TOXICOLOGY OF DICHLOROMETHANE (METHYLENE CHLORIDE)*

I. Studies on Effects of Daily Inhalation

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Dichloromethane (methylene chloride), CH_2Cl_2, is finding wide and increasing use as a solvent for cellulose esters, fats, oils, resins and rubber. It is also utilized in paint stripping operations. The compound is a colorless liquid with a boiling point of 40°–41°C., a melting point of −96.7°C., a molecular weight of 84.93 and a specific gravity of 1.336 (20°C./4°C.). It is reported (1) to be practically noninflammable and non-explosive at ordinary temperatures, but at higher temperatures under certain laboratory test conditions it is capable of forming combustible mixtures with air. Its vapor pressure at 20°C. is 348.9 mm. Hg and so the possibility exists in industry of exposures to high concentration.

The acute toxicity of dichloromethane has been studied by a number of investigators. Mice were killed by a two hour exposure to 14,500 p.p.m. (2). Nuckolls (1) determined that dichloromethane was toxic for guinea pigs. An exposure of 2 hours at a concentration of 8,000 to 10,000 p.p.m. led to marked irritation, tremors and dyspnea with slow recovery. At a level of 50,000–54,000 p.p.m. maintained for 1 hour to 1½ hours, there resulted progressive narcosis and death. Animals which died showed edema of the lungs, cloudy swelling of the kidneys and beginning yellow atrophy of the liver. Muller (4) found that the pure preparation caused no organic lesions in mice whereas the technical product caused fatty degeneration of the liver. Maloff (5) found no such degeneration in the livers of dogs even after repeated anesthesias produced by ordinary commercial preparations.

A few studies have been carried out on the effects of repeated exposures to dichloromethane. Lehmann and co-workers (3) exposed rabbits and cats 8 hours a day, 6 days a week, to concentrations of 6–7 mg./l. (1700–2,000 p.p.m.), for 4 weeks. There were no deaths and no important changes in the blood picture, but the animals did experience a reduction in body temperature and slight depression. No autopsy data were recorded. Gross (6) subjected rats to repeated 8 hour exposures at concentrations of 4–6 mg./l. (1300 p.p.m.). The animals were sacrificed at intervals of 25 days. No definite pathological changes were found until after 75 days. These consisted of slight atrophy and central fatty degeneration of the liver with sporadic round cell infiltration of the parenchyma. Similar, although somewhat greater changes were found in rabbits which had inhaled 10 mg./l. (2900 p.p.m.) for 50 and 75 days. Flury, Neumann and Miller (7) subjected cats to exposures which lasted 4 to 8 hours on 6 days a week. They used a concentration of 25 mg./l. (7200 p.p.m.) and the experiment was carried on for a period of 4 weeks. The animals showed disturbances of coordination after 6–8 hours in the exposure chamber. A temporary increase in hemoglobin and red cell count was noted (amount not stated). No abnormalities were discovered in the urine. The animals were sacrificed 1, 3, and 9 weeks after the completion of the exposure. Marked fatty infiltration without degeneration of the liver and kidney was found.

Some preliminary experiments were carried out in this laboratory which had to be discontinued due to the transfer of key personnel to the armed forces. Two rhesus monkeys, 2 dogs, 2 pups, 4 rabbits, 16 young guinea pigs and 16 young rats were simultaneously exposed to a concentration of 500 p.p.m. of dichloromethane. Exposures were carried out for 8 hours daily, 5 days a week, over a period of 15 weeks. Three of the exposed rats died within the first 6 weeks of the experiment, whereas none out of 16 control rats died. One of the exposed pigs died while there were 2 deaths among 16 control pigs. The young animals gained weight rapidly, and all of the survivors appeared in excellent condition throughout the

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ASI 000015708

test period. Blood studies were carried out at weekly intervals on the rabbits. These included red cell, white cell and differential counts and hemoglobin determinations. No significant changes were noted except for a slight increase in eosinophils after the 8th week of exposure. In a second experiment 4 cats, 4 rabbits, 16 young rats and 16 young guinea pigs were exposed 8 hours daily, 5 days a week, for 15 weeks to 1100 p.p.m. of dichloromethane. There occurred 1 death among the experimental guinea pigs, while 2 out of 16 control animals died. Two female rabbits died, both after giving birth to a litter. The young rats and guinea pigs made good weight gains. The cats, on the other hand, did not seem to thrive. No symptoms were noted except for sleepiness and reduced activity in the early part of the experiment. The rats gave birth to many litters during the test period. Blood studies were negative except that both female rabbits showed a leucopenia shortly before death.

