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Effects of Single and Repeated Exposures of Humans and Rats to Vinyl Chloride

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Rats exposed eight hours daily to vinyl chloride at concentrations of 2% for three months and 5% for 19 days exhibited changes in liver and spleen weight and in red and white cell counts. Except in the case of liver, tissue alterations did not accompany these changes. The alterations in liver morphology were within the normal range and were not pathologic in character. Because other facets of the animals' response, such as rate of growth, serum transaminase and hemoglobin, were unchanged, it is suggested that the present threshold limit value of 0.03% need not be lowered.

Introduction

THE TOXICITY of vinyl chloride has been reviewed recently.[1,2,3] Because the threshold limit value of 500 ppm is based on limited data[4] using vinyl chloride less pure than that now obtainable[5] the effects of acute exposure in man and rats and long term exposure in rats was investigated by us in 1959 and is described here. Our data do not support the conclusion of Torkelson, *et al.*[5] that the threshold limit value should be reduced ten-fold; indeed their data indicate a need for further studies prior to any revision of the threshold limit value.

Material

The vinyl chloride monomer was supplied by the Perkins Plant of Solway Process Division, Allied Chemical Corporation, in four 160 lb. cylinders with the characteristics shown in Table I, presumably differing materially from the vinyl chloride used by Torkelson, *et al.*[5] only in the presence of 60 ppm of the inhibitor, phenol. Gas chromatography of the liquid phase indicated the presence of more than 99% vinyl chloride, together with a trace of air and carbon dioxide.

TABLE I
Analysis of Vinyl Chloride Monomer

Acid	2.2 ppm
Acetylene	0.0
Aldehyde	1.9 ppm
Iron	0.0
Sulfur	0.0
Phenol	60 ppm
Non. Vol.	11 ppm
Water	170 ppm
Color	w:w
Assay	99+%

Methods

Experimental

Desired concentrations were obtained by metering air and vinyl chloride through flowmeters calibrated for these gases and passing the appropriate flows through a 2-liter mixing chamber. The concentration was also continuously monitored by a thermal conductivity meter calibrated for vinyl chloride versus air. The desired concentration of vinyl chloride was maintained with less than 5% deviation. The total gas flow was about 50 liters per minute (lpm) in all but one of the experiments.

Five experiments were conducted: (1) a five-minute exposure of human beings to concentrations of vinyl chloride ranging from 0.0 to 2.0%; (2) an exposure of rats (Sherman strain rats from Rockland Farm, New City, N. Y.) for as long as two hours with concentrations up to 15%; and (3, 4, and 5)

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...ior to putting on the mask; no suggestions ... any kind were made. The responses of ... subjects are summarized in Table II. ... apparent that the maximum concentra... causing no effect in any subject lies be... 0.8 and 1.2%. From the responses it ... evident that vinyl chloride causes clear... intoxicating symptoms which can serve ... adequate warning signs of its presence.

TABLE II
Responses of Human Subjects to Varying Concentrations of Vinyl Chloride

Per Cent ...entration	Response
...	No differences reported by Subjects 1, 2, 4, 5 and 6. Subject 3: "slightly dizzy".
...	No differences reported by *all* subjects.
...	*No differences reported by* Subjects 1, 2, 4, 5 and 6. Subject 3: "slightly heady".
...	No differences reported by Subjects 1, 3, 4 and 5. Subject 2 unsure, somewhat dizzy in middle of exposure. Subject 6, reeling, swimming head, "just like getting gas".
...	No effect reported by Subject 5. All others report various degrees of intoxication with dizziness, lightheadedness, some nausea, dulling of visual and auditory cues; these symptoms disappeared rapidly upon termination of the exposure.
...	All subjects reported intoxicating effects. Subject 1 reporting a headache that persisted for 30 minutes. These symptoms appeared earlier in the exposure than at 1.6% and the symptoms were more intense than at 1.6%.

