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Effects on Experimental Animals of Long-Term Inhalation of Trichloroethylene, Carbon Tetrachloride, 1,1,1-Trichloroethane, Dichlorodifluoromethane, and 1,1-Dichloroethylene[1]

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This is the fourth of a continuing series of toxicity studies conducted on materials of particular interest to the U.S. Navy. Previous reports on triaryl phosphate hydraulic fluid (Siegel *et al.*, 1965), nitrogen dioxide (Steadman *et al.*, 1966), and mineral spirits (Rector *et al.*, 1966) were concerned with the effects of long-term inhalation exposure to those substances.

In this study, the effects in animals of long-term inhalation exposure to various levels of dichlorodifluoromethane, carbon tetrachloride, trichloroethylene, 1,1,1-trichloroethane (methyl chloroform), and 1,1-dichloroethylene (vinylidene chloride) were examined. The objective of this investigation was the development of data pertinent to underwater vehicles where men might be exposed for prolonged periods of time on a continuous basis.

Two types of studies were conducted, namely, "continuous" 90-day exposures to simulate submarine conditions, and "repeated" daily 8-hour exposures, 5 days/week, over a period of 6 weeks to simulate exposures of the type encountered in industry.

METHODS

Materials

All materials used were of the highest purity available commercially. Because of the quantities required for these studies it was not considered feasible to redistill the liquid materials in order to remove trace impurities or inhibitors. The carbon tetrachloride,[2] trichloroethylene,[2] and inhibited 1,1,1-trichloroethane[2] had boiling ranges of 76.3–76.7°, 86.6–86.9°, and 73.3–78.1°, respectively. Further analysis showed the inhibited 1,1,1-trichloroethane to contain about 8% of inhibitors. The uninhibited vinylidene chloride[3] had a boiling point of 83.5° and a minimum purity of 98%. The dichlorodifluoromethane[4] was of 99% minimum purity.

[1] The opinions expressed herein are those of the authors and do not necessarily reflect the views of the Navy Department or the naval service at large. The experiments reported herein were conducted according to the principles enunciated in "Guide for Laboratory Animal Facilities and Care" prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences–National Research Council, Washington, D.C.

[2] Fisher Certified Reagent grade, Fisher Scientific Co., Pittsburgh, Pennsylvania.

[3] K & K Laboratories, Plainview, New York.

Exposure Equipment

A flow diagram of the chamber exposure system used in both types of studies is illustrated in Fig. 1 and was described by Fultyn (1961). The rate of air flow through the chamber was maintained at 1.23 cubic meters per minute, the relative humidity at approximately 50%, the temperature at 75–80°F, and the negative pressure at 2.0 inches of water. The "down time" necessary for feeding the animals and servicing the chambers amounted to less than 2% of the total chamber time in the continuous exposures.

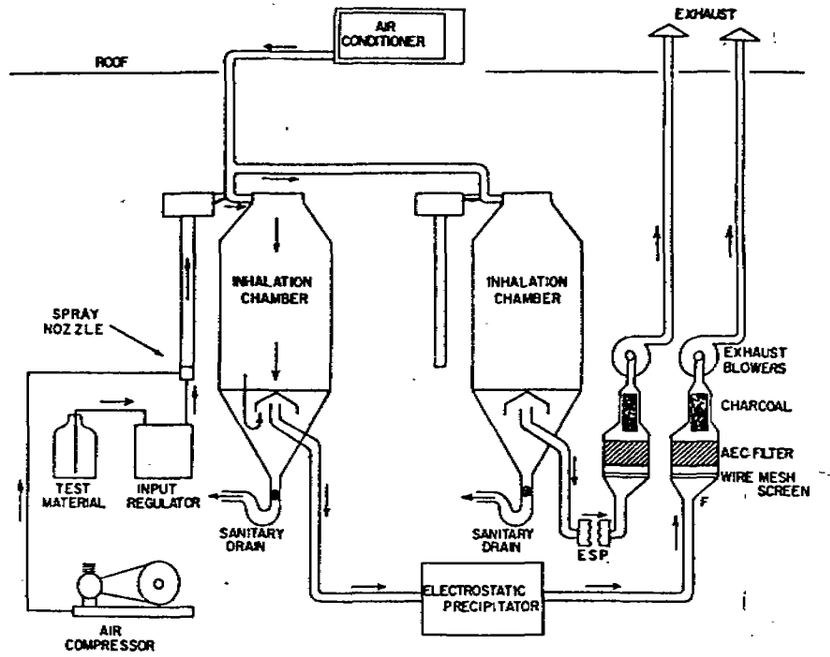

FIG. 1. Schematic diagram of a pair of chambers used for exposing animals to gases and vapors of chlorinated hydrocarbons.

The method of introducing contaminant into these chambers was dependent upon the physical and chemical properties of the substance and the concentration desired. The methods employed were of three general types.

Pump method. In the continuous exposures involving trichloroethylene and 1,1,1-trichloroethane and in all of the carbon tetrachloride exposures a metered amount of the liquid contaminant was pumped[5] from a reservoir through a

[4] Matheson Gas Company, East Rutherford, New Jersey.

[5] Accuflo Pump, Beckman Instrument Co., Palo Alto, California.

calibrated dropper into a test tube. The Venturi effect, created by high-pressure air flowing across a nozzle orifice, drew the test material up a capillary tube and to the nozzle where it was vaporized. The vapor was mixed and diluted to the desired concentration with supply air and then passed into the exposure chamber.

Free-flow method. A reservoir of contaminant was connected directly to the spray nozzle, and the quantity of liquid drawn up the capillary tube to the nozzle was controlled by the regulation of the high-pressure air stream. This method was employed in the repeated exposures involving 1,1,1-trichloroethane and trichloroethylene.

Dilution method. In the 1,1-dichloroethylene exposures, a metered stream of air was passed through a fritted-glass disk immersed in the contaminant reservoir, into an air-mixing bottle, and this diluted mixture was then drawn up into the chamber by means of the Venturi effect of the high-pressure air stream. For the dichlorodifluoromethane exposures, a stream of pure gaseous contaminant from a high-pressure cylinder was metered through a rotameter into a mixing bottle and carried into the chamber where it was diluted to the desired concentration with air.

Analytical Techniques

With the exception of two exposures, chamber concentrations were monitored continuously by means of automated analytical instrumentation. In addition to these measurements, conventional chemical analyses were performed daily and correlated with nominal input data. Three types of monitoring techniques were employed in these studies.

Infrared analysis. A stream of chamber air was drawn through a variable pathlength gas cell fitted to an infrared spectrophotometer.[6] The instrument was locked on the analytical wavelength selected for the contaminant and the percent transmission was continuously recorded. The contaminant level was then read from a graph of concentration *vs* percent transmission previously prepared by vaporizing known amounts of the test material in the gas cell.

Gas chromatography. A stream of chamber air was drawn continuously through an electrically operated sampling valve fitted on a gas chromatograph.[7] The valve was activated every 20 minutes by a recycling time-delay relay. At the beginning of the cycle the timer simultaneously activated a recorder chart drive and the sampling valve which injected a sample of chamber air into the column. A ¼-inch o.d. × 6-foot glass column, packed with 30% diisodecyl phthalate on 60/80 mesh Chromosorb W,[8] maintained at 65° was found suitable. The system remained in this activated mode for a period slightly longer than the retention time required for the appropriate peak to be recorded. At the end of this period the timer deactivated the system.

