Acute Inhalation Toxicity of Vinyl Chloride to Laboratory Animals

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Introduction

FIRST prepared in 1833, vinyl chloride has come into increased use in the last decade in the production of polyvinyl chloride resins. The fire and explosion hazard of the monomer and its narcotic properties on acute exposure are well known. As an industrial material, however, it has been regarded as of moderate toxicity. A Threshold Limit Value of 500 ppm was established by the American Conference of Governmental Industrial Hygienists.

No industrial fatalities associated with its use have previously been reported. It was therefore of special interest when two fatalities occurred in a plant handling vinyl chloride. This plant is engaged in the polymerization of the gaseous monomer with the aid of catalysts. One worker died while cleaning out a polymerization vessel and the other while working in a pit. Details of these two fatalities have been reported by one of the authors.[1]

Published information on the acute toxicity of vinyl chloride to experimental animals proved scanty. Patty, Yant and Waite[2] in 1930 reported on the acute response of guinea pigs to vinyl chloride. They found that exposure to 20 to 40 per cent in air killed guinea pigs in a short time; exposure to ten per cent was endured for several hours. Congestion and edema of the lungs with hyperemia of the liver and kidneys were noted.

Peoples and Leake[3] found the minimal anesthetic range of vinyl chloride in mice exposed for ten minutes was 5 to 12 per cent in air; 25 to 30 per cent was fatal in ten minutes. Dogs and rabbits were anesthetized within one minute when exposed to a concentration of about 18 per cent in air. Recovery was very rapid with no apparent untoward effect even after prolonged anesthesia.

Lehmann and Flury[4] noted that vinyl chloride was highly narcotic but had a wide margin between its narcotic and lethal concentrations. Its local irritating effect and its toxicity were described as very slight. Elimination from the body was very rapid. These authors quoted the work of Schaumann[5] with experimental animals. Mice, rats and dogs were able to endure repeated narcosis without any pathological evidence of liver or kidney damage. According to Schaumann, concentrations of seven to ten per cent in air would be narcotic to humans in a short time and 12 per cent would be dangerous.

Vinyl chloride has been studied as a possible anesthetic agent. Oster and associates[6] noted that incoordinated muscular activity of the extremities and cardiac arrythmias of a serious nature were observed in all six dogs used in their experiment. Carr and others[7] found that vinyl chloride produced sensitization of the myocardium in three of seven dogs in which it was administered as an anesthetic agent.

Two cases of vinyl chloride gassing occurred in a factory in Great Britain where polyvinyl chloride resin was being made. These were reported by the Chief Inspector of Factories.[8] In one case a process worker was standing outside a polymerization vessel and washing it with a water stream. After ten minutes of this he suddenly collapsed across the open manhole. Artificial resuscitation was successfully applied. Subsequent symptoms experienced by this worker were tightness of the chest, nausea, abdominal pain and headache. The second case occurred in a maintenance worker who was overcome while repairing a vinyl chloride leak. Both men were hospitalized. Another worker whose hands were accidentally sprayed with vinyl chloride liquid under pressure developed a burn. This latter case was reported by Harris.[9] Such burns may occur with other highly volatile materials when sprayed onto the hands as a liquid under pressure.

Filatova and Gronsberg[10] in 1937 reported on hygienic conditions in a polyvinyl processing plant in the U.S.S.R. The plant was engaged in the polymerization of vinyl chloride by means of a catalyst. Air sampling was don
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Experimental Materials and Pro

Vinyl Chloride: The vinyl chlo
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Specific gravity at 20°C	
Water content	
Boiling range °C	
Hydrogen chloride content	
Acetylene	
Acetaldehyde	
Iron	
Purity (per cent)	
Impurities (per cent)	

Pure vinyl chloride is a colo
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Animals: Mice, rats and guin
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a catalyst. Air sampling was done and the concentration of vinyl chloride was found to vary from about 20 ppm to about 315 ppm. (U.S.S.R. Threshold Limit Value: 1 mg/liter or about 400 ppm). A spastic type blood vessel disorder was described in workers from this plant.

Because of the recent fatalities mentioned above it was decided to undertake acute inhalation studies in laboratory animals with commercially available vinyl chloride. This was done both for comparison with earlier work and to provide information on the acute response and pathological changes in more than one species of laboratory animal.

Experimental Materials and Procedures

Vinyl Chloride: The vinyl chloride was supplied in a metal cylinder under pressure. A commercial grade with the following specifications was used:

Specific gravity at 20°C	0.963–0.985
Water content	None
Boiling range °C	−11 to −9.5
Hydrogen chloride content	None
Acetylene	10 ppm maximum
Acetaldehyde	20 ppm maximum
Iron	Essentially iron free
Purity (per cent)	99.5 maximum
Impurities (per cent)	0.5 maximum

Pure vinyl chloride is a colorless gas at room temperature; its boiling point is −13.9°C. It has a sweetish odor. Its flash point is given at −78°C and its explosive limits in air from 4 to 22 per cent. It has the molecular formula CH_2:CHCl.

Animals: Mice, rats and guinea pigs were used in the study. Test and control animals were taken from the same laboratory stock. All were fed a standard commercial diet and housed in the same way. All were of the same stage of development.

Equipment: The inhalation chamber capacity was 56.6 liters. It was equipped with a viewing window and an inlet tube. Vinyl chloride was released in gaseous form through an adjustable valve on the top of the containing cylinder, then through connecting rubber tubing and a recalibrated Fisher flow-meter. Fresh air was pumped by motor at an adjustable controlled rate through a meter. The streams of air and vinyl chloride were combined at appropriate rates of flow by a glass Y-tube leading through further rubber tubing to the animal chamber inlet, to deliver a continuing stream in the desired proportions. Calculations and adjustments were made to produce the following flow concentrations of vinyl chloride in air for delivery: 10, 20, 30 and 40 per cent. All concentrations are expressed in this report as per cent by volume in air. No determinations of vinyl chloride concentrations were done in the test chamber during the experiments.