Experiment 2

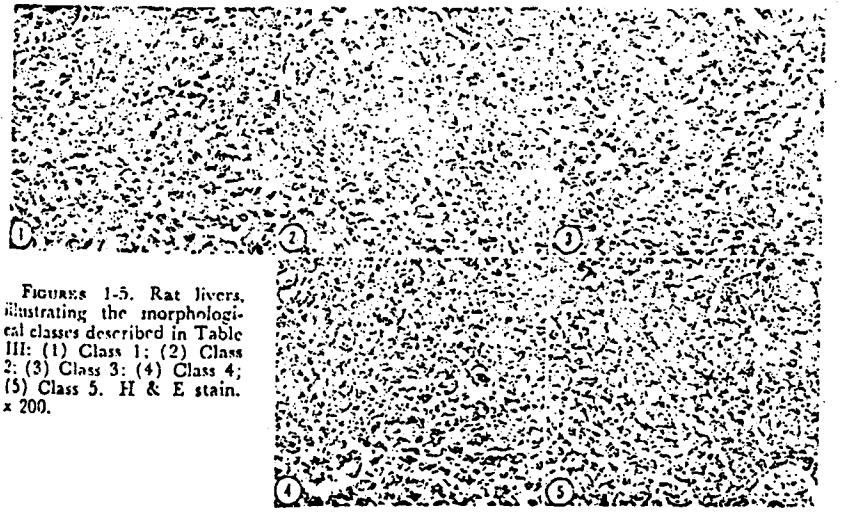
To gain further insight into the intoxicat... effects of vinyl chloride rats were ex... to varying concentrations of vinyl ... chloride for periods up to two hours. The ... gas from the mixing chamber, at ... desired concentration, was passed through ... 10-liter all-glass exposure chamber con... two rats. At a 5% concentration in... is moderate but the righting re... is lost: intoxication is more intense at ... but the righting reflex is still present. ... reflex is lost at a concentration of 7%; ... reflex disappears at a concentra... of 10%. On removal from the chamber, ... animals return to the pre-exposure state ... One animal was sacrificed after ex... to the 10% concentration and showed ... visible gross pathology. Exposure to a ... centration of 15% resulted in deep anes... within five minutes. Effusion of fluid ... the mouth preceded respiratory failure ... rat after 42 minutes; autopsy revealed ... and congestion of the lungs. The sec... rat was maintained under this deep anes... thesia for two hours; on removal to air, there was an uneventful and prompt recovery.

Experiment 3

Some notion of the distinctive and specific *pathology* that might be caused by vinyl chloride was our object in exposing rats to a concentration of *10%*.

Thirty-six rats, equally divided as to sex, were divided randomly into an experimental and a control group; the 18 experimental rats were exposed to the gas in a 1100-liter steel chamber. The concentration was initially raised rapidly to the desired level by admitting vinyl chloride without admixture with air until the effluent from the chamber attained the desired level as noted on the thermal conductivity meter. A fan within the chamber, connected by a flexible cable to an electric motor outside of the chamber (thus avoiding the hazard of an explosion), mixed the vinyl chloride with the air within the chamber. Thereafter, the effluent from the 2-liter mixing vessel was admitted to the chamber; to conserve gas, the throughput of this highest concentration was 20 *lpm*.

The experimental rats were exposed daily from 0830 to 1630 hours while the control animals were exposed from 0000 to 0800 hours. At this concentration, as already noted, rats lose consciousness, regaining it five to ten minutes after removal to air. After two consecutive 8-hour exposures, however, the appearance of the animals suggested that there would be no survivors if this concentration were maintained for the contemplated 15-day period: consequently, beginning with the third exposure, the concentration was reduced to 8%. The test was interrupted for one day after the seventh daily exposure because of a mechanical breakdown. Of the group exposed in this fashion, three female rats died, after the second, fifth and fourteenth exposures; the two animals that died earliest were replaced with substitutes for the remaining exposure period; eight female rats were thus alive at the end of the fifteenth exposure. Female rats exposed 10 and 15 times were autopsied



FIGURES 1-5. Rat livers, illustrating the morphological classes described in Table III: (1) Class 1; (2) Class 2; (3) Class 3; (4) Class 4; (5) Class 5. H & E stain. x 200.

illustrative of the class descriptions in Table III. There were no significant differences in the scoring of the groups, although there was a class "5" and no class "3" in the rats killed at the termination of the exposure, whereas there were no livers in class "5" among the control animals. There were no class "5" livers among the experimental rats killed two weeks after the exposure; the slides were evenly divided between scores of 3 and 4.

Kidney slides from the experimental animals were not graded differently than from the controls but all spleen slides from experimental animals received the highest score, differing significantly from the control spleens, although some controls also received such high scores.