In the present paper the effects on various animals produced by repeated daily exposures to dichloromethane are presented. Concentrations of 5,000 p.p.m. and 10,000 p.p.m. were employed.

Experimental Procedure

The exposure chamber used in these experiments was the same as described previously (9). It was of sufficient size (4′ x 4′ x 6′) to permit a number of animals of different species to be exposed simultaneously. A metered 400 liters of air per minute were drawn through the chamber, giving a complete change of air every seven minutes. Inside the chamber were 3 electric fans, which served to mix completely the air and dichloromethane vapors.

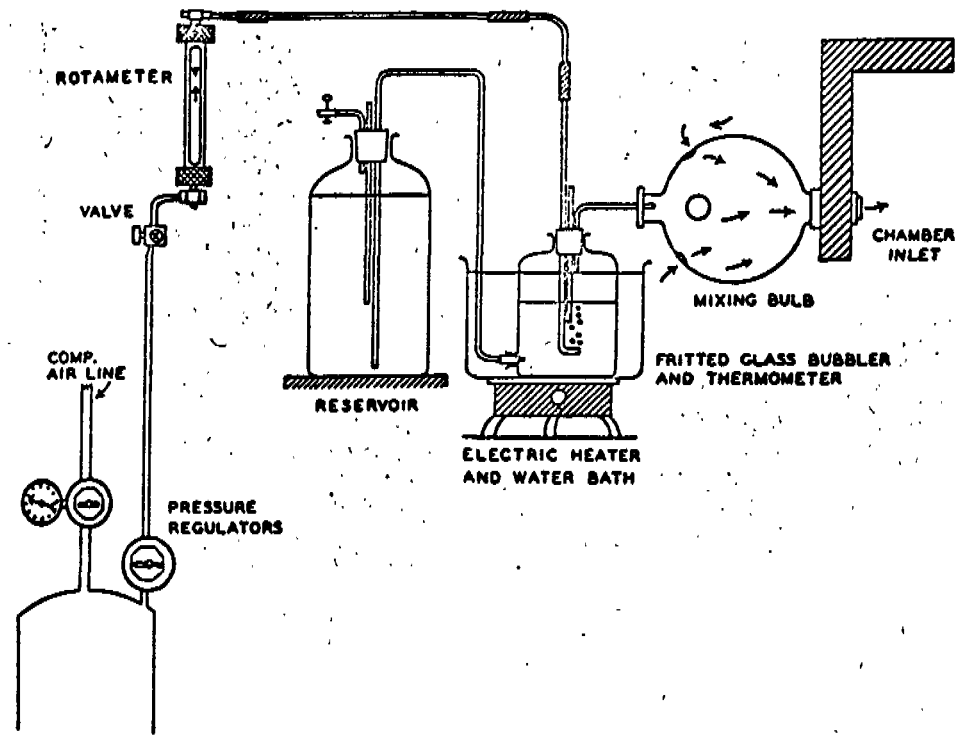

Fig. 1. Apparatus for volatilizing dichloromethane at a constant rate. Air from the storage tank passes through the rotameter and then to the fritted gas bubbler. The depth of immersion of the bubbler is kept constant. The amount of dichloromethane which evaporates is measured by noting the volume of fluid which leaves the reservoir bottle.

