For File - VCM

UNION CARBIDE

HEALTH SAFETY AND ENVIRONMENTAL AFFAIRS

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RECEIVED
SEP 6 1983
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A. A. Lang
R. W. Holland

I noticed this letter in the August 20, 1983 Lancet. You might find the comments on latency interesting.

T. A. Lincoln

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exchange, about 10% will be acutely ill and need treatment in an intensive care area.[8]

The claims for plasma exchange need constant critical evaluation, perhaps through an apheresis register.[9]

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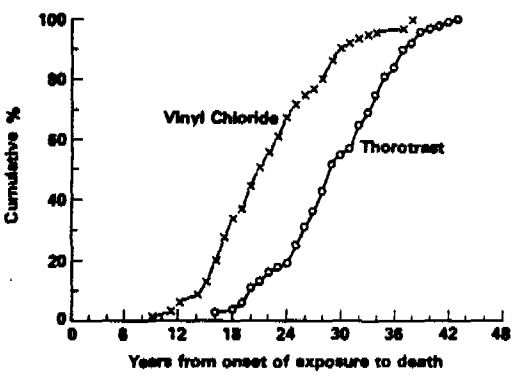
ANGIOSARCOMA AS A MODEL FOR COMPARATIVE CARCINOGENESIS

SIR,—By pooling data from several sources we have been able to contrast the latent periods for angiosarcoma of the liver induced by vinyl chloride monomer (VCM) and 'Thorotrast' (thorium dioxide). The average and minimal latent periods are probably much shorter for VCM cases than for thorotrast cases, although the hepatic neoplasms induced by these two agents are histologically the same.[1] The thorotrast-induced angiosarcomas we analysed were those reported from West Germany,[2] the UK,[3] Denmark,[4] and the United States.[5] Those attributed to VCM have been gathered by three of us (J. S., R. S., and E. D.), from personal contacts in twelve countries. The earliest case-reports involving thorotrast and VCM were published in 1947[6] and 1974,[7] respectively.

Exposure to thorotrast occurred primarily in the period 1928–55, while exposures to VCM were heaviest in the 1940s, 1950s, and 1960s. For neither agent have all cases yet occurred (table), nor are denominator data generally available. For these reasons the distributions in the figure are not definitive, but the first two decades of the experience are now sufficiently well defined to venture that angiosarcomas induced by VCM begin to appear much sooner after initial exposure than do cases caused by thorotrast. Medians for males are, respectively, 22 and 29 years, and we note that a recent report on thorotrast-induced cases in Japan gives a latent period of 33·5 years.[8] Because of the large relative risks reported for cohorts having thorotrast and VCM exposures[2,9] and the low incidence of this tumour, it seems reasonable to treat a case of angiosarcoma as if the given exposure were the cause.



Cumulative density functions of latent periods for hepatic angiosarcoma induced in males by VCM and thorotrast.

DISTRIBUTIONS OF YEAR-OF-DEATH BY AGENT AND SEX

Year-of-death	Thorotrast		VCM
	Male	Female	(male)
1955–59	2	1	2
60–64	8	2	4
65–69	19	6	9
70–74	33	6	21
75–79	32	7	45
80–81	7	2	14
Totals	101	24	95

Differences between the series in demographic composition seem clearly not to be responsible for much of the difference in latent period, and one might expect any difference between workers (VCM) and patients (thorotrast) to be reflected in a longer latent period for the healthy workers in terms of either onset or death. The exposure is different for the two agents, being continuous from the date of the injection of thorotrast and intermittent from date of first exposure to VCM. Also, we see no way of quantitating the two exposures in the same units. The limited data available on radiation-induced solid tumours suggest that latent period does not depend on dose.[10] Some part of the difference in average latent period could derive from differences in "stage of epidemic" or in completeness of ascertainment in the later decades, but these differences could hardly contribute in any important way to the differences seen in minimal latent period, especially since the thorotrast cases derive from cohorts followed up from the time of treatment. The minimum latent period for leukaemia following exposure to thorotrast is appreciably longer (8 years) than that associated with X-ray or gamma radiation (2–4 years).[11]

We believe that the distributions in the figure may well reflect differences in the mechanisms of chemical and radiation carcinogenesis, and that other comparisons of this nature would be of interest. Cohort analyses of VCM workers and thorotrast patients are clearly indicated as the effects of the exposures run their course, and animal experimentation with these compounds might provide some understanding of the differences in their carcinogenic action. Since both VCM and thorotrast are multipotential carcinogens,[2,4,12] more analytical studies of latency should carefully consider all causes of death, as well as levels of exposure.

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CAUSES

EROSION OF MULTIPLE TERMINAL PHALANGEAL TUFTS (ACRO-OSTEOLYSIS)

COMMON

1. Arteriosclerosis obliterans
2. Burn, thermal or electrical
3. Diabetic gangrene
4. Frostbite
5. Hyperparathyroidism, primary or secondary
6. Neurotrophic disease (see Gamut D-88)
7. Psoriasis
8. Raynaud's disease
9. Rheumatoid arthritis
*10. Scleroderma, dermatomyositis
11. Trauma

UNCOMMON

1. Brachydactyly B
2. Buerger's disease
3. Clubbing of fingers (see Gamut D-79)
4. Congenital (familial) acro-osteolysis (eg, Hajdu-Cheney S.)
5. Congenital indifference to pain
6. Disseminated lipogranulomatosis
7. Drug therapy (eg, dilantin, phenobarbital, ergot)
8. Ectodermal dysplasia
*9. Epidermolysis bullosa
*10. Gout
11. Leprosy

(Continued)

12. Lesch-Nyhan S. (mental defective finger biting)
13. Osteomalacia (eg, malabsorption syndromes)
14. Osteopetrosis
15. Pachydermoperiostosis
16. Pityriasis rubra
17. Plantar warts
18. Polyvinyl chloride osteolysis
19. Porphyria
20. Progeria; Werner's S.
21. Pseudoxanthoma elasticum
22. Pyknodysostosis
23. Reticulohistiocytoma (lipoid dermatoarthritis)
*24. Rothmund's S.
25. Sarcoidosis
26. Sjögren's syndrome
27. Streeter's congenital amniotic bands
28. Syringomyelia
29. Tabes dorsalis
*30. Thromboangiitis obliterans

**May be associated with calcification.*

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SUBGAMUT D-89A
ACRO-OSTEOLYSIS CONFINED TO ONE DIGIT

1. Angiomatous malformation
2. Carcinoma of nail bed
3. Epidermoid cyst
4. Fibroma
5. Giant cell tumor of tendon sheath
6. Glomus tumor
7. Infection (eg, whitlow, osteomyelitis)
8. Metastasis; lymphoma
9. Neurofibroma

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VINYL CHLORIDE

(Teratogenesis)

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Teratogenicity not proven - so must remain under review + observation —

Environmental Health Perspectives
Vol. 21, pp. 71–78, 1977

Dominant Lethal Studies with the Halogenated Olefins Vinyl Chloride and Vinylidene Dichloride in Male CD-1 Mice

by Diana Anderson,* M. C. E. Hodge,* and I. F. H. Purchase*

The mutagenic activity of vinyl chloride (VC) and vinylidene dichloride (VDC) at three exposure levels was assessed in fertile male CD-1 mice with the dominant lethal test. Each compound was assessed in a separate study.

Male mice were exposed by inhalation to VC at 3000, 10,000, and 30,000 ppm and to VDC at 10, 30, and 50 ppm for 6 hr/day for 5 days. By comparison with control males exposed to air, no mutagenic effects on any maturation stage of spermatogenesis in treated males were detected. There was no significant increase in the number of postimplantational early fetal deaths as shown by the number of females with one or more early deaths or the number of early deaths/pregnancy or the number of early deaths/total implants/pregnancy. There was no evidence of pre-implantational egg losses as indicated by the total implants/pregnant female. There was also no reduction in fertility. (The reduction in fertility at 50 ppm VDC was unproven).

The lack of effect was not due to the insensitivity of the system used, since both the VC and VDC study a mutagenic effect was clearly demonstrated in male mice dosed IP with the positive control compounds cyclophosphamide (CTX) and/or ethylmethane sulfonate (EMS). During dosing these animals were housed under similar exposure conditions to those animals exposed to the test substances but with a flow of air through the exposure chambers.

Thus, neither VC nor VDC is mutagenic in the mouse at the stated exposure levels as measured by the dominant lethal test.

Introduction

VC used in the manufacture of poly(vinyl chloride) has been found to cause tumors in rats (*1*) and man (*2*). It has also been shown to produce chromosome breaks in exposed workers (*3–6*) and causes mutations in *Salmonella typhimurium* (*7, 8*). Another chlorinated monomer, VDC is also known to cause mutation in *Salmonella typhimurium* (*7, 8*). We, therefore, carried out dominant lethal studies to determine if there were any mutagenic effects of this type in mice after VC and VDC exposure at three levels. At the same time negative control animals exposed to air and positive control animals also exposed to air and given EMS and/or CTX were assayed.

Materials and Methods

Chemicals

VC was obtained from Air Products Ltd., Worsley, Walkden, Lancs., U.K. VDC was obtained from ICI Ltd., Mond Division, Runcorn, Cheshire, U.K. EMS was obtained from Koch-Light Ltd., Colnbrook, Bucks, U.K., and CTX (Endoxana) from Ward Blenkinsop Ltd., London, U.K.

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Animals

CD-1 mice (Charles River, Manston, Kent) were used throughout the experiment. Undosed females were 8–10 weeks old when mated and male mice immediately after dosing were 10–12 weeks old. Males were caged individually and females in pairs. They received food and water *ad libitum*.

Dosing of Male Mice

Dose levels for VC and VDC were selected on the basis of preliminary toxicity studies. A dose of 30,000 ppm of VC was found to be in the toxic range and this was chosen as the highest exposure level since it was desirable that the maximum tolerated dose or higher should be used. Other levels of 10,000 and 3000 ppm were also used. The required concentrations of VC were generated by mixing known volumes of VC and compressed air using rotameters as indicators. VDC was much more toxic to the mice. Exposure levels of 50, 30, and 10 ppm were selected. The required concentrations of VDC were generated by a controlled fluid-feed/atomizer technique (9). The method involves continuously passing a known volume of the compound through a concentric jet atomizer, where it is vaporized by a calculated volume of dry clean air. The volume of air required as calculated from Eq. (1).

$$\text{Air flow (l./min)} = \frac{\text{Syringe size (ml/cm)} \times \text{density (g/ml)} \times 24 \times 10^6}{\text{Injection rate (min/cm)} \times \text{ppm} \times \text{mol. wt.}} \quad (1)$$

During dosing the mice were housed individually in chambers made of stainless steel and glass with an internal capacity of three litres.

Most of the negative control animals and all animals dosed with CTX or EMS were housed under identical conditions during the dosing period but with a flow of air through the chambers. Those dosed with CTX were injected IP on day 5 of exposure and those dosed with EMS were given an oral dose by gavage for 5 consecutive days. Both of these substances were prepared as aqueous solutions immediately before use. Some of the animals in the negative control group controlling the VDC study were housed under normal conditions. Before test mating began, groups of mice were treated as shown in Table 1.

In the VDC study, in order to obtain a group with sufficient animal numbers some of the animals which were infertile in week 0 were included in group 4. Of the 8 survivors, 5 did not mate in the fertility study and one had a higher than average number of early deaths. However some of these males mated in the main study. One male surviving 50 ppm for 2 days died in week 2 of the study. Of the 17 animals, three in the CTX treatment group also did not mate in the fertility study. Fifty animals were included in the negative control group to provide a better data base against which to compare the treated groups. There were no statistical differences between males exposed to air and housed under normal conditions.

Mating

Fertility Testing. Male mice were caged with virgin female mice, 1 male and 2 female mice in

Table 1. Treatment groups.

Group	No. survivors/ no. animals treated (mice)	Treatment
VC Study		
Group 1	20/20	Air (negative control)
Group 2	18/20	3000 ppm VC, 6 hr/day, 5 days
Group 3	19/20	10,000 ppm VC, 6 hr/day, 5 days
Group 4	9/20	30,000 ppm VC, 6 hr/day, 5 days
Group 5	15/15	200 mg CTX in water/kg body weight, once by IP injection on day 5.
Group 6	25/25	200 mg EMS in water/kg body weight orally, once/day, 5 days
VDC Study		
Group 1	50/50	15 air, 35 normal conditions (negative control)
Group 2	20/20	10 ppm VDC, 6 hr/day, 5 days
Group 3	18/20	30 ppm VDC, 6 hr/day, 5 days
Group 4	6/20	50 ppm VDC, 6 hr/day, 2 days
	2/20	75 ppm VDC, 6 hr/day for 1 day, then 50 ppm VDC, 6 hr/day, for 1 day
Group 5	17/17	200 mg CTX in water/kg body weight, once by IP injection on day 5

each cage. After 5 days the females were transferred to other cages. The female mice were killed 15 days after first introducing them to the males and examined for pregnancies. The 106 males which survived dosing in the VC study and 113 males in the VDC study and were successful in fertilizing at least 1 female in their cage were selected for continuation in the experiment.

Experimental Mating. Two virgin female mice 8–10 weeks old were put into each of the 106 and 113 cages in which the males were individually housed. After 5 days the females were removed and rehoused in pairs. A week after the initial introduction the males were caged with another two virgin females and again left for 5 days. This process was repeated until the treated male mice had been mated at weekly intervals for 8 weeks with virgin females. The males were then killed and not examined further. No attempts were made to establish whether or when mating had occurred. Instead it was assumed that most matings leading to fertilization would occur 2 or 3 days after introducing female mice to the cages containing males.

Female mice were killed 13 days after the assumed date of fertilization, i.e., 15 or 16 days after caging females with males.

Assessment

Uteri of killed mice were examined for live implantations, early deaths, and late deaths.

Statistics

The data have been statistically analyzed as reported previously (*10*).

Results

Mating weeks after treatment are represented in Tables 2–13 by numbers 1-8 and the mating week before treatment is represented by week 0. Assessment of females which became pregnant during the fertility test yielded the data for week 0 but only data from those animals that survived treatment have been included in weeks 0-8. Tables 2–7 relate to the VC study and Tables 8–13 relate to the VDC study.

Table 2. Number and percentage of male mice which survived treatment successfully mating at each week.

Week	Group 1, air (negative control)		Group 2, VC, 3,000 ppm (6 hr × 5)		Group 3, VC, 10,000 ppm (6 hr × 5)		Group 4, VC, 30,000 ppm (6 hr × 5)		Group 5, CTX, 200 mg/kg IP		Group 6, EMS, 200 mg/kg (oral) × 5	
	No. of mice	%	No. of mice	%	No. of mice	%	No. of mice	%	No. of mice	%	No. of mice	%
0	20	100	18	100	19	100	9	100	15	100	25	100
1	20	100	18	100	18	100	7	77.8	14	93.3	3*	12.2
2	20	100	18	100	19	100	9	100	14	93.3	23	92.0
3	20	100	18	100	19	100	9	100	14	93.3	23	92.0
4	20	100	18	100	19	100	9	100	15	100	25	100
5	19	95.0	18	100	19	100	7	77.8	15	100	25	100
6	20	100	18	100	19	100	9	100	14	100	24	100
7	20	100	18	100	19	100	9	100	14	93.3	25	100
8	20	100	18	100	19	100	8	88.9	13	92.9	25	100

*$p < 0.001$.

Table 3. Number of mated females becoming pregnant.

Week	Group 1, air (negative control)		Group 2, VC, 3,000 ppm (6 hr × 5)		Group 3, VC, 10,000 ppm (6 hr × 5)		Group 4, VC, 30,000 ppm (6 hr × 5)		Group 5, CTX, 200 mg/kg IP		Group 6, EMS, 200 mg/kg (oral) × 5	
	No. pregnant	No. mated	No. pregnant	No. mated	No. pregnant	No. mated	No. pregnant	No. mated	No. pregnant	No. mated	No. pregnant	No. mated
0	35	40	30	36	28	38	15	18	25	30	37	50
1	35	40	33	36	32	36	11	18	26	30	4*	50
2	36	40	36	36	33	38	15	18	25	30	31*	50
3	34	40	30	36	34	38	16	18	26	30	40	50
4	38	40	34	36	33	38	17	18	30	30	46	50
5	35	40	34	36	36	38	12	18	28	30	47	50
6	36	40	33	36	36	38	18	18	26	28	47	48
7	37	40	31	36	37	38	18	18	22	30	41	50
8	37	40	32	36	31	37	14	18	23	28	45	50

*$p < 0.001$.
†$p < 0.01$.

Table 4. Mean total implants per pregnant female.

Week	Group 1, air (negative control)	Group 2, VC, 3,000 ppm (6 hr × 5)	Group 3, VC, 10,000 ppm (6 hr × 5)	Group 4, VC, 30,000 ppm (6 hr × 5)	Group 5, CTX, 200 mg/kg IP	Group 6, EMS, 200 mg/kg (oral) × 5
0	11.9	12.6	12.0	13.0	11.3	11.8
1	12.9	13.6	13.4	16.1	8.8[a]	4.0[a]
2	12.8	12.7	13.0	13.7	10.9	8.4[a]
3	12.5	12.5	12.4	11.2	10.9	12.5
4	12.4	12.1	13.7	10.0[b]	12.6	12.7
5	13.7	12.7	14.1	12.8	12.3	12.7
6	11.7	11.9	12.5	12.6	12.5	12.1
7	11.8	11.6	11.8	12.0	11.5	12.4
8	12.7	12.1	12.6	12.1	12.5	12.5

[a]$p < 0.01$
[b]$p < 0.05$

Table 5. Number of pregnant females with one or more early deaths (ED).

Week	Group 1, air (negative control)		Group 2, VC, 3,000 ppm (6 hr × 5)		Group 3, VC, 10,000 ppm (6 hr × 5)		Group 4, VC, 30,000 ppm (6 hr × 5)		Group 5, CTX, 200 mg/kg IP		Group 6, EMS, 200 mg/kg (oral) × 5	
	0	≥ 1 ED	0	≥ 1 ED	0	≥ 1 ED	0	≥ 1 ED	0	≥ 1 ED	0	≥ 1 ED
0	18	17	21	9	16	22	10	5	22	3[a]	21	26
1	15	20	16	17	10	21	6	5	1	25[a]	1	3
2	19	17	18	18	13	20	6	9	2	23[a]	5	26[a]
3	18	16	16	14	19	15	9	6	8	18	15	25
4	16	22	17	17	20	13	9	7	14	16	19	27
5	17	18	18	16	14	22	3	9	12	16	25	22
6	17	19	11	22	21	15	7	11	13	13	24	23
7	18	19	13	18	21	16	8	10	10	12	22	19
8	13	24	14	18	15	16	8	6	13	10	22	23

[a]$p < 0.01$.

Table 6. Mean number of early deaths per pregnancy.

Week	Group 1, air (negative control)	Group 2, VC, 3,000 ppm (6 hr × 5)	Group 3, VC, 10,000 ppm (6 hr × 5)	Group 4, VC, 30,000 ppm (6 hr × 5)	Group 5, CTX, 200 mg/kg IP	Group 6, EMS, 200 mg/kg (oral) × 5
0	0.77	0.33	0.54	0.40	0.16	0.59
1	0.86	0.91	1.00	0.45	4.27[a]	3.50[a]
2	0.83	1.00	1.15	0.93	4.84[a]	2.58[a]
3	0.91	0.73	0.88	0.93	1.54	1.45
4	0.89	0.79	0.61	0.69	1.13	0.85
5	1.06	0.71	1.03	1.00	1.11	0.72
6	0.97	0.91	0.75	1.39	0.65	0.85
7	0.76	0.87	0.68	0.89	0.82	0.63
8	1.03	0.88	1.00	0.71	0.70	0.73

[a]$p < 0.01$.

Table 7. Early deaths as a percentage of total implants per pregnant female.

