was maintained at constant temperature, humidity, and atmospheric pressure. Gas chromatography was used to measure the methylene chloride concentrations.

Fodor and Winneke [37] found that in 6 subjects the CFF decreased to approximately the same end point under exposure at both 300 ppm and 800 ppm, but the response at 800 ppm was much more rapid than at 300 ppm. After 50 minutes of exposure at 800 ppm, a decrease from 36 flashes/second at the beginning to less than 33 flashes/second was observed and reported as statistically significant. At 300 ppm, a decrease approaching this magnitude was not observed until 140 minutes of exposure. In a control experiment with the same 6 subjects, the CFF did not fall below 34 flashes per second.

The ability to detect sound signals in an auditory vigilance study was reduced by exposure to methylene chloride, particularly during the

middle part of the 4-hour exposure. The authors [37] considered that during the last period of testing there was an "end-spurt" performance or extra effort by the subjects. At 800 ppm, the subjects missed up to 30% of the signals, whereas at 300 ppm they missed no more than 20%, compared to around 10% when not exposed to methylene chloride.

Four experiments with human volunteers exposed to methylene chloride at average concentrations of 317, 470, or 751 ppm for 3 or 4 hours were reported by Winneke [38] in 1973. He also reported exposures of volunteers to carbon monoxide (CO) at 50 or 100 ppm for 5 hours. All experiments took place in a 3 x 6 x 3-meter chamber. Methylene chloride or CO were metered into the chamber and mixed with air to the desired atmospheric concentrations. Methylene chloride was monitored by gas chromatography (GC) and CO by infrared (IR) absorption. The auditory vigilance task, CFF, and a battery of psychomotor tasks were studied to ascertain the effects of exposure.

Subjects exposed at concentrations of 317, 470, and 751 ppm of methylene chloride for 3-4 hours showed decreased CFF, auditory vigilance, and decreased performance in most psychomotor tasks, when compared with controls. The performances were less influenced by exposures at 317 and 470 ppm than at 751 ppm.

By contrast, the 18 volunteers exposed at 50 or 100 ppm of CO showed no impairment of CNS function as measured by these tests. [38]

In experiments reported by Stewart et al [39] in 1972, a total of 11 male subjects ranging in age from 23 to 43 years were exposed in a series of experiments to methylene chloride in concentrations from 213 ±10.4 to 986 ±104 ppm. Concentrations in the exposure chamber were continuously

monitored by IR spectrometry and periodically checked by GC.

One subject exposed for 1 hour to methylene chloride at 213 ppm (SD of 10.4 ppm) reported no unusual feelings during the experiment. [39] All 8 subjects exposed to methylene chloride at 514 ppm for 1 hour also reported no unusual feelings. However, 1 of 3 subjects exposed to methylene chloride, first at 514 ppm for 1 hour then at 868 ppm for a second hour, (experiment A) experienced light-headedness 15 minutes after the concentration was increased.

Three subjects exposed to methylene chloride at 986 ppm (SD of 104 ppm) for 2 hours (experiment B) reported its odor to be moderately strong, but experienced no sensory irritation. [39] Two subjects reported light-headedness after 1 hour of exposure. This feeling cleared 5 minutes after the exposure ceased.

CNS function was studied in experiments A and B by means of the Visual Evoked Response (VER), or change in electroencephalograms in response to a flashing light. In experiment A there were decreases in the amplitude of the response, indicating CNS depression, at the end of the first hour of exposure but not at the end of the second hour. In experiment B, 2 of the 3 subjects showed CNS depression at the end of the first hour and all 3 at the end of the second hour.

Studies of 2 groups of 7 men, 20-30 years of age, during 2 sessions, one with exposure to methylene chloride and the other while breathing control air were reported by Gamborale et al [40] in 1975. In each group the 2 study sessions were held one week apart. In one group, the control session occurred first, and in the other group the methylene chloride exposure session occurred first. Each study session was of 2 hours

duration. Except for the presence of methylene chloride, the experiments were conducted in the same way. During the methylene chloride exposure sessions, the concentration was increased at 30-minute intervals. During the first 30 minutes the concentration was 250 ppm, then 500 ppm, then 750 ppm, and during the last 30 minutes of the 2-hour session, the exposure concentration of methylene chloride was 1,000 ppm.

During the last 20 minutes at each exposure concentration 4 performance tests were conducted. These tests included an addition-reaction time test, a short-term memory test, and 2 simple reaction time tests. Responses to these tests were not affected by exposure to methylene chloride. Upon removal from exposure, the subject's evaluation of their own condition indicated that methylene chloride exerted a subjectively favorable change in their experience of calmness, relaxation, disposition, affection, alertness, and activeness.

(b) Impairment of Biological Oxidation

By chance, Stewart and co-workers [39] observed carboxyhemoglobin (COHb) concentrations of 6% and 8% on 2 occasions in a subject after he had used varnish remover containing methylene chloride. Prompted by this observation, they studied COHb and alveolar CO concentrations during and following the above mentioned experimental exposures to methylene chloride described in the previous section.

Prior to exposure, COHb, RBC count, and serum bilirubin were determined and alveolar breath samples were analyzed for CO and methylene chloride by both GC and IR methods. [39]

In a subject exposed to methylene chloride at 213 ppm for 1 hour, the COHb rose from its baseline of 0.4% to 1.5% after 30 minutes of exposure,

1.75% after 60 minutes, and after removal of the subject from exposure, COHb continued to rise to 2.4% at 3 hours after the end of exposure. Twenty hours after exposure, the COHb measurement was 1.5%. In the exposures at the higher methylene chloride concentrations (514 ppm for 1 hour, 514 ppm for 1 hour followed by 869 ppm for 1 hour, and 986 ppm for 2 hours), similar patterns of COHb build-up and disappearance were observed. The peak concentrations of COHb as well as those remaining 17-24 hours later were proportional to the methylene chloride exposure concentrations and times, as were those remaining 17-24 hours after removal from exposure.

This group of investigators have continued their studies of human exposures to methylene chloride. [41,42,43] Male subjects were exposed for 1, 3, and 7.5 hours/day, 5 days/week for 5 weeks as shown in Table III-1. The 3 subjects in group A were exposed 1 hour/day at each concentration, the 3 subjects in group B were exposed for 3 hours/day, and the 4 subjects in group C were exposed for 7.5 hours/day.

The maximum COHb values observed in nonsmokers are shown in the last column of Table III-1. In the 3-hour and 7.5-hour exposures, COHb concentrations generally reached maximum values by the end of exposure, although in a few cases, particularly on Fridays, higher values were observed one hour after exposure than at the end of exposure. In contrast, in the 1-hour exposures most of the COHb values observed 1 hour after exposure were greater than those at the end of exposure. [41]

With 7.5-hour exposures to methylene chloride at 50 ppm, the maximum observed COHb concentrations averaged 2.9% in nonsmokers; at 100 ppm methylene chloride, the maximum observed COHb concentrations in nonsmokers averaged 5.7% on the 5th consecutive day of exposure. [41]

TABLE III-1

SCHEDULE FOR EXPOSURE OF 10 MEN TO METHYLENE CHLORIDE

Exposure Week	Exposure Concentration ppm	Exposure time, daily hours	Number of Subjects	Subject Group	COHb ppm %
1	50	1	3	A	1.3
1	50	3	3	B	1.9
1	50	7.5	4	C	2.9
2	250	1	3	A	3.0
2	250	3	3	B	5.0
2	250	7.5	4	C	10.3
3	250	1	3	A	2.5
3	250	3	3	B	4.3
3	250	7.5	4	C	9.5
4	100	1	3	A	3.2
4	100	3	3	B	3.0
4	100	7.5	4	C	5.7
5	500	1	3	A	4.4
5	500	3	3	B	5.6
5	500	7.5	4	C	11.7

Derived from references 41-43

During the week, COHb values had not returned to normal on Tuesday through Saturday mornings with 7.5-hour exposures at more than 50 ppm methylene chloride. However, by Monday morning, COHb had returned to baseline values. When exposures at 100 ppm or more were of 1 or 3 hours duration, COHb appeared to have returned to baseline values daily, although the data were limited. [41]

In a similar experiment, [42] women were exposed to methylene chloride at 250 ppm on 5 consecutive days. The baseline COHb values were higher in the women (1.3%) than in the men (0.8%). With each exposure time, (1, 3, and 7.5 hours) comparably higher COHb values were found in

nonsmoking women than in nonsmoking men exposed at 250 ppm, as shown in Table III-2.

TABLE III-2

CARBOXYHEMOGLOBIN CONCENTRATIONS IN NONSMOKERS EXPOSED TO METHYLENE CHLORIDE AT 250 ppm

Exposure time, hours	Maximum observed average COHb (%)	
	men [41]	women [42]
0	0.8	1.3
1	3.0	4.0
3	5.0	5.4
7.5	9.6	10.6

COHb formed during and following experimental exposure of human subjects to methylene chloride during rest and exercise was reported by Astrand et al [44] in 1975. These investigators exposed 4 or 5 subjects to methylene chloride in 3 experiments at 250 and 500 ppm at rest and during exercise on a bicycle ergometer. In each experiment, the total exposure time was 2 hours with the 1st 30-60 minutes of exposure occurring during rest. The workloads imposed during the remainder of the exposure were equivalent to 21.7, 43.4 and 65.1 ft-lbs (50, 100, 150 watts). COHb in blood was reported as g/100 ml rather than % COHb. Considering normal Hgb as 15.4 g/100 ml, [44] 1% COHb would be equivalent to 0.154 g COHb/100 ml blood. These investigations also substracted preexposure COHb from the amounts found during exposure.