The method for obtaining the concentrations of 5,000 p.p.m. and 10,000 p.p.m. is shown in Figure 1. The solvent was vaporized at a constant rate by passing a stream of air under uniform pressure through a fritted glass bubbler which was immersed in the liquid. The temperature of the liquid was maintained within narrow limits by means of a

ASI 000015709

water bath, and a constant depth of immersion of the bubbler was achieved by means of a Mariotte flask arrangement. The vapors passed into a mixing bulb where they joined the main air stream entering the chamber.

The concentrations of dichloromethane in the exposure chamber were checked by three independent methods. First, these values were calculated from measurements of the volume of solvent volatilized each hour and the air flow through the chamber. Sample calculations of this sort have been included in a paper by Dudley and Neal (9). Another check on the concentration of dichloromethane in the chamber atmosphere was obtained by withdrawing a gas sample each day and analyzing it by thermal decomposition and determination of the inorganic chlorides (8).

TABLE 1

PHYSICAL DATA ON THE DICHLOROMETHANE WHICH WAS USED

	1ST LOT	2ND LOT	3RD LOT
Specific gravity 20°/20°.....	—	1.3287	1.3288
Index of refraction, $n_{D_{20°C.}}$	1.4241	1.4241	1.4238
Initial distillation temperature, °C....	38	38	37
Vol. per cent boiling 39°–40°	95	78	98
Vol. per cent boiling 40°–41°.....		21	
Dry point, °C.....	39.5	41	40

The third control procedure consisted of taking frequent measurements throughout the course of the day's exposure, using the Raleigh-Jeans interference refractometer.

The dichloromethane was a commercial product. Physical data on the various lots which were used is shown in Table 1. In addition, the three samples were tested for water, aldehydes, chlorides, acids and substances decomposed by sulfuric acid. The results were negative except that a faint yellow color developed in the sulfuric acid layer when the last named test was carried out. Nuckolls' directions (1) were followed.

Unfortunately, the temperature and humidity prevailing inside the chamber could not be controlled. They were measured each day. The exposures to 5,000 p.p.m. were carried out during the fall and winter months, but the second experiment using 10,000 p.p.m. was done during a warm, humid summer. Temperatures as high as 93°F. and relative humidities as high as 80 per cent were recorded.

Diets: The dogs received a mixture consisting of equal parts by weight of ground horse meat and Kippell biscuits. In addition each animal received 100 c.c. of whole milk per day. Guinea pigs and rabbits received pressed pellets of the following composition: 15 per cent oat groats, 30 per cent pulverized #1 whole wheat, 40 per cent alfalfa leaf meal US #1 sun cured, 13 per cent soy bean meal, 0.25 per cent sodium chloride and 1 per cent calcium carbonate. Small amounts of irradiated yeast and wheat germ oil were also included in the formula. Of the rats exposed to 5,000 p.p.m. of dichloromethane, half received Purina dog checkers while the remainder were fed the following ration: 20 per cent wheat flour, 20 per cent corn meal, 31 per cent rolled oats, 13.5 per cent milk powder, 3.5 per cent brewer's yeast, 6 per cent powdered hog's liver, 2 per cent dextrin and 4 per cent salts. All of the rats exposed to 10,000 p.p.m. of dichloromethane were fed Purina dog checkers. The rodents received supplements of fresh cabbage. The monkeys were fed Chim crackers, whole wheat bread, milk, eggs and a variety of fresh fruits and vegetables.

RESULTS OF VAPOR EXPOSURES

17 milligrams per liter (approx. 5,000 p.p.m.)

The first series of animals were exposed simultaneously to a concentration of 17 mg./l. (approx. 5,000 p.p.m.), 7 hours daily, 5 days a week, for periods up to 6 months. Fluctuations in the concentration of dichloromethane which prevailed in the chamber were small. A gas sample was withdrawn each day and analyzed by a combustion method (8). The average value was 17 mg./liter with a standard deviation of 2 mg./l. There was good agreement between the results of the gas analyses and the concentrations as calculated from the volume of dichloromethane which was volatilized.

Table 2 shows the number of animals exposed and the deaths which occurred.