Experimental Procedures: Different groups of five mice, five rats and five guinea pigs were placed in the chamber and exposed for thirty minutes to concentrations of 10, 20 and 30 per cent vinyl chloride in air. Similar groups of control animals were maintained but not exposed to vinyl chloride. At the end of thirty minutes exposure the test animals were immediately removed to fresh air. An additional group of five guinea pigs was exposed to a concentration of 40 per cent vinyl chloride in air. In all 65 experimental animals were involved. Observations were recorded on the animals during and after exposure.

The animals which died either during the exposure or after a delay period were autopsied soon after death. Two weeks after the exposure surviving test animals and controls were sacrificed by a blow to the head with the exception of four control rats which were killed by exsanguination.

All animals were examined for gross pathological changes. Tissues were removed from all animals for microscopic examination. The lungs, liver, kidney and heart were removed in all cases. The brain, adrenal, spleen, trachea, lymph nodes, and the eye were removed from representative animals in each group for study. The tissues were preserved in formalin and sections made for staining with hematoxylin-eosin. Special stains were made where indicated.

Observations

Control Animals: No symptoms were exhibited by the 15 control animals and no deaths occurred.

Ten Per Cent Vinyl Chloride: The response of animals exposed to this concentration is recorded below in summary form:

Exposure Time (minutes)	Response
1	Slight irritation in mice and rats.
5	Increased motor activity first in mice, then rats and guinea pigs.
10	Increased motor activity in all species; twitching of extremities in mice.
15	Pronounced tremor, unsteady gait and muscular incoordination in all species.
20	Mice and rats in side position; muscular incoordination, tremors and twitching of extremities in guinea pigs.
25	Mice and rats unconscious, guinea pigs very unsteady but still standing.
30	Mice and rats in deep narcosis; guinea pigs in side position with tremors—one unconscious.

Exposure was stopped and the animals removed to fresh air. All recovered within five minutes.

Twenty Per Cent Vinyl Chloride: The response

of animals exposed to this concentration is recorded below in summary form:

Exposure Time (minutes)	Response
1	Immediate irritation in mice and rats.
2	Muscular incoordination in mice and rats.
5	Mice and rats on their sides with marked tremors and twitching of the extremities; unsteadiness and muscular incoordination in guinea pigs.
10	Mice and rats unconscious with rapid irregular breathing; guinea pigs unconscious but showing marked twitching.
15	All animals in deep narcosis; respirations irregular and rapid.
20	Deep narcosis; breathing slow and shallow.
25	Breathing ceased in one mouse; frothing at mouth and nostrils in mice and rats.
30	All animals in deep narcosis.

Exposure was stopped and the animals removed to fresh air. Mice and rats recovered faster than the guinea pigs and appeared normal within five minutes except for one mouse which was dead. The guinea pigs continued to show muscular incoordination, unsteadiness on their feet until 20 minutes after removal from exposure.

Thirty Per Cent Vinyl Chloride: The response of animals exposed to this concentration is recorded below in summary form:

Exposure Time (minutes)	Response
—	Mice showed irritation immediately and the rats showed irritation quickly thereafter.
1	Muscular incoordination in mice and rats.
2	Mice and rats in side position with marked tremors and twitching of the extremities; muscular incoordination in guinea pigs with developing paralysis of the extremities.
5	All animals unconscious; rapid irregular respirations in mice and rats with frothing about nose and mouth; twitching of extremities still occurred occasionally in guinea pigs.
10	Respirations stopped in mice; breathing slow and shallow in rats; guinea pigs still exhibited occasional twitching movements of the extremities.
15	Breathing stopped in rats; respirations slow and shallow in guinea pigs; twitching of extremities still present in guinea pigs.
30	Mice and rats still; guinea pigs in deep narcosis with slow shallow breathing

Exposure was stopped and the animals removed to fresh air. The mice and rats were dead. The guinea pigs took 25 minutes to return to their normal appearance and activity. One guinea pig from this group died within 24 hours following exposure.

TABLE I

Number of Deaths in Different Groups of Five Mice, Rats and Guinea Pigs Exposed for Thirty Minutes to Varying Concentrations of Vinyl Chloride in Air

Vinyl chloride concentration (per cent by volume in air)	Laboratory animal			Total
	Mice	Rats	Guinea pigs	
10	0/5	0/5	0/5	0/15
20	1/5	0/5	0/5	1/15
30	5/5	5/5	1/5*	11/15
40	—	—	2/5*	2/5
Total	6/15	5/15	3/20	14/30

* A delayed death occurred within 24 hours following exposure.

Forty Per Cent Vinyl Chloride: Only five guinea pigs were exposed to this concentration. Signs of irritation were immediately apparent. Muscular incoordination appeared within seconds. After five minutes all the guinea pigs were unconscious with slow shallow breathing. At the end of the exposure period one guinea pig was dead and the remaining four were in deep narcosis. These survivors took 30 minutes to return to their normal appearance and activity, but one died within the following 24 hours.

The number of deaths occurring in different groups of five laboratory animals exposed for thirty minutes to varying concentrations of vinyl chloride in air is shown in Table I.

Pathological Findings

Gross pathological and histological studies were carried out. The findings are summarized below.

Control Animals: These animals showed no gross or microscopic evidence of damage.