[6] Model 21 Infrared Spectrophotometer, Perkin Elmer Corp., Norwalk, Connecticut.

[7] Two chromatographs were used: a Chromalab by Glowall Corp., Willow Grove, Pennsylvania, and a GC 2000 R by Micro Tek Instrument Co., Baton Rouge, Louisiana.

[8] Johns-Manville, New York, New York.

The contaminant level was then read from a plot of peak heights *vs* concentration previously prepared by sampling known amounts of contaminant vaporized in measured volumes of air.

Chemical analysis. This method utilized a conventional atmospheric sampling technique and a modified Fujiwara (1914) reaction. A 15-liter sample of chamber air was drawn at a rate of 1.0 l/min through a fritted-glass disk immersed in a bubbler containing 100 ml of toluene. This bubbler was maintained in an ice bath to retard evaporation. For trichloroethylene analysis 1.0 ml of the collecting medium was added to 10.0 ml of pyridine and 2.0 ml of 0.02 *N* NaOH and incubated at 76° for 15 minutes. The reaction produced a pink solution which was read on a spectrophotometer at 470 mμ. The amount of contaminant in the sample was determined from a standard curve prepared by reacting known amounts of the pure liquid. In the 1,1,1-trichloroethane analysis 1.0% alcoholic KOH was used in place of NaOH and the analytical wavelength was 530 mμ.

Biochemical Methods

In the later experiments several histochemical and biochemical determinations were made. Reduced nicotinamide adenine dinucleotide (NADH), reduced nicotinamide adenine dinucleotide phosphate (NADPH), and the activities of succinic dehydrogenase (SDH), lactic dehydrogenase (LDH), isocitric dehydrogenase (ICD), glucose-6-phosphate dehydrogenase (G6PD) and β-hydroxybutyric dehydrogenase (B-OHBD) were determined according to the method of Ballogh *et al.* (1961). Alkaline phosphatase activity was determined by a method described by Linhardt and Walter (1965) and reported as the number of micromoles of *p*-nitrophenylphosphate dephosphorylated per minute per gram of wet tissue at pH 10.5 and 37°. Serum glutamic-pyruvic transaminase levels were determined by a method based on that of Reitman and Frankel (1957) and are reported as the number of millimicromoles of glutamate formed per minute per milliliter of serum at pH 7.5 and 25°. Serum urea nitrogen concentrations were determined by the automated methods described by Skeggs (1957) and Marsh *et al.* (1957). Liver lipids were determined by the method described by Horwitz (1955) and are expressed as the number of grams of petroleum-ether-soluble material per 100 g of liver tissue on a dry-weight basis. All biochemical data are reported as the mean value plus or minus one standard deviation.

Experimental Animals

The typical group of animals exposed consisted of 15 Long-Evans or Sprague-Dawley rats, 15 Hartley guinea pigs, 3 squirrel monkeys, 3 New Zealand albino rabbits, and 2 beagle dogs. In several of the exposures with 1,1-dichloroethylene, the number of monkeys was increased and no rabbits were exposed. The number of animals used in each experiment is shown in Table 1. The total number of controls included 304 rats, 314 guinea pigs, 34 dogs, 48 rabbits, and 57 monkeys. These animals were maintained in

TABLE 1

MORTALITY IN ANIMALS EXPOSED TO SELECTED CHLORINATED HYDROCARBONS

Material	Concentration (mg/m³)[a]	Analytical method[b]	Type of study[c]	Mortality ratio[d]				
				Rat	Guinea pig	Rabbit	Dog	Monkey
Trichloroethylene	3,825 ± 191	IR	R	0/15	0/15	0/3	0/2	0/3
	189 ± 47	CA	C	0/15	0/15	0/3	0/2	0/3
Carbon tetrachloride	515 ± 39	GC	R	0/15	3/15	0/3	0/2	1/3
	61 ± 5.2	GC	C	0/15	3/15	0/2	0/2	0/3
	6.1 ± 0.3	GC	C	0/15	0/15	0/3	0/2	0/3
1,1,1-Trichloroethane	12,060 ± 15	IR	R	0/15	0/15	0/3	0/2	0/3
	2,059 ± 54	IR	C	0/15	0/15	0/3	0/2	0/3
	754 ± 62	CA	C	2/15	0/15	1/3	0/2	0/3
Dichlorodifluoromethane	4,136 ± 87	IR	R	1/15	0/15	0/3	0/2	0/3
	3,007 ± 112	IR	C	2/15	1/15	0/3	0/2	0/3
1,1-Dichloroethylene	995 ± 32	GC	R	0/15	0/15	0/3	0/2	0/3
	189 ± 6.2	GC	C	0/15	7/15	—	0/2	3/9
	101 ± 4.4	GC	C	0/15	3/15	0/3	0/2	2/3
	61 ± 5.7	GC	C	0/15	3/15	—	0/2	0/3
	20 ± 2.1	GC	C	2/45	2/45	—	0/6	1/21
Controls	—	—	C	7/304	2/314	2/48	0/34	1/57

[a] Mean ± SD.
[b] IR = infrared analysis; CA = chemical analysis; GC = gas chromatography.
[c] R = 30 exposures, 8 hr/day, 5 days/week; C = continuous 90-day exposure.
[d] Number that died divided by the number with which the study commenced.

exposure chambers without contaminant, but otherwise under conditions identical to those of the test animals.

The basic diet for all species was the appropriate commercially prepared dry chow. In addition, the dogs were provided daily with a meat-base canned dog food, the rabbits with lettuce, and the monkeys with bananas, oranges, and boiled eggs. Each guinea pig received one-quarter of a head of lettuce daily which provided the sole source of water. All other animals received water ad libitum.

Each test animal was weighed prior to the exposure, at monthly intervals, and at the termination of the run. Total and differential leukocyte counts, hemoglobin concentrations, and microhematocrits were obtained on all animals except the monkeys before and after the exposure. All animals were routinely checked for visible signs of toxicity, such as marked alterations in behavior, physical appearance, respiration pattern, locomotor activity, and prostration. At the termination of each study, animals were sacrificed, autopsied, and sections of heart, lung, liver, spleen, and kidney were taken for histopathologic evaluation. In several of these studies selected biochemical determinations were made in an effort to detect early alterations.

RESULTS

Mortality, hematologic, and body weight data are presented in Tables 1, 2, and 3, respectively. Normal hematologic values indicated in the table represent the average preexposure values of all animals used in inhalation studies at the U.S. Navy Toxicology Unit since 1959. Chamber controls are the average values of only those animals actually maintained in inhalation chambers as controls. Although variations were noted in pre- and postexposure values of the hematologic components studied, none of these changes was considered significant. Biochemical effects of 1,1-dichloroethylene inhalation are shown in Table 4.

