

By 00462 HCL

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**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. LÖRJINCZ 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)*

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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/m³ (~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/m³ 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-

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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/m³ (~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			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 (%)
				Live	Dead	Resorbed							
(I)													
A Air inhalation	1-9	13	54.66 ±	171	1	2	1.7	13.15 ±	3.81 ±	0.53 ±	2.3	3.71 ±	4.41 ±
Phys. sal. s.c. ^e	7-8		2.18					0.64	0.02	0.006		0.09	0.13
B VC inhalation ^f	1-9	19	55.18 ±	223	1	12	5.5†	11.68 ±	3.74 ±	0.58 ±	6.7	4.25 ±**	4.92 ±**
Phys. sal. s.c. ^e	7-8		2.09					0.38	0.03	0.006		0.09	0.09
C Air inhalation	1-9	13	55.58 ±	131	3	32	21.1††	10.15 ±	3.71 ±	0.57 ±	8.3	4.16 ±**	4.70 ±**
Trypan blue s.c. ^g	7-8		2.86					0.64	0.03	0.008		0.08	0.09
D VC inhalation ^f	1-9	18	51.12 ±	198	3	34	15.7††	11.00 ±	3.75 ±	0.54 ±	10.6	4.61 ±**	5.31 ±**
Trypan blue s.c. ^g	7-8		2.53					0.68	0.03	0.006		0.14	0.17
(II)													
A Air inhalation	8-14	14	49.38 ±	157	—	5	3.18	11.29 ±	3.96 ±	0.55 ±	6.3	4.05 ±	4.67 ±
			1.85					0.61	0.04	0.007		0.08	0.08
B VC inhalation ^f	8-14	28	51.70 ±	374	1	16	4.54	13.36 ±	3.98 ±	0.52 ±	3.21	4.26 ±*	5.03 ±*
			1.12					0.37	0.02	0.004		0.06	0.07
(III)													
A Air inhalation	14-21	18	52.72 ±	212	1	12	5.8	11.78 ±	3.65 ±	0.52 ±	11.8	3.75 ±	4.29 ±
			2.91					0.94	0.03	0.007		0.08	0.11
B VC inhalation ^f	14-21	22	40.83 ±	244	—	14	5.4	11.18 ±	3.64 ±	0.57 ±	12.3	3.60 ±	4.12 ±
			1.67					0.74	0.03	0.007		0.05	0.07
(IV)													
Untreated control	—	28	52.45 ±	315	1	10	3.37	11.25 ±	3.83 ±	0.51 ±	2.9	3.89 ±	4.50 ±
			1.23					0.54	0.02	0.005		0.08	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/m³ (~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 m³, the vertical flow rate of the air 2 m³/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/m³ (~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/m ³)	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.

age of the total number of implants was significantly increased in the group exposed to VC during the first 9 days of pregnancy ($P \approx 0.05$); 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	VC ^b	Air	VC ^b	Air	VC ^b	Air	VC ^b
	1-9	1-9	1-9	1-9	8-14	8-14	14-21	14-21
	—	—	—	—	—	—	—	—
	S.c. injection days of pregnancy							
	Phys. sal ^a	Phys. sal	Trypan b ^c	Trypan b ^c	—	—	—	—
	7-8	7-8	7-8	7-8	—	—	—	—
No. of litters examined	13	19	13	18	14	28	18	22
No. of live fetuses	171	223	131	198	157	374	212	244
External malformations								
Exencephaly	—	—	2	—	—	—	—	—
Umbilical hernia	—	—	—	1	—	—	—	—
No. of fetuses dissected	83	106	63	95	75	185	102	117
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	—
Dilatation of urinary bladder	—	3	—	6	1	8	7	—

No. of alizarin-stained fetuses	88	117	68	103	82	189	110	127	143
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. On 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/m³ (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 ($P \approx 0.05$). 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 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 to VC did not affect either the teratogenic or the embryotoxic effects of trypan blue, as no higher incidence of congenital neural malformations (exencephaly, anophthalmia, microphthalmia, aplasia of the orbit) or higher fetal losses were encountered in the group with combined VC and trypan blue treatment.

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