Ten Per Cent Vinyl Chloride: All test animals survived this exposure. They were sacrificed two weeks later. On gross examination there was evidence in mice of slight hyperemia of the lungs. This was less marked in rats. Guinea pigs showed no difference from control animals. On histologic examination the mice showed very slight engorgement of the pulmonary vessels. One mouse showed degenerative changes in the tubular epithelium of the kidney with hydropic swelling. The rats showed slight congestion of the capillaries in the lung. Lungs in the guinea pig were also slightly more hyperemic than those of the control animals.

Twenty Per Cent Vinyl Chloride: One mouse died as a result of 30 minutes exposure to this concentration. Pulmonary congestion was evident on gross examination. Microscopically, there was engorgement of the blood vessels in patchy areas of atelectasis and in The kidneys showed minimal changes in the epithelium of the tubules.

The test animals surviving this sacrificed in two weeks. On gross congestion of the lungs was present but it was more marked in the mice in the guinea pigs. Histologically, dence of pulmonary congestion in mals. Some fatty infiltration was liver of one rat. All other tissues st normal.

Thirty Per Cent Vinyl Chloride: tion of the animals which died as exposure revealed congestion of hemorrhagic areas. The mice and showed congestion of the liver an one guinea pig death was delayed. there was marked congestion w hemorrhages and the liver was diffriable. The microscopic chang which died included marked en pulmonary blood vessels with co rhages in the lungs. The trachea of superficial desquamation of the e gestion was also evident in the li of the mice and rats. The liver of which had the delayed death show degeneration of the liver contain sections stained with Sudan III.

The four surviving guinea pigs two weeks later. Marked pulmon was present with hemorrhagic ar In one case there was evidence of terial infiltration. The liver in t gave the appearance of fatty inf fat was demonstrated on frozen s

Forty Per Cent Vinyl Chlorid guinea pigs exposed to this con during the exposure and another hours. The two which died show gestion of the lungs with hemor examination. This was also evi scopic examination. The liver of the appearance of fatty infiltra could be demonstrated on frozen

The three surviving guinea pi two weeks later. Marked congest with hemorrhage was evident on and microscopic examination. T the tracheal epithelium was consi

Pathological studies which w brain, heart, spleen, adrenals, t the eyes showed no differences and animals dying as a result of tween controls and the survivin

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5	1/15
5*	11/15
5*	5/5
20	14/50

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engorgement of the blood vessels in the lung with patchy areas of atelectasis and minimal edema. The kidneys showed minimal degenerative changes in the epithelium of the convoluted tubules.

The test animals surviving this exposure were sacrificed in two weeks. On gross examination, congestion of the lungs was present in all species, but it was more marked in the mice and rats than in the guinea pigs. Histologically, there was evidence of pulmonary congestion in exposed animals. Some fatty infiltration was present in the liver of one rat. All other tissues studied appeared normal.

Thirty Per Cent Vinyl Chloride: Gross examination of the animals which died as a result of this exposure revealed congestion of the lungs with hemorrhagic areas. The mice and rats in addition showed congestion of the liver and kidney. The one guinea pig death was delayed. In this animal there was marked congestion of the lungs with hemorrhages and the liver was distended and very friable. The microscopic changes in the animals which died included marked engorgement of the pulmonary blood vessels with edema and hemorrhages in the lungs. The trachea of one rat showed superficial desquamation of the epithelium. Congestion was also evident in the liver and kidney of the mice and rats. The liver of the guinea pig which had the delayed death showed severe fatty degeneration of the liver confirmed with frozen sections stained with Sudan III.

The four surviving guinea pigs were sacrificed two weeks later. Marked pulmonary congestion was present with hemorrhagic areas and edema. In one case there was evidence of secondary bacterial infiltration. The liver in these guinea pigs gave the appearance of fatty infiltration but no fat was demonstrated on frozen section.

Forty Per Cent Vinyl Chloride: One of five guinea pigs exposed to this concentration died during the exposure and another died within 24 hours. The two which died showed marked congestion of the lungs with hemorrhages on gross examination. This was also evident on microscopic examination. The liver of one of these gave the appearance of fatty infiltration, but no fat could be demonstrated on frozen section.

The three surviving guinea pigs were sacrificed two weeks later. Marked congestion of the lungs with hemorrhage was evident on both the gross and microscopic examination. In one guinea pig, the tracheal epithelium was completely absent.

Pathological studies which were made of the brain, heart, spleen, adrenals, lymph nodes and the eyes showed no difference between controls and animals dying as a result of exposure or between controls and the surviving animals sacrificed two weeks after exposure. There was a tendency for the blood to remain unclotted in the animals dying during exposure. This feature was noted in the two human fatalities mentioned earlier but it is not a specific characteristic of vinyl chloride.

Discussion

The response of guinea pigs to inhalation of vinyl chloride was similar to that reported by Patty, Yant and Waite. The guinea pigs in our study, however, tolerated a greater exposure during the experimental period than either the mice or rats. The results in mice agreed closely with those reported by Peoples and Leake. Rats were similar to mice in their response.

The pathological changes in animals which died as a result of exposure were mainly those of vascular engorgement of the lungs with hemorrhages and edema. The severity of these changes varied with the severity of exposure. In the higher concentrations, pulmonary change was marked with severe damage to the tracheal epithelium. Congestion of the liver and kidneys also occurred in test animals. These changes are similar to those reported by Patty and associates.

Evidence of pulmonary congestion was still present in surviving animals sacrificed two weeks after exposure. Patty and associates reported that such changes had disappeared in about eight days

Degenerative changes in the tubular epithelium of the kidney were noted in one mouse dying as a result of exposure and in one mouse sacrificed two weeks after being exposed. Such changes, however, were minimal and not shown by other animals in the group.