Trichloroethylene Repeated Exposure (3825 mg/m³)

Animals were exposed repeatedly 8 hours/day, 5 days/week for 30 exposures over a 6-week period to trichloroethylene at a concentration of 3825 mg/m³. No mortality occurred, and the only sign observed was slight nasal discharge, seen in some of the rats This condition was also noted in the controls. Body weight losses were noted in the dogs. Gross pathologic findings were essentially negative with the exception of lung congestion noted in an occasional guinea pig and rat. Nonspecific inflammatory changes were found on histopathologic examination in the lungs of all animals, but there was no evidence of specific chemically induced changes in any of the organs examined which could be attributed to the exposure. Histochemical studies of enzymatic activity were performed on liver tissue from three selected experimental rats. No changes in the activities of SDH, LDH, ICD, G6PD, or B-OHBD were found.

TABLE 2

HEMATOLOGIC VALUES FOR ANIMALS SURVIVING EXPOSURE TO SELECTED CHLORINATED HYDROCARBONS

Material	Concentration (mg/m³)	Type of study	Rats[a] Leukocytes (10³/mm³)	Rats[a] Hemoglobin (%)	Rats[a] Hematocrit (%)
Trichloroethylene	3,825	R[d]	17.4 ± 5.9[c]	14.7 ± 0.7	46 ± 2.0
			13.2 ± 3.9	15.2 ± 0.9	48 ± 2.1
	189	C[e]	15.8 ± 4.7	15.0 ± 8.9	50 ± 3.8
			17.8 ± 5.3	15.2 ± 9.1	52 ± 4.0
Carbon tetrachloride	515	R	19.8 ± 6.6	14.6 ± 1.8	47 ± 4.1
			16.3 ± 4.9	14.9 ± 0.7	46 ± 3.3
	61	C	18.9 ± 4.2	15.0 ± 1.6	50 ± 4.3
			20.2 ± 4.2	15.1 ± 1.3	46 ± 2.1
	6.1	C	18.9 ± 5.9	14.0 ± 1.5	50 ± 2.4
			13.8 ± 1.8	14.7 ± 2.7	47 ± 2.2
1,1,1-Trichloroethane	12,060	R	24.4 ± 7.5	15.7 ± 1.1	48 ± 3.3
			21.2 ± 9.0	14.9 ± 1.6	47 ± 5.5
	2,059	C	17.4 ± 3.8	15.6 ± 0.8	49 ± 2.9
			17.4 ± 4.0	15.4 ± 1.0	50 ± 5.1
	754	C	14.6 ± 3.7	14.8 ± 1.7	51 ± 4.1
			10.1 ± 3.0	14.9 ± 1.7	48 ± 6.2
Dichlorodifluoromethane	4,136	R	12.3 ± 3.0	16.1 ± 1.3	51 ± 3.4
			11.6 ± 3.4	15.2 ± 1.5	47 ± 3.0
	3,997	C	12.5 ± 3.3	16.1 ± 1.8	49 ± 5.0
			10.3 ± 3.2	15.2 ± 2.8	48 ± 7.8
1,1-Dichloroethylene	395	R	25.4 ± 2.4	16.6 ± 1.4	50 ± 2.2
			15.9 ± 1.1	15.1 ± 1.7	47 ± 2.8
	189	C	18.2 ± 7.0	14.4 ± 1.1	43 ± 3.2
			15.0 ± 2.8	14.5 ± 1.0	46 ± 2.2
	101	C	23.2 ± 5.1	16.7 ± 1.2	49 ± 2.1
			21.7 ± 4.0	16.3 ± 1.3	50 ± 2.3
	61	C	21.9 ± 8.2	17.6 ± 2.0	48 ± 2.7
			14.7 ± 4.1	17.1 ± 1.7	48 ± 3.7
	20	C	12.6 ± 3.7	15.1 ± 1.0	48 ± 2.0
			14.3 ± 4.3	15.4 ± 0.9	47 ± 2.4
Normals	—	—	16.5 ± 4.7	15.0 ± 1.2	47 ± 4.0
Controls (90-day)		C	18.2 ± 6.5	15.5 ± 1.2	48 ± 3.0
			14.3 ± 4.9	15.2 ± 1.3	48 ± 3.0

[a] Mean ± SD.
[b] Mean.
[c] Upper line at each concentration represents preexposure data, lower line, postexposure data.

TABLE 2—*Continued*

Guinea pigs[a] Leukocytes (10³/mm³)	Guinea pigs[a] Hemoglobin (%)	Guinea pigs[a] Hematocrit (%)	Rabbits[b] Leukocytes (10³/mm³)	Rabbits[b] Hemoglobin (%)	Rabbits[b] Hematocrit (%)	Dogs[b] Leukocytes (10³/mm³)	Dogs[b] Hemoglobin (%)	Dogs[b] Hematocrit (%)
5.1 ± 1.7	13.1 ± 1.0	44 ± 2.4	10.3	11.5	36	10.1	16.0	49
7.3 ± 2.1	13.5 ± 0.7	45 ± 3.0	9.2	13.3	36	10.8	14.5	45
4.8 ± 4.8	15.4 ± 4.9	48 ± 2.6	7.2	13.0	41	19.9	15.0	53
5.6 ± 4.0	14.1 ± 4.5	47 ± 6.0	8.2	13.4	43	18.2	14.0	48
6.2 ± 2.2	14.5 ± 0.9	49 ± 3.9	11.1	11.6	39	15.6	16.7	50
9.7 ± 2.7	15.0 ± 0.7	48 ± 3.2	14.9	11.1	35	11.4	17.2	51
5.9 ± 1.6	12.6 ± 1.3	42 ± 4.5	14.1	12.4	41	15.0	15.5	47
10.3 ± 2.2	16.3 ± 1.7	45 ± 3.0	18.6	13.3	48	12.8	14.5	48
5.3 ± 1.7	12.6 ± 1.1	40 ± 2.7	13.1	13.3	40	16.6	15.5	51
6.8 ± 2.6	14.2 ± 0.2	47 ± 2.3	14.0	12.5	41	10.3	16.4	51
6.0 ± 1.0	16.3 ± 1.1	50 ± 2.5	5.8	12.8	37	12.5	19.5	45
7.1 ± 1.3	15.4 ± 1.2	51 ± 2.3	6.4	12.6	41	9.5	16.0	40
9.3 ± 3.3	15.2 ± 0.8	49 ± 2.8	18.1	12.8	42	13.8	14.8	45
7.6 ± 2.0	15.4 ± 1.1	50 ± 3.8	10.2	13.4	41	10.6	15.6	47
5.1 ± 1.2	14.7 ± 0.7	44 ± 1.5	8.4	13.0	41	21.6	14.5	47
6.6 ± 1.2	14.3 ± 0.6	46 ± 3.0	8.2	13.0	39	17.7	15.0	55
4.7 ± 0.8	15.9 ± 1.0	48 ± 3.8	10.2	13.2	38	11.2	13.9	38
6.3 ± 1.2	13.7 ± 1.6	42 ± 5.0	12.9	11.3	37	10.8	14.2	46
5.3 ± 1.3	15.2 ± 0.8	47 ± 2.0	9.0	11.7	38	9.2	15.0	46
8.9 ± 3.6	15.4 ± 1.2	51 ± 4.8	16.2	12.9	42	9.1	17.3	55
6.8 ± 2.1	14.3 ± 1.2	45 ± 3.2	10.5	11.9	42	14.2	15.5	51
5.6 ± 3.1	15.2 ± 1.1	46 ± 2.7	10.5	12.9	40	12.6	17.3	50
6.6 ± 1.4	14.7 ± 1.6	48 ± 2.2	—	—	—	15.2	16.0	49
7.2 ± 1.6	15.6 ± 0.9	48 ± 2.6	—	—	—	11.7	15.4	46
6.0 ± 1.8	17.2 ± 1.0	50 ± 2.1	15.8	13.3	41	18.1	17.3	48
7.5 ± 1.6	16.4 ± 0.8	45 ± 3.6	11.0	12.7	37	17.6	16.8	52
4.5 ± 1.2	14.3 ± 1.0	47 ± 3.2	—	—	—	12.7	15.3	48
6.9 ± 2.1	14.8 ± 0.8	48 ± 3.1	—	—	—	15.2	16.7	50
6.0 ± 1.1	14.5 ± 1.0	47 ± 2.5	—	—	—	16.5	16.0	49
7.5 ± 1.8	14.8 ± 1.4	47 ± 2.3	—	—	—	13.4	15.8	47
5.9 ± 1.4	14.9 ± 1.1	47 ± 3.0	9.3 ± 3	12.8 ± 1	40 ± 4	14.2 ± 4	15.2 ± 1	47 ± 4
6.4 ± 1.7	15.4 ± 1.2	48 ± 2.0	12.0 ± 4	12.5 ± 1	40 ± 2	14.6 ± 4	15.4 ± 1	47 ± 4
7.2 ± 2.0	15.2 ± 1.2	47 ± 1.0	14.8 ± 6	13.1 ± 1	41 ± 2	14.2 ± 3	16.1 ± 1	49 ± 2

