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

T. R. TORKELSON, M.S., F. OYEN, and V. K. ROWE, M.S.

Biochemical Research Laboratory, The Dow Chemical Company, Midland, Michigan

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.[7] 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.[8] 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.[8] 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.[9] 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

RSV 0016864

TABLE I

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/100 g Body Weight					
					Lung	Heart	Liver	Kidney	Spleen	Testes
Unexposed control	0	0	4/5	304	0.54	0.33	2.55	0.71	0.18	0.90
500	4.5	7	7/10	312	0.56	0.33	3.00ᵃ	0.73	0.17	0.95
Unexposed control	0	0	11/12	343	0.53	0.32	2.45	0.69	0.14	0.90
Air exposed control	6	7	10/12	356	0.52	0.32	2.52	0.70	0.15	0.76
200	6	7	9/12	341	0.52	0.32	2.58ᵇ	0.68	0.17	0.94
200	6	4	4/5	349	0.47	0.29	2.96ᶜ	0.64	0.16	0.95
200	6	2	4/5	341	0.56	0.30	2.62ᵈ	0.64	0.16	0.99
200	6	1	3/5	330	0.48	0.29	2.46	0.61	0.13	0.79
200	6	0.5	4/5	339	0.51	0.29	2.38	0.67	0.14	0.95
100	6	7	7/12	352	0.51	0.29	2.61ᵉ	0.63	0.15	0.93
100	6	4	3/5	360	0.51	0.29	2.62	0.65	0.15	0.74
100	6	2	4/5	331	0.53	0.31	2.64	0.67	0.16	0.93
100	6	1	3/5	387	0.49	0.29	2.52	0.64	0.15	0.79
100	6	0.5	5/5	363	0.45	0.30	2.42	0.64	0.16	0.79
Unexposed* control	0	0	10/12	354	0.49	0.31	2.43	0.64	0.14	0.94
Air exposed* control	6	7	9/12	393	0.50	0.30	2.41	0.63	0.15	0.79
200*	6	7	8/9	385	0.47	0.29	2.59ᵉ	0.64	0.15	0.71
100*	6	7	6/7	376	0.47	0.31	2.53ᶠ	0.65	0.14	0.76

* Pastured for 8 weeks after exposures ceased.
(a) P = 0.001
(b) P = <0.001
(c) P = 0.07
(d) P = 0.1
(e) P = 0.02
(f) P = 0.13

apparent grossly at autopsy. Microscopically their livers showed increased central lobular granular degeneration and the kidneys showed interstitial and tubular changes. The average weight increase of the livers of the male rats was statistically significant (P = 0.001), Table I. The average weight of the livers of the female rats was above that of the controls but not statistically significant, Table II. SUN, SGPT, SGOT, and alkaline phosphatase determinations were within normal limits (Table III).

Exposure to 200 ppm Vinyl Chloride

All groups exposed seven hours per day 138–144 times in 204 days were normal in appearance, mortality and growth. Hematological, SUN, SGOT, SGPT and alkaline phosphatase values (Table III) and the results of the urinalysis were within normal limits. Gross pathology was normal in all species. Micropathology was normal in the rats, guinea pigs and dogs, however, adverse effects were noted in the livers of rabbits of both sexes. In the male rabbits this was characterized by central lobular granular degeneration and necrosis with some foamy vacuolation. In the female rabbits the changes noted were central lobular granular degeneration and necrosis with periportal cellular infiltration. All organ weights were normal except for the livers of the male and female rats which were increased significantly after repeated daily 7-hour exposures (Tables I, II, IV, V, VI, and VII). In the males this increase was still apparent eight weeks after exposure ceased, although the liver weights appeared to be decreasing and returning to normal.

The male rats exposed to 200 ppm of vinyl chloride for either four or two hours per day for six months had elevated average liver weights (Table I). However, the small size of the experimental groups made the finding statistically insignificant (P = 0.07 and 0.1). The groups exposed for either 1 or 0.5 hours per day were entirely normal.

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	0.90	0.19
500	4.5	[illegible]/10	195	0.68	0.38	3.22[a]	0.81	0.20
Unexposed control	0	10/12	202	0.65	0.38	2.92	0.82	0.17
Air exposed control	5	[illegible]/12	223	0.66	0.39	2.99	0.81	0.18
200	6	10/12	205	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	5/12	223	0.61	0.37	2.67	0.82	0.18
Air exposed* control	6	9/12	240	0.58	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	0.56	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	2.89	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) $P = 0.06$

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).