Weight Changes: The adult animals appeared well nourished at all times. Two pups were born in the exposure chamber and exposed daily from birth. They made excellent weight gains. The young rats also gained weight rapidly, but the exposed guinea pigs lagged behind the control

ASI 000015710

animals. At the end of the exposure the experimental pigs had an average weight of 820 gm. compared with 1025 gms. for the control pigs. Food consumption of the exposed rats was comparable with that of the control rats, whereas the exposed guinea pigs ate somewhat less than did the control pigs.

Symptoms: The rats appeared well throughout the experimental period. They showed no evidence of narcosis while in the chamber. The rabbits also seemed to be unaffected by the repeated exposures to dichloromethane. Both females bore young once during the period of 6 months. The litters were not raised, but this

TABLE 2

ANIMALS EXPOSED TO 5,000 P.P.M.

ANIMAL	NUMBER		AVERAGE WEIGHT	NUMBER OF EXPOSURES	DEATHS
	Male	Female			
Dogs	1	5	8400	132	0
Puppies	1	1	470	115	0
Rabbits	2	2	3400	124–131	0
Guinea pigs	8		550	131	3*
Guinea pigs	6		400	65	0
Rats	11	1	146	132	1†
Rats		5	174	77	0
Rats	4		160	42	0

* Died after 35, 90 and 96 exposures. Autopsy showed extensive pneumonia associated with moderate centro-lobular fatty degeneration of the liver. No deaths occurred among 14 control pigs.

† Died shortly after giving birth to a litter (22 exposures). Autopsy showed many thrombi in renal vessels associated with marked cortical infarction. No deaths among 28 control rats.

may have been due to the disturbance resulting from daily transfers to the chamber. The surviving guinea pigs also appeared to thrive.

The dogs showed no ill effects from the exposures. Their appetite was usually excellent. Periodically they were carefully examined and special attention was paid to the appearance of the cornea, sclera, eye grounds, eye movements, gait, condition of mucous membranes, state of deep reflexes and sensory perception. Nothing abnormal was ever noted.

Mean arterial blood pressures were determined on 5 dogs at intervals of one to two weeks, using the method of direct puncture of the femoral vessel of trained, unanesthetized dogs. All of the blood pressure readings for each dog were averaged, and these averages varied from 100 to 115 mm. of mercury. Of 83 determinations, the results of all but 3 fell between the normal value of 90 and 125.

Studies on Urine and Blood: Catheterized urine specimens were examined at intervals of several weeks. They were tested for pH, specific gravity, presence of albumin, sugar, acetone, urobilin, urobilinogen and abnormal cellular constituents. The results of these examinations were uniformly negative.

After the six adult dogs had been exposed over a period of 4 months blood samples were drawn in order to measure the level of prothrombin. All values were normal. Determinations of icterus index were carried out on the sera of dogs from time to time during the experimental period of 6 months. It was never elevated.

Bromsulfalein excretion was studied after the animals had been on test for 6 months. The results were compared with those obtained with normal, unexposed dogs. Five milligrams of dye per kilogram was injected intravenously and blood samples were obtained at 15 minutes and 30 minutes after the injection. In most instances there was a retention of 4 per cent after 30 minutes for the 12 observations on the experimental animals and the 12 determinations on the control dogs.

Plasma protein levels were determined on the six adult dogs after they had been exposed to dichloromethane over a period of 6 months. Analytical methods were as follows: 1) for total protein, macro-Kjeldahl digestion, using Wong's digestion method (10); 2) separation of albumin fraction according to Kingsley's technique (11); 3) for non-protein nitrogen, nesslerization as described in Hawk and Bergeim (12). The results were comparable with those obtained upon normal dogs in this laboratory and they are similar to the levels reported in the literature.

Hematological Changes: Red and white cell counts, hemoglobin determinations and differential cell counts were carried out, first at weekly intervals and then every two weeks, during the entire exposure period on 5 of the adult dogs and on all 4 rabbits. No abnormalities were encountered in the case of the rabbits. Three of the dogs showed a tendency for the red cell count to rise but this trend was hardly significant.