Experiment 4

These preliminary tests seemed to indicate that vinyl chloride was an anesthetic gas which might also act as a lung irritant. In order to assess this feature of its action, to highlight significant pathological features and to avoid the potentiality of damage arising from anesthesia alone, five female and five male rats, matched with controls, were exposed for eight hours daily to five per cent vinyl chloride in air for 19 consecutive days. The experimental animals were placed within the chamber at 0830 and removed at 1630 and the control animals from 0000 to 0800 hours. To prevent contamination of food or water, both groups of animals were placed within the chamber in empty cages; hence experimental and control animals were deprived of food and water for eight hours daily. The requisite concentration of the vinyl chloride was attained initially as in Experiment 3, but the chamber was ventilated at 50 lpm.

Although the body weight of the experimental rats decreased initially, this trend was reversed by the fourth exposure, the rate of growth thereafter being no different than the controls. The drop in weight at the start and the subsequent resumption of growth was paralleled by an apparent increasing tolerance to the gas as the exposures continued. At the start, the intoxication of the rats was marked, instability of the hind legs being a prominent feature of the exposure. With each exposure, however, there was an obvious diminution of these symptoms, so that by the fifth or sixth exposure, it was not possible to distinguish any evident symptoms of neurological deficit.

Hemoglobin determinations during the ex-

...ess was several weeks old and probably not related to the exposure. Both experimental and control animals had parasitic liver cysts. No differences in appearance, color, consistency or degree of congestion were noted between the livers of the two groups. The other organs and tissues were within normal limits in their gross appearance, with no differences between experimental and control groups.

With the exception of the pleural adhesions in one animal, the microscopic appearance of all the organs and tissues was normal. The gross observation of parasitic liver cysts in all animals was confirmed microscopically. Liver sections from all animals were stained for fat, but none revealed evidence of increased fat nor were there any differences in intracellular fat between the two groups.

Classification of the liver slides for the morphological designations of Table III showed differences between the control and experimental groups. The mean score of the control group was 2.93 and that of the experimental group was 4.56, with only one control animal being graded "4" and no experimental animal being graded less than "4". The differences between the means was thus highly significant, yielding a "p" of less than 0.001.

No differences in kidney or spleen slides between experimental and control animals were noted.

...eriment 5

Because human exposure to vinyl chloride is unlikely at concentrations of the gas much greater than the concentration causing signs of intoxication, that is, at 1.2 to 1.6%, a long term exposure of rats was conducted at a concentration of 2.0%.

Sixty rats, each weighing about 75 grams, were separated randomly into two groups of 15 males and 15 females and placed in eight separate cages. In the week before the exposure was started, the rats were observed, weighed twice and blood withdrawn for hemoglobin determination. The experimental animals were exposed in the 1100-liter chamber to 2.0% vinyl chloride for eight hours per day (0830 to 1630) on Monday through Friday for a period of three months. The control animals were exposed to 0.0% of the test gas, that is, to a flow of 50 lpm of air, in the same chamber as the experimental group for eight hours per day on the same days of the week. No food or water was present in the cages during either exposure. All rats were weighed at approximately weekly intervals; hemoglobin determinations, from tail blood, were made at monthly intervals. In neither body weight nor in hemoglobin values were there any significant differences between the control and experimental groups.

During the course of the exposure, there were five deaths; of these, four occurred in the control group. No data from these animals are included in any of the tables.

On the 89th day blood was withdrawn from the control animals under anesthesia as previously described (Experiment 4); these animals were then killed with ether and autopsied. A similar procedure was followed on the 92nd day for the experimental animals. The livers and spleens of all animals were weighed prior to fixation in formalin. The mean values of the tissue: body weight ratios are shown in Table VII. The differences in the means were in all instances significant, the livers larger and the spleens smaller in the experimental as compared with the control animals. No significant differences between the groups appeared in the values for hematocrit and prothrombin. The serum transaminase was not determined. Monocytes and eosinophils showed no differences between the groups; values for the other blood elements are shown in Table VI.

The external appearance of all animals was normal. Parasitic liver cysts were present in all animals. There were no differences in appearance, color, consistency or degree of congestion between the livers of the two groups. All other organs and tissues were similarly normal, no differences between the groups being apparent.

All the organs and tissues examined histologically were within normal limits, no path-

groups in the 2% exposure. It must be emphasized again that the growth of the rats during the 5% exposure support the observation of the rat's behavior which indicated that there was a rapid development of tolerance to the intoxicating effects of this concentration.