[a] Thirty exposures 8 hours/day, 5 days/week.
[b] Continuous 90-day exposure

TABLE 3

BODY WEIGHT CHANGE IN ANIMALS SURVIVING EXPOSURE TO SELECTED CHLORINATED HYDROCARBONS

Material	Concentration (mg/m³)	Type of study	Rats: Starting weight[a] (g)	Rats: Change (%)	Guinea pigs: Starting weight[a] (g)	Guinea pigs: Change (%)	Rabbits: Starting weight[a] (kg)	Rabbits: Change (%)	Dogs: Starting weight[a] (kg)	Dogs: Change (%)	Monkeys: Starting weight[a] (g)	Monkeys: Change (%)
Trichloroethylene	3,825	R[c]	256 (229–303)	+41.4	305 (266–358)	+59.7	2.65 (2.61–2.70)	+28.1	10.7 (9.8–11.6)	−6.5	570 (511–630)	5.1
	189	C[b]	263 (203–356)	+20.2	408 (371–450)	+54.9	2.48 (2.20–2.55)	+4.6	7.3 (7.0–7.6)	+15.1	814 (712–915)	−4.2
Carbon tetrachloride	515	R	325 (243–410)	+13.2	399 (303–460)	−4.5	3.27 (3.05–3.60)	−2.5	10.5 (9.5–11.5)	−5.0	601 (571–631)	−6.1
	61	C	257 (220–304)	+27.1	413 (374–472)	+38.6	2.97 (2.70–3.25)	+6.4	13.9 (13.1–14.7)	0.0	724 (611–826)	2.8
	6.1	C	263 (215–334)	+10.0	417 (352–517)	+29.7	3.22 (3.20–3.25)	+13.8	10.1 (10.1–10.1)	+4.7	622 (573–653)	−6.5
1,1,1-Trichloroethane	12,060	R	275 (199–333)	+32.3	387 (345–488)	+36.4	3.57 (3.50–3.70)	−0.5	12.3 (11.6–13.0)	−1.8	518 (425–711)	−2.9
	2,059	C	249 (164–338)	+46.1	395 (346–448)	+70.8	3.33 (3.25–3.45)	+9.0	9.1 (9.1–9.1)	+6.9	694 (658–761)	−3.2
	754	C	230 (174–278)	+35.5	410 (296–478)	+48.7	2.15 (2.00–2.40)	+31.0	11.4 (10.6–12.2)	+11.9	616 (465–737)	+3.4
Dichlorodifluoromethane	4,136	R	192 (145–211)	+31.5	526 (400–662)	+22.6	2.51 (2.49–2.54)	+17.2	8.2 (7.9–8.6)	−10.3	821 (807–852)	−13.2
	3,997	C	213 (165–262)	+30.1	574 (424–655)	+20.5	2.43 (2.30–2.50)	+5.8	11.1 (10.8–11.4)	+20.3	755 (647–868)	+7.5
1,1-Dichloroethylene	395	R	268 (203–351)	+17.5	340 (234–389)	+67.0	3.80 (3.55–4.05)	−3.6	8.3 (8.0–8.6)	+9.3	606 (572–627)	+5.9
	189	C	275 (223–392)	+4.3	469 (405–534)	+50.3	–	–	9.6 (9.5–9.7)	−8.3	718 (508–820)	28.1
	101	C	234 (175–307)	+31.6	418 (360–495)	+74.0	3.35 (3.10–3.65)	−4.1	9.6 (9.5–9.7)	−5.2	759 (759)	6.1
	61	C	331 (216–450)	+7.0	447 (303–567)	+55.3	–	–	8.3 (8.0–8.6)	+13.3	667 (505–1008)	10.3
	20	C	315 (202–531)	+27.0	490 (342–622)	+58.6	3.03 (3.00–3.05)	+19.0	12.3 (9.0–14.8)	−2.2	740 (692–962)	+9.8
Controls (90-day)		C	286 (192–410)	+35.5	418 (274–646)	+69.0	2.6 (2.1–3.9)	+33.8	10.4 (7.6–14.6)	+12.4	651 (382–952)	+0.8

[a] Mean starting weight of survivors. The numbers in parentheses are ranges.
[b] Continuous 90-day exposure.
[c] Thirty exposures, 8 hours/day, 5 days/week.

TABLE 4

BIOCHEMICAL EFFECTS OF CONTINUOUS EXPOSURE TO 1,1-DICHLOROETHYLENE

Concentration (mg/m³)	Serum urea nitrogen (mg/100 ml)[a]		Liver lipids (%)[a]		Liver alkaline phosphatase (μmole/min/g)[a]		Serum glutamic-pyruvic transaminase (mμmole/min/ml)[a]	
	Rats	Guinea pigs	Rats	Guinea pigs	Rats	Guinea pigs	Rats	Guinea pigs
189	17 ± 4	20 ± 2	12.0 ± 7.7	5.9 ± 1.4	0.21 ± 0.07	0.19 ± 0.04	34 ± 13	>70
20	22 ± 2	24 ± 2	9.4 ± 2.1	9.1 ± 2.4	0.11 ± 0.04	0.08 ± 0.03	9 ± 4	11 ± 3
Controls	20 ± 4	21 ± 5	9.1 ± 1.8	11.0 ± 3.6	0.12 ± 0.05	0.08 ± 0.03	11 ± 6	10 ± 5

[a] Mean ± SD.