RSV 0016866

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 (Sigma-Frankel Unit)	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	19	19	17
#41		200	6	7	1	8.6	11	15	48
#98		100	6	7	1	4.5	16	21	13
Dog #106	F	Unexposed control	0	0	1	5.1	11	10	25
#107		Air exposed control	6	7	1	7.1	20	14	17
#103		200	6	7	1	7.9	14	16	25
#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	15.9	13.5	35	
		200	6	7	5	17.7	16.2	36	
		200	6	4	4	15.4	16.2	32	
		200	6	1	1	20.2	17.7	42	
		100	6	7	4	19.4	15.0	27	
		100	6	4	5	14.7	16.3	36	
		100	6	1	5	19.9	15.3	[illegible]	
Rat	F	Unexposed control	0	0	4	12.5	20.5	28	
		500	6	7	5	16.1	22.3	29	
		200	6	7	5	17.6	22.2	21	
		100	6	7	6	9.5	22.4	25	
Rabbit	M	Control	6	0	5	4.0	25	36	
		200	6	7	3	5.3	21	23	
		100	6	7	2	5.9	26	26	
Rabbit	F	Control	6	0	6	4.9	33	39	
		200	6	7	2	3.4	27	33	
		100	6	7	3	4.0	29	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 × 10^3	Neutrophils	Lymphocytes	Monocytes	Eosinophils
Rat	M	Air exposed control	6	4	14.5	51	15.5	25.5	71.2	2.3	0.8
Rat	M	200	6	5	14.4	50	16.7	19	77.8	0.2	2
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	12.9	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	46	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	56	25	5	11
Dog #111	M	200	6	1	16.5	58	13.8	56	41	2	1
Dog #98	M	100	0	1	15.5	52	19.9	46	46	4	4
Dog #98	M	100	6	1	16	54	15.6	61	39	0	0
Dog #106	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	33	23	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	46	51	0	2
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

RSV 0016867

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	5 10	927	0.64	0.26	3.32	0.70	0.16	0.45
M	Air exposed control	5 10	1030	0.52	0.26	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	1089	0.52	0.25	2.79(a)	0.58	0.11	0.47
M	Unexposed control	9 12	915	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	0.45
F	Unexposed control	6 8	[illegible]	0.62	0.27	3.20	0.63	0.12	
F	Air exposed control	7 8	867	0.66	0.25	3.29	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	0.59	0.26	3.50	0.64	0.12	
F	Unexposed control	9 12	725	0.78	0.34	3.41	0.69	0.18	
F	Air exposed control	9 12	854	0.65	0.26	3.64	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	0.15	2.41	0.46	0.02	0.16
M	200	3 3	3.48	0.32	0.19	2.34	0.54	0.02	0.15
M	100	3 3	3.63	0.33	0.16	2.64	0.49	0.02	0.15
M	Unexposed control	2 3	3.50	0.32	0.17	2.33	0.45	0.02	0.15
M	Air exposed control	1 3	3.57	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	4.13	0.31	0.17	2.22	0.40	0.03	
F	Air exposed control	3 3	3.91	0.30	0.18	2.18	0.41	0.04	
F	200	2 3	4.10	0.32	0.16	2.28	0.42	0.05	
F	100	3 3	4.12	0.29	0.18	1.87	0.38	0.08	
F	Unexposed control	3 3	4.31	0.30	0.17	1.94	0.46	0.04	
F	Air exposed control	2 3	3.95	0.30	0.17	1.88	0.39	0.04	
F	50	3 3	4.31	0.32	0.17	2.49	0.38	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	0.69	0.77	2.63	0.47	0.19	0.14
M	111	200	1 1	10.2	1.03	0.96	2.80	0.52	0.25	0.13
M	98	100	1 1	12.0	0.96	0.90	2.80	0.42	0.26	0.15
M	152	Unexposed control	1 1	13.3	0.90	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	162	50	1 1	9.6	0.89	0.75	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	0.73	1.89	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	0.78	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	Durationed 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	0.15	0.95
Air exposed control	6	7	12 12	347	0.51	0.32	2.41	0.70	0.16	0.91
50	6	7	12 12	339	0.50	0.31	2.49	0.68	0.17	0.87
50	6	4	8 10	348	0.51	0.33	2.59	0.69	0.16	0.91
50	6	2	6 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	0.84
Unexposed* control	0	0	8 8	336	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	0.73
50*	6	7	12 12	380	0.49	0.33	2.51	0.66	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	12.9	54	41	1	4
Dog #152	M	Unexposed control	3	1	15	52	19.4	42	50	4	4
Dog #152	M	Unexposed control	6	1	15.5	54	13.4	52	40	3	3
Dog #156	M	Air exposed control	0	1	—	—	—	—	—	—	—
Dog #156	M	Air exposed control	3	1	14.5	50	13.4	45	50	2	3
Dog #156	M	Air exposed control	6	1	14	50	11.1	55	29	5	12
Dog #162	M	50	0	1	10.5	39	13.6	49	48	2	2
Dog #162	M	50	3	1	14.5	54	12.7	49	48	3	1
Dog #162	M	50	6	1	15.5	53	11.2	43	50	4	3
Dog #151	F	Unexposed control	0	1	11	45	13.3	59	30	3	9
Dog #151	F	Unexposed control	3	1	14.5	52	23.6	67	25	0	8
Dog #151	F	Unexposed control	6	1	15.5	56	15.5	46	42	3	9
Dog #155	F	Air exposed control	0	1	13	46	14	51	37	0	2
Dog #155	F	Air exposed control	3	1	16	59	15.2	60	33	5	2
Dog #155	F	Air exposed control	6	1	15.5	56	17.0	53	33	3	11
Dog #161	F	50	0	1	10.5	35	15.3	55	40	5	0
Dog #161	F	50	3	1	15.5	55	13.4	56	42	2	0
Dog #161	F	50	6	1	15.5	56	12.3	43	42	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.