One of the guinea pigs with delayed death following exposure showed severe fatty infiltration of the liver. Changes suggestive of fatty infiltration were observed in other exposed animals but not confirmed by frozen sections stained with Sudan III.

Summary

Separate groups of laboratory animals comprising five mice, five rats and five guinea pigs were exposed in an inhalation chamber to concentrations of 10, 20 and 30 per cent vinyl chloride in air for 30 minutes. An additional five guinea pigs were exposed to a 40 per cent concentration for a similar period of time. The response by each of the three species to these concentrations is noted.

Inhalation of these relatively high concentrations produced narcosis and death. Mice were the most susceptible with guinea pigs considerably more resistant. Rats were similar to mice in their

response. Exposure to ten per cent vinyl chloride in air produced deep narcosis in mice and rats but no deaths; 30 per cent concentrations killed mice and rats. Exposure of guinea pigs to 20 per cent produced deep narcosis; but three of five guinea pigs survived exposure to 40 per cent concentration.

Animals dying as a result of exposure were autopsied shortly after death. Survivors and control animals were sacrificed two weeks later. Gross pathological and microscopic studies were done and these findings are described.

The principal pathological changes in animals dying from exposure were congestion of the lungs with pulmonary edema and hemorrhages in some, and congestion of the liver and kidneys. Failure of the blood to clot was also observed. One of the three guinea pigs which died following exposure showed severe fatty infiltration of the liver.

Survivors sacrificed two weeks after exposure showed little difference from the control animals. Pulmonary congestion was still evident but liver and kidney congestion was not.

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Introduction

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Experimental Methods and

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This paper is based on work per the United States Atomic Energy C of Rochester Atomic Energy Proje

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The Toxicity of Vinyl Chloride as Determined by Repeated Exposure of Laboratory Animals

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Groups of laboratory animals were exposed repeatedly for up to six months to either 500, 200, 100 or 50 ppm vinyl chloride in air. Detectable changes occurred at all but the lowest concentration. The results are discussed and handling precautions suggested.

Introduction

VINYL chloride (CH_2=$CHCl$) is a chemical of great industrial importance. It is used in the preparation of polyvinyl chloride resin, as a copolymer in saran and other plastics, as a chemical intermediate and as a solvent. Because of the flammability of vinyl chloride, it has been generally assumed that the greatest hazard associated with vinyl chloride is that due to its flammability rather than its toxicity.[1]

The toxicity of vinyl chloride has been reviewed by von Oettingen[2] and more recently by Mastromatteo *et al.*[3] von Oettingen concluded that the gas was anesthetic in high concentrations and that considerable interest had been shown in the use of vinyl chloride as a surgical anesthetic. However, its effect on the circulatory system has discouraged exploitation of this property. No doubt the hazard from flammability has also hindered this use. Only limited repeated exposures which were reported by Schaumann[4] were discussed by von Oettingen. These repeated exposures indicated little or no chronic effects even from anesthetic concentrations.

Mastromatteo *et al.*[3] also discussed the published data and reported the results of single exposures of mice, rats and guinea pigs which confirmed the low acute toxicity of vinyl chloride. Mastromatteo reported that only two human fatalities due to vinyl chloride had been reported.

It can be concluded from the published toxicological data that anesthesia is the only significant effect of acute exposure. Sufficient repeated exposures have not been reported to draw conclusions about chronic toxicity. The Threshold Limit Value of 500 ppm suggested by the American Conference of Governmental Industrial Hygienists (ACGIH)[5] is reported by Smyth[6] to be based on single animal exposures and human experience.

The following report summarizes the results of repeated exposures of laboratory animals to either 500, 200, 100 or 50 ppm of vinyl chloride. The significance of the results is discussed and recommendations for a threshold limit value are made.

Experimental Procedures

Materials Tested

Vinyl chloride, CH_2=$CHCl$, is a colorless gas. It has a boiling point of −13.35°C (+7.93°F) and a freezing point of −154°C (−244.82°F). The material polymerizes readily and hence is sometimes inhibited with phenol or tertiary butylcatechol. Vinyl chloride is very flammable, its flashpoint being −78°C (−108°F). The explosive limits are from 4% to 22% by volume. It has little odor although high concentrations may smell faintly sweet.

A single, uninhibited sample was used in these studies. It was shown by mass spectrographic analysis to be essentially pure CH_2=$CHCl$, air being the only impurity detected in gas phase samples.

Source and Feeding of Animals

The rats and rabbits used in this study were obtained from the stock colony of this laboratory, the guinea pigs were obtained from a commercial grower[7] and the dogs were purebred beagles obtained from a local kennel. The rats and dogs were fed Purina Laboratory Chow[8] or Famo Laboratory Ration.[9] The rabbits and guinea pigs received Famo Rabbit Breeder Ration. The guinea pig diet was supplemented with carrots.

Experimental Protocol

The experiments were conducted in three phases. In the first phase, groups of 10 male

and 10 female rats were exposed seven hours per day five days per week to 500 ppm for 4.5 months. Exposures were given in a 160-liter chamber previously described.[9] Mortality and growth records were kept. Final organ weights were obtained and tissues were saved for microscopic examination. Five male and five female rats served as unexposed controls.

The second phase consisted of repeated 7-hour daily exposures of 20–24 male and 24 female rats, ten male and eight female guinea pigs, three male and three female rabbits and one male and one female dog to either 200 ppm or 100 ppm of vinyl chloride. These animals and two groups of controls were carefully selected and matched on the basis of age, condition and weight. The first group of controls received no exposure and served as unexposed controls. The second group received repeated daily 7-hour exposures to room air in a chamber similar to the one used for exposures to the chemical. This group is referred to as the air-exposed control group. In addition to the animals receiving 7-hour daily exposures, eight separate groups of five male rats each were exposed to either 200 or 100 ppm for 4, 2, 1 or 0.5 hours per day.