TABLE 3

BODY WEIGHT CHANGE IN ANIMALS SURVIVING EXPOSURE TO SELECTED CHLORINATED HYDROCARBONS

Material	Concentration (mg/m³)	Type of study	Rats		Guinea pigs		Rabbits		Dogs		Monkeys	
			Starting weight[a] (g)	Change (%)	Starting weight[a] (g)	Change (%)	Starting weight[a] (kg)	Change (%)	Starting weight[a] (kg)	Change (%)	Starting weight[a] (g)	Change (%)
Trichloroethylene	3,825	R[c]	256 (229–303)	+41.4	305 (266–358)	+59.7	2.65 (2.61–2.70)	+28.1	10.7 (9.8–11.6)	−6.5	570 (511–630)	−5.1
	189	C[b]	263 (203–356)	+20.2	408 (374–450)	+54.9	2.48 (2.20–2.55)	+4.6	7.3 (7.0–7.6)	+15.1	814 (712–915)	−4.2
Carbon tetrachloride	515	R	325 (233–440)	+13.2	399 (303–460)	−4.5	3.27 (3.05–3.60)	−2.5	10.5 (9.5–11.5)	−5.0	601 (571–631)	−6.1
	61	C	257 (220–304)	+27.1	413 (374–472)	+38.6	2.97 (2.70–3.25)	+6.4	13.9 (13.1–14.7)	0.0	724 (611–826)	−2.8
	6.1	C	263 (215–334)	+40.0	417 (352–517)	+29.7	3.22 (3.20–3.25)	+13.8	10.1 (10.1–10.1)	+4.7	622 (573–653)	−6.5
1,1,1-Trichloroethane	12,060	R	275 (199–333)	+32.3	387 (345–488)	+36.4	3.57 (3.50–3.70)	−0.5	12.3 (11.6–13.0)	−1.8	548 (425–711)	−2.9
	2,059	C	249 (164–338)	+46.1	395 (346–448)	+70.8	3.33 (3.25–3.45)	+9.0	9.1 (9.1–9.1)	+6.0	604 (658–764)	−1.2
	754	C	230 (174–278)	+35.5	410 (296–478)	+48.7	2.15 (2.00–2.40)	+31.0	11.4 (10.6–12.2)	+11.9	616 (465–737)	+3.4
Dichlorodifluoromethane	4,136	R	192 (145–211)	+31.5	526 (400–662)	+22.6	2.51 (2.49–2.54)	+17.2	8.2 (7.9–8.6)	−10.3	824 (807–852)	−13.2
	3,997	C	213 (165–262)	+30.1	574 (424–655)	+20.5	2.43 (2.30–2.50)	+5.8	11.1 (10.8–11.4)	+20.3	755 (647–868)	+7.5
1,1-Dichloroethylene	395	R	208 (203–351)	+17.5	340 (234–389)	+67.0	3.80 (3.55–4.05)	−3.6	8.3 (8.0–8.6)	+9.3	606 (572–627)	−5.9
	189	C	275 (223–302)	+4.3	469 (405–534)	+50.3	—	—	9.6 (9.5–9.7)	−8.3	718 (508–820)	−28.1
	101	C	234 (175–307)	+31.6	418 (360–495)	+74.0	3.35 (3.10–3.65)	−4.1	9.6 (9.5–9.7)	−5.2	759 (759)	−6.1
	61	C	331 (210–450)	+7.6	447 (303–567)	+55.3	—	—	8.3 (8.0–8.6)	+13.3	667 (505–1008)	10.3
	20	C	315 (202–531)	+27.0	490 (342–622)	+58.6	3.03 (3.00–3.05)	+19.0	12.3 (9.0–14.8)	−2.2	740 (602–962)	+9.8
Controls (90-day)		C	286 (192–440)	+35.5	418 (274–646)	+69.0	2.6 (2.1–3.0)	+33.8	10.4 (7.6–14.6)	+12.4	651 (382–952)	+0.8

[a] Mean starting weight of survivors. The numbers in parentheses are ranges.
[b] Continuous 90-day exposure.
[c] Thirty exposures, 8 hours/day, 5 days/week.

TABLE 4

BIOCHEMICAL EFFECTS OF CONTINUOUS EXPOSURE TO 1,1-DICHLOROETHYLENE

Concentration (mg/m³)	Serum urea nitrogen (mg/100 ml)[a]		Liver lipids (%)[a]		Liver alkaline phosphatase (μmole/min/g)[a]		Serum glutamic-pyruvic transaminase (mμmole/min/ml)[a]	
	Rats	Guinea pigs	Rats	Guinea pigs	Rats	Guinea pigs	Rats	Guinea pigs
189	17 ± 4	20 ± 2	12.0 ± 7.7	5.9 ± 1.4	0.21 ± 0.07	0.19 ± 0.04	34 ± 13	>70
20	22 ± 2	24 ± 2	9.4 ± 2.1	9.1 ± 2.4	0.11 ± 0.04	0.08 ± 0.03	9 ± 4	11 ± 3
Controls	20 ± 4	21 ± 5	9.1 ± 1.8	11.0 ± 3.6	0.12 ± 0.05	0.08 ± 0.03	11 ± 6	10 ± 5

[a] Mean ± SD.

Trichloroethylene Continuous Exposure (189 mg/m³)

A second group of animals was exposed continuously for 90 days to an atmosphere containing 189 mg/m³ of trichloroethylene. No animals died during the course of this exposure and no visible signs of toxicity were noted. Gross pathologic findings did not differ significantly from those of the controls. The body weight gain in rabbits was lower than that found in controls. Histopathologic examination of heart, liver, lung, spleen, and kidney tissue revealed no indication of chemically induced changes.

Carbon Tetrachloride Repeated Exposure (515 mg/m³)

Animals were repeatedly exposed over a period of 6 weeks to an atmosphere containing 515 mg/m³ of carbon tetrachloride. During the course of the study 1/3 monkeys died after the 7th exposure and 3/15 guinea pigs died after the 20th, 22nd, and 30th exposures, respectively. With the exception of a few cases of slight nasal discharge, no visible toxic signs were noted in any of the animals. The guinea pigs, rabbits, dogs, and monkeys showed a body weight loss. Upon gross pathologic examination all species except dogs showed a high percentage of mottled livers. In addition, most of the guinea pigs had discolored lungs. Histopathologic examination revealed morphologic changes in the lungs and livers of all species, particularly the guinea pig, but no changes were noted in the heart, spleen, or kidney. Lungs of all species exhibited interstitial inflammation or pneumonitis. The livers of the guinea pigs exhibited fatty infiltration, fibrosis, bile duct proliferation, hepatic cell degeneration and regeneration, focal inflammatory cell infiltration, alteration of lobular structure, and early portal cirrhosis. In relation to the guinea pig, the species most adversely affected, the remaining 4 species showed fatty changes in the liver in the following order of decreasing severity: rats, rabbits, dogs, and monkeys. The hepatic changes noted were attributed to the exposure. Guinea pig liver lipid content of $35.4 \pm 10.7\%$ was markedly higher than the control value of $11.0 \pm 3.6\%$.