References

1. Manufacturing Chemists Assoc. Chemical Safety Data Sheet SD-56 (1954).
2. von Oettingen, W. F.: *The Halogenated Hydrocarbons, Toxicity and Potential Dangers.* (U. S. Public Health Service, Publication No. 414.) Government Printing Office, Washington, D. C. (1955).
3. Mastromatteo, E., A. M. Fisher, H. Christie, and D. Danziger: Acute Inhalation Toxicity of Vinyl Chloride to Laboratory Animals. *Am. Ind. Hyg. Assoc. J. 21*: 394 (1960).
4. Schachmann, O. cited by K. B. Lehman and F. Flury.

RSV 0016869

Toxicol. u. Hyg. der technischen Losungsmittel. J. Springer. Berlin. 1938 p. 130. (From the translation, *Toxicology and Hygiene of Industrial Solvents* by E. King and H. F. Smyth, Jr., 1941).

5. A.C.G.I.H. Threshold Limit Values for 1960. *A.M.A. Arch. of Envir. Health 1*: 62 (1960).
6. SMYTH, H. F., JR.: Improved Communication—Hygiene Standards for Daily Inhalation. *Am. Ind. Hyg. Assoc. J. 17*: 129 (1956).
7. Cherry Hill Farms, Camden, New Jersey.
8. Ralston Purina Company, St. Louis, Missouri.
9. Harris Milling Company, Mount Pleasant, Michigan.
10. SPENCER, H. C., V. K. ROWE, E. M. ADAMS, D. D. MCCOLLISTER, AND D. D. IRISH: Vapor Toxicity of Ethylene Dichloride as Determined by Experiments on Laboratory Animals. *A.M.A. Arch. Ind. Hyg. and Occup. Med. 4*: 482 (1951).
11. TORKELSON, T. R., M. A. WOLF, F. OYEN, AND V. K. ROWE: Vapor Toxicity of Allyl Chloride as Determined on Laboratory Animals. *Am. Ind. Hyg. Assoc. J. 20*: 217 (1959).
12. Dual Syringe Feeder Pumps, Modern Metalcraft, Midland, Michigan.

Industrial Hygiene Certification

THE AMERICAN BOARD OF INDUSTRIAL HYGIENE invites inquiries from those who are interested in applying for voluntary certification in the profession of industrial hygiene. Certificates will be issued on the basis of an examination to those who have eight years of practice, following a suitable baccalaureate degree. No examination will be required of those who have fifteen years of practice with recognized contributions to the field, following acceptable education. Certain specialists within the field will be certified when one-fourth of their time has consistently been devoted to application of their specialty to industrial hygiene. A brochure describing the certification program, and giving more details of eligibility will be sent to those who direct inquiries to Henry F. Smyth, Jr., Secretary-Treasurer, American Board of Industrial Hygiene, 4400 Fifth Avenue, Pittsburgh 13, Pennsylvania.

The Board was chartered as a non-profit corporation under the laws of the Commonwealth of Pennsylvania in September 1960. The incorporators were selected by the American Conference of Governmental Industrial Hygienists and the American Industrial Hygiene Association from among their respective memberships. They are John C. Soet, Chairman; Lester V. Cralley, Ph.D., Vice-Chairman; Henry F. Smyth, Jr., Ph.D., Secretary-Treasurer; and Edgar C. Barnes, William R. Bradley, Allan L. Coleman, Hervey B. Elkins, Ph.D., Louis F. Garber, Thomas F. Mancuso, M.D., Kenneth M. Morse, James H. Sterner, M.D., and Charles D. Yaffe.

Industrial Hygiene Engineering and Chemistry

THE DIVISION OF OCCUPATIONAL HEALTH, Public Health Service, will conduct two simultaneous courses January 8–19, 1962, at the Occupational Health Research and Training Facility, Cincinnati, Ohio. "Industrial Hygiene Engineering" is designed for industrial hygienists and engineers in the field of occupational health who have a degree in engineering or in the physical sciences. "Industrial Hygiene Chemistry" is designed for chemists in this field who have a degree in chemistry or chemical engineering, or in the physical sciences with a minor in chemistry. During the first week, trainees for both courses meet together for instruction in industrial hygiene and medicine, toxicology, and principles pertaining to evaluation of the environment. For the hygienists, the second week's work covers temperature and humidity measurements, illumination, noise measurement and control, and industrial ventilation; for the chemists, laboratory analyses for lead, free silica, and solvents, and spectroscopy, polarography, x-ray diffraction, electron microscopy, and gas chromatography. Much time in both courses is spent in the laboratory.