34 milligrams per liter (approx. 10,000 p.p.m.)

As a pilot experiment, a group of animals received a single exposure of 7 hours to 10,000

ASI 000015711

p.p.m. These included 10 rats, 9 guinea pigs, and 4 rabbits. One of the rabbits died several days after the exposure. All of the other animals remained alive and well during an observation period of one month. Sixteen mice (average weight 25 gms.) were exposed for 4 hours to 10,000 p.p.m. All but one survived.

Daily exposures on 5 days a week were then begun and these were continued for 8 weeks. The first exposure lasted for 6 hours, while all of the others were only four hours in length. A gas sample was withdrawn from the chamber each day and analyzed by the combustion method. The average value was 33.8 mg./liter with a standard deviation of 1.4 mg./liter. There was generally good agreement between the results of the gas analyses, and the concentrations as calculated from the volume of dichloromethane which was volatilized.

TABLE 3

ANIMALS EXPOSED TO 10,000 P.P.M.

ANIMAL	NUMBER		AVERAGE WEIGHT	NUMBER OF EXPOSURES	DEATHS
	Male	Female			
Dogs		4	7500	6	0
Monkeys		2	4050	36–37	0
Rabbits	5		3240	37	3*
Guinea pigs	11	1	653	38	0
Rats	9	7	190	38	2†

* Died after 1, 12, and 22 exposures. Autopsy examination of 2 of the rabbits showed pulmonary congestion and edema with focal necrosis, and splenic congestion.

† Died after 33 and 38 exposures. Autopsy on second rat showed marked pulmonary congestion and edema with focal extravasation of blood.

In Table 3 are shown the number of animals exposed and the deaths which occurred.

Weight Changes: The young rats gained weight rapidly. The exposed guinea pigs lagged behind the control animals throughout the test period, and at the end of exposure their average weight was 88 per cent of that of the control pigs.

Symptoms: The dogs were more violently affected than any of the other animals. At first they showed only mild incoordination. Within a few minutes they seemed to go into an excitement stage of anesthesia manifested by very energetic but purposeless movements of all sorts. The animals threw themselves about, bit one another, knocked their heads against the sides of the cage, and licked the shavings on the floor. Frothing appeared about their mouths and conjunctival irritation developed.

Toward the end of the 4 hour period the dogs were still engaged in these wild, aimless movements or else lay on the floor, apparently exhausted. After the animals were removed to their living cages they rapidly recovered from the effects of the anesthesia and ate their food almost at once. They were all sacrificed after 6 exposures because of the numerous bruises which they developed while tossing about in the exposure chamber.

The two monkeys showed nothing during the first hour except decreasing activity. During the second hour they developed incoordination and had trouble in getting up from a lying position. Towards the end of the 4 hour period the animals lay prostrate with barely perceptible respirations. During the last two weeks it seemed that they developed a resistance to the vapors for they were able to sit up throughout the exposure period. The animals generally ate their food, appeared well and gained in weight during the course of the experiment. This was especially surprising since one of the monkeys, at autopsy, was found to have widespread tuberculosis.

The rabbits showed excitement at the beginning of the exposure period followed by varying degrees of inactivity. Some lay on their sides while others were able to maintain an upright position. Very few signs of narcosis were displayed by the guinea pigs, which showed only increasing somnolence. There was no evidence of mucous membrane irritation. A few animals showed mild incoordination towards the end of the exposure period. The rats developed difficulty with their gait during the first half hour in the chamber. During the second hour they lay on their sides and at the end of 4 hours they were prostrate with very depressed respiration. Within one hour after removal from the chamber the animals appeared normal again.

Hematological Changes: Only a few blood counts were carried out for this experiment because the dogs were sacrificed after but a few exposures and most of the rabbits died early in the experiment. No important changes were noted for any of the animals.

PATHOLOGICAL FINDINGS

Autopsies were performed upon all of the experimental and many of the control animals. Gross

ASI 000015712

findings were negative except for pulmonary changes described below. In the case of the dogs the brain was also examined. Organ weights were obtained for the livers, kidneys, lungs, hearts, and spleens of the exposed and control animals. There were no significant differences between the two groups.

