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 0.05% 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.*[3] 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 100 lb. cylinders with the characteristics shown in Table I, presumably differing materially from the vinyl chloride used by Torkelson, *et al.*[3] only in the presence of 10 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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exposures of rats to concentrations of 8 to 10% for 15 days, 5% for 19 consecutive days and 2% for 92 days.

Pathological

The experimental and control animals were killed by ether inhalation after exposures to 2, 5 and 8 to 10% vinyl chloride were completed. Each animal was autopsied according to standard practice. In addition to the gross examination of all animals, all the livers, kidneys and spleens were examined microscopically and some animals received a complete microscopic examination. Heart, lung, liver, spleen, urinary bladder, testes, prostate, ovary, brain, spinal cord, pituitary, tibia, pancreas, stomach, small and large intestine, adrenals, kidneys, uterus, fallopian tubes, thymus, thyroid, parathyroid, eye, knee joint, bone marrow, skeletal muscle, salivary glands and skin were removed from each animal and preserved in 10% buffered formalin. All tissues were examined grossly both at autopsy and after formalin fixation. Following fixation, representative samples of all tissues were processed according to standard histological procedure and stained with hematoxylin and eosin; separate specimens of liver were stained for fat with the *Flaming Red* technique. The animals receiving such a complete examination of the tissues listed included two males and one female rat that had been exposed 15 times and one female rat exposed ten times to the 8 to 10% level; eight experimental and nine control animals surviving the 5% exposure; and 20 randomly selected rats, equally divided as to experimental and control groups and to sex, exposed to 2% vinyl chloride.

The marked increases in liver weight accompanying the exposures to 2% and 5% of the gas, the decrease in spleen weight in the 2% expsoure and the reported kidney changes in exposure to 500 ppm[3] led us to examine these particular tissues with a blind technique, thus excluding the operation of any bias or prejudice from the judgments. All slides, including duplicates, were randomly numbered so that it was impossible to distinguish, without the code, which had been experimental and which control. The slides of the liver, spleen, and kidney were examined and classified, then recoded and re-examined; in this manner a measure of the consistence and reliability of the pathologist's technique was obtained. This consistency was nearly perfect in the case of liver slides, and only slightly less so for spleen and kidney. The results of these examinations are reported in the appropriate following sections.

Results

Experiment 1

Because the main objective was the determination of the effect of long term exposure to vinyl chloride, the maximum concentration of the gas to which humans might conceivably be exposed without any immediate acute effects was determined; this concentration then became the basis for determining the concentration used during the 92-day exposure of rats.

Three men (26, 35, 50 years; 86, 78, 73 Kg.) and three women (25, 40, 55 years; 64, 52, 61 Kg.) were exposed twice each day, separated by a 6-hour interval, for three successive days to six different concentrations of vinyl chloride: 0.0, 0.4, 0.8, 1.2, 1.6 and 2.0%. The concentrations were presented in a different order to each subject to make it possible to factor out any possible adaptation to either the gas or the experimental situation; the 0.0% concentration was included so that some assessment of suggestibility could be made.

Until its conclusion, the subjects were told neither the effects to expect from the exposure nor the purpose of the experiment; nor was information vouchsafed as to the concentrations that were used at any time. The subjects each sat in a chair separated from the gas mixing equipment by a screen, a simple plastic breathing mask affixed over the face, covering the mouth and nose. The rate of air or air-gas mixture passed through the mask was sufficient (50 lpm) to prevent any dilution effects from the atmosphere. After five minutes of breathing the mixture, the exposure was terminated and the subjects were asked to compare their feelings at this time to the time immediately

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

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

Per Cent Concentration	Response
0.0	No differences reported by Subjects 1, 2, 4, 5 and 6. Subject 3: "slightly dizzy".
0.4	No differences reported by *all* subjects.
0.8	No differences reported by Subjects 1, 2, 4, 5 and 6. Subject 3: "slightly heady".
1.2	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".
1.6	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.
2.0	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 intoxicating effects of vinyl chloride rats were exposed to varying concentrations of vinyl chloride for periods up to two hours. The effluent gas from the mixing chamber, at the desired concentration, was passed through a 10-liter all-glass exposure chamber containing two rats. At a 5% concentration intoxication is moderate but the righting reflex is lost; intoxication is more intense at 6% but the righting reflex is still present. This reflex is lost at a concentration of 7%; the corneal reflex disappears at a concentration of 10%. On removal from the chamber, the animals return to the pre-exposure state rapidly. One animal was sacrificed after exposure to the 10% concentration and showed no visible gross pathology. Exposure to a concentration of 15% resulted in deep anesthesia within five minutes. Effusion of fluid from the mouth preceded respiratory failure in one rat after 42 minutes; autopsy revealed edema and congestion of the lungs. The second was maintained under this deep anesthesia 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