Week	Group 1, air (negative control)	Group 2, VC, 3,000 ppm (6 hr × 5)	Group 3, VC, 10,000 ppm (6 hr × 5)	Group 4, VC, 30,000 ppm (6 hr × 5)	Group 5, CTX, 200 mg/kg IP	Group 6, EMS, 200 mg/kg (oral) × 5
0	6.5	2.7	4.4	3.1	1.4[a]	5.1
1	6.6	6.7	7.5	2.7	49.1[a]	77.78[a]
2	6.4	7.9	9.6	6.9	44.6[a]	31.5[a]
3	7.3	5.9	7.0	7.8	14.1	11.6
4	7.2	6.5	4.5	6.5	9.0	6.7
5	7.8	5.5	7.3	7.9	9.1	5.7
6	8.2	7.6	6.0	11.1	5.2	7.0
7	6.4	7.3	5.7	7.4	7.0	5.1
8	8.0	7.2	8.1	5.9	5.7	5.9

[a]$p < 0.05$.
[b]$p < 0.01$.

Table 8. Number and percentage of male mice which survived treatment successfully: mating at each week.

Week	Group 1 (pooled negative control)		Group 2, VDC, 10 ppm (6 hr × 5)		Group 3, VDC, 30 ppm (6 hr × 5)		Group 4, VDC, 50 ppm (6 hr × 5)		Group 5, CTX, 200 mg/kg IP	
	No. of mice	%	No. of mice	%	No. of mice	%	No. of mice	%	No. of mice	%
0	50	100	20	100	18	100	8[a]	38	17[b]	76
1	50	98	20	95	18	100	8[a]	75	17[b]	65
2	50	96	20	100	18	94	7[b]	86	16	94
3	50	94	20	100	18	89	7[a]	43	17	94
4	49	98	20	100	18	89	7[a]	43	15	100
5	47	94	20	100	17	82	7[a]	57	17	100
6	49	88	20	100	17	100	7[a]	43	17	88
7	49	92	20	90	17	100	6[c]	50	16[c]	75
8	48	92	19	95	17	88	7[c]	57	19	86

[a] $p < 0.001$.
[b] $p < 0.01$.
[c] $p < 0.05$.

Table 9. Number of mated females becoming pregnant.

Week	Group 1 (pooled negative control)		Group 2, VDC, 10 ppm (6 hr × 5)		Group 3, VDC, 30 ppm (6 hr × 5)		Group 4, VDC, 50 ppm (6 hr × 5)		Group 5, CTX, 200 mg/kg IP	
	No. pregnant	No. mated	No. pregnant	No. mated	No. pregnant	No. mated	No. pregnant	No. mated	No. pregnant	No. mated
0	73	100	30	40	25	36	5[a]	16	17[b]	34
1	82	100	32	40	33	36	7[a]	16	20[b]	34
2	75	100	36	40	32	36	7[a]	14	25	33
3	82	100	34	40	26	36	5[a]	14	28	34
4	85	98	36	40	27	36	6[b]	14	26	32
5	80	94	34	40	26	34	6[a]	14	29	34
6	75	98	33	40	32	34	5[b]	14	25	34
7	77	98	33	40	28	34	7	13	19[c]	33
8	73	96	28	39	26	34	7	14	28	39

[a] $p < 0.01$.
[b] $p < 0.001$.
[c] $p < 0.05$.

Table 10. Mean total implants per pregnant female.

Week	Group 1 pooled (negative control)	Group 2, VDC, 10 ppm (6 hr × 5)	Group 3, VDC, 30 ppm (6 hr × 5)	Group 4, VDC, 50 ppm (6 hr × 5)	Group 5, CTX, 200 mg/kg IP
0	12.2	11.0[a]	12.0	11.4	11.5
1	12.3	13.1	12.8	11.7	9.2[b]
2	12.3	11.7	12.1	11.0	9.5[b]
3	12.0	12.1	13.2	13.4	11.0[c]
4	12.0	12.1	13.0	12.7	12.1
5	11.8	11.7	12.5	10.7	12.0
6	12.5	12.4	12.1	12.6	11.1[c]
7	12.6	13.0	12.5	12.7	11.7[c]
8	12.3	13.6	12.4	12.6	11.6

[a] $p < 0.01$.
[b] $p < 0.001$.
[c] $p < 0.05$.

Table 11. Number of pregnant females with one or more early deaths (ED).

Week	Group 1 (pooled negative control)		Group 2, VDC, 10 ppm (6 hr × 5)		Group 3, VDC, 30 ppm (6 hr × 5)		Group 4, VDC 50 ppm (6 hr × 5)		Group 5, CTX, 200 mg/kg IP	
	0	≥ 1 ED	0	≥ 1 ED	0	≥ 1 ED	0	≥ 1 ED	0	≥ 1 ED
0	40	33	14	16	10	15	1	4	9	8
1	41	41	18	14	19	14	6	1	0[a]	20
2	39	36	18	18	17	15	3	4	2[a]	23
3	45	37	18	16	15	11	4	1	7[b]	21
4	40	45	17	19	11	16	2	4	12	14
5	41	39	15	19	11	15	3	3	12	17
6	40	35	14	19	15	17	2	3	12	13
7	43	34	15	18	15	13	5	2	10	9
8	37	36	13	15	13	13	3	4	15	5

[a]$p < 0.001$.
[b]$p < 0.01$.

Table 12. Mean number of early deaths per pregnancy.

Week	Group 1 (pooled negative control)	Group 2, VDC, 10 ppm (6 hr × 5)	Group 3, VDC, 30 ppm (6 hr × 5)	Group 4, VDC, 50 ppm (6 hr × 5)	Group 5, CTX, 200 mg/kg IP
0	0.59	0.80	0.84	2.00	1.06
1	0.72	0.72	0.61	0.29	4.00[a]
2	0.69	0.75	0.56	0.71	4.04[a]
3	0.76	0.68	0.46	0.80	2.32[a]
4	0.79	0.83	1.00	1.00	0.85
5	0.78	0.68	0.73	0.67	0.86
6	0.88	0.91	0.94	1.00	0.64
7	0.65	0.85	0.57	0.57	0.74
8	0.85	0.75	0.85	0.71	0.30

[a]$p < 0.001$.

Table 13. Early deaths as a percentage of total implants per pregnant female.

Week	Group 1 (pooled negative control)	Group 2, VDC, 10 ppm (6 hr × 5)	Group 3, VDC, 30 ppm (6 hr × 5)	Group 4, VDC, 50 ppm (6 hr × 5)	Group 5, CTX, 200 mg/kg IP
0	4.8	10.1	7.3	17.3	9.1
1	5.7	5.3	5.1	2.2	45.6[a]
2	5.5	6.9	4.4	5.7	41.4[a]
3	6.4	6.3	3.6	5.3	21.8[a]
4	6.9	7.1	7.4	10.3	6.9
5	6.6	6.1	6.2	5.6	6.9
6	7.0	7.6	8.3	7.5	5.6
7	4.9	6.6	4.5	4.3	6.1
8	7.0	5.4	7.1	5.8	2.4

[a]$p < 0.001$.

Fertility

Successful Mating Frequency. The numbers of males successfully mating at each week are shown in Tables 2 and 8. Numbers remained high during the experiments. No statistically significant differences in the mating frequency were found between VC treatment groups and the control at any week by using a chi-squared test. There was, however, a significant difference between the EMS-treated group and the negative control group in week 1. In the VDC study, the mating frequency was high in the two groups exposed to the lowest doses of VDC in all weeks by comparison with the negative control group. The mating frequency, however, was statistically significantly lower in the high exposure group in weeks 0–8 and the positive control group in the weeks 0, 1, and 7. This effect in the VDC highest exposure group was, however, probably due to infertility of the males used.

Pregnancy Frequency. The numbers of females in each group which became pregnant at each week of mating are shown in Tables 3 and 9. In the VC study, statistical differences between treated groups and the negative control group were found only in the EMS-treated group at weeks 1 and

2 by using a chi-square test. In the VDC study there were significant differences in the highest VDC exposure group at weeks 0–6 and the positive control group in weeks 0, 1, and 7. Again this was probably due to infertility of the males.

These results indicate that VC at the three exposure levels and VDC at least at exposures of 10 and 30 ppm did not cause a reduction in fertility. Any reduced fertility at 50 ppm VDC was unproven.

Total Implantations

The mean total number of implants per pregnant female in each group is shown in Tables 4 and 10. The mean values were adjusted to take account of the unequal number of pregnant females per male and were compared statistically by using an analysis of variance and a *t*-test. In the VC study statistically significant differences were evident in week 1 in the CTX-treated group and weeks 1 and 2 in the EMS-treated group. A significant difference ($p < 0.05$) in week 4 was also found between the group exposed to the highest dose of VC (Group 4) and the negative control group. In the VDC study the CTX positive control group was statistically significantly different from the negative control groups in weeks 1, 2, 3, 6, and 7. Only the VDC group to be exposed to 10 ppm showed a significant difference from the negative control group in the pre-experimental nontreatment week.

Thus there was no indication of a preimplantation loss of eggs in either study except in week 4 after 30,000 ppm of VC.

Early Deaths

The data for early deaths have been presented in various ways.

Number of Pregnant Females with One or More Early Deaths. In the VC study, CTX and EMS treatment caused increases in the number of pregnancies with early deaths (Table 5). The effect was significant (chi-square) in weeks 1 and 2 for the CTX-treated group and in week 2 for the EMS-treated group. No differences from the negative control group were seen in the VC-treated group. Similarly in the VDC study there were only significant differences in the CTX positive control group in weeks 1, 2, and 3 (Table 11).

The Mean Number of Early Deaths per Pregnancy. A large number of low or zero values were encountered, so it was necessary to stabilize the variance prior to analysis. There were no statistically significant increases in early deaths after VC treatment (Table 6). However, differences were evident in weeks 1 and 2 with CTX treatment and with EMS treatment. In the VDC study only the CTX positive control group was significantly different from the negative control group in weeks 1, 2, and 3 (Table 12).

Early Deaths as a Percentage of Total Implants per Pregnant Female. Again, it was necessary to stabilize the variance prior to analysis. In the VC study CTX and EMS treatment groups were significantly different from the negative control groups in weeks 1 and 2, whereas VC-treated groups were not (Table 7). The CTX treatment group also showed a significant difference in the week before treatment. In the VDC study only the CTX treatment group was significantly different from the negative control group in weeks 1, 2, and 3 (Table 13).

Thus with all these different methods of analysis of the data of early deaths no statistically significant differences from the negative control groups were seen in the VC or VDC treated groups.

Late Deaths

In each study late deaths were randomly distributed throughout all the groups and did not appear to be treatment-related.

Conjoined Placentae

Conjoined placentae were seen in this strain. They were the result of very close implantation sites and were not monozygotic twins (*12*). In the experiments they were classified as double implantations. Since they occurred with equal frequency in all groups they did not appear to be correlated with treatment.

Discussion

The best indication of mutagenic activity of a substance in the dominant lethal test is an increase in the number of post-implantational foetal early deaths (*13*). From the data for early deaths there was no evidence in either study of a mutagenic effect with VC or VDC at the administered exposure levels. This did not appear to be a result of lack of sensitivity of the animals used, since there was a marked response to CTX and EMS. High doses of CTX and EMS were used in our studies to obtain a highly significant positive result. However, the dominant lethal study in our hands (*14*) is at least as sensitive as that reported elsewhere. We have shown ethyl methane sulfonate on previous occasions to give a positive result with a single IP dose of 150 mg/kg body weight, which is comparable to that reported previously (*15*) for the same mouse strain.

For reasons described earlier (*10, 11*), different evaluation methods were used for the early deaths' data and much of the data in the studies were subjected to various methods of statistical analysis.

Pre-implantation egg losses, while representing some of the mutagenic effect, are not as important as postimplantational losses, for they could also arise due to other than genetic factors (*13*). Pre-implantational egg losses have been studied by comparing values of total implants in females mated with treated males and those mated with control males, as suggested by Epstein (*16*), rather than counting corpora lutea. There was no pre-implantational egg loss by comparison of VC- or VDC-treated groups with the negative control group, except in the highest VC exposure group in week 4 ($p < 0.05$). When a treatment group is significantly different from a negative control group there is generally a uniform reduction in implants, whereas in week 4 after VC treatment the low result was due to a large extent to the result from one female. Without this female, mean values would not have been significantly different from the negative control group. Therefore, this result is not considered biologically significant. Yet another reason is that there was no corresponding increase in early deaths at this time.

Late deaths also are not considered as important as early deaths in the assessment of the mutagenic potential of a test substance (*13*). Late deaths were excluded from analysis to increase test sensitivity. Gropp and Kolbus (*17*) have shown, however, that trisomies in mouse foetuses cause death and elimination before term. It might be argued that sampling for late deaths at a later stage of pregnancy might lead to different results for late deaths. However, the randomness of their distribution in both studies would suggest otherwise.

There was no reduction in fertility as measured by the mating and pregnancy frequency at any week at the exposure levels of VC, suggesting no antifertility effect. The same was true for the VDC groups exposed at 10 and 30 ppm. However, the decreased fertility in the group exposed to 50 ppm was probably due to the infertility of the males which had to be used to establish a group of sufficient size to perform the experiment.

A fertility study was undertaken prior to the experiment also for reasons described earlier (*10, 11*). From these initial fertility data the background dominant lethality of all groups was determined primarily to ascertain that there were no initial differences between groups.

Mutagenic effects of vinyl chloride have been observed in laboratory tests and in exposed workers. In this study where exceptionally high doses of VC were used, which would never be encountered by workers, no mutational effect in the germ cells was observed. A possible explanation is that the active metabolites did not reach the germ cells. Similarly the lower but more toxic levels of VDC did not produce germ cells mutations.

It can be concluded, therefore, that VC and VDC do not cause dominant lethal mutations in male CD-1 mice at 3000, 10,000, and 30,000 ppm and 10, 30, and 50 ppm, respectively.

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Risks to the Offspring from Parental Occupational Exposures

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Risks to the offspring of workers with occupational chemical exposures may derive from mutagenic, teratogenic or carcinogenic effects of industrial agents to which the parents are exposed. Evidence for impaired pregnancies and hazards to the offspring of working populations with chemical exposures is, however, very limited. Perhaps the best documented example is increased spontaneous abortion rates in female operating room personnel who have first trimester exposure to waste anesthetic gases. Evidence is reviewed for hazards to the offspring resulting from parental occupational exposure to vinyl chloride, benzene, chloroprene, radiation and petroleum-derived hydrocarbons. It is essential in investigating the role of occupational factors that other environmental and behavioral factors with major effects on pregnancy outcome be accounted for. These include smoking, alcohol, and drug exposures. An approach to surveillance for chromosomal abnormalities in offspring of occupationally exposed parents is outlined.

Derived from the Greek word "repas," meaning a marvel, prodigy or monster, the word "teratogenic" was used by 1857 to mean the "production of monstrous formations or births."[1] The term has been refined to refer to the biological science dealing with "the causes, mechanisms, and manifestations of developmental deviations of either structural or functional nature."[2] An agent may act as a teratogen when administered in a number of ways, whether to the male or female before mating, to the female during pregnancy, or to the fetus directly.[3] Agents in the environment may produce alterations in the genome, mutations, and by that mechanism lead to abnormalities of development in the offspring. Viral, drug or chemical agents acting through common pathways may produce indistinguishable results. The outcome of such mutations may theoretically be fetal defects or predisposition to neoplasia. Agents which alter the rate of growth of the fetus or are lethal to the fetus without producing specific anatomic or functional anomalies are better termed developmental toxins than teratogens.

Cancer in the offspring of individuals exposed to a carcinogen may theoretically be the result of any of three types of exposure: prezygotic, by alteration of parental germ cells; transplacental, by passage of carcinogenic substances across the placental barrier; or postnatal, by contamination of the environment with carcinogenic substances, primarily through ingestion (including substances excreted in human breast milk) and through inhalation.

Evidence for risks to the offspring of humans occupationally exposed to potentially hazardous substances is reviewed in the present work. The term trans-generational carcinogenesis is used to describe the occurrence of cancers in the offspring which can be attributed to parental exposures. This term is proposed to encompass not only the established process of transplacental carcinogenesis, by which a substance administered to the mother during pregnancy results in cancer in the offspring, but also the controversial issue of preconception alterations in genetic matter resulting in increased teratogenic or cancer risk in the offspring. The latter effect could theoretically result from each of three distinct mechanisms: chromosome breakage, point mutation, or abnormalities in gametogenesis or fertilization.

Mutagenic and Teratogenic Characteristics of an Agent and Trans-Generational Carcinogenesis

Mutagenesis, teratogenesis and carcinogenesis are related phenomena, but the nature of their relationship is complex and occasionally controversial. It has been argued that the "same chemical that causes abortion in the early stages may produce malformations during organ development and neoplasia when exposed later in pregnancy. Therefore, screening for transplacental hazards should include, whenever possible, the entire range of fetal response, including cancers that may develop sometime after birth."[4] Teratogenic, mutagenic and carcinogenic activities are all demonstrable for a number of com-

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pounds, but transplacental carcinogenic potential has rarely been demonstrated for known teratogens and mutagens. Teratogenic effects may reflect a variety of actions involving the mother, the placenta or the feto-placental unit. These effects can be indirect and may not involve immediate action of the agent on the target fetal tissue.[5] Transplacental carcinogens may act directly on the fetal tissues which are inherently more vulnerable because of the high rate of cell division, high proportion of undifferentiated cells and immaturity of immunosurveillance mechanisms.

To appreciate potential danger from an environmental exposure to offspring of the exposed organism, the outcome of all exposed pregnancies should be considered. Early fetal wastage commonly results from abnormal fetal development. At the other extreme, transplacentally-induced tumors hardly ever appear in rodent species until the animal reaches maturity. In the only documented example of chemical transplacental carcinogenesis in humans, vaginal adenocarcinoma following diethylstilbestrol (DES) exposure, the tumor occurs decades following exposure. Thus, since transplacentally-induced tumors, unlike malformations, are rarely present at birth, one must also observe the offspring into adulthood to see the full spectrum of resulting tumors.

Environmental Threats to Pregnancy Outcome

Recognized threats to pregnancy outcome are not uncommonly encountered in the environment and must be excluded when new problems are suspected. Threats are posed by biologic, chemical and physical agents, and may derive from medical therapeutic interventions, drug and substance abuse, and environmental or occupational exposures to chemical agents.

Alcohol.—The most widespread documented threats to the fetus come not from the maternal physical environment but from the use of tobacco and alcohol during pregnancy. Only recently has the risk of alcohol ingestion in pregnancy been evaluated and the concept of the fetal alcohol syndrome refined.[7] Of infants born to recognized chronic alcoholic mothers, 83.3% had birth weights under the tenth percentile, compared with 2.3% in a comparable non-alcoholic population.[8] In addition to intrauterine growth retardation, the infants had retarded post-natal growth and intellectual development. A characteristic facies, with short palpebral fissures, hypoplastic philtrum, thin vermilion line of the upper lip and retrognathia, has also been described.

In addition to these features of the fetal alcohol syndrome, congenital malformations of various types occur with excess frequency. Recognition of an associated increase in the occurrence of congenital malformations ironically re-emphasizes ancient observations and admonitions against alcohol use during pregnancy.[9] The adverse effects on the fetus of maternal alcohol abuse during pregnancy were further quantified by Ouellette et al,[10] who classified women at the first prenatal visit into four categories according to alcohol consumption and independently evaluated pregnancy outcome. Compared to those born to abstinent or moderate drinkers, infants born to heavy drinkers had twice the risk of having an abnormality at birth. The frequency of congenital abnormalities in the offspring of heavy drinkers was extremely high—32% when minor abnormalities were included, 17% if only major anomalies were considered. Multiple abnormalities were present in 20% of the offspring of heavy alcohol users. Major, minor and multiple abnormalities occurred significantly less often among abstinent or moderate drinkers. Animal models, including chicks, rats and guinea pigs, have been developed for evaluating the effects of ethanol alone on offspring, and support the view that ethanol itself is a harmful agent, and that its teratogenic potential is common to several species.[11]

Smoking.—Another factor influencing pregnancy outcome, maternal smoking habits, should be addressed in efforts to tie environmental exposures of the parents to the survival and integrity of the fetus. Kline et al[12] suggest that the odds of spontaneous abortion among women who smoke are 1.8 times those of nonsmokers. This association remains statistically significant after maternal age, and number and outcome of previous pregnancies are taken into account.