Neither the 2% nor the 5% concentrations caused changes in the prothrombin time, hematocrit or hemoglobin values. At both the 2% and the 5% concentrations, the white cell count was lowered significantly, although still well within the normal range. The increase in red cells, although significantly elevated in the 5% exposure, was not correlated with changes in the hemoglobin content. The increase in concentration of the vinyl chloride is associated with a greater fall in the white cells and a greater (and significant) increase in the red cells at the 5% level. Although lymphocytes and neutrophils are increased, only the change at the 2% concentration unlike the previous cell changes, reaches the statistically significant level. It is difficult to know if these changes have any toxic significance, since no tissue changes were seen upon microscopic examination that would account or be associated with a drop in the white cells or an increase in red cells. The decrease in spleen: body weight ratio is in a direction opposite to that usually associated with a severe drop in white cells; although the white cell count did not suffer a severe drop, the decrease was substantial at the termination of the exposure.

The only finding suggesting a specific toxic action of vinyl chloride is the increase in liver weight on exposure to 5% for 19 days and to 2% over 92 days. The increase in liver weight is not only highly significant statistically but is also substantial, amounting to a 30% increase over the controls. It is unfortunate that no information is available as to whether this increase is reversed on discontinuing the exposure. The increase in liver weight may be interpreted as indicating alterations in water, electrolytes and protein content of the liver parenchyma, but that such changes presage the development of actual histologic lesions is not certain in view of the wide range that the liver: body weight ratio may encompass (Table VII). Rather this increase may signify a non-specific response to metabolic derangements occasioned by mild and moderate intoxication for daily 8-hour periods. From the data obtained here, it is not certain that histopathological change would have occurred had exposures been carried out for longer times.

In the paper by Torkelson *et al.*, histopathological changes in the liver and increased liver:body weight ratios are reported in male rats exposed to 500 ppm vinyl chloride for 4.5 months. That there is no causal relation between the reported histopathology and the increases in liver:body weight ratio is evident from the extensive data gathered by these investigators. Female rats exposed to 500 ppm vinyl chloride for 4.5 months showed no statistically significant increase in liver weight but are reported to have histopathological changes in the liver. Female rats exposed to lower concentrations (100 and 200 ppm) for six months had significantly increased liver:body weight ratios but no pathology. On the other hand, rabbits of both sexes exhibited liver pathology without showing any increase in liver weight after exposure for six months to a concentration of 200 ppm. The authors correctly point out that organ:body weight ratios may well be artefactual, illustrating the point by the significant decrease in kidney weight found in female rats exposed to 50 ppm for six months; such rats exhibited no changes when exposed to higher concentrations. By the same token, but overlooked by the authors, the increased liver:body weight ratio in female rats at 100 and 200 ppm is with equal reason artefact because no statistically significant increase occurred at 500 ppm. Apparently, also, species differences are of importance in the reactivity to vinyl chloride, male guinea pigs suffering a significant *decrease* in their liver:body weight ratio when exposed to 100 ppm for six months.

There are six possible combinations of the presence or absence of pathology and increases, decreases and no change in liver weight. The one combination not observed

the changes in the formed elements of the blood. The increases in liver:body weight ratios were associated with morphological alterations in the liver which appeared to have no pathological significance, while no meaningful morphological alterations were evident in either kidneys or spleens.

Because, in the long term exposures, it was impossible to attach any toxic significance to the changes noted or to observe any histopathology, and because other aspects of the animals' reactions, such as growth rate, were unimpaired, the presently accepted threshold limit value of 500 ppm for vinyl chloride, one-fortieth of the concentration tested here, seems to offer an adequate margin of safety for human exposure.

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Sources and Use of Toxicological Information

The New York University Medical Center in cooperation with the American Industrial Hygiene Association will present a course on *Sources and Use of Toxicological Information.* The course will be given October 14-18, 1963, at the Onchiota Conference Center, Sterling Forest, Tuxedo, New York.

The course is designed to acquaint those concerned with advanced methods of securing and interpreting pertinent toxicological information. Application and principles will be stressed rather than details of experimental toxicology. The design of the course eliminates the need for specialized scientific background so that all administrative and technical persons having responsibilities for use of toxicological information can benefit. Guest lecturers who are specialists in selected fields will supplement the staff of N.Y.U. Institute of Industrial Medicine for presentation of the subject material. Areas to be covered are: biological principles involved, sources of toxicological information, applications to in-plant problems, Federal regulations, and requirements of various agencies.

Inquiries and registrations should be directed to New York University Medical Center, Institute of Industrial Medicine, 550 First Avenue, New York 16, New York. The registration fee is $150 with a deposit of $25 required. Accommodations (double occupancy) at the Onchiota Conference Center for the evening (dinner) October 13 through lunch October 18 are available at $21 per day including meals (payable to the Center on departure).