TABLE III
Classification of Morphologic Changes in Livers

Class	Description
1	No swelling, no vacuoles, sinusoids visible.
2	Slight swelling of cells, only a few faint vacuoles or none, sinusoids visible but compressed.
3	Moderate swelling of cells, most cells with definite fine to medium vacuoles, sinusoids compressed.
4	Marked swelling of cells, large irregular "vacuoles" or clear spaces, compression of sinusoids. Changes focal in distribution.
5	Similar to 4 but changes more widespread and diffuse.

TABLE IV
Classification of Morphologic Changes in Kidneys

Class	Description
1	Cells well preserved, no vacuoles, glomeruli normal.
2	Vacuolization and "pyknosis" of some collecting tubules. Otherwise as 1.
3	As 2 but with more extensive vacuolization and "pyknosis" including proximal collecting tubules in cortex.
4	As 3 but with vacuolization and "pyknosis" extending to convoluted tubules.

at the termination of the exposure; the remaining six rats were autopsied 15 days later, five of these rats having been exposed 15 times and the sixth rat 13 times. The mortality was greater among the male rats: only two males survived 15 exposures, the remaining males, and their replacements, surviving only an average of eight exposures. The two male rats exposed 15 times were autopsied at the termination of the exposure.

There was no weight gain in the initial days of the exposure to this concentration, although after the ninth day some resumption of growth seemed to occur, possibly an indication of the development of tolerance to the effects of the gas as the exposure continued. Upon termination of the exposure, growth resumed promptly at the same rate as the control animals. Both at the 16th and at the 30th days, there appeared to be no differences in liver: body weight ratios between experimental and control rats, although too few livers were weighed at the termination of the experiment to make meaningful comparisons of this ratio.

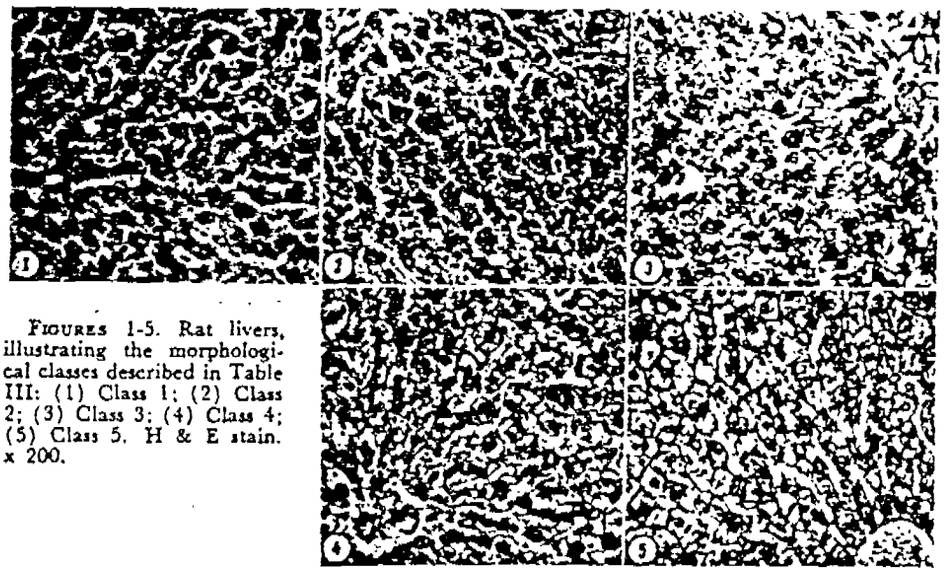
The external appearance, coat and tail, of all the animals was within normal limits. About a third of the animals had parasitic liver cysts. No differences in appearance, color, consistency or degree of congestion were observed between the livers of experimental and control animals. The lungs of three experimental animals sacrificed immediately upon cessation of the exposure had numerous focal fibrinous pleural exudates overlaying nodular yellow-brown parenchymal lesions which had the appearance of regions of acute necrotizing focal pneumonia. Of the six experimental animals sacrificed two weeks after the exposure, the lungs of two revealed a few adherent fibrous pleural adhesions which were interpreted as representing regions of healed pneumonia. All other organs and tissues were within normal limits, with no differences between experimental and control animals.