The manner in which smoking affects fetal survival is controversial. Smoking results in lowered birth weight, which may or may not be related to the increased frequency of spontaneous abortion.[13,14] Lowered birth weight may result from impaired maternal nutrition or fetal anoxia, neither of which produces an excess of chromosomal abnormalities in the conceptus. Alternate explanations for an association between smoking and spontaneous abortion have been proposed. Since spontaneous abortion serves as a means of selectively terminating abnormal conceptions and since 95% of abnormal pregnancies are believed to terminate this way, an association between an environmental factor and increased spontaneous abortion should always evoke suspicion of a teratogenic phenomenon.[15] Smoking increases the risk of spontaneous abortions by a factor of 1.8, yet the percentage of abortuses which are chromosomally abnormal is close to that expected. The absolute risk of a chromosomally abnormal fetus appears, therefore, to be higher in smoking mothers.

The relationship of karyotypic abnormalities in spontaneously aborted products of conception to maternal smoking habits is complicated by variation in the proportion of chromosomally abnormal fetuses with increasing maternal age and by the fact that a large proportion of smokers are younger women.[16] An increase in the rate of chromosomally abnormal conceptions might be masked in a proportionate ratio analysis if there was also an increased loss of conceptions without demonstrable cytogenetic abnormalities.

Evidence for an increase in congenital malformations in children of smoking mothers is limited and conflicting. In a cohort of births occurring in the first week of March, 1958, in England, Scotland and Wales, Fredrick and colleagues[17] identified a 60% increased risk of congenital heart disease among children of women who had smoked at least one cigarette per day after the fourth month of pregnancy (7.3 vs. 4.7 cases per 1000 live births). This effect remained statistically significant after adjustment for maternal age, parity and social class. An investigation of congenital defects of all systems registered in South Wales from 1964 to 1966 which took into account maternal age, parity, social class, area of residence and date of delivery did not associate maternal smoking habits with

congenital malformations in any system.[14] It is possible that moderate increases in relative risk might have gone undetected.

Analysis of data from a mailed survey of a large number of professional women in medicine identified a relative risk of up to 1.7 for spontaneous abortion and a risk of congenital abnormalities of up to 2.3 in heavy smokers after the effects of age, parity and exposure to anesthetic gases were considered.[15]

Drug Exposure.—In utero exposure to diethylstilbestrol (DES), with the subsequent development of vaginal adenocarcinoma, is the prototype for transplacental oncogenesis in humans. Appreciation of the causal role played by in utero exposure to DES followed hard upon the report of seven cases of the hithertofore exceedingly rare tumor, adenocarcinoma of the vagina, presenting in young individuals, 14 to 22 years of age.[20,21] The syndrome appears to involve not only cases of vaginal adenocarcinoma, but a spectrum of abnormalities of the vagina and cervix. Only when exposure to DES occurred during the first four months of gestation were neoplasms or abnormalities observed.

The DES vaginal adenosis-adenocarcinoma relationship is the only documented example in humans of cancer in the offspring attributable to parental environmental chemical exposure. The therapeutic agent was given in a high dose, commonly more than 10 grams in the first half of pregnancy. DES, a synthetic nonsteroidal estrogen analogue, was likely to have a direct effect on the development of the target tissue at a crucial point in embryogenesis. Since the risk to the offspring appears limited to exposures which occur during the first four months of gestation, direct exposure of the fetus is likely to be essential to the pathogenic mechanism. The long latent period, with exposure in utero and emergence of clinical sequelae in adolescence, probably reflects the importance of pubertal endogenous estrogens as promoting factors, and is a reminder of the need for an extended period of observation following suspect trans-generational carcinogenic exposures. The association between DES and vaginal adenocarcinomas, as important as it is, does not serve as a model for preconception parental occupational exposures and trans-generational carcinogenesis. Teratogenic effects of a multiplicity of drugs are now documented or suspected and these will not be discussed here further except to reiterate the need to remove the potentially confounding effects of such agents in investigations of other possible causes of impaired fetal development.[24]

Given the current status of our understanding, it is essential that in the search for exogenous causes of teratogenesis and abnormal pregnancy outcomes, the potential confounding impacts of maternal smoking, drinking, and medication intake be taken into account.

Radiation.—If preconception radiation exposure increases cancer risk in the offspring, it would heighten concern over other agents which produce chromosomal damage. Chromosomal aberrations attributed to vinyl chloride and benzene exposure, for example, are reminiscent of those produced by radiation.

While the genetic consequences of irradiation from the atomic bombings might be expected to produce some lethal mutations in the offspring of survivors, this effect has been difficult to demonstrate. Cohort studies of offspring of survivors classified by radiation exposure level did not show excess mortality in the children of the high exposure group. Neither were excess congenital malformations, increased infant mortality, nor impaired survival during the first ten years of life detected, regardless of exposure level to either parent. Thus, although animal experience strongly suggests that such effects should be manifest in offspring of individuals exposed to ionizing irradiation, studies of Japanese atomic bomb survivors have not demonstrated measurable effects.[25]

No substantial increase in leukemia risk has been detected among offspring of survivors. Analyses having a 90% chance of detecting a four-fold increase in risk of leukemia among children of heavily exposed survivors were negative. Neither did age-at-onset of observed leukemia cases nor type of leukemia in offspring differ by level of parental radiation exposure. Despite the documented persistence of chromosomal aberrations in the somatic cells of adults exposed to the atomic bomb, no measurable impact on mortality, congenital malformations or leukemogenesis has been detected in their offspring.[26]

In addition to radiation exposures related to the atomic bombings, diagnostic and therapeutic medical radiation of the parents has been investigated to assess risks to the offspring. The magnitude of leukemia risk associated with previous radiation was estimated, after adjustment for maternal age and pregnancy history, to be 1.73 times that of mothers who had not been x-rayed. Despite the suggestion of greater risk in women with higher x-ray exposure, no clear-cut dose-response gradient was demonstrated. The relative risk of leukemia in the child associated with preconception diagnostic radiation of the father (1.31) was not statistically different from unity. When both mother and father had histories of preconception diagnostic x-rays, the order of magnitude of the relative risk, 1.49, was about the same as that for children whose mothers alone had received radiation.[27]

Exposure to medical radiation has been widespread. By the early 1960's, approximately 35% of a group of mothers of healthy children reported having received diagnostic radiation prior to conceiving. Similarly, about 22% of fathers of the same children reported having had some sort of diagnostic radiation at some time prior to the child's conception. Self-reporting considerably underestimates the extent of x-ray exposures.[28] The tendency to substantially underreport x-ray exposure points up the difficulty of excluding differences in prior radiation exposure as a basis for observed differences in chromosomal aberrations. Neither is it reasonable to automatically presume that medical radiation exposures have been equally present in the study and comparison groups, especially if these groups have not been matched by age or calendar period of observations.

Occupational Exposures and Pregnancy Outcome

Vinyl Chloride.—Chromosomal abnormalities may occur more often than expected in persons exposed to vinyl chloride, especially following intense exposures of long duration. The import of such abnormalities for reproductive outcome remains ill-defined.

Chromosomal aberrations were reported in 1975 in

Swedish vinyl chloride workers. Abnormalities appeared in cells drawn from seven males with occupational exposure histories of nine to 29 years (mean 16.6 years) at vinyl chloride concentrations which had declined to 20-30 ppm by 1974.[9] The group exposed to vinyl chloride had aberrations in a total of 9.52% of all cells, compared to 1.94% for cells of three unexposed controls. Differences in the frequency of chromatid and isochromatid breaks accounted for most of the disparity. Considerable variation was exhibited, with cells considered abnormal ranging from 1.5% to 18% in the exposed workers. There was no apparent relationship between duration of exposure to vinyl chloride and the proportion of cells which were abnormal. Age, radiation exposure or other environmental factors were not evaluated, nor was it clear on what basis individuals had been selected for study.

In New York State, chromosomal aberrations were reported in somatic cells of 11 men who had worked in a polyvinyl chloride polymerization plant and who were believed to have experienced intense intermittent exposures to vinyl chloride in concentrations of over 500 ppm, as well as chronic, but unquantitated exposure for four to 28 years (mean, 15 years). Control samples were drawn from four males in the same factory who were not known to have had vinyl chloride exposure, and from six males from outside the factory. Complex chromosomal aberrations (rings, dicentrics, fragments) occurred significantly more frequently in the exposed than in the control group, a result which was properly interpreted with caution. The authors point out the absence of age-matched controls and the substantial age difference between study group and controls. The frequency of chromosome breaks and gaps was unexpectedly high in both control and study groups.[10] No relationship could be defined between dose or duration of exposure and frequency of chromosomal aberrations.

Investigators reporting from the United Kingdom studied 56 men who had had chronic exposure to vinyl chloride monomer in the course of manufacturing polyvinyl chloride and compared them with 24 unexposed individuals.[11] Vinyl chloride exposure of men employed in its manufacture could not be easily quantitated. Individuals with exposures to radiation or with recent viral infections or prolonged drug treatment were excluded. Samples were coded and read blindly. A higher proportion of cells with chromosomal aberrations, the majority of which were breakages, was found in vinyl chloride-exposed workers than in controls. Unstable and stable chromosomal aberrations and breaks were all significantly more common in cells from exposed workers.

Not all investigators have identified such changes. No differences were found in frequency of chromosomal aberrations between a group of 209 employees of a vinyl chloride plant (occupational exposures averaging 48.3 months) and a group of 295 individuals undergoing preemployment physicals.[12] No relationship was established between duration of vinyl chloride exposure and degree of chromosomal damage. The absence of blind evaluation in this study, as well as inclusion of individuals with minimal exposures in the exposed group, makes interpretation difficult. This difficulty is compounded by the substantial difference in mean age of study and control group members.

Pregnancy outcome in the wives of men exposed to vinyl chloride monomer (VCM) has been said to be less favorable than that experienced by wives of a group of polymerization and polyvinyl chloride (PVC) fabrication workers.[13,14] One comparison group, the polymerization workers, was believed not to have been exposed to VCM, wheras PVC workers had low VCM exposure. Data on pregnancies and pregnancy outcomes in the wives were derived from interviews and questionnaires administered to male workers. Participation rates for the groups queried ranged from 62% to 77%. Fetal death was defined as any known conception which did not result in a live birth and rates were adjusted by paternal age. Maternal age was not known, but was presumed to correlate closely with paternal age. Analysis of questionnaire responses suggested that the number of fetal deaths per 100 conceptions was higher in the VCM-exposed group than in the comparison group. This difference was reported only for the period following vinyl chloride exposure. Adjustments removing women who were chronic aborters eliminated statistically significant differences. While the authors felt that these observations were likely to reflect a real difference in pregnancy outcome not attributable to either interviewer or patient recall bias, the conclusions were based on indirect sources of information and could not take into account the multiplicity of maternal factors known to affect pregnancy outcome. The study design precluded documenting in even the crudest manner the validity of pregnancy histories. Without such adjustments and validation, the inferences made by Infante and colleagues[13,14] cannot be sustained and little light is shed on the possible association of abnormal pregnancy outcome with paternal occupational exposure to VCM.

Studies of pregnancy outcome (i.e., spontaneous abortion, late fetal death [stillbirth], low birth weight, neonatal death) in other settings have shown the profound and subtle effects of confounding variables such as race, socioeconomic status, maternal age, birth order, parity, smoking and alcohol exposure during pregnancy, maternal infections, and previous pregnancy outcomes. These effects may readily reverse the direction of the relationship between a suspect antecedent factor and the outcome of pregnancy.[15] Future efforts to document a relationship between impaired pregnancy outcome and occupational exposures of fathers must validate information on pregnancies and take into account known confounding factors.

Chloroprene.—Structural similarities between vinyl chloride and chloroprene have raised questions about long-term hazards resulting from chloroprene exposure. Chloroprene is mutagenic in certain systems and possibly carcinogenic. The possibility that it has induced excessive miscarriages in the wives of male workers and led to chromosomal aberrations has been asserted but not well documented.[16]

Benzene. — Benzene also may cause chromosomal aberrations following heavy occupational exposures. Twenty males working in a factory in which benzene had been used as a solvent were studied for chromosomal aberrations. Members of this group had one to 20 years of benzene exposure; 14 were known to have previously been neutropenic. Chromosomal abnormalities were reported in 2.5% of all cells from exposed workers, com-

pared to 1.0 to 1.4% for controls. Most of the excess was due to chromosomal aberrations of the unstable types. In addition to the excess of abnormal cells in the exposed group, the number of unstable alterations per abnormal cell was higher in the exposed group than in the controls.[37]

In a separate investigation, ten workers in an Italian rotogravure plant who had had sequential exposures, first to benzene and later to toluene and xylene, were studied. These workers had been subjected to concentrations of benzene ranging from 125 ppm to 532 ppm or higher. The maximal allowable concentration at the time of these exposures had been 25 ppm. Another group of 24 workers had primarily toluene exposures except for trace contamination with xylene. These 34 workers were matched with healthy controls of similar age and sex, who had been drawn from the general population and who had no history of benzene or radiation exposure. A significantly higher proportion of abnormal cells was found in the benzene-exposed individuals than in their matched controls. In the group with toluene exposure, no such excess was present. When the benzene and toluene groups were compared to each other, an excess of chromosome changes was present in the benzene group. Most of the excess was attributable to unstable chromosomal alterations.[38]

These studies and others[39] support the impression that exposure to benzene, particularly exposures intense enough to result in acute toxicity, can be followed by a measurable excess of chromosomal aberrations which may persist for years. If similar effects occur in germ cells of exposed individuals, the consequences in fetal wastage, congenital anomalies and even neoplasms might be manifest in their progeny. To date no excess of such abnormalities has been reported in offspring of benzene-exposed individuals.

Anesthetic Gases. — Occupational exposures of female operating room personnel to waste anesthetic gases have been held responsible for decreased fertility, increased rate of spontaneous abortion, low birth weight and possibly impaired development in the offspring. An excess of spontaneous abortions in wives of men professionally exposed to anesthetic gases has been suggested but not confirmed.

Among operating room nurses, 29.7% of pregnancies terminated with spontaneous abortion, compared to 8.8% among control nurses. A similar excess of spontaneous abortion was observed for female physician anesthesiologists compared to female physicians in other specialties.[40] Spontaneous abortions occurred two weeks earlier on the average among the operating room exposure groups than among controls, at an average of eight rather than ten weeks of gestation. Another survey suggested that for female nurse-anesthetists who had worked during pregnancy, the frequency of birth defects in the offspring was 16.4% compared to 5.7% for pregnancies in which the mother had not worked.[41]

Studies from Finland and the United Kingdom also suggested a deleterious effect on pregnancy outcome from maternal exposure to operating room environments. An increase in frequency of early spontaneous abortions, as well as low birth weight, has been reported for Finnish female operating room staff.[42] Among female physicians in England and Wales, greater incidence of congenital defects, lower birth weights and higher stillbirth rates, but not higher spontaneous abortion rates, were reported from pregnancies of women holding anesthesiology appointments than from those of other women physicians.[43]

The effects of paternal exposure to anesthetic gases are uncertain. Congenital anomalies were reported to be increased by 25% in the offspring of male anesthesiologists compared to offspring of male members of the American Academy of Pediatrics, but no difference was reported for spontaneous abortion rates among wives of male operating room personnel compared to controls.[44] Questionnaires completed by 5119 married male physicians in the United Kingdom suggested an increase in minor but not in major congenital anomalies, and no difference in frequency of infertility, spontaneous abortion or cancer in the offspring of male anesthesiologists compared to control physicians.[45]

Hydrocarbons. — Two studies offer conflicting evidence with respect to the risk of cancer in children of men whose work might lead them to be exposed to petroleum-derived hydrocarbons. Fabia and Thuy[46] reviewed death certificates in Quebec during 1965 to 1970 to identify children who had died from malignant disease while under five years of age. For 386 of 402 such children, birth certificates were also found in the Quebec population register. A control group of 772 children (two for each cancer death) was selected using the birth registration record preceding and following that of each case in the official files. This effectively matched for season, calendar period, and province of birth. Occupation of the father at the time of birth was taken from the birth certificate. An industrial hygienist independently grouped the fathers into three levels of probable exposure to petroleum-derived hydrocarbons. Occupation of the father was unknown for 30 cases and 56 controls. The distribution of occupation of the father at birth differed for cases and controls. Most of this difference was the result of an excess among cases of paternal occupations considered to be hydrocarbon-related. The relative odds of cancer in the children of men holding hydrocarbon-related jobs at the time of the child's birth were 3.1, an increase which was unlikely to have occurred by chance. Most of the excess in exposed occupations was accounted for by motor vehicle mechanics, machinists, miners and painters. When similar analyses were conducted by the type of cancer in the child, the excess of hydrocarbon-related occupations prevailed for the following groupings: leukemia-lymphoma, nervous system malignancy, other tumors. Fathers of four of five cases of Letterer-Siwe disease were in the hydrocarbon-related work groups. No such excess was present for children with Wilms' tumor. The differences observed could not be attributed to differences in parental age or in geographic residence at the time of birth. The investigation was based on deaths in children under five, and it is possible that social class bias may have been introduced in that fashion. If, of those with a childhood neoplasm, children of more affluent parents are more likely than children of poorer parents to survive beyond age five, they would not be included in the study group. This would lead to a higher proportion of children with parents of lower socio-economic status in

the cancer death group and would bias the distribution of father's occupation. Moreover, it was not possible to confirm that fathers in the so-called "exposed" group did, in fact, have contact with hydrocarbon-derived chemicals. While not ideal, the death certificate probably is a valid source of diagnostic information for cancer deaths in young children. The cancer groupings employed in the analysis – "leukemias and lymphomas," "nervous system" and "others" – were quite broad and included many histopathologic entities. Occupational association was not restricted to specific entities within the broader categories. Because the epidemiologic features of the various histopathologic types of leukemias, lymphomas, and nervous system tumors are distinctive, a specific chemical exposure or class of exposures, if assumed to be of causal significance, would more likely be linked with specific histopathologic as well as organ system effects.

A study with a similar design but different findings was conducted in Finland.[47] Children under 15 years of age who developed cancer were identified from the Finnish Cancer Registry, 1959-1968. Of 1409 cancer cases so identified, the final series consisted of 852 pairs for whom birth records which included father's occupation were available. Each case was matched with a child whose birth date immediately preceded that of the case and who was born in the same maternity welfare district. This procedure matched effectively by calendar period, season and domicile at the time of birth. Father's occupation was drawn not from birth registration records but from records of the free, nationwide antenatal care system in operation in Finland. Father's occupation was classified by likelihood of hydrocarbon exposure in a manner which was shown to be comparable with that employed in the study done in Quebec. No excess risk for hydrocarbon-exposed fathers was detected for any class of neoplasms either for children whose cancers occurred under five or under 15 years of age.

The two studies differed primarily in the source of information on father's occupation. For the Finnish study, that information was drawn from antenatal records compiled for the most part in the first trimester of gestation. This may be a better index of exposure at the time of conception than information recorded at the time of birth in the birth certificate itself. Although a large number of pairs were discarded in Finland because father's occupation was not recorded, these came from a circumscribed calendar period. Since a matched pairs design was maintained throughout the analysis, removal of these cases should not influence the result. The Finnish study used incident cancer cases and looked at age groups up to 15 years at the time of cancer diagnosis. The Quebec group identified only cancer deaths up to five years of age. Variation in terminology of occupational classification might also contribute to the differences, although efforts were made to establish their comparability. At present, the contradictory findings of the two studies are not readily reconciled.

Surveillance of Spontaneous Abortions as a Strategy of Environmental Monitoring

The relationship between mutagenic, teratogenic and carcinogenic effects in the offspring of persons exposed to noxious environmental agents is a complex one. Surveillance of spontaneous abortions may have a number of advantages as a prospective means of monitoring for such effects since defective conceptions are aborted selectively. As a result, studies of teratogenesis focusing on spontaneous abortions may be considerably more efficient than those conducted in newborns. Because the frequency of abnormalities is higher in spontaneous abortions, the sample size needed to demonstrate a change in risk is much smaller than that required for a parallel query addressed to defects recognized at birth. The magnitude of this difference can be dramatic. If chromosomal defects diagnosable on the appearance of the newborn are considered alone, the sample size required may be hundreds of times that required in an investigation examining prevalence of chromosomal anomalies in early abortion.[48] In addition to sample size considerations, study of spontaneously aborted conceptions offers a lead time of at least six months over studies of live births. The abortion specimen can be studied with care and thoroughness, permitting detection of anomalies lethal to the fetus which might escape detection in studies of live births.