Because of the design of the experiment, the effects of methylene chloride concentration, exercise level, and exposure time were not clearly seperate. However, it appears from the data that exercise during exposure resulted in lower COHb values at the end of 2 hours of exposure than have been found by others with 2 hours of exposure at rest. COHb continued to rise for up to 3 hours after removal from exposure with exercise, compared to up to 1 hour in experiments previously reported by others. [41,42]

Effects of exposure to a paint remover during rest and exercise were reported by Stewart and Hake [45] in 1976. The volatile components of the paint remover were 80% methylene chloride and 20% methanol by weight. The volume of the exposure chamber was 2,680 cu ft. During a 3-hour experiment, 1 quart of the paint remover was applied to, and scraped from, a baby crib. One subject applied and scraped the paint remover, while another subject sat in the chamber so the results of exposure at work and at rest could be compared. The experiment was conducted 4 times at different ventilation rates and the methylene chloride vapor concentration in the breathing zone of the subjects was continuously monitored by IR. The average breathing zone concentrations of methylene chloride in the 4 experiments were 216, 368, 654, and 788 ppm. Methanol concentrations measured at the end of each hour of exposure averaged 77, 115, and 186 ppm, prespectively in the first 3 experiments, and was not measured in the other.

In these experiments, [45] there were small differences in the COHb values of the active and inactive subjects at the end of exposure, but the differences increased after exposure as the COHb continued rise for up to 4 hours in the active subjects. In all subjects the return of COHb to preexposure values was delayed in comparison to subjects exposed to only

methylene chloride. [41,42] The authors [45] proposed that this prolonged maintenance of elevated COHb was an effect of methanol on methylene chloride uptake and metabolism.

The affinity of 4 subjects' hemoglobin for oxygen was determined during the 5th to 7th hour of exposure on Thursday or Friday of each exposure week. [43] The affinity of Hgb for oxygen was increased. That is, the ability of the red blood cells to give up oxygen to the tissues was decreased. The average oxygen tensions required to saturate 50% of the Hgb are summarized in Table III-3. The data show a progressive decrease in the oxygen tension with increasing methylene chloride exposure concentration, and some recovery toward normal in the week following the last exposure.

The physiological significance of this change in the oxygen binding property of hemoglobin may be minor since arterial blood lactate following exercise was only slightly elevated when the exposure was to methylene chloride at 500 ppm, and not at all elevated when the exposure was at 100 ppm. [43]

TABLE III-3

OXYGEN TENSION FOR 50% HEMOGLOBIN SATURATION
IN SUBJECTS EXPOSED TO METHYLENE CHLORIDE

Exposure week	Exposure concentration ppm	Oxygen tension mm Hg
1st	0	26.7
2nd	50	26.3
5th	100	24.7
3rd	250	24.4
4th	250	24.0
6th	0	25.9

Derived from Forster et al [43]

(c) Absorption

Absorption of inhaled methylene chloride in 2 subjects was reported by Lehmann and Schmidt-Kehl [46] in 1936. The methylene chloride in the room air and in the exhaled air was collected in alcohol and analyzed by alkaline hydrolysis. The percent absorbed was determined from the ratio of the concentration in exhaled air to the concentration in room (inhaled) air. Both subjects absorbed similar amounts; the authors averaged and reported the data as shown in Table III-4.

TABLE III-4

ABSORPTION OF METHYLENE CHLORIDE BY HUMAN SUBJECTS

Experimental day	Inhalation Concentration mg/liter	ppm	Exposure time, min	Abosrbed %
1	2.3	662	20	74
2	2.8	806	30	75
3	4.0	1,152	30	72
4	4.1	1,181	30	70

Derived from Lehmann and Schmidt-Kehl [46]

Other investigators [47,48] reported that lesser amounts of methylene chloride were absorbed. In 1966, Riley et al [47] reported absorption of methylene chloride in 1 subject exposed at 100 ppm for 2 hours as ranging from 70% at the beginning of exposure to 31% at the end. The exposures took place in a specially constructed room where methylene chloride concentrations were measured with a continuously recording hydrocarbon analyzer. The subject was seated and not required to carry out any

physical work.

During exposures at 100 and 200 ppm in the experiments reported by DiVincenzo et al, [48] 50-66% of inhaled methylene chloride was absorbed. (Tables XII-2, XII-3, and XII-4). Astrand et al [44] found that during 30-minute periods of exposure at 250 and 500 ppm at rest about 55% of the inhaled methylene chloride was absorbed. During 30-minute periods of exposure during exercise, the percent absorbed decreased but, because of the greater amount inhaled, the total amount absorbed was increased 2-3 times by exercise.

Stewart and Dodd [49] studied absorption of methylene chloride through the skin of the thumb. Four subjects each immersed 1 thumb in methylene chloride for 30 minutes. Breath concentrations of methylene chloride at 10 and 30 minutes of exposure and at 10, 30, 60, 120, and 300 minutes after exposure are shown in Table III-5.

TABLE III-5

ALVEOLAR AIR CONCENTRATIONS OF METHYLENE CHLORIDE FROM THUMB IMMERSION

After beginning of exposure	
Time, min	Concentration, ppm
10	1.4-2.4
30	2.3-3.6
After end of exposure	
10	2.1-4.1
30	1.1-6.6
60	0.6-4.1
120	0.26-1.7
300	<0.1

Derived from Stewart and Dodd [49]

(d) Excretion and Blood Concentrations

In the experiments reported by DiVincenzo et al, [48] concentrations of methylene chloride in the blood and its excretion in the breath and urine were studied in 11 healthy human volunteers, 28-60 years of age. Exposures were either at 100 or 200 ppm for 2 hours, or at 100 ppm for 4 hours. To establish exposure room concentrations, liquid methylene chloride was delivered at constant flow over a heated surface. The resulting vapor was carried in a continuous flow of nitrogen into the intake mixture throughout the chamber where day to day variations in methylene chloride concentrations were ±5-10% of the intended concentration. During exposure, the subjects were usually seated in a 5 x 6.5 x 11-ft chamber. In some experiments conducted in the same chamber, the subjects exercised intermittently during the exposure.

Exhaled breath samples, not alveolar air samples, were collected in plastic bags and 25-ml aliquots were injected into the gas chromatograph. Two-milliliter samples of venous blood were transferred to 25-ml heparinized Erlenmeyer flasks, and urine was collected in amber bottles from which 2-ml aliquots were transferred to 25-ml Erlenmeyer flasks for analysis. The Erlenmeyer flasks containing the urine and the blood samples were heated at 75-80 C for 5 minutes, and head space vapor was drawn off directly into the chromatograph for methylene chloride analysis.

DiVincenzo et al [48] in 1972 graphically summarized the data on excretion of methylene chloride in the breath. The tabular data, subsequently supplied to NIOSH by the authors, are presented in Tables XII-2 to XII-6. The subjects, while at rest, were exposed either at 100 or 200 ppm of methylene chloride for 2 hours (Tables XII-2 and XII-4). The

magnitude of exhaled breath concentrations was directly proportional to the magnitude of exposure. After the exposure ceased, the concentration of methylene chloride in the breath decreased rapidly and by 5 hours the concentration was less than 1 ppm.

With a given concentration, DiVincenzo et al [48] found that increasing the exposure time from 2 hours to 4 hours did not double the concentrations of methylene chloride in exhaled breath after exposure, but doubling the exposure concentration did. The postexposure breath concentrations for the 2-hour and 4-hour exposures at 100 ppm of methylene chloride are shown in Tables XII-2 and XII-3.

Individuals who exercised during exposure at 100 ppm absorbed more methylene chloride than those who did not exercise, as evidenced by elevated postexposure excretion (Table XII-6). [48]

The concentrations of methylene chloride in blood during and after the 2-hour exposure at 200 ppm, and after the 2-hour exposure at 100 ppm, are presented in Tables XII-7 and XII-8. [48] The concentrations in blood at the end of exposure were proportional to the initial exposure concentration. Upon cessation of exposure, blood methylene chloride decreased rapidly at first, then more slowly.

The urinary excretion of methylene chloride by 4 individuals exposed at 100 ppm for 2 hours and by 7 individuals exposed at 200 ppm for 2 hours was studied. [48] An average of 22.6 μg of methylene chloride was excreted in 24 hours by the group exposed at 100 ppm (Table XII-9) and an average of 81.6 μg by the group exposed at 200 ppm (Table XII-10). Fluid consumption was not regulated.

Venous blood concentrations found by Astrand et al [44] in subjects exposed at 250 ppm for 2 hours who had exercised for 1.5 hours averaged 3.6 mg/kg compared to a maximum of 2.2 mg/liter reported by Divincenzo et al [48] for their subjects who had been exposed at rest. Four hours after removal from exposure the concentration of methylene chloride in the subjects reported by Astrand et al [44] was 0.8 mg/kg compared to a maximum of 0.155 mg/liter in the subjects reported by Divincenzo et al. [48]

Based on data from a series of exposure concentrations (44-680 ppm) and exposure times (2-150 minutes), Riley et al [47] developed a model for estimating exposure concentrations from methylene chloride concentrations in the breath after exposure. They had an opportunity to test the model when a workman was accidently anesthetized by a 4-hour exposure to methylene chloride. Breath samples collected 3.5, 19, and 44 hours after the workman was removed from the exposure area contained approximately 500, 30, and 3 ppm methylene chloride, respectively. Using the model, they estimated that the man had been exposed at 8,000-10,000 ppm of methylene chloride.

(e) Occupational Exposures

One of the earliest reports of methylene chloride intoxication in industry was by Collier [35] in 1936. Four painters had been removing paint from the walls of a large room with closed windows. A paint remover which contained approximately 96% methylene chloride was used to soften the paint which was then scraped off by hand. Concentrations of methylene chloride to which the men were exposed were not determined. All 4 men complained that while using the paint remover they became faint, giddy, and "stupid." They also stated that "the stupor passed off after a few hours,"

that they "felt better when not at work," that the "stuff upset their appetites," and that they "did not care for food." Two of the 4 men were sufficiently ill to have to leave work.

One of them was 42 years old and had been a painter for 13 years. He complained of irregular but severe pains in the legs and arms, "hot flushes," headache, vertigo, "stupidity" while working with paint remover, poor eyesight at night, anorexia, precordial pain, rapid pulse, shortness of breath, great fatigue on exertion, and attacks of rapid heart beat.

The second painter examined by Collier [35] was 45 years of age, had been a painter for 20 years, and had been using the paint remover indoors extensively for the last 2 years. He claimed to be drowsy at work and disinclined to do anything in the evenings. He was irritable and had pains in his head and tingling in his hands and feet. In this case, the blood picture was essentially normal and showed no punctate basophilia. Collier [35] found that the patient had a peptic ulcer and had suffered intermittently for 2 years from methylene chloride intoxication. No specific laboratory studies to confirm methylene chloride poisoning were reported.