The experimental animals sacrificed at the termination of the exposure received a complete histologic examination. The lesions in the lungs of the three animals in this group showing gross pleural pathology were seen to be due to acute focal necrotizing pneumonia in varying stages of organization. Some pulmonary edema was present as well as diffuse infiltrates of mononuclear cells in the alveolar septae. Some areas of metaplasia were present in and near the regions of pneumonia and were interpreted as secondary to the pneumonia.

The parasitic liver cysts seen grossly were confirmed microscopically. The liver sections stained for fat revealed individual variation of some degree but no significant deviation from normal. The coded slides of the livers were classed according to the descriptions in Table III while kidney and spleen were judged according to the criteria in Tables IV and V; Figures 1, 2, 3, 4, and 5 are

TABLE V
Classification of Morphologic Changes in Spleen

Class	Description
1	Average size follicles, little or no congestion, balance between lymphocytes, germinal epithelium and interstitial tissue.
2	Obvious congestion, no change in follicles or epithelium, volume of germinal centers less than volume of peripheral lymphocytes.
3	Germinal centers approximately same volume as peripheral lymphocytes, moderate congestion.
4	Increased number of lymphocytes, hyperplasia of germinal centers (greater than volume of peripheral lymphocytes).
5	Active hyperplasia: 2 classes of lymphocytes, (1) a zone immediately around germinal centers containing young lymphocytes surrounded by (2) a peripheral zone of small dark lymphocytes.
6	Lymphocytic hyperplasia extending to include most of the interstitium.



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-

TABLE VI
Formed Elements of the Blood at Termination of Exposure to 5 and 2% Vinyl Chloride

	RBC $\times 10^6$/mm³		WBC $\times 10^3$/mm³		Lymphocytes %		Neutrophils %	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Control—5% (9)	5.13	0.49	12.67	2.75	89.4	3.7	9.4	3.2
Expt.—5% (8)	5.87	0.75	7.96	1.85	92.3	3.5	6.3	3.1
"p" less than	0.05		0.01		NS		NS	
Control—2% (26)	6.60	0.83	11.84	2.63	84.8	4.80	12.62	4.71
Expt.—2% (29)	6.82	0.83	9.12	3.08	89.7	3.84	8.02	4.94
"p" less than	NS		0.01		0.01		0.01	

posure period revealed no difference between experimental and control groups.

On the twentieth day experimental and control animals were anesthetized with diethyl ether, blood was drawn by cardiac puncture and 1/9 volume of 0.1*M* sodium oxalate was added to the blood. The animals were then killed with an overdose of the anesthetic and autopsied. Measurements of hemoglobin, prothrombin time, hematocrit, red cells, white cells, differential white cells and serum transaminase were performed on the blood drawn. Serum transaminase, hematocrit values and prothrombin times were normal for both groups. Table VI lists the values for some of the formed elements of the blood. Monocytes and eosinophils formed only a small proportion of the white cells; no differences between control and experimental animals occurred. The red cells were somewhat elevated and the white cells lower in the experimental group. Table VII shows that the liver : body weight ratio of the experimental animals was significantly elevated.

All five male experimental animals exposed to 5% vinyl chloride had coats which were somewhat thinner than normal; the tails of these animals were scaly. The three female experimental animals and all the control animals had normal coats and tails. One male experimental animal had fibrous pleural adhesions on the left side; the fibrous nature of these adhesions suggested that the