The problem of power and sample size is only one of the issues which beleaguer investigators of associations between exposures to parents and outcomes of pregnancy. Related factors are the timing of the exposure, maternal or paternal, in relation to conception and/or gestation, and the specific measures of pregnancy outcome.[49] Paternal exposures may act in two ways – by contamination of the maternal environment resulting in secondary maternal exposures, or directly by affecting paternal germinal tissue. Since spermatogenesis is a continuous process, paternal exposures occurring shortly before conception are those requiring the most investigative attention. Cumulative or delayed effects are more likely to be of importance in maternal exposures. By focusing on early pregnancy wastage, particularly if the frequency of chromosomal abnormalities in the aborted product of conceptions can be determined, the objectives of study are better focused and achieved with reduced sample size, and the confounding effects of the maternal in utero environmental factors are minimized.

In conclusion, while the potential clearly exists for teratogenesis and trans-generational carcinogenesis in the offspring of workers exposed to mutagenic and carcinogenic agents, such effects have been difficult to demonstrate conclusively in humans. Future investigations must take into account a variety of environmental and behavioral factors which can affect fetal development if causal associations between occupational exposures and pregnancy outcome are to be identified.

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CARCINOGENIC, MUTAGENIC AND TERATOGENIC RISKS ASSOCIATED WITH VINYL CHLORIDE

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Summary

The data presented demonstrate clearly that vinyl chloride (VC) is related to a significant excess of mortality from cancer of the liver, lung and brain among workers occupationally exposed to VC. The risk of dying from cancer of the lymphatic and hematopoietic system also appears to increase with an increase in latency. These cancer sites could have been predicted by the animal bioassay conducted by Maltoni. With regard to the liver, even the histopathologic type of cancer (angiosarcoma) was observed first in experimental animals. A study of cancer mortality among populations residing proximate to VC polymerization facilities also demonstrated an increased risk of dying from CNS and lymphatic cancer. These latter findings raise cause for concern about out-plant emissions of VC, but without further study these cancers obviously cannot be interpreted as being related to out-plant exposure to VC.

Various test systems now have elicited a positive mutagenic response to VC. Thus, our observations of a significant excess of fetal mortality among the wives of males, who were occupationally exposed to VC, raise public health concern that VC may be mutagenic in humas.

With regard to the teratogenicity of VC, observations of a significant excess of children born with birth defects were reported among populations residing proximate to VC polymerization facilities. Additional epidemiologic study is needed to determine whether a repeated pattern of excessive numbers of children born with birth defects can be observed in other communities with VC polymerization facilities.

Introduction

In 1930, the first adverse health effects of vinyl chloride (VC) were reported [22]. Since then, numerous investigators have reported the toxic effects of VC on the central nervous system, the liver, the bones of the fingers and the lungs

[2,5,9—12,18.20,28]. More recently, the study of VC toxicity has broadened to assess the spectrum of carcinogenesis [2,15,16.21,26,29,30], mutagenesis [1,3,4,6,13,14,23,24]. and teratogenesis [8]. These efforts were stimulated by the work of Viola et al. [29] who, in 1971, reported the induction of tumors of the skin, lungs and bones in rats exposed by inhalation to 30,000 ppm of VC over a period of twelve months. Widespread concern for the carcinogenic activity of VC, however, did not occur until early 1974, when Creech and Johnson [2] reported four deaths from angiosarcoma of the liver among workers employed in the manufacture of polyvinyl chloride (PVC) resins. It was later learned that Maltoni had previously demonstrated VC-induced hepatic angiosarcomas, lung adenomas, brain neuroblastomas, lymphomas and various other tumors in mice, rats and hamsters [15,16].

With regard to mutagenicity, several investigators have induced mutations via microbial test systems [1,14,24]. Also, VC metabolites have induced mutations in mammalian cells [6]. In addition, reports from several countries have demonstrated significant excesses of chromosomal aberrations among workers exposed to VC as contrasted with those not exposed [3,4,13,23]. Because of these observations and of the widespread exposure to VC among both workers and the general population, the National Institute for Occupational Safety and Health (NIOSH) in the U.S.A. undertook an epidemiologic program to evaluate the magnitude and spectrum of VC toxicity to humans. This program was 4-fold in nature and sought to evaluate: (1) The site-specific risk of cancer among workers exposed to VC [30]. (2) The risk of some cancers among populations residing proximate to VC polymerization facilities [8]. (3) The site specific risk of congenital anomalies among populations residing proximate to VC polymerization facilities [8]. (4) The risk of fetal wastage among the wives of workers occupationally exposed to VC [7].

Cancer risk among workers exposed to VC

To assess the neoplastic risk of workers exposed to VC, a population consisting of employes from four VC polymerizing facilitates was selected for cohort mortality study [30]. Since occupationally induced cancers often take many years to become manifest, the study cohort was restricted to workers who had achieved five or more years of employment and for whom at least 10 years had lapsed since initial employment. Thus, the exposure period was five or more years and the latency period was 10 or more years. Follow-up for study cohort members was greater than 99%.

Table I shows the total mortality experience among the study cohort. The expected numbers are based on United States mortality data applied to NIOSH's modified life-table method. For all malignant neoplasms, there were 35 observed vs 23.5 expected, the SMR was 149. This excess was significant at the $P < 0.05$ level of confidence. For total mortality, 136 deaths were observed vs 126.3 expected. Selecting a cohort with at least five years work experience and 10 years latency eliminated the "healthy worker effect" [19] and so there were more total deaths than expected and this was mostly at the expense of cancer mortality.

Table II shows cancer mortality experience by greater than 10 and 15 year latency periods. At the greater than 10 year latency period, only biliary and

TABLE I

MORTALITY EXPERIE

Cause of death

All malignant neoplasms
Heart
Non-malignant respirator
Cirrhosis
All other causes

ICD, International Cla
SMR, standardized mo
Significant at $P < 0.05$

liver cancer death
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TABLE II

CANCER MORTALIT
OF VINYL CHLORIDE

Site of malignancy

All malignant neoplasm

Brain and CNS cancer

Respiratory system car

Biliary and liver cance

Lymphatic and hemat

All other malignant n

Observed.
Expected.
SMR, standardized r
Significant at $P < 0.$
Significant at $P < 0.$

TABLE I

MORTALITY EXPERIENCE AMONG COHORT WORKERS EXPOSED TO VINYL CHLORIDE

Cause of death	ICD code [a]	Observed	Expected	SMR [b]
All malignant neoplasms	(140—205)	35	23.5	149 [c]
Heart	(400—443)	57	54.7	104
Nonmalignant respiratory diseases	(470—527)	6	3.4	176
Cirrhosis	(581)	2	4.0	50
All other causes		36	40.7	88

[a] ICD, International Classification of Diseases, 7th revision.
[b] SMR, standardized mortality ratio.
[c] Significant at $P < 0.05$.

liver cancer deaths were significantly in excess; however, when the sub-cohort with 15 years of latency since initial employment was used, deaths from three categories of cancer were significantly in excess. The excess in mortality from cancer of the lymphatic and hematopoietic systems was not significant; however, the SMR increased from 159 to 176 with an increase in latency. These comparisons show the importance of latency when looking for occupationally-induced cancers.

TABLE II

CANCER MORTALITY BY INTERVAL SINCE INITIAL EXPOSURE AMONG WORKERS EXPOSED TO VINYL CHLORIDE

Site of malignancy		10+ years		15+ years
All malignant neoplasms	Obs. [a]	35		31
	Exp. [b]	23.5		16.9
	SMR [c]	149 [d]	<	184 [e]
Brain and CNS cancer	Obs.	3		3
	Exp.	0.9		0.6
	SMR	329	<	498 [d]
Respiratory system cancer	Obs.	12		11
	Exp.	7.7		5.7
	SMR	156	<	194 [d]
Biliary and liver cancer	Obs.	7		7
	Exp.	0.6		0.4
	SMR	1155 [e]	<	1606 [e]
Lymphatic and hematopoietic system cancer	Obs.	4		3
	Exp.	2.5		1.7
	SMR	159	<	176
All other malignant neoplasms	Obs.	9		7
	Exp.	11.7		8.4
	SMR	77	<	83

[a] Obs., observed.
[b] Exp., expected.
[c] SMR, standardized mortality ratio
[d] Significant at $P < 0.05$.
[e] Significant at $P < 0.01$.

In laboratory studies, Maltoni has induced hepatic angiosarcomas, lung adenomas, brain neuroblastomas and lymphomas [16]. Therefore, the predictive value of animal bioassay studies can be demonstrated, not only by the observation of a significant excess of angiosarcoma of the liver among VC workers, but also by significant excesses of lung and brain tumors.

Cancer risks in populations residing near VC polymerization facilities

The risk of some cancers among adult populations residing in the only three Ohio communities with VC polymerization facilities was assessed [8]. All three communities had at least one facility in operation by 1954 and Painesville had a second plant in operation by 1967. The community population sizes varied from 12,000–24,000. Between 1960–70 the population had remained stable in Painesville and Ashtabula, but had increased by 30% in Avon Lake. As reported previously, for the three communities taken as a whole, there were no apparent differences in racial origin or family income as compared to the average for the State [8].

As a result of previous findings [26], cancers of the central nervous system, lymphatic and hematopoietic systems, were selected for study. Because of possible error in death certificate data in terms of metastases from other primary sites, data for lung and liver cancers were not analyzed.

Table III shows data for observed versus expected CNS cancer deaths in the white population by sex for the period 1958–73. North Ridgeville is the only community shown which does not have a PVC polymerization facility. It was included in the analyses of the cancer data because it is located contiguous to Avon Lake and because it had a high incidence of children born with birth defects [8]. If North Ridgeville had not been included in the analyses of cancer mortality, little difference in the results would have been observed. Expected values are based on the occurrence in the balance of the counties over the same period of time. As shown in Table III, there was a significant excess of CNS cancer deaths in males. The excess was greatest in Painesville and North Ridgeville. With all communities combined, there were 27 CNS cancer deaths observed in males versus 14.1 expected; the SMR was 191. The difference was significant at $P < 0.01$.

In females, with all groups combined, there was only a slight excess. With sex groups combined, the excess was significant at $P < 0.01$. It may be noteworthy that one father-daughter combination for CNS tumor deaths occurred in Painesville. The daughter died of a papillary ependymoma in 1963 at the age of 16. The father died two years later of a glioblastoma multiforme at the age of 58. It would be difficult to determine whether this observation is the result of genetic or environmental factors.

Table III also shows deaths from lymphomas. Although the differences between observed and expected deaths in males were not significant, there was a consistent excess in each community. In females, there were excess lymphoma deaths in two of the four communities. With sex groups combined, a significant excess of lymphoma deaths was observed in Ashtabula. With sex groups and communities combined, the number of observed deaths was 61 versus 48.7 expected. The SMR was 125. This was significant at

TABLE III

OBSERVED AND EXPECTED DEATHS FOR THREE TYPES OF CANCER FOR RESIDENTS 45 YEARS AND OLDER IN THE OHIO COMMUNITIES WITH VINYL CHLORIDE POLYMERIZATION FACILITIES, 1958–73

Expected numbers of cancer deaths based on occurrence over the same period of time in the balance of the counties in which the communities are located.

	Males		Females		Sexes combined	
	Obs./Exp.	SMR	Obs./Exp.	SMR	Obs./Exp.	SMR
	CNS cancer (191–192)[a]					
[illegible]	7 6.1	115	6/ 3.8	158	13/ 9.9	131
[illegible]ville	12 3.8	316 [d]	2/ 2.8	71	14/ 6.6	212 [b]
Avon Lake	2 2.3	87	1/ 1.8	56	3/ 4.1	73
[illegible]ville	6 1.9	316 [b]	2/ 1.5	133	8/ 3.4	235 [b]
Communities combined	27 14.1	191 [c]	11/ 9.9	111	38/24.0	158 [c]
	Leukemia and aleukemia (204–207)[a]					
[illegible]	13 14.7	88	11/ 8.6	128	24/23.3	103
[illegible]ville	8 7.1	113	7/ 6.8	103	15/13.9	108
Avon Lake	1 3.6	28	5/ 2.2	227	6/ 5.8	103
[illegible]ville	2 3.2	63	0/ 1.9	0	2/ 5.1	39
Communities combined	24 28.6	84	23/19.5	118	47/48.1	98
	Lymphomas (200–203)[a]					
[illegible]	18 12.6	143	14/ 5.8	241 [c]	32/18.4	174 [c]
[illegible]ville	12 10.4	115	4/ 8.3	48	16/18.7	86
[illegible] Lake	4 3.3	121	5/ 2.9	172	9/ 6.2	145
[illegible]ville	3 2.8	107	1/ 2.6	38	4/ 5.4	74
Communities combined	37/29.1	127	24/19.6	122	61/48.7	125

[a] International Classification of Diseases, 8th revision codes.
[b] $P < .05$.
[c] $P < .01$.
[d] $P < .002$

$0.05 < P < 0.10$. Data were then analyzed for leukemia and aleukemia mortality. As shown in Table III, the observed versus expected mortality was virtually identical.

Birth defects among populations residing near VC polymerization facilities

The occurrence of birth defects was studied [8] for the same three Ohio communities with VC polymerization facilities. Birth data for residents of North Ridgeville were not combined with data for the three index communities in the initial analyses (Table IV) because the high incidence of birth defects in North Ridgeville, which lies contiguous to Avon Lake, was identified in subsequent analyses [8]. Data for North Ridgeville, however, were included in subsequent analyses (Table V) for specific birth malformations. The observations among community residents were compared to both the occurrence in

TABLE IV

RESIDENT BIRTHS, MALFORMATION RATE PER 1000 LIVE BIRTHS IN OHIO AND IN THREE SELECTED COMMUNITIES AND OBSERVED VERSUS EXPECTED NUMBERS OF MALFORMATIONS IN EACH CITY, YEARS COMBINED, 1970–73

Malformations are based on codes 740–759 of the International Classification of Diseases, 8th revision, 1968.

Area	Births	Malformations			
		Rate/10^3	Number observed	Number expected [a]	χ^2
Entire state of Ohio	719, 287	10.1	7295	—	—
Ashtabula city	1900	17.4	33	19.3	9.78 [b]
Painesville city	1381	18.1	25	14.0	10.29 [b]
Avon Lake city	738	20.3	15	7.5	7.56 [b]
All three communities combined	4019	18.2	73	40.8	27.13 [c]

[a] Expected numbers are based on state rate per 1000 live births.
[b] $P < 0.01$.
[c] $P < 0.001$.

the balance of the counties in which the communities are located and to the occurrence in the State for the period 1970–73. Between 1970–73, the rate for birth defects changed from 9.3 to 11.3 per 1000 live births for the balance of the counties, from 9.6 to 10.8 for the entire state, whereas, the rate for the three index cities combined changed from 17.0 to 22.5. Thus, the incidence of birth defects for children born in the index cities was almost twice as great as the incidence in the balance of the counties or in the entire State, and the differences appear to be increasing with time.

Table IV shows data for malformation rates in each index community versus the rate for the entire State. These differences were all highly significant. The rates ranged from 17.47 in Ashtabula to 20.33 in Avon Lake, as compared to 10.14 for the entire State. When the community experience was compared to the occurrence in the balance of the counties in which the communities were

TABLE V

OBSERVED, EXPECTED AND RELATIVE RISK FOR SPECIFIC CONGENITAL ANOMALIES IN INDEX AREAS INCLUDING N. RIDGEVILLE, 1970–73 [a]

Defect category	Number of defects		RR [b]
	Observed	Expected	
All defects (740–756, 758, 759) [c]	109	56.0	1.95
Central nervous system (740–749)	17	5.6	3.02
Cleft palate and lip (749)	10	6.5	1.53
Genital organs (752)	16	8.4	1.90
Clubfoot (754)	23	8.2	2.79
All other defects	43	27.2	1.58

[a] Excludes skin, hair and nails (757).
[b] RR, relative risk (observed/expected).
[c] International Classification of Disease codes, 8th revision, are shown in parentheses.

located, the differences remained significant. Therefore, whether you compare the occurrence in the communities to the expected, based on the average for the State, or for the balance of the counties, the excess of children with congenital anomalies in the communities appears to be significant.

Table V shows observed versus expected numbers and the relative risk for total and selected malformations. Although an increase in most organ systems was observed, the greatest excess of severe defects included malformations of the CNS, cleft lip and palate, club foot and genital organs. These observations have been reported previously in more detail [8].

Fetal mortality among wives of workers exposed to VC

Since VC had elicited a positive mutagenic response via microbial test systems [1,6,14,24] and also had been associated with significant excesses of chromosomal aberrations in the lymphocytes of workers occupationally exposed to VC [3,4,13,23], concern was expressed that VC may induce germinal mutations. Thus, a questionnaire-interview survey was conducted to ascertain the incidence of fetal loss (defined as any product of conception not born alive) among wives of workers exposed to VC [7a]. All current VC polymerization and polyvinyl chloride (PVC) fabrication workers were included for study together with a similar number of current rubber workers selected from work areas relatively free from known toxic materials and matched as a group to the VC workers by age. No interviews were conducted with workers' wives and no data were obtained concerning maternal age, except indirectly through paternal age. Group participation rates ranged from 62—77 percent. Data for the wives of VC polymerization workers (study group) were contrasted with data for the wives of PVC fabrication and rubber workers ("controls"), who were known to have had very low or no VC exposure, respectively.

The data in Table VI show the paternal age distribution for fetal deaths according to the husband's exposure. Prior to husband's exposure, the crude fetal death rates for the control and study group were 6.9 and 10.1%, respectively. Subsequent to husband's exposure, the crude rates were 8.8 and 16.5%, respectively.

As can be seen in Table VI, the excess in fetal mortality subsequent to husband's exposure, was associated with younger-aged husbands. For husbands 30 years of age and older, the rates for the control group 17/142 (12.0%) and primary VC exposure group 9/69 (13%) were about the same; whereas, for husbands less than 30 years of age, the rates for the control group 7/131 (5.3%) and primary VC exposure group 14/70 (20.0%) were significantly different, $P < 0.001$, $\chi^2 = 10.52$, with one degree of freedom (df). The excess of fetal mortality among wives of younger-aged husbands may be a reflection of a practice of placing newly hired personnel because of little or no seniority, in jobs where occupational exposures to VC may have been worse. This hypothesis, however, needs further assessment in other working populations. Since parental age was positively correlated with fetal mortality in this population and in a previous study [25], fetal mortality rates for the study group were adjusted to the age-distribution of the control group.

TABLE VI

PATERNAL AGE DISTRIBUTION FOR FETAL DEATHS ACCORDING TO HUSBAND'S VC EXPOSURE

Paternal age group (years)	"Controls"			Primary VC Exposure		
	Pregnancies	Fetal deaths		Pregnancies	Fetal deaths	
	(N)	(N)	(%)	(N)	(N)	(%)
	Prior to husband's exposure					
<20	31	2	6.5	7	0	0.0
20–24	80	4	5.0	44	2	4.5
25–29	38	4	10.5	56	7	12.5
30–34	6	1	16.7	27	5	18.5
≥35	4	0	0.0	14	1	7.1
All ages crude rate	159	11	6.9	148	15	10.1
Mean paternal age at conception (year)	23.0			26.4		
Age-adjusted [a] rate			(6.9)			(6.1)
	Subsequent to husband's exposure					
>20	1	0	0.0	0	0	0.0
20–24	43	4	9.3	22	3	13.6
25–29	87	3	3.4	48	11	22.9
30–34	87	7	8.0	36	3	8.3
≥35	55	10	18.2	33	6	18.2
All ages crude rate	273	24	8.8	139	23	16.5
Mean paternal age at conception (year)	30.4			30.2		
Age-adjusted [a] rate			(8.8)			(15.8)

[a] Fetal mortality rates for primary VC exposure group are direct age-adjusted to the paternal age-distribution of the pregnancies in the control group (shown in parentheses).

TABLE VII

MEAN PATERNAL NUMBER OF PREGNANCIES AND AGE-ADJUSTED FETAL DEATH RATES ACCORDING TO HUSBAND'S VC EXPOSURE

	"Controls" [a]	Primary VC exposure [b]
	Prior to husband's exposure	
Number of families	95	70
Mean paternal age at conception (years)	23.0	26.4
Number of fetal deaths among wives	11	15
Number of pregnancies	159	148
Age-adjusted fetal deaths/100 preg. [c]	6.9	6.1
	Subsequent to husband's exposure	
Number of families	113	62
Mean paternal age at conception (years)	30.4	30.2
Number of fetal deaths among wives	24	23
Number of pregnancies	273	139
Age-adjusted fetal deaths/100 preg. [c]	8.8	15.8 [d]

[a] Rubber and PVC fabrication workers.