A report of 4 cases of acute exposure to methylene chloride was made by Moskowitz and Shapiro [50] in 1952. The exposures occurred in a factory in which methylene chloride was used to extract an oleoresin from plant material. The operations in the plant were mainly in closed systems, but there was some opportunity for methylene chloride vapor to escape into the workroom. Workers were instructed to leave the plant as soon as they could detect the odor of the solvent. The exposure took place during the night shift in the winter. The exposure times were known to have been from less

than 1 hour to about 3 hours, but the concentrations of methylene chloride in the work atmosphere were not known. The 4 men were found unconscious, either on the first floor or in the basement. Two of the 4 workers had contusions and lacerations of the head. They were all removed to a hospital where 1 worker was found to be dead on arrival and the others recovered. The 3 survivors remained unconscious for about 2.5 hours after they were removed from the plant. [50]

Two of the men were hospitalized for 4 days, and the other for 8 days. None of them remembered smelling methylene chloride. They exhibited signs of either eye, lung, or respiratory tract irritation. They had low hemoglobin values (76-79%) and low RBC counts (3,550,000-3,950,000). All other findings appeared normal. The individual who died had been employed in the plant for 7 months prior to the accident. On necropsy the dura mater was found adherent to the skull and the veins of the pia-arachnoid were conspicuously engorged. The authors reported that there was no evidence of skull fracture. The lung tissue contained 0.1 ml of methylene chloride per 500 g of tissue. [50]

A 19-year-old laborer was the subject of a report by Hughes [51] in 1954. The man was exposed to methylene chloride for 4 hours while degreasing copper gaskets in a small, poorly ventilated room. Concentrations of methylene chloride were not measured. He reported that he noticed an oppressive odor and that his eyes were irritated. During the latter part of the shift he complained of excessive fatigue, weakness, sleepiness, lightheadedness, chilly sensations, and nausea. He was admitted to the hospital with shortness of breath, substernal pain of 8 hours duration, weakness, a dry nonproductive cough, temperature of 100 F,

pulse of 100, and respiratory rate of 42. Rales were heard at the base of his right lung and occasionally elsewhere over the chest. A chest x-ray showed diffuse spongy infiltration of both lung fields, characteristic of pulmonary edema. The patient was treated with penicillin, and all symptoms subsided 18 hours after exposure to methylene chloride. Chest x-rays made after intervals of 3 and 5 days were reported as normal.

Christensen and Huizinga [52] reported on the case of a 17-year-old male who was found dead in a turret where he had been using a mixture of 80% methylene chloride and 14.9% methanol to remove paint. The autopsy showed slight enlargement of the liver and heart and voluminous lungs. The alveoli contained enlarged nodules of bloody fluid. Barbiturate derivatives were found by thin-layer chromatography in the blood, urine, brain, and stomach contents. Death was ascribed to the combination of barbiturates and methylene chloride.

Stewart and Hake [45] reported on a fatality following exposure to a paint stripping formulation that contained 80% methylene chloride. Other components of the formulation were not reported. Although this was not an occupational exposure, it was the result of an application of methylene chloride that does occur occupationally. The 66-year-old man used the paint stripper in his basement on 3 occasions for 2-3 hours each time. On each occasion he developed severe radiating retrosternal pain. On the 1st 2 occasions he was hospitalized. The patient's 1st hospital course was uncomplicated, but during hospitalization for the next acute myocardial infarction that occurred with the 2nd exposure 2 weeks later, cardiogenic shock, dysrhythmia, and heart failure occurred. The fatal exposure occurred 6 months after discharge from this hospitalization. On this final

occasion, the man developed chest pain, collasped, and died 2 hours after working slowly with the paint stripper.

(f) Phosgene Hazards from Methylene Chloride

Phosgene is a combustion product of methylene chloride, [34,53] and two cases resembling phosgene poisoning in persons using methylene chloride paint remover in the presence of kerosene flames were reported by Gerritsen and Buschmann [53] in 1960. A 52-year-old painter, working in a small room with the windows closed and a kerosene stove in use, experienced a burning sensation in his throat. He continued working and after a few hours had feelings of tightness in his chest. Later that day, he was admitted to the hospital after having been found extremely dyspneic and cyanotic. He died a few hours after arrival at the hospital. The diagnosis was influenzal pneumonia with pulmonary edema and cardiac decompensation. Extensive degenerative changes were found in the epithelium of the trachea, bronchi, and bronchioli together with hemorrhagic edematous focal pneumonia. Confirmatory microbiolgial studies were not performed. While there is no basis to question the physician's diagnosis, it seems that an equally tenable diagnosis, from the data presented, was phosgene poisoning.

In the second case [53], a 38-year-old woman in the 7th month of pregnancy used a paint remover containing methylene chloride in a cellar heated by a portable kerosene stove. The exposure occurred during a 3-hour period in the afternoon. In the evening, she had a feeling of tightness in her chest. She expectorated some blood-stained sputum. The next morning she felt much worse with symptoms of dyspnea and cyanosis. Her pulse was 120 and her temperature was 101 F. A chest x-ray revealed opacities similar to those seen in cases of pulmonary edema. The patient was treated

and discharged 8 days later. Two months later, she gave birth to a healthy child. [53]

Another case resembling phosgene poisoning was reported in 1964 by English. [54] A 67-year-old interior decorator used methylene chloride in the presence of a kerosene stove in a small, unventilated room. After 8 hours he experienced breathlessness, headache, giddiness, and a tight feeling across the chest. On admission to the hospital the next morning, the breathlessness had increased. The patient was cyanotic, sweating, and tachypneic, with extensive coarse rales in both lungs. He had extreme right hypochondrial tenderness. He had nausea with vomiting, dyspnea, hypotension, and a history of chronic bronchitis and a quiescent duodenal ulcer. Anorexia and intractable retrosternal and epigastric pain persisted for 4 weeks after admission. After 5 weeks of hospitalization the patient was discharged, but he experienced lassitude, weakness, and hypochondriasis for 3 additional months. Since the man had previously used paint strippers containing methylene chloride without such effects, the diagnosis was phosgene poisoning from methylene chloride decomposition in the presence of heat from the kerosene stove. [54]

Epidimiologic Studies

A study of 33 workers, 17 women and 16 men, who used methylene chloride in the production of cellulose acetate film foils, was reported by Kuzelova and Vlasak [55] in 1966. The film foils were produced from a solution of 13% cellulose triacetate, 78% of which was methylene chloride, and the remainder a mixture of methanol, triphenylphosphate, and dibutylphthalate. The methylene chloride also contained impurities of

0.25% methyl chloride, 0.25% chloroform, and 1% ethyl alcohol. The concentrations of methylene chloride in the air of the workplace were determined for 13 locations within the plant, but the method of analysis was not stated. The concentrations ranged from 30 to 5,000 ppm (0.1-14 mg/liter) (Table XII-11). [55]

Most of the workers had been exposed for an average of 2 years. Many of the exposed workers reported experiencing a sweet taste, mild intoxication, and heart palpitations. The authors [55] reported that 72% of the workers complained of headache and 50% of increased fatigue, that 49% had irritation of the upper respiratory tract and conjunctiva, that 30% had digestive disorders and that neurasthenic disorders were found in 50% of the exposed persons. Clinical and laboratory examinations revealed no abnormalities. On the assumption that methylene chloride was metabolized to formaldehyde and formic acid, the investigators [55] studied formic acid in the urine. Although formic acid was found in the urine of most exposed persons, there was no correlation between the intensity of exposure to methylene chloride and the concentration of formic acid in the urine. [55]

Fifty-nine workers chronically exposed in Russian chemical plants to mixtures of chlorinated methanes including methylene chloride were studied by Fokina [56] and reported in 1965. The maximum permissible concentrations (MAC) of individual components were exceeded at times, but detailed exposure data were not presented. The Russian MAC for the chlorinated methanes were: methyl chloride, 2.42 ppm; carbon tetrachloride, 3.2 ppm, methylene chloride, 14.4 ppm; chloroform, not established at that time. [57] Thirty-three of the workers showed signs of autonomic dysfunction, including deterioration or disappearance of the

corneal reflexes, dissociation between the deep (exaggerated) and superficial (sluggish) reflexes, marked persistent dermographism, general hyperhydrosis, acrohyperhydrosis, blotchiness of the skin on hands and forearms, tenderness when pressure was applied to specific cervical points, and arterial hypotension. In 18 workers there were signs of "diencephalic" disturbances including 13 with "narcoleptic-like" attacks of "irresistible somnolence," 4 with "autonomic vascular crises," and one with "cataleptic" type numbness. Signs of autonomic-sensory neuritis were found in 8 additional workers. Autonomic dysfunction was mainly found in workers employed for less than 3 years. Workers employed for more than 5 years showed autonomic polyneuritis and diencephalic disturbances. [56] The meaning of this study is difficult to interpret or apply because the exposure concentrations were not reported in sufficient detail and the terminology used to describe the worker responses was not clear.

A 39-year-old chemist in a pharmaceutical factory was judged by Weiss [58] in 1967 to have developed toxic encephalosis from chronic exposure to methylene chloride by inhalation and skin absorption. The chemist had worked several hours a day for 5 years in an unventilated 100-cubic meter room where sodium chloride was rinsed in methylene chloride and recrystallized. Methylene chloride in the workroom air arose from the distillation of the salt solution into 2 open containers, from opening the distillation apparatus several times each day to remove the salt, and by evaporation from the salt which was spread on filters to dry. Measurements of methylene chloride in the workroom air were 660 ppm above the container, 900 ppm in the breathing zone above the open distillation equipment, and 3,600 ppm at 20 cm above the ground. The method of analysis was not given.

[58] The hands and arms of the chemist were also exposed to liquid methylene chloride when the salt was removed from the distillation apparatus.