The procedures and equipment used were basically the same as reported previously.[11] Growth and mortality records were kept on all groups. The livers of the dogs were biopsied prior to exposure and after 3½ months of exposure, hence the liver of each dog served as its own control. Pre-exposure and terminal hematological determinations were made on all dogs and terminal determinations on representative groups of rats. Urine samples were collected from the dogs and representative groups of rats. At the termination of the experiment, part of the rats were starved overnight and then these and all the guinea pigs and rabbits were killed by decapitation on the day after their last exposure. The dogs were killed by exsanguination after anesthesia with thiopental, sodium (Pentothal, Abbott). Samples of blood were taken of representative groups of animals for determination of alkaline phosphatase, serum-urea-nitrogen (SUN), serum-glutamic-pyruvic-transaminase (SGPT), and serum-glutamic-oxalacetic-transaminase (SGOT). The organs were weighed and tissues fixed for histopathological examination. The rats which were not killed the day after exposures were stopped were pastured for eight weeks and then sacrificed in the previously described manner.

The third phase of this experiment consisted of repeated daily 7-hour exposures of 24 male and 24 female rats, 12 male and 12 female guinea pigs, 3 male and 3 female rabbits and one male and one female dog to 50 ppm vinyl chloride. Equal matched groups served as unexposed and air-exposed controls. Three additional groups of 10 male rats each were exposed for 4, 2 or 1 hour per day to 50 ppm.

The procedures followed were essentially the same as used for exposures to 200 and 100 ppm except that hematological examinations were made on the dogs after three months and liver biopsies were made only prior to exposure. Urine was collected from the rabbits as well as the rats and dogs. The rats which were not sacrificed at the end of the exposures were pastured for six weeks and then sacrificed.

Equipment Used

The exposure equipment used has been described previously.[11] However, in order to gain additional data about the results of repeated short daily exposures, it was necessary to devise a method of introducing additional groups of rats. This was done without opening the doors of the chamber by dropping the rats through chutes made of 3.25-inch stainless steel tubing. These chutes were closed with a rubber stopper except during the brief time it took to insert the rats. The chutes, which were sloped at a 45° angle, ended in covered, screened cages inside the chamber. The additional groups were started 3, 5, 6 and 6.5 hours after the 7-hour exposures started. Since all animals were removed after the 7-hour exposure was completed, this routine resulted in exposures lasting 4, 2, 1 or 0.5 hours.

The vinyl chloride was metered from a saran plastic bag which served as a reservoir for the gas. Metering of the gas was done by Dual Syringe Pumps.[11] Total air flow through the chambers was measured by means of calibrated flow meters.

Analysis of the Chamber Atmospheres

Analyses of the air were made by direct combustion of a known volume of air in a heated quartz tube. The resulting chloride was trapped in 1% sodium formate—1% sodium carbonate solution and subsequently titrated by a micro-Volhard technique. The results of the individual analyses were within 15% of the theoretical concentrations during the exposures to 200, 100 and 50 ppm. The average concentrations recovered were 197, 100.5 and 48.7 ppm respectively.

Results of Repeated Exposures

Exposure to 500 ppm Vinyl Chloride

The rats exposed repeatedly at 500 ppm for 4.5 months, grew normally and no changes were

TABLE II

Summary of Average Body and Organ Weights of Female Rats Receiving Repeated 7-Hour Exposures to Vinyl Chloride 5 Days per Week

Concentration in ppm	Months on Exposure	Ratio of Survival	Final Average Body Weight, g	Organ Weights, g/100 g Body Weight				
				Lung	Heart	Liver	Kidney	Spleen
Unexposed control	0	5/5	194	0.82	0.41	3.06	0.60	0.19
500	4.5	9/10	195	0.85	0.35	3.22[a]	0.81	0.20
Unexposed control	0	10/12	202	0.65	0.35	2.92	0.82	0.17
Air exposed control	6	9/12	223	0.66	0.39	2.99	0.81	0.18
200	6	10/12	208	0.63	0.38	3.39[b]	0.84	0.18
100	6	11/12	221	0.59	0.39	3.35[c]	0.79	0.20
Unexposed* control	0	8/12	233	0.61	0.37	2.67	0.82	0.18
Air exposed* control	6	9/12	240	0.59	0.37	2.97	0.83	0.18
200*	6	12/12	233	0.61	0.38	3.06	0.81	0.20
100*	6	12/12	235	0.63	0.40	3.17	0.83	0.21
Unexposed control	0	11/12	211	0.58	0.39	3.02	0.83	0.20
Air exposed control	6	12/12	202	0.59	0.38	2.93	0.84	0.20
50	6	9/12	206	0.61	0.37	3.02	0.84	0.20
Unexposed** control	0	11/12	195	0.66	0.40	2.88	0.91	0.22
Air exposed control**	6	9/12	221	0.63	0.40	3.01	0.87	0.19
50**	6	11/12	233	0.63	0.38	2.83	0.82[d]	0.20

*Pastured for 6 weeks after exposures ceased.
** Pastured for 6 weeks after exposures ceased.
(a) P = 0.17
(b) P = 0.03
(c) P = 0.01
(d) P = 0.05

Exposure to 100 ppm Vinyl Chloride

All species exposed to 100 ppm of vinyl chloride, seven hours per day, 138–144 times in 204 days, were judged normal on the basis of the following criteria: appearance, mortality, growth, hematological examination, SUN, SGOT, SGPT, alkaline phosphatase determination, urinalysis and gross and microscopic examination of tissue. However, slight increases were found in the average weights of the livers of male and female rats (Tables I and II). Although not significant statistically, an increase was also seen in the average weight of the livers of the male rats exposed to 100 ppm for either four or two hours per day. The groups exposed for either 1.0 or 0.5 hour per day were entirely normal.