[b] VC polymerization workers.

[c] Rates age-adjusted to "control" group paternal age distribution.

[d] Subsequent to husbands' exposure, the frequency of fetal deaths among wives was significantly greater in the primary VC exposure group as compared to the "controls" ($P < 0.05$) or to the frequency in the study group prior to husband's exposure ($P < 0.02$) by age-adjusted Chi-square testing.

The data in Table VII show fetal death rates per 100 pregnancies for the wives of male workers in the control and study groups. Prior to their husband's exposures, the rates were 6.9% and 6.1% for the control and study groups, respectively. Subsequent to husband's exposure, however, the rates were 8.8% for the control group versus 15.8% for the study group. This difference was significant at the $P < 0.05$ level by Mantel-Haenszel Chi-square testing [17] ($\chi^2 = 4.84$, df = 1). Further, the before and after exposure comparisons indicated changes in rates from 6.9 to 8.8% for the control group as compared to a change from 6.1 to 15.8% for the study group. The rates for before and after husband's exposure in the study group were significantly different ($P < 0.025$, $\chi^2 = 5.78$, df = 1). For the study group, it may be noted that before exposure age-adjustment reduced the crude rate from 10.1 to 6.1%; whereas, after exposure the crude rate was reduced from 16.5 to 15.8%. The greater reduction in rates for the before exposure age-adjustment resulted from a difference in the age distribution of the husbands in the two groups. As can be seen in Table VII, prior to husband's exposure, the mean paternal age in the study group was 26.4 years as compared to only 23.0 years for the control group, whereas, subsequent to husband's exposure, the mean ages of the two groups were virtually the same, i.e., 30.4 years versus 30.2 years. In both situations mean age was a good measure of central tendency.

To determine whether women who had chronically experienced abortions might have weighted the results in favor of a higher fetal mortality rate in the primary VC exposure group, data for the pregnancies of women who had two, three or four or more spontaneous abortions were eliminated. The data were then recalculated to determine whether or not the trend of a greater miscarriage rate could be maintained. As shown in Table VIII, the trend was maintained for each analysis.

TABLE VIII

NUMBER OF PREGNANCIES AND AGE-ADJUSTED FETAL DEATH RATES ACCORDING TO HUSBAND'S VC EXPOSURE EXCLUDING PREGNANCIES IN WOMEN WITH ≥2, 3 OR 4 FETAL DEATHS

Rates for the primary VC exposure group are age-adjusted to the Control group

	Controls		Primary VC exposure	
	Number of pregnancies	Fetal death rate	Number of pregnancies	Fetal death rate
	≥2 Fetal deaths excluded			
Before husband's exposure	155	5.8%	126	1.7%
After husband's exposure	255	4.7%	111	6.2%
	≥3 Fetal deaths excluded			
Before husband's exposure	159	6.9%	141	3.1%
After husband's exposure	265	6.8%	120	10.8%
	≥4 Fetal deaths excluded			
Before husband's exposure	159	6.9%	142	5.8%
After husband's exposure	265	6.8%	127	11.8%

As reported previously [7a], additional analyses suggested that the significant excess in fetal mortality after the husband's exposure would not seem to be the result of bias from interviewers nor from respondents. Because of the highly volatile nature of vinyl chloride [27], carry-home exposure to the wife would seem unlikely. Therefore, the leading possibility for the mechanism involved would seem to be germ-cell damage in the male through direct VC exposure.

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TOXICOLOGY AND APPLIED PHARMACOLOGY 39, 497–513 (1977)

The Effects of Maternally Inhaled Vinyl Chloride on Embryonal and Fetal Development in Mice, Rats, and Rabbits[1,2]

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The Effects of Maternally Inhaled Vinyl Chloride on Embryonal and Fetal Development in Mice, Rats, and Rabbits. JOHN, J. A., SMITH, F. A., LEONG, B. K. J., AND SCHWETZ, B. A. (1977). *Toxicol. Appl. Pharmacol.* 39, 497–513. These studies evaluated the effects of inhaled vinyl chloride on mouse, rat, and rabbit embryonal and fetal development. Groups of pregnant CF-1 mice, Sprague–Dawley rats and New Zealand white rabbits were exposed to 500 ppm of vinyl chloride 7 hr daily during the period of major organogenesis. Subsequently, other groups of mice were similarly exposed to 50 ppm of vinyl chloride and rats and rabbits were exposed to 2500 ppm of vinyl chloride. While maternal toxicity was observed, vinyl chloride alone did not cause significant embryonal or fetal toxicity and was not teratogenic in any of the species at the concentrations tested. Maternal toxicity was more prominent among mice than among rats and rabbits. Simultaneous exposure of some of the pregnant animals to vinyl chloride by inhalation plus 15% ethanol in the drinking water resulted in toxic effects greater than those associated with exposure to vinyl chloride alone in the three species. The maternal toxicity was enhanced to an extent greater than the embryotoxicity.

Vinyl chloride is widely used in the preparation of polyvinyl chloride resin, as a copolymer in plastics, and, to a lesser extent, as a solvent and as a chemical intermediate. A report of the effect of single exposures of mice, rats, and guinea pigs to vinyl chloride by Mastromatteo *et al.* (1960) indicates that this compound has very low acute toxicity. Anesthesia is the primary significant effect of acute exposure to high concentrations (75,000–100,000 ppm). The effect of repeated exposure of laboratory animals to vinyl chloride has been reported by Torkelson *et al.* (1961). Repeated exposure for 6 months to 200 ppm resulted in histologic changes in the centrilobular area of the livers of rabbits, but not in rats, guinea pigs, or dogs. In a study reported by Viola *et al.* (1971), rats were exposed to 30,000 ppm of vinyl chloride vapor for 12 months. Findings on

[1] The majority of this study was supported by the companies sponsoring research on vinyl chloride and was administered by The Manufacturing Chemists Association.

[2] This work was reported, in part, at the 14th Annual Meeting of the Society of Toxicology, March 10, 1975, Williamsburg, Virginia.

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these rats were reported to include severe chronic hepatitis, interstitial pneumonia, as well as tumors of the skin, lungs, and bones. Maltoni and Lefemine (1974) reported the oncogenic effects of repeated exposure to inhaled vinyl chloride in rats and mice. Angiosarcomas, zymbal gland carcinomas, and nephroblastomas developed in rats exposed to concentrations of vinyl chloride ranging from 50-10,000 ppm, 4 hr/day, 5 days/week for 12 months and subsequently maintained and observed until death. Pulmonary adenomas, mammary carcinomas, and liver angiosarcomas were observed among mice exposed to the same range of concentrations for 7 months. Similarly, Keplinger *et al.* (1975) reported neoplasms in mice and the tentative diagnosis of tumors in hamsters and rats exposed to 50, 200, or 2500 ppm of vinyl chloride.

The carcinogenic potential of inhaled vinyl chloride has also been studied by exposing animals *in utero* (Maltoni, 1975), but observations to determine effects which are more in line with a classical teratologic study were not made. Reports on the embryotoxic potential of vinyl chloride in laboratory animals have not been found in the literature. Thus, the purpose of the studies described in this report was to assess the potential of inhaled vinyl chloride to have a deleterious effect on embryonal and fetal development in mice, rats, and rabbits.

Since previous studies in this laboratory suggested that the primary metabolic pathway for vinyl chloride is blocked by ethanol (Hefner *et al.*, 1975), it was considered possible that administration of ethanol in the drinking water of animals exposed to vinyl chloride might alter its metabolism in a manner which would enhance its toxic or teratogenic potential. To assess this possibility, some of the vinyl chloride-exposed animals were given 15% ethanol in their drinking water during the days of exposure to vinyl chloride. The teratogenic potential of 15% ethanol in the drinking water in mice, rats, and rabbits was previously studied in this laboratory and is summarized in Table 11 of this report (unpublished data, The Dow Chemical Co.).

METHODS

Animals and test material. Female CF-1 mice[4] weighing 25 to 30 g, Sprague–Dawley rats[5] weighing approximately 250 g, and New Zealand white rabbits[6] weighing 3.5 to 4.5 kg were used in this study. The day on which a vaginal plug was observed or the day on which sperm were seen in a vaginal smear was considered to be Day 0 of pregnancy for mice and rats, respectively. The day of mating was considered to be Day 0 for rabbits. Between daily exposures, animals were housed in wire-bottom cages in a room controlled for temperature, humidity, and light cycle. Commercial laboratory animal food[7] and water were available. Food consumption was measured at 3-day intervals for mice and rats and at 2-day intervals for rabbits.[8]

Exposure of bred animals was conducted in stainless steel chambers of 3.7 m^3 volume under dynamic airflow conditions. The atmosphere of vinyl chloride was generated by

[4] Mice were obtained from Carworth, Portage, Michigan.
[5] Rats were obtained from Spartan Research Animals, Inc., Haslett, Michigan.
[6] Rabbits were obtained from Langshaws Rabbitry, Augusta, Michigan.
[7] Ralston Purina Co., St. Louis, Missouri.
[8] Ethanol consumption was measured during a separate study in which animals were given only 15% ethanol in the drinking water; these results will be reported separately (unpublished data, The Dow Chemical Company).

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diluting gaseous vinyl chloride with filtered room air at a rate calculated to give the desired concentration. Samples of inhibited vinyl chloride monomer (chloroethylene)[9] were used for the exposures. The actual concentration was measured with an infrared spectrophotometer (Perkin–Elmer 12A or Miran I) with a multipath gas cell.

Experimental design. In the initial experiment, groups of 30–40 bred mice, 20–35 bred rats, and 15–20 bred rabbits were exposed to 500 ppm of vinyl chloride for 7 hr daily on Days 6–15 (mice and rats) or 6–18 (rabbits) of gestation. Subsequently, additional groups of mice were exposed to 50 ppm of vinyl chloride. Additional groups of rats and rabbits were exposed to 2500 ppm of vinyl chloride. As summarized in Table 1, some of the animals which were exposed to vinyl chloride were also given 15% ethanol in their drinking water on Days 6–15 (mice and rats) or Days 6–18 (rabbits) of gestation.

TABLE 1

TERATOLOGIC STUDIES WITH VINYL CHLORIDE[a]

	Vinyl chloride (ppm)	Ethanol (%)
Mice	500	0
	500	15
	50	0
	50	15
Rats	2500	0
	2500	15
	500	0
Rabbits	2500	0
	2500	15
	500	0
Mice, rats, and rabbits	0	0

[a] Mice and rats were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–15 of gestation. Some of the mice and rats were given ethanol in their drinking water (15%, v/v) on Days 6–15 of gestation. Rabbits were exposed to vinyl chloride on Days 6–18 of gestation. Some of the rabbits were given ethanol in their drinking water on Days 6–18 of gestation.

Maternal and fetal observations. All animals were observed daily throughout pregnancy and maternal body weights were recorded on gestation Days 6, 12, 15, and 18 for mice and on Days 6, 10, 16, and 21 for rats. Maternal body weights for rabbits were recorded on Days 6, 12, 18, 22, and 29 of gestation. Pregnant mice and rats were sacrificed by carbon dioxide inhalation on Days 18 and 21 of gestation, respectively. Pregnant rabbits were sacrificed on Day 29 of gestation. The uterine horns were exteriorized through a midline incision in the abdominal wall and the number and position of live, dead, and resorbed fetuses were noted. After being weighed, measured (crown–rump length), and sexed (mice and rats), the fetuses were examined for external ano-

[9] Vinyl chloride was obtained from Matheson Gas Products, Joliet, Illinois.

malies. One-third of each litter was immediately examined for evidence of soft tissue anomalies by dissection under a low-power microscope. Rabbit fetuses were sexed on the basis of examination of internal genitalia. All fetuses were then eviscerated, preserved in alcohol, and subsequently cleared and stained with alizarin red-S (Dawson, 1926) for examination of skeletal anomalies.

Statistical evaluation. The Fisher exact probability test (Siegel, 1956) was used to evaluate the incidence of resorptions among litters. Maternal and fetal body weights and body measurements and maternal liver weights were analyzed statistically by an analysis of variance and the Dunnett test (Steel and Torrie, 1960). The incidence of fetal anomalies was analyzed by the Wilcoxon test as modified by Haseman and Hoel (1974).

Controls. The group of animals which was exposed only to vinyl chloride served as the control for those animals which were exposed to vinyl chloride in combination with 15% ethanol in the drinking water. The controls for animals exposed to vinyl chloride alone were exposed concurrently to filtered room air.

RESULTS

Maternal toxicity. Among mice exposed to 500 ppm of vinyl chloride by inhalation, there was a decrease in maternal weight gain and food consumption during gestation and in the absolute liver weight at the time of cesarean section compared to control values (Table 2). These effects were not observed among mice exposed to 50 ppm of vinyl chloride. Mice exposed to a combination of 500 ppm of vinyl chloride by inhalation and 15% ethanol in their drinking water also showed a decrease in weight gain during gestation and in liver weight (absolute and relative) on Day 18 of gestation. Ethanol in combination with 50 ppm of vinyl chloride also resulted in a decrease in maternal weight gain during gestation and a decrease in the absolute liver weight on Day 18 of gestation. Food consumption throughout gestation was decreased for mice exposed to both concentrations of vinyl chloride in combination with ethanol.

Except for an apparent decrease in maternal weight gain in rats (Table 3) and a decrease in food consumption for rabbits (Table 4), no signs of toxicity were observed in the adult rats or rabbits during exposure to 500 ppm of vinyl chloride. The apparent decrease in maternal weight gain among rats exposed to 500 ppm is most likely due to the lower body weight of the control animals on Day 6 and, subsequently, a higher weight gain of these animals during the later days of gestation. Among rats exposed to 2500 ppm, both the absolute and relative liver weights were significantly increased on Day 21 of gestation, but maternal weight gain was no different from that of rats exposed to filtered room air. Maternal food consumption was, however, lower than among control rats in this group. The relative liver weight on Day 21 of gestation was significantly increased among rats exposed to 2500 ppm of vinyl chloride and 15% ethanol. Maternal weight gain for this group of rats was significantly decreased during gestation. Food consumption during the exposure period was further decreased among these rats which were exposed to vinyl chloride in combination with ethanol in the drinking water.

No effect on maternal weight gain, liver weight, or food consumption was observed among rabbits exposed to 2500 ppm of vinyl chloride by inhalation. A decrease in maternal weight gain was observed among rabbits during exposure to 2500 ppm of vinyl chloride in combination with 15% ethanol (Days 6–18 of gestation), but total

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TABLE 2

MATERNAL WEIGHT GAIN, LIVER WEIGHTS, AND FOOD CONSUMPTION OF MICE EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration[b]			High concentration[b]		
	0 0	50 0	50 15	0 0	500 0	500 15
Number of dams	21	20	16	26	19	7
Body weight on gestation Day 6[c]	30 ± 2	31 ± 2	31 ± 2	29 ± 2	29 ± 3	30 ± 3
Weight gain during gestation Days 6–18[c]	16 ± 4	17 ± 6	11 ± 7[d]	20 ± 3	17 ± 4[e]	10 ± 7[d]
Liver weight on gestation Day 18[c]						
Absolute	2.75 ± 0.26	2.76 ± 0.40	2.37 ± 0.52[d]	2.75 ± 0.31	2.49 ± 0.29[e]	1.78 ± 0.36[d]
Relative[f]	59.5 ± 8.7	57.8 ± 4.5	56.6 ± 7.3	55.5 ± 5.5	54.4 ± 4.2	45.8 ± 5.1[d]
Food consumption during gestation Days 6–15[c]	6 ± 1	6 ± 1	4 ± 2[d]	6 ± 1	5 ± 1[e]	3 ± 1[d]

[a] Mice were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–15 of gestation. Some of the mice were given ethanol in their drinking water (15%, v/v) on Days 6–15 of gestation.
[b] Top row, vinyl chloride in drinking water (ppm); bottom row, percentage ethanol in drinking water.
[c] Grams, mean ± SD.
[d] Significantly different from vinyl chloride alone by an analysis of variance, $p < 0.05$.
[e] Significantly different from control by an analysis of variance, $p < 0.05$.
[f] Milligrams of liver per gram of body weight.

TABLE 3

MATERNAL WEIGHT GAIN, LIVER WEIGHTS, AND FOOD CONSUMPTION OF RATS EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration[b]		High concentration[b]		
	0 0	500 0	0 0	2500 0	2500 15
Number of dams	28	31	19	16	16
Body weight on gestation Day 6[c]	258 ± 26	277 ± 20[d]	288 ± 27	274 ± 19	272 ± 14
Weight gain during gestation Days 6–21[c]	148 ± 11	125 ± 19[d]	127 ± 15	138 ± 23	120 ± 15[e]
Liver weight on gestation Day 21[c]					
Absolute	14.81 ± 1.79	15.00 ± 1.14	14.27 ± 1.38	15.55 ± 1.23[d]	16.52 ± 1.50
Relative[f]	36.5 ± 4.1	37.1 ± 2.6	34.4 ± 3.3	37.8 ± 2.6[d]	42.1 ± 2.4[e]
Food consumption during gestation Days 6–15[c]	21 ± 2	22 ± 2	22 ± 2	21 ± 2[d]	13 ± 2[e]

[a] Rats were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–15 of gestation. Some of the rats were given ethanol in their drinking water (15%, v/v) on Days 6–15 of gestation.

[b] Top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.

[c] Grams, mean ± SD.

[d] Significantly different from control by an analysis of variance, $p < 0.05$.

[e] Significantly different from vinyl chloride alone by an analysis of variance, $p < 0.05$.

[f] Milligrams of liver per gram of body weight.

TABLE 4

MATERNAL WEIGHT GAIN, LIVER WEIGHTS, AND FOOD CONSUMPTION OF RABBITS EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration[b]		High concentration[b]		
	0 0	500 0	0 0	2500 0	2500 15
Number of dams	18	20	11	5	16
Body weight on gestation Day 6[c]	3.85 ± 0.23	3.82 ± 0.25	4.08 ± 0.21	4.39 ± 0.30[d]	4.02 ± 0.47
Weight gain during gestation Days 6–29[c]	0.05 ± 0.19	0.01 ± 0.19	0.06 ± 0.27	0.01 ± 0.13	−0.14 ± 0.42
Liver weight on gestation Day 29[c]					
Absolute	96 ± 19	89 ± 14	102 ± 16	122 ± 25	116 ± 30
Relative[e]	24.6 ± 3.6	23.2 ± 2.9	24.7 ± 2.7	27.7 ± 5.9	30.0 ± 6.3
Food consumption during gestation Days 6–18 (g)	98 ± 30	76 ± 29[d]	91 ± 36	89 ± 26	15 ± 9[f]

[a] Rabbits were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–18 of gestation. Some of the rabbits were given ethanol in their drinking water (15%, v/v) on Days 6–18 of gestation.
[b] Top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.
[c] Kilograms, mean ± SD.
[d] Significantly different from control by an analysis of variance, $p < 0.05$.
[e] Grams of liver per kilogram of body weight.
[f] Significantly different from vinyl chloride alone by an analysis of variance, $p < 0.05$.

weight gain was not different from that among rabbits exposed to 2500 ppm of vinyl chloride alone (Table 4). Food consumption was significantly decreased among the rabbits exposed to 2500 ppm plus ethanol.

Observations made at the time of cesarean section. Observations made at the time of cesarean section of mice, rats, and rabbits are presented in Tables 5–7. Maternal deaths were observed among mice exposed to 500 ppm of vinyl chloride alone and in combination with ethanol (Table 5). There was an increase in the incidence of resorptions and the fetal body weights were lower than in controls for the 500-ppm vinyl chloride group. Litter size was also reduced. These effects were augmented among mice exposed to 500 ppm of vinyl chloride in combination with 15% ethanol. Two litters in this group were totally resorbed. In addition, the number of implantation sites per dam, fetal crown–rump length, and percentage pregnancy were significantly lower in the ethanol group as compared to mice receiving 500 ppm of vinyl chloride alone. Among mice exposed to 50 ppm, the fetal crown–rump length was significantly greater than among controls; a decrease, however, in fetal body weight and crown–rump length was observed in the 50-ppm vinyl chloride plus ethanol group. The incidence of resorptions was not significantly greater among mice treated with 50 ppm of vinyl chloride in combination with ethanol, although two litters were totally resorbed.