Microscopic examination was done for most of the animals exposed to 5,000 p.p.m. Examination was done on each of the 7 dogs, 4 rabbits, 14 guinea pigs and 21 rats. Unexposed animals taken from stock at the same time as those on experiment served as controls; these included 4 rabbits, 10 guinea pigs, and 10 rats. In the group exposed to 10,000 p.p.m., examination was done on each of the 4 dogs and 2 monkeys, and on 4 of the 5 rabbits, 6 of the 12 guinea pigs and 6 of the 16 rats. The 4 rabbits included the 2 which died after 12 and 22 exposures and the 2 which survived. The 6 guinea pigs studied represented a sample of the group of 12, all of which survived. The 6 rats examined included the one which died after the 38th exposure, together with a sample of 5 from the 14 which survived. Four unexposed rats and 4 unexposed guinea pigs were used as controls.

Only the liver was examined from the 4 dogs exposed to 10,000 p.p.m., but from all other animals on both exposures, the heart, lungs, liver, spleen and kidneys were studied. In addition, the adrenals from the guinea pigs and skeletal muscle from the rats were examined. All tissues were fixed in a 10 per cent solution of formalin and routine sections were stained with eosin-methylene blue. Sections from the liver, spleen and kidneys were stained for hemosiderin and frozen sections from the liver and kidneys were stained for fat.

Among the animals exposed to 5,000 p.p.m. of dichloromethane, there were no lesions which appeared to be related to the exposure. The only animals showing pathological changes of any significance were the 2 guinea pigs which died after 90 and 96 exposures respectively, and the rat which died after giving birth to a litter. Each of these guinea pigs showed an extensive pneumonia associated with moderate centrolobular fatty degeneration of the liver. The rat exhibited many thrombi in renal vessels associated with marked certical infarction.

Among those animals exposed to 10,000 p.p.m., no lesions were seen in the livers of 2 of the 4 dogs. The other 2 showed moderate centrolobular congestion with narrowing of liver cell cords and slight to moderate fatty degeneration. The fat droplets were of small size and were observed chiefly in liver cells in congested areas and, to a lesser extent, in irregularly scattered macrophages.

One of the 2 monkeys revealed disseminated tuberculosis lesions but no other histopathologic alterations were found in either of the two.

The 2 rabbits which survived 37 exposures were negative, but the 2 which died showed marked pulmonary congestion and edema with focal extravasation of blood. One of these also exhibited marked congestion of the spleen with considerable pyknosis and karyorrhexis of the lymphoid cells of the follicles.

Four of the 6 exposed guinea pigs revealed slight to moderate fatty degeneration of the liver characterized by fine fat droplet accumulation in the liver cells in centrolobular areas. The remaining 2 were normal. One of the 4 controls exhibited moderate fatty degeneration of the liver, but the fat droplets were moderate in size and diffusely distributed.

TABLE 4

ANALYSES OF BLOOD OBTAINED FROM DOGS IMMEDIATELY FOLLOWING EXPOSURE TO 5,000 P.P.M. OF DICHLOROMETHANE FOR 7 HOURS

DOG NUMBER	NO. SAMPLES	MGS. CH_2Cl_2/100 ML.	
		Average	Range
1	7	9.6	8.1–11.7
2	7	10.5	9.5–12.6
3	7	8.7	7.4–12.0
4	7	9.5	7.2–13.2
5	7	10.7	8.7–12.5

The rat which died after the 38th exposure showed marked pulmonary congestion and edema with focal extravasation of blood. Similar but less marked changes were seen in the lungs of one of the rats which survived, but there were no significant lesions in the others.

Analyses of Volatile Chloride in Blood and Urine From Animals Exposed to Various Concentrations of Dichloromethane

The volatile chloride was determined by the combustion method developed in this laboratory (13). Volatile chloride released by the aeration of blood or urine and decomposed by passing over heated platinum was absorbed in an alkaline sulfite solution and titrated by a standard method.