After 3 years of exposure the chemist complained of a burning pain around the heart, a feeling of pressure, palpitations, and restlessness. He also complained of forgetfulness, insomnia, and a feeling of drunkenness. At first these disturbances disappeared after he left the workplace, but later he claimed that he needed a few days away from the job to recover. During the last few months of the 5 years during which the worker had been exposed to methylene chloride, he developed auditory and visual hallucinations. Examination of the man did not show any organic disorder, especially no sign of liver trouble. The ECG showed no circulatory problems and there were no changes in hemoglobin or RBC count. There was slight erythema on the skin of the hands and underarms. Several days after removal from exposure the man felt better and eventually recovered. [58]

Ratney et al [59] studied concentrations of CO in their own alveolar air and that of 4 workers engaged in manufacturing plastic film. A commercial instrument that utilized a 3-electrode electrochemical cell was used to measure CO concentration. The workmen were 20, 21, 26, and 33 years of age, and the 3 members of the investigative team were 33, 33, and 41 years of age. Two of the workers were smokers who agreed not to smoke from 12 hours before the first alveolar air samples were taken and until the final samples were collected. The employees had worked in the plant 8 hours/day, 6 days/week for several years. The investigators and the workers were exposed to methylene chloride in the factory air on the day

prior to and on the day of the alveolar breath sampling.

In the manufacturing process, methylene chloride was the main component of the solvent mixture which also contained chloroform and toluene. Workroom air concentrations of methylene chloride were measured periodically for 42 hours, beginning 18 hours prior to alveolar breath sampling. [59] The environmental concentrations of methylene chloride are summarized in Table XII-12. (LD Pagnotto, written communication, December 1973) On the first day, the concentrations at the location where workers spent most of their time ranged from 210-471 ppm. During the day on which alveolar air samples were taken, methylene chloride concentrations at the same location, determined by collection on charcoal and analysis by gas chromatography, ranged from 159 to 219 ppm with a mean of 183 ppm. The authors [59] reported that there was no measurable CO in the workroom air.

Alveolar air samples were taken from the 7 subjects at regular intervals during a 24-hour period beginning 18 hours after environmental sampling had started. All of the breath samples were analyzed for CO and some for methylene chloride. Methylene chloride was detected qualitatively in the breath of the subjects. The authors [599] estimated COHb percentages from alveolar CO concentrations from the formula:

%COHb = 230 (PCO2) x (%O2Hb/PO2), where:

PO2 is assumed to be 98 mmHg, and

% O2Hb is assumed to be (99-%COHb).

The alveolar CO concentrations and the estimated COHb percentages derived from them for the 7 subjects are presented in Table XII-13. COHb, estimated from the breath sample CO measurements at the time the employees arrived at work on the day of the alveolar air sampling, averaged 4.9% with

a range of 3.3-5.3%. Eight hours after the onset of exposure, the mean estimated COHb was 8.3% with a range of 5.7-12.0%; at the beginning of the next workday, COHb ranged from 3.6 to 4.9%.

Animal Toxicity

(a) Acute Inhalation Exposures

In 1931, Flury and Zernik [60] reported their previously unpublished experiments in which they exposed animals to methylene chloride. Mice exposed for 2 hours developed narcosis at 10,000 ppm and died at 14,500 ppm. Dogs and rabbits exposed at 4,000 ppm for 6 hours developed light narcosis after 2.5 and 6 hours, respectively. After removal from exposure, recovery was "soon." During exposure to methylene chloride at 6,000 ppm for 6 hours, light narcosis was reached in 3/4 hour in rabbits and cats, 2 hours in dogs, and 2 to 2.5 hours in guinea pigs. All the experimental animals recovered from narcosis in 15-60 minutes after removal from exposure. The rabbits died 24 hours later, but no deaths were reported in the other species. The number of animals used in these experiments was not given.

Muller [61] in 1925 gave "fatal" concentrations for mice as 17,000 ppm (63 mg/liter) and Lazarew [62] in 1929 determined them at 14,500 ppm (50 mg/liter). Svirbely et al [63] exposed groups of 20 mice each to a series of methylene chloride concentrations (13,000-17,000 ppm) for 7 hours and estimated the LC50 to be 16,188 ±98 ppm within 8 hours after exposure. The mice exposed by Svirbely et al [63] were restless and had muscular twitchings, uncoordinated movements, labored respiration, and narcosis.

Von Oettingen et al [64] studied the effects of methylene chloride on dogs breathing through a tracheal cannula. At 15,000 and 20,000 ppm, pupillary and corneal reflexes disappeared after 10-20 minutes when methylene chloride concentrations in the blood were 33 mg%. Complete muscular relaxation developed after 25-35 minutes at which time methylene chloride concentrations in the blood were 42 mg %. At 40,000 ppm, corneal and pupillary reflexes also disappeared after 10-20 minutes, but complete muscular relaxation developed after 16 minutes when the methylene chloride concentrations in the blood were 46-50 mg %. Of the 5 dogs exposed at 40,000 ppm, 1 died after 2.5 hours, 1 after 4.5 hours, and 1 after 5 hours. No deaths occurred in the dogs exposed at either 15,000 or 20,000 ppm.

Other effects of methylene chloride on the central nervous system were studied by Berger and Fodor [65] who exposed rats to methylene chloride at concentrations ranging from 2,800 to 28,000 ppm. Electrodes were implanted on albino rats in order to study electroencephalograms (EEG) and electromyograms (EMG). In these exposures, an initial excitement period was followed by a deep narcosis, a decrease in muscular tonus detected by EMG and a reduction of EEG activity. This was followed by breathing difficulties, tremors, and a continued, gradual decrease in electrical amplitudes. At concentrations between 25,000 and 28,000 ppm, electrical activity stopped after 1.5 hours, and after 6 hours at concentrations between 16,000 and 18,000 ppm. Wheather the decreases in electrical amplitudes and the later cessation of electrical activity were due to EEG or EMG was not explained.

At concentrations ranging from 5,000 to 9,000 ppm, long sleeping periods occurred without desynchronization phases which usually appear every few moments with normal sleep. [65] Measurable changes in EMG and EEG patterns were not found with concentrations below 5,000 ppm, but when rapid eye movements (REM) were then examined as a criterion of sleep pattern, changes were found. A 14-hour inhalation of 2,800 ppm methylene chloride decreased the proportion of REM sleep in relation to the entire sleeping time. During the subsequent 24-hour period, the investigators [65] found an increase in wake periods and a normalization in the proportion of REM phases.

Sleeping patterns as indicators of CNS function were also studied by Fodor and Winneke [37] in albino rats exposed to methylene chloride. Sleep-wakefulness behavior was assessed by EEG and EMG activity on 3 consecutive days in 20 female albino rats weighing 180-220 g. On the first and third days, the animals inhaled air in a 16 x 16 x 25-inch test chamber. On the second day, the rats were exposed to methylene chloride at either 500, 1,000, or 3,000 ppm. Under nonexposure conditions the animals slept approximately 56% of the time, of which 16% was REM sleep. During exposure, total sleep time and the time between 2 successive REM periods increased in proportion to the methylene chloride concentration. At 500 ppm, the effect on total sleep time was small and REM sleep was almost identical to that in controls.

Running activity of male rats in activity cages was studied by Heppel and Neal. [66] Data for all the animals are shown in Table XII-14. Five young adult male rats were selected for this experiment on the basis of running activity. They were housed in regular stock colony cages and were

fed dry dog food and cabbage. The rats were put in activity cages in the evenings and allowed to adjust to their environment for 30 minutes. Then the number of revolutions of the drum was recorded for 1 hour. The rats were then removed from the chamber to feed for 90 minutes. Twenty preexposure determinations were made for each rat. The rats were then exposed to methylene chloride on 5 alternate days as follows: the exposure chamber was charged with 5,000 ppm methylene chloride, the rats were placed in the exposure chamber for 30 minutes to adjust to the environment, the running activity was measured for 1 hour, the vapor flow was turned off and 30 minutes were allowed to pass, then the activity was measured for another hour. On the intervening days, activity was measured as during the preexposure period. [66]

The average numbers of revolutions for the 5 rats during the preexposure period were 186, 482, 518, 725, and 928. These running scores were generally greater than those during the exposure period. For example, the rat which had a score of 725 during preexposure conditions had scores of 1, 136, 0, 276, and 232, respectively, during exposures to methylene chloride. During the exposure period, the rats ran more on the days when there was no exposure. On the exposure days they ran more during the postexposure hour than during the exposure hour. [66]

Excretion of methylene chloride in fasted male beagle dogs, 6 years of age, was studied by DiVincenzo et al. [48] In a 1-cubic meter inhalation chamber, dogs were exposed at 100, 200, 500, or 1,000 ppm for 2 or 4 hours. Methylene chloride was introduced into the chamber by means of a dual action syringe pump. Postexposure breath samples were collected with a tracheal tube or with a latex mask equipped with a 2-way valve. Venous

blood was withdrawn from the femoral vein. Samples were analyzed by gas chromatography. [48]

As with their human subjects, the serial breath excretion curves in the dogs were proportional to the initial concentration. [48] Methylene chloride was more concentrated in the breath of dogs than in that of humans exposed at the same concentrations for the same time, but humans eliminated methylene chloride at a faster rate. The concentrations of methylene chloride in the blood were parallel to those in the breath. The half-life of methylene chloride in the blood was found to be 1.5 hours. As in humans, only a slight increase in methylene chloride concentration in the breath occurred when the exposure time was doubled.

Carbon monoxide concentrations in the blood of a large number of rats exposed to methylene chloride, CO, and combinations of the two were reported by Fodor et al [67] in 1973. Carbon monoxide was determined by gas analysis methods and COHb percentages were estimated from the blood CO concentrations. The control air contained 0.5-2 ppm CO, and its inhalation by the rats for 3 hours gave control CO concentrations in the blood of 1.0 10.2 μg/ml of blood, and an estimated 0.4% COHb. Carbon monoxide in the blood following exposure at 100 ppm of CO for 3 hours was 27.4 μg/ml and the estimated COHb was 10.9%. Measurements of CO in the blood of rats exposed to methylene chloride for 3 hours were elevated as shown in Table III-6.