Carbon Tetrachloride Continuous Exposure (61 mg/m³)

At the 61 mg/m³ level, 3/15 guinea pigs died on days 47, 63, and 71. All 3 monkeys had an emaciated appearance and a loss of hair; otherwise, no apparent signs of toxicity were noted. All species exhibited depressed growth curves compared to those of the control animals. Gross pathologic examination upon autopsy revealed a high incidence of enlarged and/or discolored livers in rats, guinea pigs, monkeys, and rabbits. No gross pathologic abnormalities were noted in the dogs. Histopathologic study revealed liver changes of varying degrees in all species; these were most pronounced in the rats and guinea pigs, and included fatty changes associated with mononuclear cell infiltrates, fibroblastic proliferation, collagen deposition, hepatic cell degeneration and regeneration, and alteration in the structure of the liver lobule. These liver changes were considered to be chemically induced by the exposure. Sections of the affected livers were taken from three rats

and three guinea pigs for histochemical enzymatic activity studies. The NADH, NADPH, SDH, LDH, and G6PD enzymes and coenzymes were studied, and the only abnormality observed was a moderately reduced activity of succinic dehydrogenase in the guinea pigs. All other systems appeared normal.

Carbon Tetrachloride Continuous Exposure (6.1 mg/m³)

The delivery rate necessary for the generation of 6.1 mg/m³ was beyond the lower limit of the pump being used, and it was therefore necessary to dilute the carbon tetrachloride. A solution of carbon tetrachloride in *n*-octane made it possible to generate this low concentration (6.1 mg/m³ of carbon tetrachloride in 61 mg/m³ of *n*-octane) with a higher and more stable pump rate.

During the course of this study no visible signs of toxicity were noted in any of the species and no animals died. At termination of the exposure all species except the rat showed less body weight gain than the controls. Histopathologic examination revealed nonspecific inflammatory changes in the lungs of all species. Nonspecific inflammatory changes of the liver, kidney, and heart were also observed in several animals, but no specific pathologic changes attributable to the exposure were noted. The lipid content of the guinea pig livers of $9.7 \pm 2.4\%$ compared favorably with the control value of $11.0 \pm 3.6\%$. Serum urea nitrogen concentrations in rats and guinea pigs of 20 ± 2 and 23 ± 3 mg/100 ml, respectively, compared favorably with control values of 20 ± 4 and 24 ± 5 mg/100 ml, respectively.

A duplicate exposure was conducted with an identical set of animals and chamber conditions, using an atmosphere containing 62 mg/m³ of *n*-octane. During the course of this exposure no animals died and no visible signs of toxicity were noted. Weight patterns and hematologic data were within normal limits. Gross pathologic and subsequent histopathologic evaluation of tissues from the experimental animals did not reveal any abnormalities attributable to the *n*-octane.

1,1,1-Trichloroethane Repeated Exposure (12,060 mg/m³)

In this 30-day exposure no animals died and no visible toxic signs were noted. Rabbits and dogs showed a body weight loss. Gross and histopathologic examination of brain, heart, lung, liver, spleen, and kidney did not reveal any abnormalities that could be attributed to the exposure. Serum urea nitrogen determinations on guinea pigs revealed no elevation (25 ± 4 mg/100 ml *vs* 24 ± 5 mg/100 ml for controls).

1,1,1-Trichloroethane Continuous Exposure (2059 mg/m³)

Exposure at this level did not result in death or visible toxic signs in the test animals after 90 days. There was less body weight gain in the dogs and rabbits than in control animals. Gross and histopathologic examination did not reveal any abnormalities attributable to the exposure. One rat had gray nodules on the lower lobe of the left lung and one rabbit had

grapelike sacs containing clear fluid on the abdominal wall and adjacent organs. Microscopic examination of tissue showed nonspecific inflammatory changes in the lungs of all species. Guinea pig serum urea nitrogen concentration of 23 ± 4 mg/100 ml was again similar to the control value of 24 ± 5 mg/100 ml.

1,1,1-Trichloroethane Continuous Exposure (754 mg/m³)

In this exposure 2/15 rats died on days 27 and 77, and 1/3 rabbits died on day 38; the survivors exhibited no toxic signs. Body weight patterns were normal. Varying degrees of lung congestion and pneumonitis were noted in all species and in a number of the controls. In view of the 3 deaths and the pneumonitis present in the surviving animals, no positive conclusion could be drawn as to whether the effects were associated with the exposure. It should be noted that in the higher exposure at 2059 mg/m³ no deaths occurred and no pathologic abnormalities were noted.

Dichlorodifluoromethane Repeated Exposure (4136 mg/m³)

During the course of the exposure at 4136 mg/m³, 1/15 rats died but no signs of toxicity were observed in the survivors. Dogs and monkeys lost weight. Gross examination revealed that most rabbits and monkeys, and several rats and guinea pigs, had varying degrees of lung congestion but other organs appeared normal. Histopathologic examination of tissues showed nonspecific interstitial inflammatory changes in the lungs of both experimental and control animals. Several experimental guinea pigs were found to have focal necrosis or fatty infiltration of the liver while one monkey had heavy pigment deposits in the liver, spleen, and kidney. These changes appeared to be related to the exposure in view of similar but more severe effects noted in the continuous study.

Dichlorodifluoromethane Continuous Exposure (3997 mg/m³)

In this continuous exposure 2/15 rats and 1/15 guinea pigs died, but no other visible signs were noted. Body weight gains of the rabbits and guinea pigs were depressed, but it might be noted that the starting weights of the guinea pigs were higher than those of the controls. Gross examination revealed a high incidence of varying degrees of lung congestion in rabbits, monkeys, rats, and guinea pigs. Histopathologic study of selected tissues revealed nonspecific inflammatory changes in the lungs of all species. One guinea pig showed a focal giant cell pneumonitis. Slight to extensive fatty infiltration of the hepatic cells was noted in all guinea pig liver sections examined. Several sections exhibited focal or submassive necrosis of the liver. These hepatic changes were considered to have been induced by the exposure.

1,1-Dichloroethylene Repeated Exposure (395 mg/m³)

No animals died and no visible signs of toxicity were observed during this exposure. The rabbits and monkeys lost weight. Gross examination revealed normal organs in all animals with the exception of one rat that had a gelatinous material on the kidney and bloody urine in the bladder. Histopathologic study revealed nonspecific inflammatory changes in the lungs of all species, but these were not attributed to the exposure. Pneumonitis and congested lungs were found in an occasional control rat and guinea pig. Guinea pig serum urea nitrogen concentration was 23 ± 3 mg/100 ml, which compared favorably with 24 ± 5 mg/100 ml obtained on control animals.