TABLE VII
Liver and Spleen Weights of Rats Exposed to Vinyl Chloride

Group	Vapor Conc. (%)	Days on Expt.	Number of Animals	Sex	% of Body Weight			
					Liver		Spleen	
					Mean	S.D.	Mean	S. D.
Control	0	89	14	F	3.65	0.36	0.49	0.160
Expt.	2	92	14	F	4.76[a]	0.80	0.88[a]	0.068
Control	0	89	12	M	3.73	0.19	0.38	0.062
Expt.	2	92	15	M	4.74[a]	0.30	0.58[d]	0.060
Control	0	19	5	F	5.10	0.23		
Expt.	5	19	3	F	6.95[a]	0.61		
Control	0	19	4	M	5.17	0.46		
Expt.	5	19	6	M	6.71[b]	0.40		
Control*	0		4	F	4.98	0.20		
Expt.*	8-10	See Text	6	F	5.13[c]	0.35		
Control*	0		5	M	5.45	0.57		
Expt.*	8-10	See Text	4	M	6.09[a]	1.08		

*Pastured for two weeks
[a] "p" < 0.001
[b] "p" < 0.01
[c] "p" < 0.05
[d] "p" < 0.02
[e] "p" not significant

process 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.

Experiment 5

Because human exposure to vinyl chloride seems unlikely at concentrations of the gas much greater than the concentration causing signs of intoxication, that is, at 1.2 to 1.6%, the 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 5 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-

ology being evident in either experimental or control animals.

The parasitic liver cysts seen grossly were confirmed microscopically. Liver sections stained for fat revealed normal variation, but no animals had increased intracellular fat nor were there differences between the experimental and control animals. Graded in accord with the morphology in Table III, the liver slides revealed differences between the groups. The mean score of the control group was 1.58, that of the experimental group 3.63. No liver in the control group scored more than 2 and none in the experimental group scored less than 3; one liver scored 5. Because there was no overlap the differences between the means are highly significant.

There were no differences in score for the spleens in the two groups, but the kidney slides of the experimental animals scored significantly less than their controls.

Discussion

The data from the present investigation confirm the acute effects to be expected from various concentrations of vinyl chloride: concentrations below 1%, when exposure is limited to five minutes, cause no observable intoxicating effects; five minutes, however, is shorter than the time necessary to reach an equilibrium level in the circulation; from behavioral observations in the rat, it may be estimated that in five minutes some two-thirds of the equilibrium level is reached; consequently a concentration of 0.6 to 0.7%, if long continued would not produce intoxication. As the concentration rises above this level the intensity of the intoxicating signs increases until at a concentration of 7% the righting reflex is lost; at 10% the corneal reflex disappears and at concentrations of 15% and above respiratory failure takes place. Vinyl chloride thus acts as an anethetic gas, its depressant action increasing with increasing concentration of the gas in the air breathed, the corresponding increasing neurological deficits ending in death at concentrations greater than 15%. The purpose in exposing rats to concentrations causing anesthesia (8 to 10%) was an attempt to produce some singular or characteristic pathology. It cannot be said that this goal was achieved, the results being questionable and uncertain. Lung lesions were certainly present but these could not be ascribed with certainty to any irritant properties of the gas since they might well have arisen from the long-continued anesthesia; there was an apparent regression of these lesions in rats allowed a 14-day recovery period. The presence of pneumonia certainly raises the possibility of an acute toxic effect on lung tissue at these concentrations; however, the pneumonia could just as well be caused by secondary infection during the severe central nervous system and respiratory depression, an interpretation favored somewhat by the diffuse lesions and by the irregular occurrence of the pneumonia.

The kidney and liver changes described by Mastromatteo, *et al.*[3] in rats exposed for 30 minutes to 20, 30 and 40% of vinyl chloride, were not observed here, although 15 repeated 8-hour exposures to an anesthetic concentration is also a relatively severe stimulus. The findings in the lungs agree with the relative lack of effect found by Mastromatteo *et al.* in rats exposed for 30 minutes to 10% vinyl chloride, except that continued exposure, for days, does result in mortality. The relative lack of pathology as the result of 30 minute exposure at 10% found by Mastromatteo *et al.* would seem to support the view that lung lesions found after repeated exposures could well arise from the anesthesia and not from some action peculiar to vinyl chloride. There was no indication at either the 2% or 5% level of any untoward or other effects upon the lung tissue.

As an overall measure of general health, body weight and rate of growth are sensitive indicants. Even in the exposure to a concentration of 5%, which at first produces a marked and severe intoxication, the sharp drop in the weight of male experimental animals seen at the start of the exposure was soon reversed so that by the end of the 19-day exposure there was no difference between experimental and control rats of either sex. Body weight and rate of growth similarly showed no differences between the

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

by Torkelson, *et al.* is histopathological change associated with a decrease in liver weight. Obviously, if five of the six possible combinations have been observed in a relatively small sample, no causal connection can be said to exist between these two measures.