One maternal death was observed among rats exposed to 2500 ppm of vinyl chloride by inhalation (Table 6). There was no significant effect on litter size, the number of implantation sites per dam, or the incidence of resorptions among any of the exposed groups of rats. The pregnancy wastage was significantly lower among rats exposed to 500 ppm than among the control rats. Percentage pregnacy was unaffected among rats exposed to vinyl chloride alone or in combination with 15% ethanol in the drinking water. Fetal body weight and crown–rump length were significantly reduced among rats exposed to 2500 ppm of vinyl chloride in combination with ethanol. A significant reduction in fetal body weight was also observed among rats exposed to 500 ppm of vinyl chloride alone, but not among those exposed to 2500 ppm. Significant decreases in the number of corpora lutea per dam were observed among rats exposed to 500 ppm of vinyl chloride alone and among those exposed to 2500 ppm in combination with 15% ethanol. A significant increase, however, in the number of corpora lutea per dam was observed among rats exposed only to 2500 ppm of vinyl chloride. Since the number of corpora lutea is established prior to Day 6 of gestation, these differences are not considered to be a treatment-related effect, but rather a measure of the reproductive status of the animals prior to the beginning of the experiment.

Among rabbits, a significant increase in the incidence of resorptions was observed in the high concentration (2500 ppm) plus ethanol group, in which seven litters were totally resorbed (Table 7). Exposure of rabbits to either 500 or 2500 ppm of vinyl chloride alone did not alter the incidence of resorptions. A decrease in the number of live fetuses per litter was observed among rabbits exposed to 500 ppm of vinyl chloride alone, but not among those exposed to 2500 ppm of vinyl chloride alone or in combination with 15% ethanol in the drinking water. Since the decrease in litter size was associated with a decrease in the number of corpora lutea which is established prior to Day 6 of gestation, this effect is probably not due to exposure to vinyl chloride. Pregnancy wastage in rabbits was unaffected by exposure to vinyl chloride. No differences in fetal body weight or crown–rump length were observed in any of the exposed groups of rabbits.

TABLE 5

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TABLE 5

OBSERVATIONS MADE AT THE TIME OF CESAREAN SECTION OF MICE EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration			High concentration		
	0 0	50 0	50 15	0 0	500 0	500 15
Number of litters	21	20	16	26	19	7
Implantation sites/dam[b]	12 ± 2	12 ± 4	11 ± 4	14 ± 2	13 ± 2	10 ± 6[c]
Live fetuses/litter[b]	10 ± 4	11 ± 4	10 ± 4	12 ± 2	11 ± 2[d]	8 ± 6[c]
Implantations resorbed (%)	15 (40/261)	8 (18/238)	11 (19/172)	7 (26/351)	13 (33/248)[e]	19 (13/69)
Litters with resorptions (%)	67 (14/21)	55 (11/20)	69 (11/16)	58 (15/26)	79 (15/19)	86 (6/7)
Litters totally resorbed	1	0	2	0	0	2
Resorptions/litters with resorptions	2.9 (40/14)	1.6 (18/11)	1.7 (19/11)	1.7 (26/15)	2.2 (33/15)	2.2 (13/6)
Sex ratio, M:F	50:50	50:50	48:52	54:46	52:48	64:36
Fetal body weight (g)[f]	1.00 ± 0.11	1.02 ± 0.10	0.84 ± 0.14[c]	1.07 ± 0.06	0.99 ± 0.11[d]	0.78 ± 0.15[c]
Fetal crown–rump length (mm)[f]	23.0 ± 1.9	24.2 ± 0.8[d]	22.4 ± 1.5[c]	23.7 ± 1.2	23.6 ± 1.0	21.2 ± 1.5[c]
Maternal deaths/treated dams (%)	0 (0/37)	0 (0/27)	0 (0/28)	0 (0/30)	17 (5/29)[e]	13 (4/30)
Percentage pregnancy[g]	57 (21/37)	74 (20/27)	57 (16/28)	88 (28/32)	72 (21/29)	31 (9/29)[h]

[a] Mice were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–15 of gestation. Some of the mice were given ethanol in their drinking water (15%, v/v) on Days 6–15 of gestation. Low and high concentrations; top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.

[b] Mean ± SD.

[c] Significantly different from vinyl chloride alone by an analysis of variance, $p < 0.05$.

[d] Significantly different from control by an analysis of variance, $p < 0.05$.

[e] Significantly different from control by the Fisher exact probability test, $p < 0.05$

[f] Mean of litters ± SD.

[g] Based on the presence of fetuses and/or resorption sites observed by gross examination at the time of Cesarean section.

[h] Significantly different from vinyl chloride alone by the Fisher exact probability test, $p < 0.05$.

TABLE 6

OBSERVATIONS MADE AT THE TIME OF CESAREAN SECTION OF RATS EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration		High concentration		
	0 0	500 0	0 0	2500 0	2500 15
Number of litters	28	31	19	16	16
Corpora lutea/dam[b]	15 ± 3	13 ± 2[c]	14 ± 2	15 ± 2[c]	14 ± 2[d]
Implantation sites/dam[b]	12 ± 2	13 ± 2	12 ± 2	14 ± 2	12 ± 2
Pregnancy wastage[b,e]	3 ± 2	0.4 ± 1[c]	1 ± 1	2 ± 1	2 ± 2
Live fetuses/litter[b]	12 ± 2	12 ± 2	12 ± 2	13 ± 2	12 ± 2
Implantations resorbed (%)	1 (4/342)	3 (11/398)	4 (9/238)	3 (6/220)	4 (7/195)
Litters with resorptions (%)	14 (4/28)	29 (9/31)	32 (6/19)	25 (4/16)	25 (4/16)
Litters totally resorbed	0	0	0	0	0
Resorptions/litters with resorptions	1.0 (4/4)	1.2 (11/9)	1.5 (9/6)	1.5 (6/4)	1.8 (7/4)
Sex ratio, M:F	52:48	50:50	49:51	53:47	51:49
Fetal body weight (g)[f]	5.67 ± 0.29	5.44 ± 0.38[c]	5.59 ± 0.27	5.62 ± 0.29	5.34 ± 0.32[d]
Fetal crown–rump length (mm)[f]	42.6 ± 1.2	43.6 ± 0.8[c]	43.6 ± 1.5	43.3 ± 1.1	42.4 ± 0.9[d]
Maternal deaths/treated dams (%)	0 (0/29)	0 (0/33)	0 (0/20)	6 (1/17)	0 (0/17)
Percentage pregnancy[g]	96 (28/29)	94 (31/33)	95 (19/20)	100 (17/17)	94 (16/17)

[a] Rats were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–15 of gestation. Some of the rats were given ethanol in their drinking water (15%, v/v) on Days 6–15 of gestation. Low and high concentrations: top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.

[b] Mean ± SD.

[c] Significantly different from control by an analysis of variance, $p < 0.05$.

[d] Significantly different from vinyl chloride alone by an analysis of variance, $p < 0.05$.

[e] The number of corpora lutea minus the number of implants.

[f] Mean of litters ± SD.

[g] Based on the presence of fetuses and/or resorption sites observed by gross examination at the time of Cesarean section.

TABLE 7

OBSERVATIONS MADE AT THE TIME OF CESAREAN SECTION OF RABBITS EXPOSED TO VINYL CHLORIDE BY I

[c] Significantly different from control by an analysis of variance, $p < 0.05$.
[d] Significantly different from vinyl chloride alone by an analysis of variance, $p < 0.05$.
[e] The number of corpora lutea minus the number of implants.
[f] Mean of litters ± SD.
[g] Based on the presence of fetuses and/or resorption sites observed by gross examination at the time of Cesarean section.

TABLE 7

OBSERVATIONS MADE AT THE TIME OF CESAREAN SECTION OF RABBITS EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration		High concentration		
	0 0	500 0	0 0	2500 0	2500 15
Number of litters	18	19	11	5	16
Corpora lutea/dam[b]	9 ± 1	8 ± 1[c]	10 ± 2	10 ± 7	10 ± 2
Implantation sites/dam[b]	9 ± 1	8 ± 1[c]	8 ± 2	8 ± 4	9 ± 2
Pregnancy wastage[b,d]	0.4 ± 1	1 ± 1	2 ± 1	2 ± 3	1 ± 1
Live fetuses/litter[b]	8 ± 1	7 ± 2[c]	6 ± 3	6 ± 4	4 ± 4
Implantations resorbed (%)	6 (10/162)	9 (14/150)	22 (19/88)	24 (10/42)	53 (79/149)[c]
Litters with resorptions (%)	44 (8/18)	32 (6/19)	64 (7/11)	80 (4/5)	88 (14/16)
Litters totally resorbed	0	1	2	1	7
Resorptions/litters with resorptions	1.2 (10/8)	2.3 (14/6)	2.7 (19/7)	2.5 (10/4)	5.6 (79/14)
Sex ratio, M:F	53:47	50:50	61:39	[c]50:50	43:57
Fetal body weight (g)[f]	35.23 ± 4.82	34.13 ± 4.17	36.46 ± 4.82	33.77 ± 4.48	32.48 ± 5.88
Fetal crown–rump length (mm)[f]	91.0 ± 4.2	92.6 ± 5.0	92.6 ± 4.7	87.1 ± 5.2	87.7 ± 6.3
Maternal deaths/treated dams (%)	0 (0/18)	0 (0/20)	0 (0/11)	14 (1/7)	16 (3/19)
Percentage pregnancy[g]	100 (18/18)	95 (19/20)	100 (11/11)	86 (6/7)	95 (18/19)

[a] Rabbits were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–18 of gestation. Some of the rabbits were given ethanol in their drinking water (15%, v/v) on Days 6–18 of gestation. Low and high concentrations: top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.
[b] Mean ± SD.
[c] Significantly different from control by an analysis of variance, $p < 0.05$.
[d] The number of corpora lutea minus the number of implants.
[e] Significantly different from vinyl chloride alone by the Fisher exact probability test, $p < 0.05$.
[f] Mean of litters ± SD.
[g] Based on the presence of fetuses and/or resorption sites observed by gross examination at the time of cesarean section.

Incidence of anomalies. The incidence of anomalies among litters of mice, rats, and rabbits exposed to vinyl chloride by inhalation is indicated in Tables 8, 9, and 10, respectively. The incidence of gross anomalies observed by external examination of fetuses from mice was not significantly greater than among control litters (Table 8). One fetus among the litters of mice exposed to 500 ppm of vinyl chloride in combination

TABLE 8

INCIDENCE OF ANOMALIES AMONG LITTERS OF MICE EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration			High concentration		
	0	50	50	0	500	500
	0	0	15	0	0	15
	[Number examined, fetuses (litters)]					
Gross anomalies	221 (20)	220 (20)	153 (14)	325 (26)	215 (19)	56 (5)
Soft tissue anomalies	74 (20)	75 (20)	50 (14)	107 (26)	73 (19)	19 (5)
Skeletal anomalies	221 (20)	220 (20)	153 (14)	325 (26)	215 (19)	56 (5)
Bones of the skull[b]	147 (20)	145 (19)	103 (14)	217 (26)	142 (19)	37 (5)
Gross anomalies	[Fetuses affected (%) (litters affected, %)]					
Exencephaly	0	0	0	1 (8)	1 (10)	2 (20)
Anopthalmia	0	0	0	0	0	2 (20)
Cleft palate	1 (10)	1 (10)	2 (21)	0	1 (5)	4 (40)
Soft tissue anomalies						
Small thymus	0	0	4 (7)	0	0	0
Skeletal anomalies						
Sternebrae						
Unfused	3 (20)	3 (25)	13 (57)[c]	2 (19)	9 (42)[d]	34 (80)[c]
Delayed ossification	7 (50)	4 (35)	44 (100)[c]	1 (12)	6 (42)[d]	43 (100)[c]
No. 5 sternebra missing	0	0	3 (21)	0	1(10)	7 (40)[c]
Ribs						
Extra	4 (30)	5 (30)	0.6 (7)	3 (31)	3 (32)	14 (60)[c]
Spurs	4 (35)	5 (40)	2 (21)	4 (31)	3 (21)	14 (80)[c]
Vertebrae						
Forked atlas	0.4 (5)	1 (10)	4 (36)[c]	0	0	4 (20)
Missing cervical centra	0	0	0	0	1 (10)	38 (60)[c]
Delayed ossification of cervical arches	0	0	1 (14)	0	0	5 (40)[c]
Skull						
Delayed ossification	9 (35)	8 (37)	40 (100)[c]	13 (54)	30 (58)[d]	70 (100)[c]
Unfused occipital	0	0.7 (5)	24 (50)[c]	1 (12)	5 (21)	11 (20)

[a] Mice were exposed to vinyl chloride or filtered room air by inhaltion 7 hr daily on days 6–15 of gestation. Some of the mice were given ethanol in their drinking water (15% v/v) on days 6–15 of gestation. Low and high concentrations: top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.

[b] Calculations of the incidence of anomalies of bones of the skull are based on approximately two-thirds of the total population of fetuses. The remainder of the fetuses are decapitated during the soft tissue examination. Among litters of mice which were exposed to 50 ppm of vinyl chloride, one litter consisted of only one fetus, which was decapitated during the soft tissue examination.

[c] Significantly different from vinyl chloride alone by the modified Wilcoxon test, $p < 0.05$.

[d] Significantly different from control by the modified Wilcoxon test, $p < 0.05$.

[e] $p = 0.057$, modified Wilcoxon test.

with 15% ethanol exhibited anopthalmia. The incidence of cleft palate in this group was slightly higher than that observed among mice receiving vinyl chloride alone.

No soft tissue anomalies occurred at an incidence significantly greater than control in mice. Two fetuses from one litter among mice exposed to 50 ppm of vinyl chloride plus ethanol exhibited a small thymus. No skeletal anomalies were observed at an incidence significantly greater than in controls among mice exposed to 50 ppm of vinyl chloride. Significant increases in the incidence of delayed ossification of sternebrae

(No. 5) and bones of the skull were observed among litters of mice exposed to 500 ppm of vinyl chloride. The incidence of unfused centers of ossification of sternebrae was also significantly higher in this group. Among mice exposed to vinyl chloride in combination with 15% ethanol, several skeletal anomalies occurred at an incidence significantly greater than among mice exposed to vinyl chloride alone. Among litters of mice exposed to 50 ppm of vinyl chloride plus ethanol, increases in the incidence of delayed ossification of bones of the skull and sternebrae (Nos. 4–6) were observed upon skeletal examination. The incidences of unfused occipital, unfused sternebrae (Nos. 5 and 6),

TABLE 9

INCIDENCE OF ANOMALIES AMONG LITTERS OF RATS EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration		High concentration		
	0	50[6]	0	2500	2500
	0	0	0	0	15
	[Number examined, fetuses (litters)]				
Gross anomalies	339 (28)	387 (31)	229 (19)	214 (16)	188 (16)
Soft tissue anomalies	113 (28)	129 (31)	76 (19)	73 (16)	63 (16)
Skeletal anomalies[b]	337 (28)	387 (31)	229 (19)	214 (16)	188 (16)
Bones of the skull[c]	225 (28)	259 (31)	153 (19)	141 (16)	125 (16)
Gross anomalies	[Fetuses affected (%) (litters affected, %)]				
Omphalocele	0	1 (3)	0.4 (5)	0	0.5 (6)
Soft tissue anomalies					
Micropthalmia	0	0	0	0	2 (6)
Dilated ureter (unilateral or bilateral)	2 (7)	2 (6)	5 (10)	27 (50)[d]	5 (19)[d]
Small kidney	0	0	0	0	2 (6)
Skeletal anomalies					
Sternebrae					
Unfused	0	1(6)	3 (32)	0.5 (6)[d]	1 (12)
Ribs					
Spurs	1 (4)	9 (52)[d]	14 (68)	12 (69)	35 (69)[e]
Vertebrae					
Missing cervical centra	0.3 (4)	2 (16)	7 (53)	4 (50)	21 (81)[e]
Skull					
Delayed ossification	16 (61)	12 (61)	18 (58)	6 (31)[d]	3 (25)
Unfused	0	0	53 (90)	3 (12)[d]	2 (12)

[a] Rats were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–15 of gestation. Some of the rats were given ethanol in their drinking water (15%, v/v) on Days 6–15 of gestation. Low and high Concentrations: top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.

[b] Among litters of the low concentration control rats, two fetuses were misplaced and were not available for examination for skeletal anomalies.

[c] Calculations of the incidence of anomalies of bones of the skull are based on approximately two-thirds of the total population of fetuses. The remainder of the fetuses are decapitated during the soft tissue examination.

[d] Significantly different from control by the modified Wilcoxon test, $p < 0.05$.

[e] Significantly different from vinyl chloride alone by the modified Wilcoxon test, $p < 0.05$.

and forked atlas were also significantly increased in the 50-ppm plus ethanol group. Among litters of mice exposed to 500 ppm plus ethanol, increases in the incidence of delayed ossification of bones of the skull, sternebrae (Nos. 2–6), and arches of the cervical vertebrae were observed. Significant increases in the incidence of lumbar spurs, missing centra of the cervical vertebrae, unfused sternebrae, and missing fifth sternebrae were observed in this group as well. The incidence of extra ribs was slightly, but not significantly, increased compared to the controls.

In rats, no gross anomalies occurred at an incidence significantly greater than among control animals (Table 9). Among litters of rats exposed to 2500 ppm, the incidence of

TABLE 10

INCIDENCE OF ANOMALIES AMONG LITTERS OF RABBITS EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration[b]		High concentration[b]		
	0 0	500 0	0 0	2500 0	2500 0
	[Number examined, fetuses (litters)]				
Gross anomalies	152 (18)	136 (18)	69 (9)	32 (4)	70 (9)
Soft tissue anomalies	50 (18)	47 (18)	24 (9)	10 (4)	25 (9)
Skeletal anomalies	152 (18)	136 (18)	69 (9)	32 (4)	70 (9)
Gross anomalies	[Fetuses affected (%) (litters affected, %)]				
Cleft palate	0	0	0	0	1 (11)
Soft tissue anomalies					
Dilated renal pelvis	0	0	0	0	8 (11)
Dilated cerebral ventricle	0	0	0	10 (25)	0
Enlarged right atrium of heart	0	0	0	0	8 (11)
Skeletal anomalies					
Sternebrae					
Delayed ossification No. 5	28 (77)	38 (94)[c]	20 (44)	16 (75)	24 (67)

[a] Rabbits were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–18 of gestation. Some of the rabbits were given ethanol in their drinking water (15%, v/v) on Days 6–18 of gestation.

[b] Top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.

[c] Significantly different from control by the modified Wilcoxon test, $p < 0.05$.

dilated ureter was significantly higher than among control litters. The incidence of dilated ureter was significantly lower, however, among litters of rats exposed to 2500 ppm of vinyl chloride in combination with 15% ethanol. Among litters of rats exposed to vinyl chloride, only minor skeletal variations were observed at an incidence higher than that of controls. The incidence of lumbar spurs was increased among litters exposed to 500 ppm. No increases in the occurrence of skeletal anomalies were observed among litters of rats exposed to 2500 ppm. The incidences of delayed ossification of bones of the skull and unfused centers of ossification of skull and sternebrae were significantly decreased among litters of this group. The incidences of lumbar spurs and missing centra of the cervical vertebrae were, however, significantly increased among

litters of rats exposed to 2500 ppm of vinyl chloride in combination with 15% ethanol in the drinking water.

In rabbits, no gross anomalies were observed at an incidence greater than among control litters, although a cleft palate was observed in one fetus among litters of rabbits exposed to 2500 ppm of vinyl chloride plus ethanol (Table 10). Dilated renal pelvis was also observed in two fetuses from one litter among rabbits exposed to 2500 ppm plus ethanol. Two fetuses from this group also exhibited an enlarged right atrium of the heart. Among litters of rabbits exposed to 500 ppm, the incidence of delayed ossification of the fifth sternebra was significantly higher than that of control litters. Delayed ossification of sternebrae did not occur at a significantly higher incidence than control among litters of rabbits exposed to 2500 ppm of vinyl chloride alone or in combination with 15% ethanol in the drinking water.