Exposures to 50 ppm Vinyl Chloride

The increase in weight of the rat livers, the only significant finding in animals exposed to 100 ppm, did not occur in rats exposed repeatedly to 50 ppm of vinyl chloride, 130 times in 189 days. All groups of animals were judged normal in all other respects. See Tables II, V, VI, VII, VIII and IX. A statistically significant decrease in kidney weight which was seen in the female rats (Table II) was considered to be an artifact since it was not seen at higher concentrations.

Discussion

Repeated exposure to vinyl chloride at concentrations considerably below the level that has been considered safe for human exposure has been shown to have an effect on the liver and kidney of laboratory animals. Histopathological changes and increased liver weights in male and female rats were noted after repeated exposure at 500 ppm. Repeated exposure at 200 ppm for six months resulted in an increase in the average weight of the livers of male and female rats and micropathological changes in the livers of the male and female rabbits. The only effect noted after repeated daily 7-hour exposures to 100 ppm for six months was a slight increase in the average weight of the rat livers. Repeated 7-hour exposures to 50 ppm for six months had no effect on any species studied. Hence, the highest concentration without detectable effect on any species was 50 ppm.

It is interesting to speculate on the apparent discrepancy between the effects observed at 100 ppm in this experiment and the lack of reported injury in humans using a threshold limit of 500 ppm. Several factors may be contributory. First, the effects noted in animals at 500 ppm were only slight to moderate; growth, mortality, and general appearance were unaffected. Hence, it is possible that if humans were exposed repeatedly at 500 ppm slight injury might occur but due to the lack of subjective symptoms the injury would escape detection. Secondly, the injury was apparently reversible. The liver weights of both male and female rats which had shown definite increases after exposures at 200 and 100 ppm were normal or approaching normal after 6–8 weeks of recovery (Tables I, II and VIII).

TABLE III

Summary of Average Biochemical Values Determined for Animals Receiving Repeated Exposures to Vinyl Chloride 5 Days per Week

Species and Dog No.	Sex	Concentration in ppm	Months on Exposure	Duration of Daily Exposure, hours	Number of Animals	Alkaline Phosphatase King-Armstrong Units	Blood Urea Nitrogen, mg/100 ml	SGPT	SGOT
								Sigma-Frankel Unit	
Dog #101	M	Unexposed control	0	0	1	12.2	24	18	43
#113		Air exposed control	6	7	1	4.1	10	19	17
#41		200	6	7	1	8.5	11	13	48
#98		100	6	7	1	4.5	15	21	19
Dog #106	F	Unexposed control	0	0	1	5.1	11	10	26
#107		Air exposed control	6	7	1	7.1	20	14	17
#103		200	6	7	1	7.9	14	16	26
#110		100	6	7	1	5.6	10	10	14
Rat	M	Unexposed control	0	0	5	32.9*	20.6*	30	
		500	4.5	7	4	18.9	13.8	35	
		200	6	7	5	17.7	16.2	35	
		200	6	4	4	18.4	16.2	32	
		200	6	1	3	20.2	17.7	42	
		100	6	7	4	19.4	15.0	27	
		100	6	4	3	14.7	16.3	38	
		100	6	1	3	19.0	15.3	30	
Rat	F	Unexposed control	0	0	4	12.1	20.5	38	
		500	6	7	3	16.1	22.3	26	
		200	6	7	5	13.6	22.2	21	
		100	6	7	6	9.5	22.8	23	
Rabbit	M	Control	6	0	5	4.0	25	36	
		200	6	7	3	5.3	21	23	
		100	6	7	2	5.0	26	26	
Rabbit	F	Control	6	0	6	4.9	33	38	
		200	6	7	2	5.6	27	35	
		100	6	7	3	4.0	26	35	

* Because of two unusually high individual values in this group these average values are elevated and do not correspond with the values obtained on similar control groups in our laboratory. Normal control values are in the same ranges as those of the experimental groups listed in this table.

TABLE IV

Summary of Average Hematological Values for Animals Receiving Repeated 7-Hour Exposures to Vinyl Chloride

Species or Dog #	Sex	Concentration in ppm	Months on Experiment	No. of Animals	Hemoglobin g/100 g	Hematocrit	WBC X 10^3	Neutrophils	Lymphocytes	Monocytes	Eosinophils
Rat	M	Air exposed control	6	4	14.5	51	15.8	20.5	71.2	1.5	0.8
Rat	M	200	6	5	14.4	50	16.7	19	77.8	0.2	3
Rat	F	Air exposed control	6	4	14.0	49.5	13.5	22.5	74.5	0.2	2.8
Rat	F	200	6	5	13.5	47.4	12.8	24.8	70.2	1.0	4
Dog #101	M	Unexposed control	0	1	—	—	—	—	—	—	—
Dog #101	M	Unexposed control	6	1	15	53	14.2	40	51	0	3
Dog #113	M	Air exposed control	0	1	14.5	50	13.5	61	23	5	11
Dog #113	M	Air exposed control	6	1	14.5	52	13.2	58	34	4	4
Dog #111	M	200	0	1	13	47	16.2	50	28	3	13
Dog #111	M	200	6	1	10.5	53	15.8	50	41	2	1
Dog #98	M	100	0	1	15.5	52	19.8	40	46	4	4
Dog #98	M	100	6	1	16	54	18.6	61	39	0	0
Dog #100	F	Unexposed control	0	1	14.5	51	21.8	42	37	3	18
Dog #106	F	Unexposed control	6	1	15.5	54	16.5	54	41	1	4
Dog #107	F	Air exposed control	0	1	15	53	14.4	55	33	4	8
Dog #107	F	Air exposed control	6	1	16	57	11.7	66	23	4	7
Dog #103	F	200	0	1	14	50	17.5	44	48	3	5
Dog #103	F	200	6	1	16	54	17.3	40	51	0	3
Dog #110	F	100	0	1	12.5	44	16.3	61	37	2	0
Dog #110	F	100	6	1	15.5	54	14.4	49	50	1	0