ASI 000015713

TABLE 5

ANALYSES OF BLOOD AND URINE OBTAINED FROM 2 DOGS FOLLOWING A 3 HOUR EXPOSURE TO 5,000 P.P.M. OF DICHLOROMETHANE

SAMPLE OBTAINED	DOG #6		DOG #7	
	Mgs. CH_2Cl_2/100 ml.			
	Blood	Urine	Blood	Urine
Immediately after exposure	13.7	3.1	5.1	1.1
2 hours after exposure	5.7	1.9	—	—
4 hours after exposure	—	—	1.5	0.6
6 hours after exposure	0.2	0.2	—	—

TABLE 6

ANALYSES OF BLOOD OBTAINED FROM RABBITS FOLLOWING EXPOSURE TO 10,000 P.P.M. OF DICHLOROMETHANE

ANIMAL NO.	DURATION OF EXPOSURE	TIME SAMPLE WAS OBTAINED AFTER DISCONTINUING EXPOSURE	MGS. CH_2Cl_2/100 ML.
	hours		
7	1	Immediately	8.7
13	1	Immediately	9.0
9	2	Immediately	14.9
10	2	Immediately	12.5
12	2	Immediately	17.0
7	3	Immediately	14.5
10	3	Immediately	13.9
9	4	Immediately	17.1
2	4	15 minutes	13.4
2	4	15 minutes	14.5
3	4	15 minutes	14.6
11	4	10 minutes	19.9
1	4	30 minutes	7.3
11	4	1 hour	5.8
7	4	1 hour	10.7
3	4	1 hour	7.0
10	4	1 hour	6.6
3	4	2 hours	6.2
11	4	2 hours	3.7
9	4	2 hours	6.0
2	4	3 hours	3.6
7	4	3 hours	3.0
9	4	4 hours	1.7
10	4	4 hours	2.6
10	4	5 hours	1.1
3	4	5 hours	1.1
3	4	6 hours	1.5
3	4	7 hours	1.1
3	4	8 hours	0.9

Dog blood was withdrawn from the jugular vein and rabbit blood from the heart. Whenever necessary the samples were stored in the refrigerator in aeration tubes or glass stoppered volumetric flasks. Uncontaminated blood containing volatile chloride has been stored five days without any loss of volatile chloride. The results are shown in Tables 4, 5, and 6. The figures usually represent the average of 2 determinations.

Blood withdrawn from a dog after a six hour exposure to 10,000 p.p.m. of dichloromethane had a concentration of 21.8 mg. dichloromethane in 100 ml. while blood from 3 rabbits had the following concentrations: 18.6, 20.4 and 16.4 mg./100 ml.

One rabbit exposed to 10,000 p.p.m. of dichloromethane for four hours was bled ½, 1½, 2½, and 3½ hours after the exposure was discontinued. The following concentrations of dichloromethane were found in the blood at these intervals: 10.4, 9.0, 4.1, and 2.6 mg./100 ml.

A urine specimen was obtained from a rabbit three hours after a four hour exposure to 10,000 p.p.m. dichloromethane. The concentration of dichloromethane in this urine was 3.4 mg./100 ml.

EFFECTS OF SKIN APPLICATIONS IN RABBITS

A pledget of cotton was soaked in dichloromethane and then applied for one minute to the ear. Applications were made once a day, 5 days a week for 4 weeks.

The response to dichloromethane seemed to be that which would result from mild simple irritation maintained by repeated exposures. It had a fat solvent action which made the hair follicles become more prominent. Then there was noted hyperemia followed by a scaly exfoliation.