1,1-Dichloroethylene Continuous Exposure (189 mg/m³)

During this exposure, 7/15 guinea pigs died between day 4 and day 9 of exposure, and 3/9 monkeys died on days 26, 60, and 64. The surviving animals exhibited no visible signs of toxicity. Dogs and monkeys lost weight while the rats gained less than the controls. Gross examination revealed mottled livers in a majority of the experimental animals. Histopathologic examination was performed on sections of heart, lung, liver, spleen, and kidney from all species as well as on sections of brain, spinal cord, and adrenal gland from dogs and monkeys, and thyroid gland from dogs. Sections of liver from dogs, monkeys, and rats showed morphologic changes which consisted of fatty metamorphosis, focal necrosis, hemosiderin deposition, lymphocytic infiltration, bile duct proliferation, fibrosis and pseudo-lobule formation. These changes were most severe in dogs. Sections of kidney from all rats showed nuclear hypertrophy of the tubular epithelium. One adrenal gland from a dog contained a cortical adenoma composed of cells of the zona glomerulosa type. There were nonspecific inflammatory changes in the lungs of a majority of the animals. The hepatic changes in dogs, monkeys, and rats and the renal changes in rats are considered to be a direct result of the exposure. Liver alkaline phosphatase activity was determined in surviving rats and guinea pigs and showed a slight elevation in both species when compared with control animals. Serum glutamic-pyruvic transaminase activity was also increased in both species with a more marked increase seen in guinea pigs. Mean values for liver lipid content did not reveal marked alterations from controls; however, two rats had elevated liver lipid contents of 34.4 and 20.0%. Serum urea nitrogen concentrations in both species did not differ significantly from those of controls. These data are presented in Table 4.

1,1-Dichloroethylene Continuous Exposure (101 mg/m³)

No visible toxic signs were noted in any of the surviving animals although 3/15 guinea pigs died between the third and sixth exposure days and 2/3 monkeys on days 39 and 47. Weight patterns indicated a loss in body weight for the rabbits, dogs, and monkeys. On gross examination white or bluish-gray spots and nodules were found on the lungs of a number of guinea pigs and rats. Histopathologic study revealed nonspecific inflammatory changes in the lungs of all animals, but no changes were observed that could be attributed to the exposure. Guinea pig serum urea nitrogen concentration of 24 ± 3 mg/100 ml compared favorably with the control value of 24 ± 5 mg/100 ml.

1,1-Dichloroethylene Continuous Exposure (61 mg/m³)

In this exposure, 3/15 guinea pigs died on the third and fourth days of exposure, but no visible signs of toxicity were noted in any of the surviving animals. The monkeys showed a loss in body weight, and the rats gained less than the controls. Gross examination revealed mottled livers and/or spleens in several animals of all species. Histopathologic examination showed nonspecific inflammatory changes in all species which were most marked in the lungs but were also observed to a lesser degree in the liver and kidneys. These changes were not considered to have been induced by the exposure. Rat and guinea pig serum urea nitrogen levels of 20 ± 4 and 26 ± 3 mg/100 ml, respectively, were similar to the control values of 20 ± 4 and 24 ± 5 mg/100 ml, respectively.

1,1-Dichloroethylene Continuous Exposure (20 mg/m³)

Three separate exposures were run at 19.5, 19.7, and 20.5 mg/m³ and the results were combined for the purpose of reporting as an exposure to an average concentration of 20 mg/m³. During the course of these exposures 2/45 rats, 2/45 guinea pigs, and 1/21 monkeys died but no visible toxic signs were noted in the survivors. The dogs lost weight while the rats gained less than control animals. Gross examination revealed mottled livers in about one-third of the animals of all species. Histopathologic examination revealed nonspecific inflammatory changes in the lungs of all species and in the kidneys and livers of monkeys. No changes were noted in the heart or spleen in any species. None of the pathologic changes noted were considered to have been caused by the exposure. The same biochemical studies were performed on animals in this group that were done on those exposed to 189 mg/m³. Urea nitrogen concentrations in rat and guinea pig sera again compared favorably with controls. Liver lipid values in both species fell within control limits. The elevations in liver alkaline phosphatase and serum glutamic-pyruvic transaminase activities that were found in the 189 mg/m³ exposure could not be demonstrated in this study.

DISCUSSION

Trichloroethylene

Due to its extensive use in industrial degreasing processes and in anesthetic procedures, the toxicity of trichloroethylene has come under the scrutiny of many investigators. The literature review by von Oettingen (1955) indicated that the major effect noted in acute inhalation toxicity studies was depression of the central nervous system. This effect was less predominant in long-term repeated studies at lower concentrations. Adams *et al.* (1951) exposed rats and rabbits to 3000 ppm for 7 hours/day for 27 exposures in 36 days. The rats displayed minor disturbances of equilibrium, increased salivation, and hyperexcitability but recovered quickly upon removal from the chamber. The weights of the liver and kidney in both species were found to have been

significantly increased but histopathologic examination of the tissues revealed no significant changes. The blood urea nitrogen levels in both species were found to be normal. They also exposed rats, guinea pigs, rabbits, and monkeys 7 hours/day, 5 days/week for approximately six months to various levels of trichloroethylene and found that the maximum concentration that could be endured without any adverse effect was 400 ppm for monkeys, 200 ppm for rats and rabbits, and 100 ppm for guinea pigs. Effects observed in animals exposed to levels above these maximums were concentration dependent and included growth depression and increased liver and kidney weights. Repeated exposure of all these species to 400 ppm of trichloroethylene failed to produce any abnormalities in either hematologic or biochemical data (blood nonprotein and urea nitrogen, serum phosphatase, plasma prothrombin time, and total lipid, phospholipid, neutral fat, and free and esterified cholesterol of the liver). Histopathologic examination of tissues from the exposed animals failed to reveal any adverse effects due to the exposure.

The results of the repeated exposure to trichloroethylene at a level of 3825 mg/m³ (730 ppm) reported in this paper appeared to parallel the results of previous workers. The exposure did not produce any deaths or hematologic or histopathologic changes. Histochemical studies revealed no alterations in the enzymes and cofactors studied. The only significant response was the growth depression noted in the dogs.

The 90-day continuous exposure at 189 mg/m³ (35 ppm) gave essentially the same results as the repeated exposures at the higher concentration. Neither deaths nor visible toxic signs were noted. Gross pathologic observation, hematologic and histopathologic study did not reveal any significant abnormalities in any species. Body weight data revealed a very slight growth depression in all species except the dog. However, there were only two dogs and both had preexposure weights below that of the controls.

Carbon Tetrachloride

Widespread use of carbon tetrachloride has prompted many investigations into the harmful effects of this solvent. The literature survey by von Oettingen (1955) reported that the primary effect of chronic exposure to the solvent was serious impairment of liver and kidney function, although other organs may also be injured. Irish (1963a) in his review on aliphatic halogenated hydrocarbons stated that the predominant response of humans to high-level carbon tetrachloride exposures was depression of the central nervous system. At lower concentrations nervous system effects consisting of dizziness, vertigo, headache, depression, mental confusion, and loss of consciousness were observed. He further stated that long-term chronic exposures to low concentrations of the solvent resulted in serious impairment of both liver and kidney functions. Adams *et al.* (1952) reported on the effects noted in rats exposed to 400 ppm for 7 hours/day for 127 days in a 173-day period, and on guinea pigs exposed similarly for 168 days in a 232-day period. A 70% mortality was experienced in the rats and 83% of the guinea pigs died. In both species the liver was found to be enlarged,

and histopathologic examination revealed central fatty degeneration and cirrhosis. Kidney weights of both species were increased and there was slight degeneration of the tubules. They concluded that the maximum vapor concentration that could be endured without adverse effects for 7 hours daily, 5 days/week was 25 ppm for the monkey, 10 ppm for the rabbit, and 5 ppm for the rat and guinea pig.