TABLE V

Summary of Terminal Average Body and Organ Weights of Guinea Pigs Receiving Repeated Daily 7-Hour Exposures to Vinyl Chloride 5 Days per Week for 6 Months

Sex	Concentration in ppm	Ratio of Survival	Final Average Body Weight, g	Organ Weights, g/100 g Body Weight					
				Lung	Heart	Liver	Kidney	Spleen	Testes
M	Unexposed control	8/10	927	0.64	0.28	3.32	0.70	0.10	0.48
M	Air exposed control	5/10	1030	0.52	0.28	3.36	0.61	0.10	0.48
M	200	7/10	1044	0.53	0.27	3.01	0.60	0.09	0.44
M	100	7/10	1059	0.52	0.25	2.79[a]	0.55	0.11	0.47
M	Unexposed control	8/12	918	0.60	0.29	3.24	0.68	0.12	0.52
M	Air exposed control	11/12	936	0.61	0.27	3.31	0.64	0.12	0.50
M	50	10/12	1001	0.63	0.26	3.14	0.61	0.12	0.48
F	Unexposed control	8/8	599	0.62	0.27	3.20	0.63	0.12	
F	Air exposed control	7/8	867	0.66	0.25	3.28	0.67	0.11	
F	200	7/8	897	0.74[b]	0.27	3.60[c]	0.66	0.13	
F	100	7/8	949	0.58	0.26	3.50	0.64	0.12	
F	Unexposed control	9/12	723	0.78	0.34	3.41	0.69	0.15	
F	Air exposed control	8/12	854	0.65	0.26	3.84	0.65	0.14	
F	50	11/12	906	0.69	0.28	3.44	0.64	0.14	

(a) P = 0.05
(b) P = 0.24
(c) P = 0.23

TABLE VI

Summary of Terminal Average Body and Organ Weights of Rabbits Receiving Repeated Daily 7-Hour Exposures to Vinyl Chloride 5 Days per Week for 6 Months

Sex	Concentration in ppm	Ratio of Survival	Final Average Body Weight, kg	Organ Weights, g/100 g Body Weight					
				Lung	Heart	Liver	Kidney	Spleen	Testes
M	Unexposed	3/3	3.61	0.39	0.21	2.27	0.43	0.03	0.16
M	Air exposed control	2/3	3.90	0.31	0.18	2.41	0.40	0.02	0.16
M	200	3/3	3.48	0.32	0.19	2.31	0.54	0.02	0.15
M	100	3/3	3.65	0.33	0.18	2.64	0.49	0.02	0.15
M	Unexposed control	2/3	3.60	0.32	0.17	2.33	0.45	0.02	0.15
M	Air exposed control	1/3	3.87	0.37	0.18	3.18	0.53	0.03	0.14
M	50	3/3	3.70	0.36	0.20	2.46	0.47	0.03	0.17
F	Unexposed	3/3	4.18	0.31	0.17	2.22	0.40	0.03	
F	Air exposed control	3/3	3.91	0.30	0.16	2.18	0.41	0.04	
F	200	3/3	4.10	0.32	0.16	2.20	0.42	0.05	
F	100	3/3	4.12	0.29	0.15	1.87	0.35	0.03	
F	Unexposed control	3/3	4.31	0.30	0.17	1.91	0.46	0.04	
F	Air exposed control	2/3	3.05	0.30	0.17	1.88	0.39	0.04	
F	50	3/3	4.31	0.32	0.17	2.40	0.39	0.04	

TABLE VII

Summary of Final Body and Organ Weights of Dogs Receiving Repeated Daily 7-Hour Exposures to Vinyl Chloride 5 Days per Week for 6 Months

Sex	Dog. No.	Concentration in ppm	Ratio of Survival	Final Body Weight, kg	Organ Weights, g/100 g Body Weight					
					Lung	Heart	Liver	Kidney	Spleen	Testes
M	101	Unexposed control	1/1	13.0	0.66	0.62	2.46	0.38	0.32	0.15
M	113	Air exposed control	1/1	8.5	0.63	0.77	2.03	0.47	0.19	0.14
M	111	200	1/1	10.2	1.03	0.96	2.50	0.52	0.25	0.13
M	98	100	1/1	12.0	0.56	0.80	2.50	0.42	0.20	0.15
M	152	Unexposed control	1/1	13.3	0.80	0.75	2.69	0.53	0.23	0.11
M	156	Air exposed control	1/1	10.6	0.77	0.87	3.05	0.45	0.36	0.14
M	102	50	1/1	9.6	0.89	0.78	2.40	0.45	0.23	0.12
F	106	Unexposed control	1/1	9.6	0.71	0.65	2.67	0.43	0.21	
F	107	Air exposed control	1/1	8.7	0.77	0.80	3.15	0.43	0.33	
F	103	200	1/1	10.5	0.78	0.71	1.85	0.45	0.29	
F	110	100	1/1	11.9	0.99	0.78	2.14	0.43	0.31	
F	151	Unexposed control	1/1	8.6	0.99	0.82	4.10	0.45	0.43	
F	155	Air exposed control	1/1	11.3	0.70	0.84	3.31	0.40	0.25	
F	161	50	1/1	7.4	0.87	0.76	2.48	0.47	0.33	