It should be noted that applications for the course or requests for information should be addressed to the Chief, Training Program, Robert A. Taft Sanitary Engineering Center, 4676 Columbia Parkway, Cincinnati 26, Ohio, or to a DHEW Regional Office Director.

Toxicology, 11 (1978) 45—54

EFFECTS OF VINYL CHLORIDE EXPOSURE ALONE AND IN COMBINATION WITH TRYPAN BLUE — APPLIED SYSTEMATICALLY DURING ALL THIRDS OF PREGNANCY ON THE FETUSES OF CFY RATS

GY. UNGVÁRY, ARANKA HUDÁK, ERZSÉBET TÁTRAI, M. LŐRINCZ and G. FOLLY

Departments of Experimental Pathology and Chemistry, State Institute of Occupational Health, Budapest, H-1450 Budapest P.O.B. 22 and Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, H-1450 Budapest P.O.B. 67 (Hungary)

(Received December 29th, 1977)
(Revision received May 22nd, 1978)
(Accepted May 23rd, 1978)

SUMMARY

Vinyl chloride (VC) has been shown to be present in the fetal and maternal blood as well as in the amniotic fluid after the exposition of pregnant CFY rats to VC at an atmospheric concentration of 5500, 18 000 or 33 000 mg/m^3 (~2000, 7000 or 12 000 ppm) for 2.5 h on the 18th day of pregnancy, indicating the permeability of the placenta to the agent.

Teratological investigation of the offspring of pregnant rats exposed continuously to VC at an atmospheric concentration of 4000 mg/m^3 air (1500 ppm) during the first, second or last third of pregnancy has shown that VC has no teratological effect in the rat and has no embryotoxic effects either, when applied during the second or last third of pregnancy in the above concentration. Exposition to VC during the first third of pregnancy resulted in an increased fetal mortality and in the manifestation of embryotoxic effects. Fetal losses and induction of central nervous system malformation due to trypan blue administration were not potentiated by a combined exposure of pregnant rats to VC and the dye.

INTRODUCTION

VC is ranking 23rd from among the 50 most widely used industrial chemi-

Supported, in part, by the Scientific Research Council, Ministry of Health, Hungary. 6-11-0401-03-1/MU.
Abbreviation: VC, vinyl chloride.

cals. Carcinogenicity of the compound has been reported in rats, mice and hamsters [1—3]. In man the occurrence of hemangiosarcoma — a rare malignant neoplasm — of the liver has been brought into possible causal relationship with VC exposure [4—8]. VC or its metabolite, chloroethylene oxide, have been shown to induce mutagenic effects [9—14]. Chromosome aberrations have also been described in plant workers after VC exposure of various lengths [15—17]. However there are only a few data on the teratogenicity of VC. The results of the epidemiological studies are controversial. Infante [18] reported an increase in the incidence of congenital malformations of the central nervous system among people living near chemical plants working with VC. Further epidemiological study [19] did not confirm this finding. Infante et al. [20] found higher mortality in families where the father was subject to occupational exposure to VC. Paddle [21], however, questioned methodological aspects of this approach. The only study in experimental teratology with VC was conducted by John et al. [22]. They found no teratogenic effect after inhalation of VC at an atmospheric concentration of 50, 500 or 2500 ppm for 7 h daily, during organogenesis in S-D rats, CF-1 mice or N-Z rabbits.

The large scale production of VC and the wide usage of the polymer PVC, the great number of people exposed, the reported hazardous effects, mutagenicity and carcinogenicity of the compound as well as controversial data of epidemiologic studies of teratogenicity call for a detailed study of experimental teratology of the compound.

The present work was aimed to answer the following questions:

(1) Does VC, inhaled by the pregnant animal, cross the placenta and result thus in a direct intrauterine exposure of the fetus?

(2) Does VC in itself possess teratogenic or embryotoxic effects?

(3) When given in combination with a known teratogenic agent does VC potentiate the teratogenic effect of the former compound?

MATERIALS AND METHODS

Female CFY rats of 240—280 g body wt. were mated in a harem system. The day of finding sperm in the vaginal smear was considered as the first day of gestation. The animals were kept on a standard rat pellet [LATI*, Gödöllő] and tap water ad lib. Body weights were recorded once a week.

Groups of 3 rats were exposed to VC for 2.5 h on the 18th day of gestation at 5500, 18 000, 33 000 mg/m^3 (~2000, 7000, 12 000 ppm) atmospheric concentrations. At the end of the exposure the animals were sacrificed and maternal and fetal blood and amniotic fluid samples were collected for VC determination performed by the method of Lőrincz [23].