TABLE III-6

CARBON MONOXIDE IN RAT BLOOD AFTER METHYLENE CHLORIDE EXPOSURE

CH_2Cl_2 Concentration, ppm	μg CO/ml blood	Estimated COHb, %
50	7.6 ±1.3	3.2
100	15.3 ±2.4	6.2
500	26.1 ±3.3	10.5
1,000	31.1 ±3.7	12.5

Derived from Fodor et al [67]

With exposure for 3 hours to a mixture of 100 ppm CO and 100 ppm methylene chloride, CO in the blood measured 40.8 ±2.5 μg/ml, and the estimated COHb percentage was 16.4. When the exposure concentration of CO was 100 ppm and methylene chloride was 1,000 ppm, the measured CO in the blood was 47.3 ±5.5 μg/ml and the estimated COHb percentage was 19.0. [67]

Concentrations of COHb in the blood of rabbits exposed for 20 minutes at 2,000-12,000 ppm methylene chloride were found by Roth et al [68] to be a linear function of methylene chloride exposure concentration, approximately 5.5% at 2,000 ppm methylene chloride and 13% at 12,000 ppm. The rabbits were exposed individually by face mask. In 4-hour exposures at about 7,000 ppm methylene chloride (6,850-7,320 ppm) steady state COHb concentrations of 14% were reported. These reported COHb concentrations were the determined values minus the preexposure values, which averaged 0.75%. COHb was determined spectrophotometrically from absorbance at 3 wave lengths on a double beam spectrophotometer. [68]

wave lengths on a double beam spectrophotometer. [68]

(b) Chronic Intermittent Inhalation Exposures

Effects of daily inhalation of methylene chloride on several species of animals were reported by Heppel et al [69] in 1944. In a preliminary experiment, 2 monkeys, 2 dogs, 2 pups, 4 rabbits, 16 guinea pigs, and 16 young rats were exposed to methylene chloride at 500 ppm for 8 hours/day, 5 days/week for 15 weeks. Three of the 16 experimental rats died within the first 6 weeks; none of the 16 controls died. One exposed, and 2 of the 16 control guinea pigs died. All other exposed animals appeared to remain in excellent condition. Rabbits showed a slight increase in eosinophils after the 8th week of exposure; other hematologic measurements were not changed.

In a second preliminary experiment reported by Heppel et al, [69] 4 cats, 4 rabbits, 16 young rats, and 16 young guinea pigs were exposed to methylene chloride at 1,100 ppm for 8 hours/day, 5 days/week for 15 weeks. One experimental and 2 control guinea pigs died and 2 rabbits died postpartum. Cats "did not seem to thrive," but young rats and guinea pigs made good weight gains and the rats gave birth to many litters during the experiment.

Heppel et al [69] then exposed dogs, rabbits, guinea pigs, and rats in a 4 x 4 x 6-ft chamber at 5,000 ppm for 7 hours/day, 5 days/week for up to 6 months. Three of the 14 guinea pigs and one of the 20 rats died. The 2 female rabbits had litters during the experiment, but did not raise them. All 6 dogs, 2 puppies born in the exposure chambers, and 4 rabbits survived. The 2 pups gained weight normally. The exposed guinea pigs ate less than the controls and, at the end of the experiment, weighed 820 g compared to the control weight of 1,025 g.

There were no hematologic changes in rabbits, but RBC counts rose slightly in 3 of 5 dogs. Many other observations were made on the dogs without any abnormal findings. These observations included: the appearance of the cornea, sclera, eye grounds, eye movements, and mucous membranes; the states of deep reflexes and sensory perception; arterial blood pressure; bromosulfonephthalein (BSP) excretion, and icteric index; plasma proteins; and studies of the urine including pH, specific gravity, albumin, sugar, acetone, urobilin, urobilinogen, and cellular constituents.

Four dogs, 2 monkeys, 5 rabbits, 12 guinea pigs, and 16 rats were exposed to methylene chloride at an average of 10,000 ppm for 4 hours/day, 5 days/week for 8 weeks. [69] All animals became inactive during each exposure, but some underwent an initial excitement stage. Because of the severity of the excitement stage, dogs were not exposed more than 6 times. Three rabbits died, 1 each after 1, 12, and 22 exposures; 1 rat died after 33 exposures and another after 38.

All experimental animals exposed at 5,000 and 10,000 ppm and some of the controls were necropsied. Only pulmonary changes such as congestion and edema were found on gross examination. At 5,000 ppm, no microscopic lesions were found which appeared to be related to exposure. [69] At 10,000 ppm, moderate centrilobular congestion with narrowing of liver cell cords and slight to moderate fatty degeneration were found in 2 of the 4 dogs. No other organs were examined in the dogs. Slight to moderate fatty degeneration of the liver was found in 4 of the 6 exposed, and one of the 4 control guinea pigs. No microscopic lesions attributable to the exposure were found in the monkeys, rats, or rabbits. [69]

(c) Continuous Inhalation Exposures

Interest in possible exposure of astronauts to methylene chloride evaporated from construction materials in space cabins prompted research on continuous 90-day exposures of animals. Several reports of this research have been published by members of the investigative team. [70-74]

The effects on the livers of female mice exposed continuously at 5,000 ppm of methylene chloride for 7 days (168 hours) were studied by Weinstein et al. [71] Two "Thomas type" exposure domes were used as exposure chambers, one serving as a control chamber. For all experiments, mice were randomly separated into groups consisting of 13-20 mice. The groups of animals were maintained in the exposure chambers for the desired times, and then were killed by cervical dislocation within 5 minutes after removal from the chamber. The concentration of methylene chloride was maintained by a pressure-activated induction system and was continuously monitored with an automatic hydrocarbon analyzer. The atmospheric concentration of methylene chloride was 5,000 ±170 ppm. [71]

After the "first few hours" of exposure, physical activity and food and water intake decreased. Beginning at 24 hours of exposure, the mice became lethargic, assumed a hunched posture, and developed yellow, greasy, rough appearing hair coats. After 96 hours of exposure, normal activity resumed, eating and drinking increased and improvement was seen in postures and hair coats. At the end of the 168 hours of exposure, the abnormal signs had disappeared except for the appearance of emaciation and dehydration. [71] The body weights of the control mice remained fairly constant throughout the experiment, but the body weights of the exposed mice decreased from approximately 24 g to a mean of 18.0 g during the 7

days of exposure. The ratios of liver weight to body weight were significantly higher in animals from the exposed group throughout the experiment (Table XII-15). [71] Although the liver weights were not reported, the average weights calculated from the body weights and the liver/body weight ratios (Table XII-15) indicate an increased weight in the first days of the experiment, with a return to control values. This is in accord with the information on fat content. The concentrations of liver triglycerides were significantly greater in the experimental animals than in the controls on all days of exposure. In controls, the concentrations were about 10 mg/g wet weight throughout the experimental period. In the exposed animals, peak concentrations between 90 and 100 mg/g wet weight were reached after 72 hours of exposure. The concentrations then decreased to an average of 20 mg/g wet weight on the 6th and 7th days of exposure. [71]

With these exposures at 5,000 ppm, the earliest lesion in the liver dectected by the electron microscope was observed after 12 hours of exposure and consisted of dissociation of polyribosomes and swelling of the rough endoplasmic reticulum. Fatty infiltration was first noticed after 24 hours and involved the entire lobule. Hepatocytes in the central half of each lobule contained one or more large lipid droplets, while periportal hepatocytes contained many small droplets. On the 2nd day, centrilobular cells showed severe hydropic degeneration. On the 4th day, a few necrotic hepatocytes were observed as well as mild accumulations of lipid in Kupffer cells. On the 7th day, centrilobular fatty infiltration was found in the livers of the exposed mice. [71]

Incorporation of tritium labeled leucine into liver proteins was studied in 8 exposed and 8 control mice by Weinstein et al. [71] At exposure intervals of 4, 12, 24, and 48 hours, mice of both groups were given 20 μci/g body weight of the labeled L-leucine via the tail veil and killed 45 minutes later. Uniform labeling of hepatocytes was shown for all controls and mice exposed for 4 and 12 hours. Reduced labeling was observed after 24 hours of exposure and a further reduction was noted at 48 hours.

Fatty changes were found in the renal tubules of exposed mice on days 5 through 7. [71]

Continuous, 24-hour a day exposures of animals at either 1,000 or 5,000 ppm of methylene chloride for 14 weeks were studied by MacEwen et al [73] and reported in 1972. Concentrations of methylene chloride were continuously monitored with a flame-ionization hydrocarbon analyzer. Three exposure chambers [75] were used, 1 for control and 2 for exposure. Four female rhesus monkeys, 8 female beagle dogs, 20 male Sprague-Dawley rats, and 400 female ICR mice were placed in each. The chambers were operated at 50% relative humidity (±10%), 72 F (±5%), and 275 torr, or a little over 1/3 of an atmosphere.

At 5,000 ppm, animals of all species became relatively inactive in the first 2 days. Mice began to die on the 2nd day of exposure and 23 had died by the end of the 3rd day. The total number of mice that died during the first 35 days (as approximated from a graph) was 118. A monkey died after 10 days of exposure and 4 dogs died, 1 each on days 16 and 21, and 2 on day 22. Because of the severity of the responses, the exposures were discontinued after 35 days, except for 10 rats.

At 1,000 ppm, 6 of the 8 dogs died, 1 each on days 34, 38, 41, and 46, and 2 on day 48. Ten mice died; the monkeys and rats all survived the exposure.

The average concentration of methylene chloride in the blood of dogs exposed at 5,000 ppm for 16 days was 183 mg/liter, compared to 36 mg/liter in dogs exposed at 1,000 ppm. Methylene chloride found in the urine of 2 dogs exposed at 5,000 ppm was 51 mg/liter after 6 hours of exposure and 33 mg/liter after 48 hours.

Hematocrit, hemoglobin concentrations, RBC counts, and the activities of serum glutamic-pyruvic transaminase (SGPT) and isocitric dehydrogenase (ICDH) were all elevated and BSP retention was increased in dogs exposed to methylene chloride at 1,000 ppm for 4 weeks (Table XII-16). Changes in these items were not as great nor as consistent in monkeys exposed to methylene chloride at 1,000 ppm as they were in dogs. [73]

Dogs that died during exposure showed fatty livers, icterus, pneumonia, and splenic atrophy and in the 4 dogs that died from exposure at 5,000 ppm, edema of the brain or meninges was also present. In addition to fatty changes in the liver, vacuolar changes in the renal tubules were found by histological examination.