TABLE VIII

Summary of Terminal Average Body and Organ Weights of Male Rats Receiving Repeated Exposures to Vinyl Chloride 5 Days per Week

Concentration in ppm	Months on Exposure	Duration of Daily Exposure, hours	Ratio of Survival	Final Average Body Weight, g	Organ Weights, g/100g Body Weight					
					Lung	Heart	Liver	Kidney	Spleen	Testes
Unexposed control	0	0	8/8	319	0.50	0.33	2.67	0.75	0.18	0.98
Air exposed control	6	7	12/12	347	0.51	0.32	2.41	0.70	0.16	0.81
50	6	7	12/12	339	0.50	0.31	2.49	0.68	0.17	0.87
50	6	4	8/10	346	0.51	0.33	2.59	0.69	0.16	0.81
50	6	2	8/10	322	0.49	0.32	2.57	0.70	0.16	0.80
50	6	1	9/10	348	0.47	0.32	2.51	0.73	0.17	0.81
Unexposed* control	0	0	8/8	330	0.53	0.32	2.37	0.72	0.16	0.83
Air exposed* control	6	7	12/12	370	0.53	0.30	2.42	0.64	0.14	0.75
50*	6	7	12/12	360	0.49	0.33	2.51	0.68	0.15	0.82

* Pastured for 6 weeks after exposures ceased.

Thirdly, it is doubtful whether it would be possible for humans to be exposed continuously at 500 ppm in production plants since this high a general concentration would indicate severe leaks in the equipment and extremely high concentrations

TABLE IX
Summary of Average Hematological Values for Dogs Receiving Repeated 7-Hour Exposures to Vinyl Chloride

Dog #	Sex	Concentration in ppm	Months on Experiment	No. of Animals	Hemoglobin g/100 g	Hematocrit	WBC × 10^3	Neutrophils	Lymphocytes	Monocytes	Eosinophils
Dog #152	M	Unexposed control	0	1	11	43	13.8	51	41	1	4
Dog #152	M	Unexposed control	3	1	15	52	19.4	42	30	4	4
Dog #152	M	Unexposed control	5	1	15.5	54	15.4	52	40	5	3
Dog #158	M	Air exposed control	0	1	—	—	—	—	—	—	—
Dog #158	M	Air exposed control	3	1	14.5	50	13.4	45	50	2	3
Dog #158	M	Air exposed control	5	1	14	50	11.1	55	28	5	12
Dog #162	M	50	0	1	10.5	39	13.6	48	48	2	2
Dog #162	M	50	3	1	14.5	51	12.7	48	48	3	1
Dog #162	M	50	5	1	15.5	53	11.2	43	50	4	3
Dog #151	F	Unexposed control	0	1	11	45	13.3	50	30	3	9
Dog #151	F	Unexposed control	3	1	14.5	52	23.6	67	25	0	8
Dog #151	F	Unexposed control	5	1	15.5	56	15.5	46	42	3	9
Dog #155	F	Air exposed control	0	1	13	45	14	61	37	0	2
Dog #155	F	Air exposed control	3	1	16	59	18.2	60	33	5	2
Dog #155	F	Air exposed control	5	1	15.5	56	17.0	53	31	5	11
Dog #161	F	50	0	1	10.5	35	15.3	53	40	5	0
Dog #161	F	50	3	1	15.5	55	13.4	56	42	2	0
Dog #161	F	50	5	1	15.5	56	12.3	43	43	3	11

adjacent to the leaks. The flammability of vinyl chloride precludes such severe leaks if this hazard of vinyl chloride is to be controlled.

Industrial Hygiene Standard

The data presented indicate that little likelihood of injury would be expected if repeated daily 7- to 8-hour exposures are limited to 100 ppm or less.

Although the level of 100 ppm may not appear to offer any margin of safety since rats exposed repeatedly to this level were very slightly affected, the vast amount of human experience that is available while operating under an MAC of 500 ppm indicates that injury is not likely. Likewise the effects of even rather severe overexposure are not serious, hence this level seems reasonable.

A time-weighted average for all exposure should probably not exceed 50 ppm.

Summary

Vinyl chloride (CH_2=$CHCl$) is a monomer used in very large quantities in the production of plastics. Repeated exposures of laboratory animals to several concentrations of vinyl chloride in air were conducted to determine the chronic toxicity of this material towards animals in order to assess the hazard to humans. Vinyl chloride was found to have a slight capacity to cause liver and kidney injury on repeated exposures. Male and female rats showed micropathological changes after repeated daily 7-hour exposures at 500 ppm for 4.5 months. Repeated 7-hour exposures at 200 ppm for six months resulted in micropathological changes in the livers of rabbits and statistically significant increases in the average weight of the livers of male and female rats but no detectable changes in dogs and guinea pigs. Repeated 7-hour exposures at 100 ppm resulted in slight increases in the average weight of rat livers, the other species were not affected. All species studied tolerated repeated daily 7-hour exposures to 50 ppm for six months with no detectable injury.

Repeated daily 1-hour exposures at 200 and 100 ppm of vinyl chloride were without effect, longer exposures caused a slight increase in liver weight.

The standard for evaluating regular daily 7- to 8-hour exposures may be defined as the concentration below which practically all analytical results must fall. The value of 100 ppm is suggested as this standard for vinyl chloride, with a time-weighted average for all exposures not to exceed 50 ppm.

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