Allocation of other pregnant rats to experimental groups can be seen in Table I. Groups IA, IC, IIA and IIIA inhaled air in an inhalation chamber for 24 h/day on the days of pregnancy 1—9, 8—14 and 14—21, respectively. Groups IB, ID, IIB and IIIB were exposed to VC in an atmospheric concen-

* Institute of Laboratory Animals, Gödöllő.

TABLE I

SUMMARIZED DATA OF EXPERIMENTAL GROUPS OF PREGNANT CFY RATS EXPOSED TO VINYL CHLORIDE, TRYPAN BLUE OR BOTH

Treatment 24 h/day	Days of pregnancy	No. of litters	Maternal weight gain[a] (%)	No. of fetuses: Live	Dead	Resorbed	Fetal loss[b] (%)	Mean litter size	Mean fetal weight (g)	Mean placental weight (g)	Weight retarded[c] fetuses (%)	Liver wt./body wt. ratio (%)	Liver wt./reduced body wt.[d] ratio (%)
(I)													
A Air inhalation Phys. sal. s.c.[e]	1–9 7–8	13	54.66 ± 2.18	171	1	2	1.7	13.15 ± 0.64	3.81 ± 0.02	0.53 ± 0.006	2.3	3.71 ± 0.09	4.41 ± 0.13
B VC inhalation[f] Phys. sal. s.c.[e]	1–9 7–8	19	55.18 ± 2.09	223	1	12	5.5†	11.69 ± 0.38	3.74 ± 0.03	0.58 ± 0.006	6.7	4.25 ±** 0.09	4.92 ±** 0.09
C Air inhalation Trypan blue s.c.[g]	1–9 7–8	13	56.58 ± 2.86	131	3	32	21.1††	10.15 ± 0.64	3.71 ± 0.03	0.57 ± 0.008	8.3	4.18 ±** 0.08	4.70 ±** 0.09
D VC inhalation[f] Trypan blue s.c.[g]	1–9 7–8	18	51.12 ± 2.53	198	3	34	15.7††	11.00 ± 0.68	3.75 ± 0.03	0.54 ± 0.006	10.6	4.61 ±** 0.14	5.31 ±** 0.17
(II)													
A Air inhalation	8–14	14	49.38 ± 1.85	157	—	5	3.18	11.29 ± 0.81	3.96 ± 0.04	0.55 ± 0.007	6.3	4.05 ± 0.08	4.67 ± 0.08
B VC inhalation[f]	8–14	28	51.70 ± 1.12	374	1	16	4.54	13.36 ± 0.37	3.98 ± 0.02	0.52 ± 0.004	3.21	4.26 ±* 0.06	5.03 ±* 0.07
(III)													
A Air inhalation	14–21	18	52.72 ± 2.91	212	1	12	5.8	11.78 ± 0.94	3.65 ± 0.03	0.52 ± 0.007	11.8	3.76 ± 0.08	4.28 ± 0.11
B VC inhalation[f]	14–21	22	40.83 ± 1.67	244	—	14	5.4	11.18 ± 0.74	3.64 ± 0.03	0.57 ± 0.007	12.3	3.60 ± 0.05	4.12 ± 0.07
(IV)													
Untreated control	—	28	52.45 ± 1.23	315	1	10	3.37	11.25 ± 0.54	3.83 ± 0.02	0.51 ± 0.005	2.9	3.89 ± 0.08	4.50 ± 0.09

[a] In per cent of starting body weight.
[b] In per cent of total implantation sites.
[c] Per cent of living fetuses weighing less than 3.3 g.
[d] Maternal weight -- (total weight of fetuses + placentas)
[e] 0.5 ml/100 g body wt./day
[f] 4000 mg/m³ (1500 ppm)
[g] 0.5 ml/100 g body wt./day of 1% (w/v) solution.
* $P < 0.05$; ** $P < 0.01$; (t-test)
† $P < 0.05$; ††: $P < 0.01$; (Mann Whitney U test); ± S.E.M.

tration of 4000 mg/m^3 (~1500 ppm) for the same length during the same periods of gestation as their respective controls. The volumes of the inhalation chambers were 0.13 m^3, the vertical flow rate of the air 2 m^3/h at a regulated temperature of 24–25°C and 50–55% relative humidity. VC concentration in the inhalation chamber was determined by means of a type 5840 A Hewlett Packard digital gaschromatograph [24]. The rats in groups IC and ID were given subcutaneously 2 injections of 50 mg/kg body wt. trypan blue (1% solution) on the 7th and 8th day of gestation. Group IV was kept in the animal quarters during the whole period of gestation.