Monkeys exposed at 1,000 ppm showed no gross lesions, but microscopically some abnormal fat accumulation around the hepatic veins was observed in 3 of 4 monkeys. After 4 weeks of exposure at 5,000 ppm mild-to-moderate fatty changes, mild atrophy, and swelling of the livers were found. Bifrontal encephalomalacia was found in the monkey that died. [73]

The livers of mice that survived exposure at 1,000 ppm for 14 weeks were soft, atrophied, slightly pale in color, and had irregular surfaces.

Microscopic findings in the liver included concentration of pigment including hemosiderin around portal areas, ductal proliferation into portal area with focal collapse involving a few cells, occasional pyknotic cells, and some cytoplasmic and nuclear degeneration. Some hemosiderin was also found in some renal tubules of half the mice examined. [73]

Hepatic changes similar to those noted in mice were found in tissues from rats exposed at both 1,000 and 5,000 ppm of methylene chloride. Iron pigmentation and cell degeneration were noted in renal cortical tubular cells of rats exposed at either concentration for 14 weeks.

The studies with lower concentrations, 25 and 100 ppm of methylene chloride, were reported by Haun et al [70] in 1972. Two hundred mice were exposed continuously at 100 ppm of methylene chloride and 200 were exposed continuously at 25 ppm for up to 2 weeks. Mice were periodically added to, and removed from, the exposure chamber in groups of twenty to get different exposure times. Concentrations of triglycerides in the liver were determined and the livers were examined by electron microscopy. Then 2 groups of 100 mice each were exposed continuously at either 25 or 100 ppm for various exposure times up to 2 months. Following this, 3 groups of 170 mice, 20 rats, 4 monkeys, and 16 dogs each were exposed to methylene chloride at 0, 25, or 100 ppm. Most of the animals in the final experiment were used for special tests, but 20 rats, 20 mice, 4 monkeys, and 4 dogs in each group were exposed continuously for 100 days.

Haun et al [70] reported that no animals showed any overt signs of toxic stress during exposure. Body weight gains were not affected by the exposures at these concentrations. No significant differences were found between groups of mice in hexobarbital sleep-time determinations.

Cytochrome P-450 was reduced in the livers of mice by exposure at 100 ppm methylene chloride. Cytochromes b5 and P-420 were also reduced at 30 days, but were elevated at 90 days of exposure. Mice exposed at 25 ppm did not show significant differences from controls in any of the cytochromes. The data are presented in Table XII-17. [70]

Positive fat stains and cytoplasmic vacuolization were found in livers of mice exposed at 100 ppm of methylene chloride, and positive Oil-Red-O stains were found in livers of mice exposed at both concentrations. Only nonspecific tubular degenerative and regenerative changes were found in the kidneys of rats exposed at 25 and 100 ppm of methylene chloride. The authors [70] reported no significant differences in organ weights between the exposed and the control rats and no significant findings in hematologic, chemical, or microscopic examinations of dogs and monkeys exposed to methylene chloride.

Monkeys had less methylene chloride in the blood than dogs exposed to the same concentrations as shown in Table III-7.

TABLE III-7

METHYLENE CHLORIDE IN BLOOD OF DOGS AND MONKEYS

Exposure Time wks	Exposure Concentration ppm	Monkeys CH2C1 2 µg/ml	Dogs CH2C1 2 µg/ml
6	25	0.6	1.1
	100	3.1	5.1
13	25	1.0	1.8
	100	2.7	4.0

Derived from Haun et al [70]

Significantly elevated COHb was found in exposed dogs and monkeys. The 100-ppm monkeys showed the highest COHb percentages, followed by the 100-ppm dogs, then the 25-ppm exposed monkeys. Dogs exposed to methylene chloride at 25 ppm did not have significantly elevated COHb. [70]

Exploratory studies of spontaneous activity of mice continuously exposed to methylene chloride at 25 and 100 ppm were reported by Thomas et al. [72] Baseline measurements of spontaneous activity were made daily during 2-hour recording sessions for 2 weeks before exposure to methylene chloride. During the 14 weeks of exposure to methylene chloride, the activity of the mice exposed at 100 ppm did not differ from that of controls, but the activity of the mice exposed at 25 ppm was always greater than either the controls or those exposed at 100 ppm.

The light and electron microscopic studies of the livers of exposed ICR strain female mice exposed continuously to methylene chloride at 100 ppm for up to 10 weeks were reported by Weinstein and Diamond [74] in 1972. Liver triglyceride concentrations and liver and body weights were also reported, as noted below.

Twelve groups of 16 mice each were maintained in the exposure chambers for 3 days or for 1, 2, 3, 4, or 10 weeks. The body and liver weights of the exposed mice were consistently less than the controls during the experiment. The fat content of the livers of the exposed group increased for 3 weeks and then decreased. The maximum concentration was 27.88 mg/g liver after 3 weeks of exposure; it declined to 11.80 mg/g liver after 10 weeks of continuous exposure. The fat content of control livers varied between 6.10 and 8.23 mg/g. [74]

All livers from exposed mice which were examined by light microscopy after 7 days of exposure showed a decrease in hepatocyte glycogen and an infiltration of many small fat droplets in centrilobular hepatocytes. At 3, 4, and 10 weeks many hepatocyte nuclei appeared enlarged, but hydropic degeneration and hepatocyte necrosis were not observed. With electron microscopy, small autophagic vacuoles containing clumped membranous debris were found at 3, 4, and 10 weeks of exposure. At 10 weeks of exposure, large autophagic vacuoles were also found in a few centrilobular hepatocytes. Other organelles, including both the smooth and rough endoplasmic reticulum, appeared normal. [74]

(d) Skin Exposure

In 1960, Schutz [76,77] reported studies of absorption of methylene chloride through skin of rats. The hair was shaved from the stomach in a 4.5 x 5.5-cm rectangular patch, to a length of 0.5 mm, 3 days before the experiment. At the beginning of the experiment, each rat was intubated with 40 ml/kg of tap water, then the shaved area was covered with an inverted beaker containing 2 ml of liquid methylene chloride. After exposures of 2, 5, 10, 15, or 20 minutes, the skin was blotted dry and the rats were placed in separate cages for urine collection during the following 3 hours.

The volume of urine voided during 3 hours decreased as exposure time increased; controls voided slightly more than 8 ml, those exposed for 2 minutes voided 8 ml, and those exposed for 20 minutes voided less than 1 ml. There was no hemoglobin in the urine of control animals. Hemoglobin was found in the urine of 34 of the 50 exposed rats; in half of those exposed for 2 and 20 minutes and in 8, 9, and 7, respectively of the 10

rats in each of the groups exposed for 5, 10, or 15 minutes. [76,77]

(e) Teratogenicity

Schwetz et al [78] exposed 19 pregnant rats and 9 pregnant mice for 7 hours daily to methylene chloride at 1,250 ppm on days 5 through 15 of gestation. There was some evidence of both maternal and fetal toxicity in both species. Maternal liver weights were significantly increased in both species. In mice both the absolute and relative maternal liver weights were increased. Extra sternebrae were found in 6 (50%) of the litters of mice from exposed dams and in 3 or 14% of control litters. Cleft pellets and rotated kidneys were each found in 2 experimental litters and no control litters. 'In fetal rats there were increased incidences of dilated renal pelvis and delayed ossification of the sternebra. Other measurements were normal.

(f) Metabolism and Mechanism of Action

The chloromethanes have similarities in their metabolism and mechanism of action. [79,80] Chloroform can be formed from carbon tetrachloride and methylene chloride can be formed from chloroform. [80] Carbon dioxide is a metabolic product of both carbon tetrachloride, [80] chloroform, [80] and methylene chloride. [81]

Reynolds and Yee [82] administered carbon-14 labeled chloromethanes (methyl chloride, methylene chloride, chloroform, and carbon tetrachloride) to rats in oral doses of 8.3 and 26 mM/g body weight. (It is noted that in this paper [82] the legends to Figures 10 and 11 are reversed.) Labeled carbon from carbon tetrachloride and chloroform appeared in the amino acid fraction corresponding to methionine, whereas the labeled carbon from methylene chloride and methyl chloride appeared in the amino acid fraction

corresponding to serine. In electrophoretic lipid analysis, the label from carbon tetrachloride was generally distributed over all lipid fractions, the label from chloroform was limited to phospholipids, and the labels from methylene chloride and methyl chloride were limited to only the slower moving phospholipids.

Heppel and Porterfield [83] found that protein fractions from liver catalyzed the hydrolysis of methylene chloride to hydrogen ion, halide ion and formaldehyde. Kuzelova and Vlasak [55] found formic acid in the urine of workers exposed to methylene chloride, indicating that formaldehyde would be in the pathway of methylene chloride metabolism by man. DeVincenzo and Hamilton [81] did not find labeled formaldehyde in rat serum 2 hours following ip injection (1 ml/kg) of carbon-14 labeled methylene chloride. However, when labeled formaldehyde was fed to rats by Reynolds and Yee, [82] the labeling pattern of proteins and fats was similar to the labeling patterns of ingested methylene chloride and methyl chloride. According to the authors, [82] the similarity of methylene chloride and formaldehyde label patterns into serine suggested that methylene chloride was heterolytically cleaved into 2 ions.