DISCUSSION

The results of these studies indcate that exposure of pregnant mice, rats, or rabbits to vinyl chloride by inhalation at concentrations sufficiently high to cause maternal toxicity was not teratogenic in any of the three species. The responses of mice, rats, and rabbits are summarized in Table 11. Less maternal toxicity was observed among rats and rabbits than among mice during exposure or at the time of cesarean section. Among rats, one maternal death and an increase in liver weight were observed at 2500 ppm and a decrease in maternal weight gain was observed at 500 ppm. Among rabbits, one maternal death was observed at 2500 ppm and there was a decrease in food consumption at 500 ppm. In comparison, 500 ppm was quite toxic to pregnant mice, as evidenced by the significantly decreased weight gain and food consumption and by the occurrence of a number of maternal deaths.

Exposure to vinyl chloride alone was not consistently embryotoxic in the three species studied. Among mice, at 500 ppm, the incidence of resorptions was significantly higher (13%) than among the concurrent controls (7%). Since the incidence of resorptions among the mice which served as controls for the 50-ppm group was 15% and the incidence among groups of control mice from recent studies in our laboratory was 10% (193/1895), the apparent increase at 500 ppm was probably due to the unusually low incidence of resorptions among their concurrent control group. Resorptions among exposed rats and rabbits occurred at a frequency comparable to controls. Some decreases in fetal body weight and crown–rump length were observed in rats and mice, but not in rabbits. A teratogenic response to maternally inhaled vinyl chloride was not observed in mice, rats, or rabbits. With the exception of unilateral and bilateral dilated ureter among litters of rats exposed to 2500 ppm of vinyl chloride, no external or soft tissue anomalies were observed at an incidence significantly higher than control in any of the three species. Examination of the skeletons revealed only minor skeletal variations; no major skeletal malformations were found at an incidence significantly greater than in the control groups.

Ingestion of 15% ethanol in the drinking water enhanced some of the effects of inhaled vinyl chloride. In each of the three species tested, maternal weight gain and food consumption were lower than among animals exposed to vinyl chloride alone. The percentage resorptions was slightly but not significantly increased among mice exposed

TABLE 11

SUMMARY—VINYL CHLORIDE TERATOLOGIC STUDIES

	Mice[a]					Rats[a]				Rabbits[a]			
	50		500		0	500	2500		0	500	2500		0
	0	15	0	15	15	0	0	15	15	0	0	15	15
Gestation days of treatment	6–15		6–15		6–15	6–15	6–15		6–15	6–18	6–18		6–18
Maternal deaths	No	No	Yes	Yes	No	No	Yes	No	No	No	Yes	Yes	No
Percentage pregnancy	—[b]	—	—	Dec	—	—	—	—	—	—	—	—	—
Number of litters examined	20	16	19	7	21	31	16	16	19	19	5	16	14
Maternal weight gain	—	Dec	Dec	Dec	—	Dec	—	Dec	—	—	—	—	—
Maternal food consumption	—	Dec	Dec	Dec	Dec	—	Dec	Dec	Dec	Dec	—	Dec	Dec
Maternal liver weight													
Absolute	—	Dec	Dec	Dec	—	—	Inc	—	—	—	—	—	—
Relative	—	—	—	Dec	—	—	Inc	Inc	Inc	—	—	—	—
Implantation sites/dam	—	—	—	Dec	—	—	—	—	—	Dec	—	—	—
Percentage resorptions	—	—	Inc[c]	—	—	—	—	—	—	—	—	Inc	—
Litters totally resorbed	0/20	2/16	0/19	2/7	0/21	0/31	0/16	0/16	0/19	1/19	1/5	7/16	2/14
Litter size	—	—	Dec	Dec	—	—	—	—	—	Dec	—	—	—
Fetal body weight	—	Dec	Dec	Dec	Dec	Dec	—	Dec	Dec	—	—	—	—
Fetal crown–rump length	Inc	Dec	—	Dec	Dec	Inc	—	Dec	—	—	—	—	—
External anomalies	—	—	—	—	—	—	—	—	—	—	—	—	—
Visceral anomalies	—	—	—	—	—	—	Inc[d]	—	—	—	—	—	—
Skeletal anomalies	—	Inc	—	Inc	Inc	—	—	—	Inc	—	—	—	—

[a] Top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.
[b] —, No change; Dec, decrease; Inc, increase as compared to control values.
[c] This apparent increase was due to a lower than normal incidence of resorptions among the control group; see Discussion for details.
[d] Dilated ureter.

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to vinyl chloride in combination with 15% ethanol in the drinking water. A statistically significant increase in the percentage of resorptions was observed among rabbits exposed to vinyl chloride plus ethanol. Fetal body measurements were lower among litters of mice and rats which received ethanol and vinyl chloride compared to vinyl chloride alone. The combination of ethanol and vinyl chloride did not cause a teratogenic response in the three species tested, although higher incidences of some skeletal variations were observed among litters of mice and rats. Certain malformations were observed among litters of mice, rats, and rabbits which also received ethanol, but their incidence was not statistically different than among litters of animals exposed to vinyl chloride alone. The effect of simultaneous ingestion of ethanol on the disposition of vinyl chloride in these animals seemed to enhance maternal toxicity to an extent greater than embryotoxicity.

In summary, the results of these studies indicate that exposure of pregnant mice, rats, and rabbits to vinyl chloride by inhalation was not teratogenic at the concentrations tested. Mice were more susceptible to the toxic effects of vinyl chloride than either rats or rabbits. Simultaneous exposure to vinyl chloride by inhalation and 15% ethanol in the drinking water resulted in toxic effects greater than those associated with exposure to vinyl chloride alone in the three species. Exposure to vinyl chloride alone or in combination with 15% ethanol in the drinking water did not cause a significant teratogenic response in mice, rats, or rabbits.

ACKNOWLEDGMENTS

The authors are grateful to Mr. K. D. Nitschke, Ms. H. D. Ioset, and Ms. M. F. Balmer for their assistance in all aspects of this study and to T. R. Torkelson for advice and assistance in the prepartion of this report.

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[a] Top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.
[b] —, No change; Dec, decrease; Inc, increase as compared to control values.
[c] This apparent increase was due to a lower than normal incidence of resorptions among the control group; see Discussion for details.
[d] Dilated ureter.

at days 12 and 13; 8 micro Mol/L. at day 14 produced 65% of clefts.
IN-VITRO. Fusion of the isolated 14 day foetal palatal shelves was inhibited by a level of retinyl palmitate in the culture medium of the same order as that found to prevent fusion in the 14 day foetus "in utero".
Correlation of the morphology of experimental clefts, the quantitative parameters and the types of cleft presenting in man will be demonstrated and thus give a quasi-quantitative basic model of the human malformation.
This investigation was supported by the Medical Research Council of Great Britain and the Scientific and Research Committee of the Newcastle University Hospitals.

JENSH, R.P., J. LUDLOW*, I. WEINBERG*, W.H. VOGEL*, T. RUDDER*, and R.L. BRENT. Departments of Anatomy, Pharmacology, and Radiology, Thomas Jefferson University, Philadelphia, Pennsylvania. Studies concerning the effects of protracted prenatal exposure to a non-thermal level of 2450 MHz microwave radiation in the pregnant rat.

Twelve pregnant Wistar rats were exposed to 2450 MHz microwave radiation in an anechoic chamber 8 hours daily throughout pregnancy (mean = 115 hours). Since previous studies had indicated that power intensities to 20 mW/cm^2, inclusive, were non-thermal, i.e., did not cause an increase in body temperature throughout the test period as measured by a rectal thermocouple probe, this dosage level was used. Concurrent control animals were placed in the chamber for similar time periods. All animals were killed on the 22nd day of gestation, their uteri exteriorized, and fetal positions and resorption sites recorded. All fetuses were dissected using Wilson's cross-sectional technique. Data were analyzed and compared with historic baseline control groups. No significant differences among groups were observed for the following parameters: initial maternal weight; term maternal weight; maternal weight gain; embryonic and fetal resorption rates; abnormality rate; and term fetal and placental weights. Using these parameters as indices, the present results indicate that exposure of pregnant rats to a non-thermal but threshold level of 2450 MHz microwave radiation 8 hours daily throughout gestation does not adversely affect the offspring.
(Supported by NIH Grant #ESO-1121)

John*, J. A., F. J. Murray*, F. A. Smith*, and B. A. Schwetz, Toxicology Research Laboratory, Dow Chemical U.S.A., Midland, Michigan. Teratologic evaluation of vinyl chloride, vinylidene chloride, and styrene in laboratory animals.

Teratologic studies on a series of monomeric substituted vinyl-chemicals widely used in the plastics industry have been conducted in laboratory animals. The chemicals, routes of administration, species, and dose levels are as follows: vinyl chloride - inhalation - mice, rats, rabbits - 50, (mice only), 500, 2500 (not mice) ppm: vinylidene chloride - inhalation - rats, rabbits - 20 (rats only), 80, 160 ppm, drinking water - rats - 200 ppm; styrene - inhalation - rats, rabbits - 300, 600 ppm, gavage - rats - 180, 300 mg/kg/day. The current TLV's for these chemicals are 1, 20 and 100 ppm, respectively. Each chemical was administered throughout major organogenesis. Teratogenic effects were not observed in any species with any of these monomers.

KALTER, H., Children's Hospital Research Foundation, Cincinnati, Ohio. Failure of testosterone to modify the development of a spontaneous eyelid abnormality in mice.

An attempt was made to modify the pronounced sex difference in the frequency of spontaneous open eyelid in A/JKt mice by hormonal means. Primiparous females were anesthetized with nembutal (0.05 mg/g, ip) on the 15th day of gestation (about 2 days before the lids usually close), an incision was made through the skin of the nape, and about 1/3 of a 75-mg pellet of testosterone (Creton, Schering) was inserted subcutaneously and the wound closed with surgical staples. The same procedure was followed in controls except no testosterone was inserted. The females were killed 4 days later, i.e., 1 day after the lids usually close and 2 days before expected parturition, and the offspring were removed, examined, and sexed by inspection of the gonads. Female offspring were externally masculinized, the anogenital distance in them being indistinguishable to the eye from that in males. The resorption rate was similar in the experimental and control groups (17.0 and 19.4%). The hormone had no effect on eyelid development. The overall frequency of open eyelid was 15.4% in the experimental group and 14.0% in the control; and was 21.1% in males and 8.9% in females in the former; and 20.8% in males and 7.7% in females in the latter. Perhaps earlier treatment or administration of a larger dose would be successful.

KERR, K.S., and B.G. SHAH*, Bureau of Chemical Safety, Health and Welfare Canada, Ottawa, Ontario. Failure of zinc acetate to reduce teratogenicity of ethylenethiourea in pregnant rats.

Ethylenebisdithiocarbamate fungicides, their degradation and a probable metabolic product-ethylenethiourea(ETU), and maternal zinc deficiency during pregnancy, have all produced strikingly similar anomalies in rats. Mechanism of

TRANSLATION;

Mirkova, E., A. Mikhailova, and M. Nosko: EMBRYOTOXIC AND TERATOGENNIC ACTION OF VINYL CHLORIDE. (Emgriotoksichno i teratogenno deistvie na vinilkhlorid.) *Khigiena a Zdraveopazvane,* Vol. 23, No. 5, pp. 440-443, 1977. Institute of Hygiene and Occupational Diseases, Sofia.

E. Mirkova, A. Mihailova, M. Nosko — Embryotoxic and Teratogenic Action of Vinylchloride

Summary. The authors examined the embryotoxic and teratogenic action of vinylchloride on 40 pregnant white rats of the strain Wistar. The experiments were carried out under the conditions of daily inhalatory poisoning during the whole gestation at mean daily concentration of vinylchloride (6.15 mg/m³).

There was a manifested embryotoxic and teratogenic effect of vinylchloride-elevated total embryonal mortality, lowered weight of the fetuses, induction of external and internal anomalies in the development of the fetus. The authors propose a correction of temporary acting threshold value of concentration in Bulgaria (10 mg m³) on the basis of the obtained results.

It is known that a number of chemical agents used in industry have an unfavorable effect on the development of the fetus and progeny [G. Goncharuk, 1968; A. Dyban et al., 1965; L. Ivanova-Chemishanska, 1969; E. Mirkova, 1975, etc.]. One of the most important tasks before public health experts and toxicologists is therefore to determine the causes of the later effects of poisoning with harmful industrial chemicals, and in particular the necessary evaluation of the risk of an smbryotoxic and tetratogenic action. Unfortunately, studies on these specific forms of the biological action of industrial chemicals in cases of exposure to low concentrations are still very inadequate. Studies at these exposure levels make it possible to determine the threshold of the embryotoxic effect, and are the basis for substantiating preventive measures for the protection of the prenatal period of human development [I. Sanotskii, 1972].

The subject of our experimental study was testing the embryotoxic and teratogenic activity of vinyl chloride during inhalation exposure in a concentration of 6.15 mg/m^3 -- lower than the accepted provisional Bulgarian standard in force (10 mg/m^3).

MATERIAL AND METHODS

The experiments were carried out on 40 pregnant white Wistar rats under conditions of daily inhalation exposure throughout gestation at an average daily vinyl chloride concentration of 6.15 mg/m^3. The animals were sacrificed by decapitation on the 21st day of pregnancy. During the autopsy, the number of implantation sites in the uterus were counted, the resorptions and autolyses, the number of corpera lutea of pregnancy [corpora lutea vera? -- *Tr. Ed.*] in the ovaries, and the number of live and dead fetuses were recorded, and the weight and craniocaudal dimensions of these latter were determined. The fetuses were macroscopically examined for the presence of external malformations, after which the routine teratologic method of J. Wilson [1965] as modified by A. Dyban et al. [1970] and A. Dawson [1926] was used to look for developmental anomalies in the internal organs and defects in skeletal ossification. The lethal effect of the vinyl chloride was calculated from the method of A. Malashchenko and I. Egorov [1967]. Using a biological test, a first generation was bred from mothers treated while pregnant and control fathers. Its postnatal development was monitored until the onset of sexual maturity; in evaluating the health of the progeny both the generally accepted criteria of physical development -- weight gain in the newborn and postnatal mortality -- and sensitive functional methods of investigation were used. The status of the detoxifying function of the liver was studied based on the length of hexabarbital sleep, while the excretory function was examined from the bile cholic acid level (Reinhold method, [1970]), and the activity of the bile enzymes LAP and GGTP (Fermognost tests).

The results were statistically treated using the Student-Fisher t criterion at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

The prenatal effect of vinyl chloride on the fetus is characterized by a marked embryotoxic action. The level of the overall embryonal mortality in the experimental group is double the control value. A breakdown of this indicates that the increase is due to an elevated postimplantation lethality. The lethal action of vinyl chloride is directed toward the stages of embryogenesis which occur immediately after blastocyst implantation. This is indicated by the more than eight-fold increase in the incidence of early postimplantation mortality (embryonal resorption), while the level of late postimplantation lethality is unchanged under the action of the test chemical. When pregnant animals are exposed to vinyl chloride at the earliest stages of embryogenesis, there is a tendency toward an increase in preimplantation mortality, but the changes recorded are statistically insignificant.

Vinyl chloride's embryotoxic action is also manifested in a reduction in fetal weight. In all probability, the suppression of overall development is due to a disturbance in homeostasis in the mother's body, and particularly to the impaired condition of the liver, resulting in a deficit of the plastic [nutrient? -- *Tr. Ed.*] and energy resources necessary for the vital activity of the developing embryo [A. Mikhailova, E. Mirkova, unpublished data].

The negative effect of vinyl chloride on the intrauterine development of the fetus is also manifested by a sharp -- eight-fold -- increase in general anomalies (hematomas) in comparison with the control. These vary in severity over a wide range, and are probably due to primary toxic damage to the fetal vessels.

Under the studied exposure conditions, vinyl chloride also had a teratogenic effect. External developmental anomalies, malformations of the internal

organs, and defects in skeletal ossification are induced. The defects in fetal development which arise do not cover a broad spectrum, and involve only morphogenesis and the brain tissue, the vascular walls, and sternal ossification. The disturbance in morphogenetic processes in the brain leads to the formation of internal hydrocephalus, combined with intracerebral hematomas in 54.4% of the fetuses examined, and to the isolated development of hematomas in 14.3%. External brain malformations (encephaloceles) and anomalies in sternal ossification (presence of additional ossification centers) are characterized by a low incidence -- 2.53% and 2.8% of the fetuses, respectively.

In the study of the postnatal development of the generation exposed in utero to vinyl chloride, no deviation in overall physical development and survival rate were found. The data from the functional studies conducted indicate a disturbance in the adaptive capacity of the progeny, however. Impairment of the liver's detoxifying function is observed in the progeny at the age of one month, as evidenced by prolonged hexabarbital sleep. The changes are persistent and are recorded in the second month of the postnatal period as well, after the onset of sexual maturity; the degree of their expression is the same in both sexes. As a result of the prenatal exposure to vinyl chloride, the excretory function of the liver is impaired as well. The reduction in bile cholic acid levels which was recorded is an early sign of developing toxic hepatopathy [A. Mikhailova, 1975]. The data obtained on the reduced rate of bile secretion, the reduction in the overall quantity of bile, and the decrease in the activity of the bile enzymes -- LAP and GGTP -- are also evidence of disturbances in the hepatobiliary system of the progeny as a consequence of intrauterine exposure to vinyl chloride.

CONCLUSIONS

1. When rats are exposed to it during pregnancy, vinyl chloride has a marked embryotoxic and teratogenic effect at a concentration of 6.15 mg/m^3. The overall embryonal mortality is increased, general physical development of the fetus is suppressed, and external and internal brain malformations and defects in sternal ossification are induced.

2. The postnatal development of the first generation of rats exposed in utero to vinyl chloride is disturbed. The changes recorded in the detoxifying and excretory functions of the liver are persistent.

3. The existence of a marked embryotoxic and teratogenic of vinyl chloride at a concentration of 6.15 mg/m^3 -- lower than the provisional Bulgarian health standard in force (10 mg/m^3) -- as well as the data on the impaired adaptive capacities of the progeny during the postnatal period, are the basis for reviewing this provisional MAC and indicate the need for lowering it.

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Tox. Appl. Pharm. Vol. 33, 1975

once daily for 7 days. Since pretreatment with 1254 at this dose caused loss of body weight and striking enlargement and pallor of the liver, lower doses were included. Controls were given H_2O by mouth. On the eighth day, after a 16-hr fast, animals were either sacrificed for determination of microsomal cytochromes b_5, P-448, P-450, NADPH-P-450 reductase and NADH-cytochrome-c reductase, oxidative-*N*-demethylase, and glucose-6-phosphatase, or exposed to 5% VCM for 6 hr and sacrificed 24 hr later. Liver injury as indicated by centrolobular vacuolization, focal midzonal necrosis, and increased serum transaminase activity was not apparent in H_2O-, SNL-, 3-MC-, or PCN-pretreated rats, while slight, moderate, and severe injury were consistently found in HCB-, PB-, and 1254-pretreated animals, respectively. In animals pretreated with 150 μmol 1254/kg, a dose which nearly tripled P-450, VCM produced the most severe injury of the combinations tested. Although liver injury was only found in animals pretreated with an agent which induced P-450, induction of P-450 alone did not correlate with injury. PCN which induced P-450 did not potentiate VCM injury. Other cellular components in addition to P-450 may be important in the potentiation of VCM toxicity. (Supported by NIH Grants ES-00002, OH-00315, AM-16183, and HL-06370.)

29. *Results of a Vinyl Chloride–Teratology Study in Mice, Rats, and Rabbits.* B. A. Schwetz, B. K. J. Leong, F. A. Smith, M. Balmer and P. J. Gehring, Toxicology Research Laboratory, Health and Environmental Research, The Dow Chemical Company, Midland, Michigan.