The repeated exposure study at 515 mg/m³ (80 ppm) reported in this paper resulted in the death of 3/15 guinea pigs and 1/3 monkeys, and severe liver damage in all species. Surviving guinea pigs showed a marked increase in liver lipid content when compared with controls. This hepatic response confirms the observations reported by previous investigators.

A continuous exposure at 61 mg/m³ (10 ppm) resulted in the deaths of 3 guinea pigs, as well as growth depression and liver damage in the survivors of all species. A second continuous exposure at 6.1 mg/m³ (1 ppm) did not cause deaths or visible toxic signs in any species. All species except the rat exhibited slight growth depression, but no hematologic or histopathologic evidence of toxicity, at 6.1 mg/m³.

1,1,1-Trichloroethane

1,1,1-Trichloroethane is widely used as a substitute for carbon tetrachloride and consequently has become the object of many toxicity investigations. Stewart (1963) reported no injury to man following repeated exposures to concentrations of less than 500 ppm. Torkelson *et al.* (1958) reported that rats, guinea pigs, rabbits, and monkeys were unaffected after 6 months of repeated exposures 7 hours/day, 5 days/week at a concentration of approximately 500 ppm of inhibited 1,1,1-trichloroethane.

Our repeated exposures conducted at a level of 12,060 mg/m³ (2200 ppm) did not result in any deaths or visible signs of toxicity, although body weight loss was observed in rabbits and dogs. The continuous exposure at 2059 mg/m³ (370 ppm) did not cause any deaths, visible toxic signs, significant growth depression, biochemical, hematologic, or pathologic changes. A continuous study at 754 mg/m³ (135 ppm) resulted in 3 deaths but no visible toxic signs or growth depression in any of the survivors. Autopsy and subsequent histopathologic examination of the experimental animals revealed lung congestion and pneumonitis which may have been severe enough to have caused the deaths of the 2 rats and the rabbit but were not considered attributable to the exposure.

Dichlorodifluoromethane

Dichlorodifluoromethane has received less attention than the solvents previously discussed. Sayers *et al.* (1930) reported distinct symptoms but no signs of severe poisoning in dogs, monkeys, and guinea pigs exposed 7 hours/day for 12 weeks to 20 volumes percent (200,000 ppm) in air. Lester and Greenberg (1950) noted muscular twitching in rats exposed for 2 hours to levels ranging from 20 to 40 volumes percent. They also reported that exposures ranging from 4 to 6 hours in duration at a level of 80 volumes percent produced deep anesthesia in rats, but no mortality. Neither permanent effects nor pathologic changes were noted in their studies.

In our repeated study at 4136 mg/m³, focal necrosis of the liver was noted but it was concluded that this finding could not definitely be attributed to the exposure. In the continuous study at 3997 mg/m³, submassive necrosis of the liver was noted in surviving guinea pigs. This toxic response may be due to the continuous nature of the exposure or to the high order of susceptibility of the guinea pig noted by Rector *et al.* (1966) in their studies with mineral spirits.

1,1-Dichloroethylene

Extensive literature searches yielded virtually no significant information on the effects of long-term exposure to this material. The lack of reliable data has been the cause of widely varying estimates of its toxicity. Irish (1963b) reported preliminary evidence which indicated that 1,1-dichloroethylene had approximately the same order of toxicity as carbon tetrachloride. On the other hand, Fairhall (1957) reported the vapor toxicity to be of the same order as that of 1,2-dichloroethylene.

Repeated exposure to 395 mg/m³ (100 ppm) produced no deaths or visible toxic signs. Hematologic and growth data did not reveal any evidence of a toxic response, and histopathologic findings were negative in all species. No toxic effects were obtained in this exposure at 395 mg/m³ whereas severe liver damage was found in animals repeatedly exposed to 515 mg/m³ (80 ppm) of carbon tetrachloride. On this basis it appears that 1,1-dichloroethylene is somewhat less injurious than carbon tetrachloride in repeated exposures.

Continuous exposure of animals to 1,1-dichloroethylene at levels of 189, 101, 61, and 20 mg/m³ resulted in mortality as shown in Table 1. Body weight data for the continuous studies contain several deviations from the normal, but only the 189 mg/m³ study resulted in definite growth depression in all species. Serum urea nitrogen concentrations were within control limits for all exposures. Two rats that were used in the 189 mg/m³ study had liver lipid contents of 20.0 and 34.3% which were definitely elevated when compared with controls. Increases in the activity of liver alkaline phosphatase and serum glutamic-pyruvic transaminase were observed in rats and guinea pigs after exposure to 189 mg/m³, but could not be demonstrated in the same species after continuous exposure to 20 mg/m³. Histopathologic examination of tissues from the surviving experimental animals revealed that only the 189 mg/m³ study resulted in definite morphologic alterations in liver and kidney which were considered to be a direct result of the exposure. Since no detectable liver or kidney damage was noted in animals exposed to 101 mg/m³ or below of 1,1-dichloroethylene, while liver damage in rats and guinea pigs was detected following exposure to 61 mg/m³ of carbon tetrachloride, it would appear that 1,1-dichloroethylene is somewhat less toxic than carbon tetrachloride upon continuous exposure. However, this is not borne out by the mortality data, which would tend to indicate that the toxicity of both materials is approximately of the same order.

SUMMARY

Rats, guinea pigs, dogs, rabbits, and monkeys were exposed to trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluoromethane, and 1,1-dichloroethylene. Two types of inhalation experiments were conducted: continuous exposure for 90 days and 8-hour exposures, 5 days a week, for a total of thirty exposures. The parameters studied included mortality, visible signs of toxicity, and hematologic, biochemical, pathologic, and body weight changes. Throughout this entire study which encompassed 17 separate exposures over a period of nearly four years, no visible signs of toxicity were noted in any species exposed to these materials. Significant mortality was found in both the repeated (515 mg/m³) and continuous (61 mg/m³) exposures to carbon tetrachloride as well as in the continuous exposures to 1,1-dichloroethylene at 189, 101, and 61 mg/m³. Growth depression in varying degrees was found in all continuous exposures involving trichloroethylene, carbon tetrachloride, and 1,1-dichloroethylene and in the repeated exposures to carbon tetrachloride. No significant hematologic alterations were noted in any of the studies.

No biochemical studies were done in the earlier years and hence no biochemical data were obtained from the animals exposed to trichloroethylene and dichlorodifluoromethane. Serum urea nitrogen levels were within control limits in all of the exposures to carbon tetrachloride, 1,1-dichloroethylene, and 1,1,1-trichloroethane in which determinations were made. Liver lipid contents in guinea pigs were found to be significantly elevated following repeated exposure to 515 mg/m³ of carbon tetrachloride. Significant elevations of serum glutamic-pyruvic transaminase and liver alkaline phosphatase activities were found in rats and guinea pigs following continuous exposure to 189 mg/m³ of 1,1-dichloroethylene.

Histopathologic study revealed liver damage following continuous exposures to high levels of dichlorodifluoromethane (3997 mg/m³), and to lower levels of carbon tetrachloride (61 mg/m³), and 1,1-dichloroethylene (189 mg/m³). Similar liver damage was also found in the repeated exposures to the two latter materials at 515 mg/m³ and 395 mg/m³, respectively.

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