By contrast to the evidence for heterolytic cleavage of methylene chloride, evidence exists that carbon tetrachloride and chloroform undergo homolytic cleavage to 2 free radicals each. [84,85] Carbon tetrachloride [86] and, to a lesser extent, chloroform [87] cause lipoperoxidation and necrosis of the liver. This action was related to metabolism o, these compounds, and gave evidence of their homolytic cleavage. [88,89] Methylene chloride has not been shown to cause either lipoperoxidation or liver necrosis, [90] lending support to the evidence of heterolytic cleavage of

Methylene chloride combined with reduced cytochrome P-450 to cause a spectral shift similar to that caused by carbon tetrachloride. [91,92] These shifts were also similar to, but clearly at longer wavelengths than, the shift caused by CO. The reaction of carbon tetrachloride with cytochrome P-450 was associated with a reduced ability of the enzyme systems to oxidatively demethylate ethylmorphine and aminopyrine, and to hydroxylate hexobarbital. Methylene chloride did not inhibit these enzyme reactions. [93,94]

With methylene chloride, cytochrome P-450 was not decreased 3 hours after a single oral dose of 1.25 ml/kg as it was with carbon tetrachloride. [94] However, upon continuous exposure at 100 ppm methylene chloride, but not at 25 ppm, cytochrome P-450 was decreased when measured after 30, 60, and 90 days of exposure. [70]

Chloroform was shown to combine with equine, rabbit, bovine, and human ferrihemoglobin, and similar combination of carbon tetrachloride and methylene chloride was confirmed with equine ferrihemoglobin. [95,96] In the case of equine ferrihemoglobin, all 3 compounds caused similar spectral shifts with significant absorption in the range of 390 to 430 nm. Chloroform did not give a spectral change with reduced equine hemoglobin or rabbit oxyhemoglobin; these studies were not carried out with methylene chloride. Further evidence of the interaction of methylene chloride with equine methemoglobin was given by a shift in the specific rotation trough minimum from 232.5 to 220 nm, indicative of structural changes in the protein. [95,96]

Crystals of sperm whale metmyoglobin were found to bind methylene chloride at a binding site in the interior of the myoglobin molecule,

approximately equidistant from one of the pyrrole rings of the heme group and the ring of heme-linked histidine. [97] The binding site corresponded to that of xenon and cyclopropane which increase the affinity of myoglobin for CO. [97]

In 1972, Stewart et al [39] reported finding elevated COHb concentrations in the blood and CO in the breath of subjects exposed to methylene chloride. Breath samples were analyzed for methylene chloride and CO by GC and IR. Blood samples were routinely analyzed for COHb with an automated spectrophotometer, and the authors reported that the readings were cross-checked by the gas chromatographic method of Porter and Volman. [98]

Following the report by Stewart et al, [39] other reports of CO in the blood or breath of man and animals during and after methylene chloride exposure appeared. [59,67,68,70,81,99,100] In some of these studies the analytical methods were not given in sufficient detail for evaluation. [70,59]

Fassett [99] described an experiment where 1 ml/kg labeled methylene chloride was administered ip to rats. The animals were killed serially at 30-minute intervals, and COHb was determined by the procedure of Van Slyke et al. [101] Radioactivity was present in the liberated gas. Fassett [99] stated that the CO apparently came from methylene chloride. DiVincenzo and Hamilton [81] found that within 24 hours after ip administration of carbon-14 labeled methylene chloride most of it was eliminated unchanged in the breath, and that about 3% was exhaled as carbon dioxide, 2% as CO, and 1% as an unidentified metabolite.

Carlsson and Hultengren [102] exposed 10 rats at 550 ppm of radioactive methylene chloride for 1 hour and determined the COHb and radioactive CO formed. A correlation coefficient of about 0.9 was found between COHb and radioactive CO.

Kubic et al [100] studied COHb concentrations in blood of rats following administration of dihalomethanes. In all cases they found elevated COHb during 1 to 6 hours following ip injections of 3 mM/kg. With methylene chloride doses of 1.5, 3.0, and 6 mM/kg, maximum COHb measurements of about 5%, 8%, and 9%, respectively, were found. The COHb was measured by GC. [103]

Kubic et al [100] found that pretreatment with either phenobarbital or 3-methylcholanthrene did not alter the amount of COHb after ip administration of methylene chloride and Roth et al [68] found that this was the case when rabbits were exposed to methylene chloride by inhalation. These experiments [68,100] indicate that microsomal hydroxylating enzymes are not involved in metabolism of methylene chloride to CO. However, repeated administration of methylene chloride may stimulate activity of the enzymes involved in the transformation. Kubic et al [100] reported that when methylene chloride was administered to rats ip daily for 5 days, more COHb was found after administration of the 5th dose than after administration of the first dose, [100] but the data were not adjusted for baseline values. Evidence that repeated inhalation of methylene chloride by rabbits led to increased amounts of COHb was not found by Roth et al, [68] when their data were adjusted for baseline values.

Using carbon-13 labeled methylene chloride, Kubic et al [100] found that the IR spectrum of the labeled hemoglobin resembled the spectrum of

COHb obtained from exposure to similarly labeled CO.

These studies provide information that methylene chloride is metabolized to CO in man and several animal species, and some information that formaldehyde and formic acid are other metabolites. Kassebart and Angerer [104] proposed a mechanism by which both formic acid and CO could form from methylene chloride. They proposed formaldehyde formation from hydrolytic dehydrochlorination and oxidation of the formaldehyde to formic acid which would be oxidized to carbon dioxide and water and dehydrated to CO and water. Such a mechanism would be compatible with most of the data presented in this section.

Correlation of Exposure and Effect

(a) CNS and Behavioral Effects

Methylene chloride was initially used as an anesthetic and was found to have a number of side effects; not all were undesirable. A number of investigators [31-33] reported that with methylene chloride an anesthetic stage of analgesia and unconsciousness could be obtained without the loss of muscle tone. As recently as 1950, Grasset and Gauthier [33] reported that when methylene chloride was administered to women during labor, muscle tone was maintained, and the patients experienced retrograde amnesia afterward. Exposure of humans and experimental animals [31,32] at concentrations of methylene chloride capable of producing narcosis often resulted in epileptiform attacks while subjects were anesthetized.

Moskowitz and Shapiro [50] reported that in a plant where methylene chloride was used as an extractant, the 4 workers on the night shift all became unconscious. One of them died. The workers were exposed for 1-3

hours, but no concentration measurements were obtained. None of the men remembered detecting the odor of methylene chloride. The individual who died had been working in the plant for 7 months. Autopsy revealed that the dura mater was adherent to the skull and the veins of the pia-arachnoid were conspicuously engorged. The methylene chloride content of the brain was 0.1 ml/500 g tissue. Two of the 4 workers had contusions and lacerations of the head suggesting that this was either the result of falling when unconscious or of muscular movements while unconscious.

Weiss [58] described the effects of methylene chloride on a 39-year old chemist who worked 5 years in a pharmaceutical factory where sodium chloride was dissolved in methylene chloride then recrystallized after evaporation of methylene chloride. The man was exposed at vapor concentrations of methylene chloride ranging from 660 to 3,600 ppm for several hours a day, and his hands and arms were often exposed to liquid methylene chloride. After 3 years, the man complained of forgetfulness, insomnia, and a feeling of drunkenness. During the last few months of his 5th year of exposure, he complained of auditory and visual hallucinations.

In 1966, Kuzelova and Vlasak [55] studied workers in a factory where cellulose acetate films were manufactured using methylene chloride as the principal solvent. Other components of the solvent mixture were methanol, triphenyl phosphate, and dibutyl phtalate. The concentrations of methylene chloride in the air ranged from approximately 25 to 5,000 ppm in 14 locations (Table XII-11). Of the 33 workers who had been exposed for 1.5 to 2 years, slightly more than 72% complained of headaches, about half complained of increased fatigue, and approximately 50% of nervous disorders.

In experimental exposures of humans to methylene chloride, Stewart et al [39] reported that 2 of 3 subjects exposed at 986 ppm for 2 hours became lightheaded after 1 hour. This feeling cleared 5 minutes after the exposure ceased. Similar results occurred in subjects exposed at 868 ppm. Subjects exposed at 986 showed alterations in VER indicative of the initial phases of CNS depression at both 1 hour and 2 hours of exposure. The subjects showed the same response when exposed at 514 ppm for 1 hour, but in this experiment the response was not maintained during a 2nd hour of exposure at 868 ppm.

Fodor and Winneke [37] and Winneke [38] reported that human subjects showed decreased behavioral performance on certain controlled tests. In 12 female subjects exposed at 317 ppm methylene chloride by Fodor and Winneke, [37] the CFF was decreased by a statistically significant amount after 100 minutes of exposure. At 751 ppm the effect occurred after 50 minutes of exposure. In exposures at 317 and 470 ppm, auditory vigilance was decreased by similar amounts. At 751 ppm, auditory vigilance was decreased much more. [38] Winneke [38] reported significant impairment on a battery of psychometer tests during exposure at 751 ppm for 4 hours.

Gamborale et al [40] found no decrement in performance on the 4 psychomotor tests they used during 2 hours of exposure in which the concentration of methylene chloride increased at 30-minute intervals from 250 to 1,000 ppm. The tests used were addition-reaction time, memory, and two simple reaction time tests.

No CNS or behavioral effects of methylene chloride were reported by the Massachusetts Department of Labor and Industries among workmen exposed to methylene chloride. (LD Pagnotto, written communication, December 1973)

Atmospheric concentrations in the casting room, where most of the workers spent their time were between 100 and 280 ppm.

(b) Effects on Internal Organs

At lower concentrations, eg 25 ppm for 90 days continuous exposure, no effects were evident on liver and kidneys of mice, rats, monkeys or dogs. The magnitude of the liver and kidney effects increase in such a way that extremely high doses of methylene chloride, taking concentrations and duration of exposure into account, can produce toxic effects. [69,70-72]

A variety of animals were exposed at 10,000 ppm by Heppel et al [69] for 4 hours/day, 5 days/week for 8 weeks. Necropsies were performed on all of the exposed animals. Gross examination revealed pulmonary congestion and edema with focal necrosis and focal extravasation of blood in rabbits and rats. Microscopic examination of liver tissue revealed moderate centrilobular congestion and fatty degeneration of the liver in dogs and guinea pigs. In a similar experiment at 5,000 ppm, no gross or microscopic abnormalities were found. [69]

Weinstein et al [71] exposed mice to methylene chloride continuously at 5,000 ppm for 7 days. Throughout the experiment the ratio of liver weight to body weight was significantly higher for the exposed animals. The concentrations of liver triglyceride were also significantly greater in the experimental animals. All animals were killed. Microscopic examination of the tissues showed fatty changes in the renal tubules; in the livers, centrilobular fatty infiltration and hydropic degeneration of hepatocytes were found.

Fourteen-week continuous exposures at 5,000 ppm resulted in more severe toxic effects to mice, rats, dogs, and monkeys in the experiments

reported by MacEwen et al. [73] There was high mortality in all species. Fatty livers, icterus, pneumonia, splenic atrophy, and vacuolar changes in the renal tubules were found. With an exposure concentration of 1,000 ppm for the same period of time, MacEwen et al [73] reported elevated liver enzymes and bilirubin levels, centrilobular fat accumulation, and elevated BSP retention time.