On the 21st day of gestation the animals were anesthetised with ether. Abdominal wall and uterine horns were cut open and the number, position of fetuses living, dead or resorbed were noted. Fetuses and placentae were excised, weighed and macroscopic investigation was carried out. Half of the fetuses of each mother were put into Bouin's fixative and dissected after fixation under the stereomicroscope [25]. Organs with macroscopic abnormalities were embedded and hematoxylin-eosin stained sections were studied further. Histological investigation of representative other organs was also carried out. In order to investigate the skeletal system the other half of the fetuses were fixed in alcohol and stained with alizarin-red-S [26]. The mothers were dissected and their livers were processed in routine histology.

Arithmetic means and standard errors were calculated, Student's *t*-test was used for statistical comparison. The litter was regarded as the experimental teratological unit [27]; affected over total fetus ratios were calculated. Mann-Whitney U-test was used for the statistical comparison of the ratios obtained.

RESULTS

Considerably high VC concentrations were found in the blood of pregnant rats as well as their fetuses, when the mothers were exposed to VC at an atmospheric concentration of 5500, 18 000 or 33 000 mg/m^3 (~2000, 7000, 12 000 ppm) for 2.5 h on the 18th day of pregnancy. The presence of VC in the amniotic fluid was also detectable (Table II).

Maternal loss was not encountered in the experiments. No difference in the weight gain of pregnant rats expressed as percentage of the starting body weight was found with the exception of group IIIB exposed to VC during the third week of pregnancy. The weight gain in this group was lower, than in the other groups (Table I).

The maternal liver weight and liver weight/body weight ratio increased in response to trypan blue as well as to VC applied in the first or second week of pregnancy ($P < 0.01$ and $P < 0.05$, respectively) while no difference was seen in these parameters after VC exposure during the third week of pregnancy (Table I). No pathological change was observed in the liver of VC treated mothers at the light microscopic level. There was a marked periportal histiocytic reaction in the liver of trypan blue injected animals.

The number of resorbed fetuses as well as the fetal loss taken as percent-

TABLE II

VINYL CHLORIDE LEVELS IN MATERNAL AND FETAL BLOOD AND AMNIOTIC FLUID OF CFY RATS ON THE 18TH DAY OF PREGNANCY AFTER 2.5 H OF EXPOSURE

Inhalation chamber (mg/m³)	Maternal blood (μg/ml)	Fetal blood (μg/ml)	Amniotic fluid (μg/ml)
0	0 ± 0	0 ± 0	0 ± 0
5500 (~2000 ppm)	19.02 ± 1.70	12.80 ± 2.92	4.27 ± 0.42
18000 (~7000 ppm)	32.40 ± 2.12	22.67 ± 2.75	4.93 ± 0.18
33000 (~12000 ppm)	48.43 ± 1.95	30.52 ± 3.77	13.50 ± 2.99

±, S.E.M.

ige of the total number of implants was significantly increased in the group exposed to VC during the first 9 days of pregnancy ($P \cong 0.05$); there was a tendency of increased resorption and fetal loss, though not significant in the group exposed to VC during the second week of pregnancy ($P < 0.1$) and no difference in the parameters was seen after an exposure to VC during the third week of pregnancy. There was an increase in the number of resorbed fetuses as well as fetal loss in the group injected with trypan blue ($P < 0.01$). Combined trypan blue administration and VC exposure was not more effective than the dye injection alone. The number of dead fetuses was increased by the trypan blue treatment (Table I).

A slight tendency of increase in the ratio of retarded fetuses was seen in the groups exposed to VC during the first 9 days of gestation or injected with trypan blue, but no significant change in the mean weight of fetuses or placentae and in the ratio of fetuses with weight retardation (less than 3.3 g) was seen no matter during which time of gestation the VC exposure occurred. Although the ratio of retarded fetuses was higher in the groups inhaling air, or VC in the inhalation chambers during the third week of gestation, this difference was not significant and probably may be due to the stressor effect of novel environment.

The findings of the dissection and skeletal investigation of fetuses are shown on Table III. None of the malformations or anomalies could be attributed to VC.

DISCUSSION

Continuous exposure of rats to VC results in a permanent elevation of its blood level [28]. A permanent increase in blood level and the low molecular weight of VC facilitate a rapid extravascular distribution of the chemical [28]. One can assume, that during a continuous exposure of pregnant rats