The potential of inhaled vinyl chloride monomer (VCM) to have a deleterious effect on the developing embryo and fetus of laboratory animals has been evaluated. In an initial experiment, CF-1 mice, Sprague–Dawley rats, and New Zealand white rabbits were exposed to 500 ppm VCM 7 hr daily during organogenesis. Some of the VCM-exposed mice were also treated with 15% ethanol (EtOH) in the drinking water as their only source of liquid to determine if concomitant ingestion of ethanol would alter the toxicity and/or teratogenicity of VCM. No signs of toxicity were observed in the adult rats or rabbits during exposure or at the time of necropsy and caesarean section. Among the rats, there was a significant decrease in fetal body weight but the incidence of external, soft tissue and skeletal malformations was not different than among control litters. There was no effect on fetal body measurements or the incidence of external, soft tissue or skeletal malformations in rabbits. Mice were more susceptible to VCM than either of the other two species. There was a slight decrease in the weight gain among mice and maternal deaths were observed (6 deaths/30 exposed mice). The percentage of pregnancy among mice was slightly lower than among controls. There was a slight increase in the incidence of resorptions and fetal body weights were slightly lower than control values but the incidence of external, soft tissue and skeletal malformations was not different than among controls. Concomitant treatment of mice with EtOH in the drinking water accentuated some of the toxic effects associated with exposure to VCM alone. The maternal weight gain and the pregnancy rate was further decreased, but the incidence of maternal deaths was not increased by concomitant EtOH ingestion. The incidence of cleft palate and certain skeletal anomalies was significantly increased among mice receiving EtOH plus VCM. Thus, based on the results of this initial study, exposure of bred mice, rats, and rabbits to 500 ppm VCM during organogenesis did not result in a teratogenic response. Mice were slightly more susceptible to the toxic effect of VCM than either rats or rabbits. Simultaneous exposure of mice to VCM by inhalation and EtOH in the drinking water resulted in toxic effects greater than that observed among mice exposed to either one alone. These results are being used to design additional studies to assess the toxicity of VCM.

30. *A Laboratory Aerosol System for Conducting Inhalation Toxicity Studies.* B. S. Brar, C. E. Trattor, C. R. Boshart and J. F. Noble, Toxicology Research Section, Lederle Laboratories Division of American Cyanamid Company, Pearl River, New York.

An aerosol system has been developed for the safety evaluation of drugs to be used in the respiratory tract. Design features of the equipment include: (1) a spinning disc generator

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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

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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 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-

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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/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-

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TABLE 1

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

Treatment 21 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	1–9	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
Phys. sal. s.c.[e]	7–8												
B VC inhalation[f]	1–9	19	55.18 ± 2.09	223	1	12	5.5†	11.68 ± 0.38	3.74 ± 0.03	0.58 ± 0.006	6.7	4.25 ±** 0.09	4.92 ±** 0.09
Phys. sal. s.c.[e]	7–8												
C Air inhalation	1–9	13	55.58 ± 2.86	131	3	32	21.1††	10.15 ± 0.64	3.71 ± 0.03	0.57 ± 0.008	8.3	4.16 ±** 0.08	4.70 ±** 0.09
Trypan blue s.c.[g]	7–8												
D VC inhalation[f]	1–9	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
Trypan blue s.c.[g]	7–8												
(II)													
A Air inhalation	8–14	14	49.38 ± 1.85	157	–	5	3.18	11.20 ± 0.61	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	3.75 ± 0.08	4.29 ± 0.11
B VC inhalation[f]	14–21	22	40.83 ± 1.67	241	–	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/m³ (~1600 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 C
FLUID O
EXPOSUF

Inhalation (mg/m³)
0
5500 (~2000
18000 (~7000
33000 (~12000

±, S.E.M.

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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 ($\mu g/ml$)	Fetal blood ($\mu g/ml$)	Amniotic fluid ($\mu g/ml$)
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 ($P \cong 0.05$); 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	–	–
No. of skeletal examined fetuses	–	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	4	3				
Shortness of [illegible]									

No. of alizarin stained fetuses	88	117	68	103	82	189	110	127	113
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 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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TRANSLATION:

Ungváry, Gy., A. Hudák, E. Tátrai, M. Lőrincz, and G. Folly: STUDY OF THE TERATOGENIC AND EMBRYOTOXIC EFFECT OF VINYL CHLORIDE IN CFY RATS*. (A vinilklorid teratogén és embriotoxikus hatásának vizsgálata CFY patkányokon.) *Egészségtudomany*, Vol. 21, pp. 363-369, 1977. National Institute of Occupational and Industrial Hygiene and Hungarian Academy of Sciences Research Institute of Experimental Medicine, Budapest.

Gy. Ungváry, Aranka Hudák, Erzsébet Tátrai, M. Lőrincz, G. Folly: Study of the Teratogenic and Embryotoxic Effect of Vinyl Chloride in CFY Rats

CFY rats in the 8—14 days of gestation were kept in inhalation chambers which were continuously ventilated vertically with air containing 4000 mg/m³ (cc 1500 ppm) vinyl chloride. Two control groups of the same gestation time were used. The first group was kept in a chamber ventilated with clean air and the second one under normal animal-house conditions. The animals were sacrificed on the 21st day of gestation and the offsprings were subjected to teratological studies. Groups of 3 CFY rats on the 18th day of gestation were subjected to inhalation with vinyl chloride of 5.500, 18.000 and 33.000 mg/m³ concentration respectively for two and a half hour and the vinyl chloride concentration was determined in the blood of the mothers and offsprings and in the amniotic fluids. The vinyl chloride failed to have teratogenic effect. Applied in the middle third period of gestation the above mentioned concentration did not induce embryotoxic effect. The vinyl chloride gets through the placenta of the rat foetus and it is demonstrable in the foetal blood and amniotic fluid.

Vinyl chloride (VC) is in 23rd place on the list of the 50 most widely used industrial chemicals. The yearly vinyl chloride production is ca. 8 billion kg (Teynolds et al. [20]) -- 2.3 billion kg in the U.S.A. (Boden [2]), and several million kg in Hungary. VC has a carcinogenic effect in rats, mice, and hamsters (Viola et al. [26], Maltoni and Lefemine [15, 16]). The first human case of hepatic angiosarcoma for which a causal relationship with exposure to VC could be demonstrated was autopsied in 1973 and published in 1974 [Creech and Johnsson [3]). In the WHO International Agency for Research on Cancer International Technical Report, 14 reported cases of hepatic angiosarcoma were mentioned in June 1974, and already 43 reported cases were mentioned in January 1975 [27, 29]. The occurrence of hepatic angiosarcoma due to exposure to VC

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has been corroborated by several authors (Thomas and Popper [25], Makk et al. [13], Smith et al. [22], Zimmermann and Eck [31], etc.).

After the confirmation of the carcinogenic effect, light was soon shed on the mutagenic effect of VC as well (more exactly, of chloroethylene oxide, a metabolite of VC) (Bartsch et al. [1], Malaveille et al. [14], Huberman et al. [9], Loprieno et al. [11], Rannug et al. [19], Barro et al. [7], etc.). Chromosome aberrations in persons exposed to VC for variable lengths of time have also been reported (Ducatman et al. [4], Funes-Cravioto [6], Szentesi et al. [24]).

Data on the teratogenic effect of VC are scanty, and the results of the epidemiological surveys are inconclusive. An increased incidence of malformations of the central nervous system due to VC in the vicinity of PVC polymerizing plants was reported by Infante (cited by Edmonds et al. [5]). Edmonds et al. [5], rechecking the relationship with VC, were unable to confirm these data. Infante et al. [10], who examined the wives of workers handling VC, observed increased fetal loss; this report was not considered accurate by Paddle [18] from a methodological point of view. Experimental studies were reported only by Schwetz et al. [21]: short-term exposure to VC at a concentration of 500 ppm for 7 hours/day during organogenesis failed to elicit a teratogenic effect in S-D rats, CF-1 mice, and N-Z rabbits.

Exact and detailed experimental teratological studies are definitely justified by the production of large amounts of VC, its wide use in the form of PVC, by the large number of persons exposed to VC, as well as by the demonstrated carcinogenic and mutagenic effects of the substance, and by the controversial epidemiological results.

In our study, we wished to examine the teratogenic or embryotoxic effect

of VC in rats. We also wished to determine if the VC inhaled by the mother is able to pass into the fetus through the placenta, thereby causing direct fetal exposure.

Female CFY rats weighing 240-280 g were mated in a harem system. We considered the day on the morning of which sperm were found in the vaginal smears taken daily from the animals was considered day 1 of the pregnancy. The animals were kept on standard LATI feed and tap water ad libitum throughout the experiment. The animals were weighed weekly.

From day 8 to 14 of the pregnancy, 28 animals were kept in two chambers with a volume of 0.13 m^3 each, through which air containing 4,000 mg/m^3 VC was circulated in a vertical direction at a rate of 2 m^3/hour. The temperature was 24-25°C and the humidity was 50-55% in the chambers. The VC concentration in the air of the chambers was measured by means of a Hewlett-Packard 5840 A digital gas chromatograph.

Fourteen control animals were kept in a similar chamber, through which only clean air was circulated, between days 8 and 14 of the pregnancy. Twenty-eight animals were used as untreated controls.

On day 21 of pregnancy the animals were killed by ether anesthesia. After opening of the abdominal wall, the uterine horns were opened, and the position and number of the live, dead, and resorbed fetuses were recorded. The fetuses and placentae were removed one by one, examined, and weighed. Half of the fetuses from each mother were fixed in Bouin's fluid and then autopsied under a steromicroscope (Wilson [29]). The other half of the fetuses were stained with alizarin S after fixing with alcohol (Staples and Schnell [23]) for the examination of the skeletal system. The mothers were autopsied and the livers removed and weighed.

Groups of 3 animals were exposed to VC concentrations of 5,500, 18,000, and 33,000 mg/m^3, respectively, for 2.5 hours on day 18 of pregnancy. We determined the VC levels in the maternal and fetal blood and in the amniotic fluid to clarify the transplacental passage of this substance into the fetuses. The method described by Lörincz [12] was used to determine the VC.

The data obtained were subjected to statistical analysis: we calculated the arithmetic means and standard error and employed the "two-tailed" [unsure of this -- *Tr. Ed.*] *t* test. We considered the litter (pregnant female) as a teratological unit (Haseman and Hogan [8]); we determined the incidence of all affected fetuses per litter. The statistical decisions were made by means f the Mann-Whitney *U* test performed with these incidences.

RESULTS

There was no maternal mortality. The gain in weight during pregnancy, expressed in % of the initial body weight, showed no differences among the three groups (Table 1).

The number of resorbed fetuses, as well as the fetal loss, expressed in % of the total number of implants, showed no statistically significant differences among the three groups, even though the number of resorbed fetuses was higher in the group which had inhaled VC; the number of implanted fetuses was also higher in this group (Tables 1 and 2). There were only two dead fetuses in the experiments: one in the group exposed to air, and another in the untreated control group. The average fetal and placental weights, as well as the ratio of the underweight fetuses (below 3.3 g) showed no significant differences among the three groups (Table 1).

The liver of the mothers inhaling VC was enlarged in terms of both the

TABLE 1. DISTRIBUTION OF THE MOTHERS AND FETUSES IN THE GROUPS AND SOME CHARACTERISTIC PARAMETERS. KEY: (a) treatment; (b) number of mothers; (c) gain in weight[1], %; (d) number of fetuses; (e) fetal loss[2], %; (f) average number of fetuses/mother; (g) average fetal weight, g; (h) average placental weight, g; (i) underweight fetuses[3], %; (j) inhalation of air between days 8 to 14 of pregnancy; (k) inhalation of vinyl chloride between days 8 to 14 of pregnancy; (l) live; and (m) resorbed, dead.

Kezelés (a)	Anyák száma (b)	Testsúly-gyarapodás[1] (c) %	magzatok száma (d)		magzati veszteség[2] % (e)	átlag magzat-szám/anya (f)	átlag magzat-súly g (g)	átlag placenta-súly g (h)	súlyelmaradást magzatok[3] % (i)
			élő (l)	resorbeált halott (m)					
—	28	52,45± 1,23	315	11	3,37	11,25± 0,64	3,85± 0,02	0,51± 0,005	2,90
levegő inhalációja a terhesség 8—14. napján (j)	14	49,38± 1,85	157	5	3,18	11,29± 0,61	3,96± 0,04	0,55± 0,007	0,30
vinilklorid inhalációja a terhesség 8—14. napján (k)	28	51,70± 1,12	374	17	4,54	13,36± 0,37	3,98± 0,02	0,52± 0,004	3,21

[1]Expressed in % of the initial body weight.

[2]Number of resorbed and dead fetuses, expressed in % of the total number of implantations (live + dead + resorbed fetuses).

[3]Number of fetuses weighing less than 3.3 g, expressed in % of all live fetuses.

TABLE 2. INCIDENCE OF FETAL RESORPTION. KEY: (a) number of implanted fetuses per litter; (b) number of resorbed fetuses per litter; (c) untreated controls; (d) inhalation of air; and (e) exposure to vinyl chloride.

Implantálódott magzatok száma családonként (a) / Resorbeált magzatok száma családonként (b)	kezeletlen kontroll (c)			levegő inhaláció (d)			vinilklorid inhaláció (e)		
	0	1	2	0	1	2	0	1	2
3		1							
4									
5		1							
6									
7				1					
8	1			1					
9	1			1					
10	2			1			1		
11	5			3			1	1	
12	4				1		1	2	1
13	5	2	1	2	1		2		
14	1		1		1		4	4	
15	1	1		1		1	3		2
16							1	2	1
17							2		

absolute liver weight and the liver weight expressed in % of the body weight. The latter value was also calculated for the adjusted body weight of the mothers (difference between the maternal body weight and the total weight of the fetuses and placenta). The difference proved to be significant in this case as well (Table 3).

Macroscopically visible developmental malformation was seen in only 1 out of the 847 embryos examined, in the group inhaling VC. The vertebral column of this underweight (3.0 g) fetus was excessively curved. Staining with alizarin S revealed the absence of thoracic vertebrae V, VI, and VII, and irregular, malformed vertebrae above and below the gap. Left ribs Nos. 5, 6, and 7 and right ribs Nos. 5 and 6 were also missing, and ribs 7 and 8 showed a blade-shaped fusion.

Autopsy of the fetuses revealed the following internal anomalies: thymus

TABLE 3. EFFECT OF VINYL CHLORIDE ON THE BODY WEIGHT AND LIVER WEIGHT OF THE ANIMALS. KEY: (a) treatment; (b) number of mothers; (c) body weight at time of sacrifice, g; (d) liver weight, g; (e) liver weight/body weight, %; (f) liver weight/ /adjusted[1] body weight, %; (g) inhalation of air between days 8 to 14 of pregnancy; and (h) inhalation of vinyl chloride between days 8 to 14 of pregnancy.

Kezelés (a)	Anyák száma (b)	Ölési testsúly (c) g	Májsúly (d) g	Máj/testsúly (e) %	Máj/redukált[1] testsúly (f) %
—	14	399,0± 6,20	15,51± 0,31	3,89± 0,06	4,50± 0,09
levegő inhaláció a terhesség (g) 8—14. napján	14	380,0± 8,86	15,42± 0,57	4,05± 0,08	4,67± 0,08
vinilklorid inhaláció a terhesség (h) 8—14. napján	28	395,0± 3,78	16,85±* 0,31	4,26±* 0,06	5,03±** 0,07

[1]Maternal weight -- (weight of fetuses + placentas).

*p <0.05.

**p <0.01.

with appendage, thymic hypoplasia, hydrocephalus internus, wide renal pelvis, dilated ureter, and dilated urinary bladder. All these anomalies were seen in the group inhaling VC at the same frequency as in the control group.

The fetal skeletal system was examined by alizarin S staining. We found the following anomalies. Signs of skeletal retardation: broad cranial fusions, large fontanels; two or more of the 6 ossification centers of the sternum are missing, or the majority of the ossification centers are hypoplastic; the cores of the ventral vertebral bodies are dumbbell-shaped or reduplicated, and rib No. 13 is of reduced length. Skeletal anomales: fused, irregular ossification centers in the sternum; supernumerary ribs or rib pairs. While the frequency of the signs of retardation was numerically higher in the group inhaling air than in the untreated controls and in the group inhaling VC, there

was no statistically significant difference either in the frequency of these signs or in the frequency of the anomalies (Table 4).

TABLE 4. INCIDENCE OF SIGNS OF SKELETAL RETARDATION. KEY: (a) number of affected/examined fetuses per litter; (b) number of litters; (c) untreated controls; (d) inhalation of air; and (e) inhalation of vinyl chloride.

(a) Családonként érintett/vizsgált magzatok száma	(b) családok száma		
	kezeletlen kontroll (c)	levegő inhaláció (d)	vinilklorid inhaláció (e)
0/9			1
0/8			4
0/7	4	2	8
0/6	11	4	8
0/5	7	1	5
0/4	2	1	
0/2	1		
0/1	1		
1/8			1
1/6		2	
2/5		2	
2/4		1	
3/8		1	
4/7			1

Multiple skeletal developmental malformation was found in a single fetus from the VC group, which was already mentioned.

A considerable VC concentration was found in the blood of the mothers exposed to VC concentrations of 5,500, 18,000, or 33,000 mg/m^3 for 2.5 hours on day 18 of pregnancy, as well as in the blood of their fetuses. VC was also detectable in the amniotic fluid (Table 5).

DISCUSSION

To investigate the effect of VC on organogenesis, we exposed the rats t ca. 1,500 ppm (4,000 mg/m^3) VC in the air continuously between days 8 and 14 of pregnancy. This concentration is 80 times higher than the MAC effective in

TABLE 5. VINYL CHLORIDE CONCENTRATIONS.
KEY: (a) chamber, mg/m^3; (b) maternal blood, µg/ml; (c) fetal blood, µg/ml; and (d) amniotic fluid, µg/ml.

Kamra mg/m³ (a)	Anyai vér (b) µg/ml	Magzati vér (c) µg/ml	Magzatvíz (d) µg/ml
0	0 ± 0	0 ± 0	0 ± 0
5 500	19,02 ± 1,70	12,80 ± 2,92	4,27 ± 0,42
18 000	32,40 ± 2,12	22,67 ± 2,75	4,92 ± 0,18
33 000	48,43 ± 1,95	30,52 ± 3,77	13,50 ± 2,99

Hungary (MAC value in Hungary: 50 mg/m^3 = ca. 20 ppm), i.e., it is a high concentration. Yet this high VC concentration failed to cause any noticeable change in the animals' behavior: their feed and water consumption was the same as that of the controls, and there was, in principle, no reason to expect the so-called "self-protective mechanism"-inducing effect of VC, either (Reynolds et al. [20]). We should mention, however, that the liver weight of the animals exposed to VC was increased, and the cause is currently unknown. Continuous exposure generates a steady VC level in the blood of rats (Withey [30]). This steady blood level and the rapid interstitial migration of the low-molecular-weight VC (Withey [30]) suggest that not only the mothers, but also the fetuses are exposed to VC steadily. This is also supported by the results reported in this paper: VC was detectable in the blood of the mothers exposed on day 18 of pregnancy, and also in the fetal blood, as well as in the amniotic fluid. The hemoendothelial placenta of the rat represents no barrier to VC.

These examinations, carried out on a large number of fetuses, have basically yielded a negative result. Yet two findings which, though not significant statistically, show a numerical difference, deserve to be analyzed briefly:

1. The number of resorbed fetuses was increased in the group inhaling VC; the number of implantations as well as the number of the live fetuses per mother were also higher in this group. The increased resorption may possibly be attributed to the higher number of implantations.

2. The frequency of the signs of skeletal retardation was numerically higher in the group inhaling air than in the group exposed to VC; this value was higher in both these groups than in the untreated control group. This phenomenon can be explained by the stressful effect of the unusual environment in the inhalation chambers. The stress effect was fended off partially by the VC, having a narcotic effect.

Consequently, it can be said in the light of these results that VC as such is not teratogenic in CFY rats, nor does it have an embryotoxic effect in the concentration applied in this species. Schwetz et al. [21] also arrived at a similar conclusion. However, they drew their conclusion on the basis of examinations following exposures during short, arbitrarily chosen periods of organogenesis. We wish to emphasize the importance of exposure of the animals to the test substance at all relevant moments of organogenesis in experimental teratology. This is especially important in the case of such rapidly excreted inhaled toxic substances as VC. Therefore, we believe that the teratogenic effect of this substance can be ruled out only on the basis of examinations following exposure covering the entire duration of organogenesis, as was employed in our experiment.

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