Weinstein and Diamond [74] exposed mice at 100 ppm continuously for 10 weeks to determine the hepatotoxicity of methylene chloride as determined by tissue triglyceride concentrations, liver and body weights, and microscopic examination of the liver. The liver and body weights were essentially the same for exposed and control animals, but liver triglyceride levels were elevated after 2 weeks of exposure to methylene chloride. There was centrilobular fatty infiltration, vacuolization in liver cells, and a decrease in hepatocyte glycogen of the exposed mice.

The results of experiments by Haun et al [70] showed similar findings. Mice exposed continuously at 100 ppm for periods of 2 weeks to 2 months had altered levels of the cytochromes P-450, P-420, and b5, fatty infiltration of the liver, and vacuolization in liver cells. Only nonspecific tubular degenerative and regenerative changes were found in the kidneys. Animals exposed at 25 ppm continuously showed no overt signs of toxicity, and only nonspecific degenerative and regenerative changes were found in the renal tubules.

(c) Effects on the Skin and Mucous Membranes

Stewart and Dodd [49] studied effects of methylene chloride in 4 subjects each of whom immersed one thumb in methylene chloride for 30 minutes. All subjects experienced an intense burning sensation within 2

minutes following immersion of the thumbs in methylene chloride. After 10 minutes the burning sensation was mixed with a numb or cold feeling. The slightest movement of the thumb triggered intense pain. A slight degree of white scale was noted on the thumb as well as a mild secretion. The erythema and paresthesia subsided within 1 hour. In these experiments, it was shown by breath analysis that methylene chloride was absorbed through the skin.

Effects of methylene chloride applied to shaved skin of rats were reported by Schutz. [76,77] Fifty rats were each subjected to 2 ml methylene chloride applied to the skin for 2, 5, 10, 15, or 20 minutes. As application time increased, the urine voided in 3 hours decreased from approximately 8.0 ml to 1.0 ml. Thirty-five of the 50 exposed rats, but none of the controls, had hemoglobin in the urine.

In a number of occupational studies where workers have been exposed to methylene chloride there have been reports of irritation of the eyes and respiratory tract. [50,51,55] Kuzelova and Vlasak [55] reported that 16 of 33 workers exposed at a range of 25-5,000 ppm for an average of 2 years experienced irritation of the respiratory tract and conjunctiva.

A laborer degreasing copper gaskets in a small and poorly ventilated room experienced nausea and eye irritation. [51] The exposed man had been working for 4 hours; the concentrations of methylene chloride were not measured.

Three of 4 workers rendered unconscious by unknown concentrations of methylene chloride were studied by Moskowitz and Shapiro [50] and all three exhibited some signs of either eye, lung, or respiratory tract irritation.

(d) Blood CO and COHb Measurements

Several investigators [39,41,43,59,67,68,70,100] reported COHb in blood and CO in exhaled breath with exposure to methylene chloride. The response was proportional to both exposure time and concentration of methylene chloride in the exposure atmosphere.

These data need to be evaluated cautiously because it has not been unequivocally proven that either CO or COHb was totally responsible for all the measured responses. Methylene chloride was shown by Bucher [95] in 1968 to combine with methemoglobin. The combination absorbs light in the analytical range for COHb (390-430 nm) and may interfere with COHb measurements determined spectrophotometrically. Other investigators [39,58,67,68,70,100] have apparently not been aware of this phenomenon which was first reported in 1971. [96] In several studies [39,41-43] COHb was estimated from analysis of CO in exhaled breath samples, and the reports did not give details about the conversion factor for CO to COHb. In one report, the authors [39] indicated that both CO and methylene chloride in the breath samples were analyzed by GC and IR but the data to compare the 2 methods were not presented.

Haun et al [70] found elevated measurements of COHb in dogs when exposed continuously to methylene chloride at 100 ppm, but not when exposed at 25 ppm. In the same experiment, monkeys showed elevated COHb measurements which were proportional to the exposure concentration. The authors noted that the dogs had higher methylene chloride concentrations in the blood than the monkeys, but the monkeys had the higher COHb percentage measurements, suggesting that the monkeys' metabolic processes were more effective in converting methylene chloride to CO.

Fodor et al [67] measured CO content of the blood during 3-hour exposures of rats to methylene chloride at 40, 100, 500, and 1,000 ppm. They also exposed groups of rats for 3 hours to CO at 100 ppm and to combinations of 100 ppm CO with 100 and 1,000 ppm methylene chloride. At 1,000 ppm of methylene chloride, the rats developed 12.5% COHb, at 100 ppm they developed 10.9% COHb, and with the combination exposure at 1,000 ppm of methylene chloride and 100 ppm CO, they developed 19.0% COHb. Fodor et al [67] gave no data on how they determined CO or COHb, except to say "the blood CO concentration was determined by gas analysis methods." Since exposures to the combination of CO and methylene chloride, each at 100 ppm, resulted in COHb values that could not be attained by exposure at 100 ppm CO, it would appear that the affinity of hemoglobin for CO was increased by exposure to methylene chloride.

Carlsson and Hultengren [102] found a high correlation (r=0.9) between COHb and radioactive CO of rats exposed to labeled methylene chloride at 550 ppm for 1 hour.

Kubic et al [100] studied COHb in rats injected with methylene chloride ip. Their method of determining blood CO was by GC, [103] similar to the method of Porter and Volman [98] that was used by Stewart et al. [39] The COHb was estimated by dividing blood CO content by the product of 1.39 and the total hemoglobin concentration determined by the cyanomethemoglobin method. [101] When rats were given 0.125, 0.25, and 0.5 g/kg of methylene chloride, maximum COHb percentages were 5, 8, and 9, respectively. These data were derived from single animals because, as the authors stated, "although multiple experiments were conducted in virtually all cases, animal-to-animal variation made averaging and statistical processing of curves difficult."

On the assumption that metabolism of methylene chloride to CO was mediated through hydroxylating enzyme systems, Kubic et al [100] pretreated rats with the enzyme inducers, phenobarbital and 3-methylcholanthrene, and with the enzyme inhibitor 2-diethylaminoethyl 2,2-diphenylvalerate HCl. Pretreated rats did not produce measurably different amounts of CO when subsequently given methylene chloride than did the controls when given methylene chloride without the enzyme modifiers, indicating that hydroxylating enzymes were not involved. Roth et al [68] obtained similar results by inhalation exposure to methylene chloride in rabbits. Rats injected with methylene chloride on 5 consecutive days had higher COHb measurements on the 5th day than controls, [100] but a similar response was not found in rabbits from repeated inhalation exposures. [68] Kubic et al [100] did not measure COHb on the 5th day before injection of methylene chloride.

Finally, Kubic et al [100] studied the IR spectrum of rat blood equilibrated with CO labeled with carbon-13, and compared this with the absorption spectrum of rat blood taken 2.5 hours after injection of similarly labeled methylene chloride. The absorption spectra appeared identical, but the absorption of rat blood treated in vitro with labeled methylene chloride was not determined. Also, no quantitative analysis was made of the amount of CO that might have been formed.

Extensive studies of COHb in human subjects experimentally exposed to methylene chloride have been conducted. [39,41-43] Exposure concentrations of 50, 100, 250, and 500 ppm each with exposure times of 1, 3, and 7.5 hours were employed. Each combination was repeated on 5 consecutive days. In addition, effects of single 2-hour exposures at

approximately 700 and 1,000 ppm were studied. [39] In some cases, COHb percentages (measured spectrophotometrically) were greater 1 hour after the end of exposure than at the end of exposure. This occurred more often with the shorter exposure times, higher exposure concentrations, and on Fridays. [39,41] Maximum COHb percentages determined sprectophotometrically in nonsmokers are presented in Table III-8.

TABLE III-8

MAXIMUM COHb PERCENTAGES OBSERVED IN NONSMOKERS EXPOSED TO METHYLENE CHLORIDE

Exposure Concentration, ppm	Exposure Time, Hours			
	1	2	3	7.5
50	1.3		1.6*	2.9
100	3.2*		3.0*	5.7
200	2.4			
250	3.0		4.3	9.6
500	4.4		5.6	11.4
700		8.0		
1,000		15.0		

* End exposure values, all others are 1-hour after exposure
Derived from Stewart et al [41]

The studies reported by Astrand et al [44] in 1975, and by Stewart and Hake [45] in 1976 indicate that the COHb values encountered in employees exposed to methylene chloride would be related to the work load. These studies also indicate that the maximum COHb values of exposed workers

might occur 3-4 hours after leaving work.

With 3- and 7.5-hour daily exposures at 100, 250, and 500 ppm, COHb percentages remained elevated above baseline values on the mornings following exposure. However, after 5 consecutive 7.5-hour days of exposure at either 100 or 250 ppm, they returned to normal by Monday morning. [41]

In the subjects exposed for 7.5 hours daily, the oxygen dissociation curves were distorted in proportion to exposure concentration. Following exercise the blood lactate in the subjects exposed at 500 ppm, but not those exposed at 100 ppm, became slightly elevated above control values. [43]

Concentrations of CO in the breath of 7 individuals exposed to methylene chloride in a working environment were studied by Ratney et al. [59] The CO was determined by an electrochemical method not equilibrated with air mixtures of CO and methylene chloride. The workroom concentrations of methylene chloride were measured periodically during the workday. They were greater in the afternoon (219-610 ppm) than in the morning (159-172 ppm). All individuals had been exposed to higher concentrations of methylene chloride on the previous day (300-966 ppm). Four of the 7 subjects were employees who had been exposed to methylene chloride for years. The measured concentrations of CO in exhaled air of the subjects ranged between 24 and 32 ppm at the beginning of the workday. The concentrations, measured hourly, gradually increased during the day, and at the end of the workday they ranged from 36 to 77 ppm. The COHb percentages derived from these breath data ranged from 3.3 to 5.3 at the beginning of the day and from 5.7 to 12.0 at the end of the day. The following morning, the measured CO concentrations in the exhaled breath

ranged from 17-29 ppm and the derived COHb percentages ranged from 3.6 to 4.9. Measurements of methylene chloride exhaled by the subjects were not reported. [59]

(e) Summary

Data to correlate exposure and effect are summarized in Table XII-18.