Changes in the liver:body weight ratio may well have some toxic significance, but if they are unaccompanied by histopathological alterations, increased fat content or serum transaminase changes it is impossible to conclude that taken alone they signify much. Reference to Table VII will show that the liver:body weight ratios of the rats used as control animals in the 5% exposure were significantly higher than the experimental animals in the 2% exposure. This illustrates well the fact that though control animals are used, unknown and non-specific changes in the environment, time of the year, temperature, diet, etc., may be responsible for changes in organ:body weight ratio without at the same time producing pathological alterations in the organ.

Similar considerations apply to the decrease in spleen weight; here, not only was pathology not observed, but there was no difference in the morphological character at either the 2% or 5% level between the experimental and control groups.

Unlike the interstitial and tubular changes in kidneys of rats exposed to 500 ppm of vinyl chloride reported by Torkelson, *et al.*, was the lack of any pathology in our animals exposed to 2% and 5% and the fact that the only morphological alteration in which a significant difference between control and experimental animals occurred (kidney at 2%) indicated that the control animals were further from "normal". Because it is unreasonable to attach toxic significance to changes associated with a control air exposure, it is our belief that the morphological alterations we have observed should not be interpreted as manifestations of pathology.

The concentrations to which the rats were subjected in these experiments were at least 40 times those used by Torkelson, *et al.* If any reliance is to be placed in a dose-effect relationship, pathology of some considerable degree should have been found in our experiments. Yet only morphological alterations of the character already described and pictured were seen, which, in our knowledge and experience, are of no pathological significance. Whether the explanation resides in a difference between the rat strains used by us and by Torkelson *et al.* or elsewhere is not known, but without additional data it is impossible to resolve the contradiction.

On the basis of the present data, and the seeming unimportance of the liver weight changes seen by Torkelson *et al.*, and without further evidence, a change of the present threshold limit value of 500 ppm seems unwarranted.

Summary

From 5-minute exposures of human subjects to concentrations of vinyl chloride ranging from 0.0 to 2.0%, it is estimated that a prolonged exposure to a level of more than 0.6% is necessary to produce minim symptoms of intoxication. Rats exposed up to two hours to higher concentrations exhibited moderate intoxication at 5%, lost their righting reflex at 7% and the corneal reflex at 10%. Respiratory failure occurred at 15%. If the exposure to 10% was long continued (two 8-hour daily exposures), mortality increased; death was apparently caused by a pneumonic process, but it was impossible to decide whether this was the result of a primary action or secondary to the anethesia.

Rats exposed eight hours daily to 5% for 19 days or to 2% over 92 days did not show any lung involvement. These levels had no effects upon growth rate, hemoglobin, hematocrit or prothrombin time. At both concentrations there were increases in the liver:body weight ratio and decreases in the white cells; at 2% the spleen:body weight ratio decreased and at 5% there was an increase in red cells.

No gross or microscopic changes were found in any tissue that was correlated with

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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References

1. von Oettingen, W. F.: *The Halogenated Hydrocarbons: Toxicity and Potential Dangers.* U. S. Public Health Service, Publication 414, Government Printing Office, Washington, D. C. (1955).
2. Mastromatteo, E., A. M. Fisher, H. Christie, and H. Danziger: Acute Inhalation Toxicity of Vinyl Chloride to Laboratory Animals. *Amer. Ind. Hyg. Assoc. J. 21:* 394 (Oct. 1960).
3. Torkelson, T. R., F. Oyen, and V. K. Rowe: The Toxicity of Vinyl Chloride as Determined by Repeated Exposure of Laboratory Animals. *Amer. Ind. Hyg. Assoc. J. 22:* 354 (Oct 1961).
4. Smyth, H. F., Jr.: Improved Communication—Hygiene Standards for Daily Inhalation. *Amer. Ind. Hyg. Assoc. J. 17:* 129 (1956).
5. Patty, F. A., W. P. Yant, and C. P. White: Acute Response of Guinea Pigs to Vapors of Some New Commercial Organic Compounds. V. Vinyl Chloride. *Public Health Reports (U.S.) 45:* 1963 (Aug. 1930).

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).