DISCUSSION

The data presented here confirm other reports on the low toxicity of dichloromethane for various experimental animals. A concentration of 5,000 p.p.m., one half per cent in the atmosphere, had no discernible ill effect on dogs, rabbits, rats or guinea pigs, with one exception. In the case of the guinea pigs there was an adverse effect upon rate of growth. Reproductive function was unimpaired and young rats and puppies grew normally. This is remarkable when one considers that the animals were in the exposure chamber for 7 hours daily, 5 days a week, for 6 months. There was no

ASI 000015714

indication from the urine examinations of renal irritation nor did microscopic sections of the kidneys show any lesions. Absence of liver damage in the dogs is indicated by the following evidence: 1) repeated normal values for the icterus index of blood serum, 2) normal levels of plasma protein and normal A/G ratios after 6 months of exposure, 3) normal prothrombin levels after 4 months of exposure, 4) absence of urobilinogen in the urine on repeated examinations, 5) normal bromsulfalein excretion values, 6) absence of pathological changes in the liver.

Even when the animals were repeatedly exposed for periods of 4 hours to 10,000 p.p.m. of dichloromethane, which caused light to moderate anesthesia, there were few residual ill effects. Recovery was rapid and appetite was generally unimpaired. Autopsy examination of the few animals which died showed pulmonary congestion and edema. It is possible that the high temperatures and humidities which prevailed during this part of the work may have been partly responsible for these deaths. Two out of 4 dogs showed slight to moderate fatty degeneration of the liver after 6 exposures. Four out of 6 guinea pigs also developed liver injury. There was no microscopic evidence of liver damage for the 2 monkeys, 2 rabbits and 6 rats which received almost 40 exposures.

Concentrations as high as 5,000 p.p.m. would probably not be tolerated for very long by human beings. According to Lehmann and Flury (6) a concentration of 310 p.p.m. can just be detected by smell; 2300 p.p.m. causes nausea after 30 minutes; 7200 p.p.m. causes paresthesias of the extremities after 8 minutes, as well as congestion in the head, a sense of heat and slight irritation of the eyes.

The analyses of volatile chloride in blood and urine show that appreciable quantities of dichloromethane are excreted by the urinary route. The concentration in the blood of dogs after an exposure of 7 hours to 5,000 p.p.m. is approximately the same as that reported for rabbits by Moran (13). The data give some idea as to the rate of elimination of dichloromethane. It would be desirable to carry out a detailed study of the rate and extent of absorption and elimination of dichloromethane by various routes. Such an investigation must be deferred, however, until the sensitivity of the method of analysis has been increased.

Summary

1). Several species of animals were simultaneously exposed to 17 mg./l. (5,000 p.p.m.) of dichloromethane. Repeated 7 hour exposures, 5 days a week, for 6 months were tolerated without evidence of toxic reaction by rats, rabbits and dogs. In the case of guinea pigs there was an adverse effect upon rate of growth, but the animals appeared in good condition. The mean arterial blood pressure of the dogs remained within normal limits. No significant changes were observed in the blood picture of dogs or rabbits. No evidence of liver damage was given by the following studies on dogs: 1) serum icterus index, 2) plasma total protein level and A/G ratio, 3) testing for urobilinogen in the urine, 4) bromsulfalein excretion tests, 5) pathological examination of the liver, 6) prothrombin level. Repeated clinical examinations of the urine were negative. Microscopic sections of various organs were normal.

2). Repeated 4 hour exposures, 5 days a week, for 7½ weeks to 10,000 p.p.m. produced no microscopic evidence of liver damage in monkeys, rabbits or rats. Two out of 4 dogs showed slight to moderate fatty degeneration of the liver after 6 exposures. Four out of 6 guinea pigs also developed liver injury. This concentration of dichloromethane causes light to moderate narcosis. Several of the animals died, apparently because of acute pulmonary congestion and edema.

3). Repeated applications of dichloromethane to the ears of albino rabbits lead to hyperemia followed by scaly exfoliation.

4). Data are presented on the concentration of dichloromethane in the blood and urine of various exposed animals.

5). These experiments confirm other reports on the low toxicity of dichloromethane compared with other halogenated hydrocarbon solvents. No data are available concerning the effects on human subjects of repeated exposures. From these animal experiments, it is tentatively suggested that the maximum allowable concentration for 8 hours daily exposure is 500 p.p.m.

ASI 000015715

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