TABLE III

FINDINGS OF DISSECTION AND SKELETAL INVESTIGATION OF FETUSES

	Treatment								
	Inhalation 24 h/day of pregnancy								
	Air 1–9	VC[b] 1–9	Air 1–9	VC[b] 1–9	Air 8–14	VC[b] 8–14	Air 14–21	VC[b] 14–21	— —
	S.c. injection days of pregnancy								
	Phys. sal[a] 7–8	Phys. sal 7–8	Trypan b[c] 7–8	Trypan b[c] 7–8	— —	— —	— —	— —	— —
No. of litters examined	13	19	13	18	14	28	18	22	28
No. of live fetuses	171	223	131	198	157	374	212	244	315
External malformations									
Exencephaly	—	—	2	—	—	—	—	—	—
Umbilical hernia	—	—	—	1	—	—	—	—	—
No. of fetuses dissected	83	106	63	95	75	185	102	117	166
Internal malformations									
Internal hydrocephalus	—	—	1	1	1	—	1	—	—
Anophthalmia	—	1	2	2	—	—	—	—	—
Microphthalmia	—	1	—	2	—	—	—	—	—
Polycystic lungs	—	—	2	—	—	—	3	1	—
Thymus with processus	4	6	9	9	6	9	9	9	—
Pyelectasia	2	4	5	2	6	20	6	—	3
Dilatation of urinary bladder	—	3	—	6	1	8	7	—	—

Skeletal retardation signs									
Poorly ossified sternebrae	2	7	1	4	7	3	9	7	—
Bipart. vertebra centra	8	6	9	6	3	1	11	12	—
Shortness of 13th rib.	—	—	—	—	1	4	—	—	—
Skeletal anomalies									
Fused sternebrae	—	—	—	—	2	2	1	—	1
Supernumerary ribs.	2	—	2	1	1	2	4	2	2
Skeletal malformations									
Missing orbita	—	—	—	1	—	—	—	—	—
Multiplex	—	—	—	—	—	1	—	—	—

[a] 0.5 ml/100 g body wt./day.
[b] 4000 mg/m³ (1500 ppm).
[c] 0.5 ml/100 g body wt./day of 1% (w/v) solution.

to VC the fetuses like the mothers are also permanently exposed. Our detection of VC in the amniotic fluid and in the blood of fetuses of pregnant rats exposed on the 18th day of gestation (Table II) justify this assumption.

In order to study the possible teratogenic and embryotoxic effects of the compound, rats were exposed to VC at 4000 mg/m^3 (1500 ppm) atmospheric concentration continuously during the first, second or third week. of pregnancy. In spite of the exposure at this very high level of concentration no obvious alteration in the behaviour of experimental animals was observed; their food and water consumption and weight gain did not differ from that of the controls and activation of self protective mechanisms [29] was not seen either.

Although our study of the great number of fetuses gave essentially negative results, some tendencies found — not reaching the level of significance — might deserve further attention.

There was an increase in the number of resorbed fetuses in the groups subjected to VC during the first and second week of pregnancy. Though the increase in the number of implantations and higher birth rate might explain this difference in the group exposed during the second week; no increase in the number of implants was seen in the rats exposed during the first week. Thus it is most probable that the toxic effects of VC might explain the increased fetal loss close to the level of significance ($P \cong 0.05$). This is all the more probable, because toxic agents independent of their chemical nature have been shown to result in similar embryotoxic effects, when applied during the first third of pregnancy [30].

Among the offspring of mothers exposed to VC during the first week of pregnancy one case of microphthalmia and an other case of anophthalmia occurred. In spite of the fact that this was not consistent with a significant increase in the incidence of congenital malformations, these cases deserve further attention for the following reasons. None of these malformations was observed in the group exposed to air in the chamber, or in the untreated controls. Both malformations are related to the central nervous system, and an increased incidence of congenital malformations of the central nervous system have been brought into causal relationship with VC exposure by Infante [18].

On the basis of our results VC exposure in itself has no teratogenic effect in CFY rats, but an embryotoxic effect of VC exposure during the early stages of pregnancy at high atmospheric concentrations should be taken into consideration.

A similar lack of teratogenic effect of VC has been reported by John et al. [22]. Their conclusion is based on studies of the effect of VC applied during arbitrarily chosen short periods of organogenesis. One could emphasize here that it is a minimal requirement of experimental studies aimed to reveal the teratogenic effect of any particular chemical that the pregnant mothers are exposed to the chemical in such a way as to provide continuous exposure of the fetuses during the whole period of organogenesis. The fulfilment of this requirement is particularly important with VC and other toxic agents taken up by inhalation and exhaled rapidly. In our view the teratogenic effect of a chemical cannot be excluded in studies using shorter exposure times than the whole period of organogenesis.

We are not aware of any data on the effect of VC in early pregnancy. Even if the results of our animal experiments cannot be applied directly to the human and considering that an exposure to VC at 250 ppm atmospheric concentration would result in the saturation of the metabolizing capacity of VC [31], the hazards of an occupational exposure of women in the fertile age requires thorough consideration.

Infante [18] claims that the high incidence of congenital malformations of the central nervous system in the neighbourhood of PVC producing plants is due to VC. Edmonds et al. [19] were not able to confirm this view. In this context we studied the possibility that even if VC has no teratogenic effect to induce malformations of the central nervous system it might potentiate a teratogenic effect of other agents. Trypan blue has been reported to be embryotoxic and bring about malformations of the nervous system [32—34]. Negative results have been obtained in this respect; a concomittant exposure :o VC did not affect either the teratogenic or the embryotoxic effects of rypan blue, as no higher incidence of congenital neural malformations exencephaly, anophthalmia, microphthalmia, aplasia of the orbit) or higher etal losses were encountered in the group with combined VC and trypan lue treatment.

:KNOW EDGEMENT

The nical assistance of Mr Gy. Krasznai, Miss Á. Csonka, Mrs Gy. omolanyi, Mrs J. Nyilas is gratefully acknowledged.

ERENCES

P.L. Viola, A. Bigotti and A. Caput, Cancer Res., 31 (1971) 516.
C. Maltoni and G. Lefemine, Environ. Res., 7 (1974) 387.
C. Maltoni and G. Lefemine, Ann. N.Y. Acad. Sci., 246 (1975) 185.
I.L. Creech and M.N. Johnson, J. Occup. Med., 16 (1974) 150.
.B. Thomas and H. Popper, Ann. N.Y. Acad. Sci., 246 (1975) 268.
. Makk, F. Delmore, J.L. Creech, Jr., L.L. Ogden, II., E.H. Fadell, C.L. Songster, Clanton, M.N. Johnson and W.M. Cristopherson, Cancer, 37 (1976) 149.
M. Smith, D.M.J. Williams and D.M.D. Evans, Bull. N.Y. Acad. Med., 52 (1976) 17.
Zimmermann and H. Eck, Virchows Arch. A. Pathol. Anat. Histol., 368 (1975)
Bartsch, C. Malaveille and R. Montesano, Int. J. Cancer, 15 (1975) 429.
Malaveille, H. Bartsch, A. Barbin, H.M. Camus, R. Montesano, A. Croisy and P. quinan, Biochem. Biophys. Res. Commun., 63 (1975) 363.
Huberman, H. Bartsch and L. Sachs, Int. J. Cancer, 16 (1975) 639.
Loprieno, R. Barale, S. Baroncelli, C. Bauer, G. Bronzetti, A. Cammellini, G. ignani, C. Corsi, G. Gervasi, C. Leporini, R. Nieri, A.M. Rossi, G. Stretti and urchi, Mutat. Res., 40 (1976) 85.

13 U. Rannug, R. Göthe and C.A. Wachtmeister, Chem.-Biol. Interact., 12 (1976) 251.
14 A.J. Carro, J.B. Guttenplan and P. Milvy, Mutat. Res., 38 (1976) 81.
15 V. Ducatman, K. Hirschorn and I.J. Selikoff, Mutat. Res., 31 (1975) 163.
16 F. Funes-Cravioto, B. Lambert, J. Linsten, L. Ehrenberg, A.T. Natarajan and S. Osterman-Golkar, Lancet, i (1975) 459.
17 J. Szentesi, E. Hornyák, Gy. Ungvary, A. Czeizel, Z. Bognár and M. Timár, Mutat. Res. 37 (1976) 313.
18 P.F. Infante, Lancet, ii (1975) 1098.
19 L.D. Edmonds, H. Falk and J.E. Nissim, Lancet, ii (1975) 1098.
20 P.F. Infante, J.K. Wagoner, A.J. McMichael and R.J. Waxweiler, Lancet, i (1976) 734.
21 G.M. Paddle, Lancet, i (1976) 1079.
22 J.A. John, F.A. Smith, B.K.J. Leong and B.A. Schwetz, Toxicol. Appl. Pharmacol., 39 (1977) 497.
23 M. Lörincz, Munkavédelem, 24 (1978) 47.
24 NIOSH Manual of Analytical Methods, Method No.P and CAM-127 (1974).
25 J.G. Wilson and F. Warkany, Methods for administering agents and detecting malformations in experimental animals, in Teratology: Principles and Techniques. The University of Chicago Press, Chicago, 1965.
26 R.E. Staples and V.L. Schnell, Stain. Technol., 39 (1964) 62.
27 J.K. Haseman and M.D. Hogan, Teratology, 12 (1975) 165.
28 J.R. Withey, J. Toxicol. Environ. Health, 1 (1976) 381.
29 E.S. Reynolds, M.T. Moisen, S. Szabó and R.J. Jaeger, Res. Commun. Chem. Pathol. Pharmacol., 12 (1975) 685.
30 J. Elis, Proc. Eur. Soc. Toxicol., 16 (1975) 133.
31 H.M. Bolt, R.J. Laib, H. Kappus and A. Buchter, Toxicology, 7 (1977) 179.
32 L. Denker, Teratology, 15 (1977) 179.
33 J. Gillmann, C. Gilbert, I. Spence and T. Gillmann, S. Afr. J. Sci., 13 (1948) 47.
34 J. Warkany, J.G. Wilson and J.P. Geiger, J. Comp. Neurol., 109 (1958) 35.

RSV 0016880