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

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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

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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

*$p < 0.001$.
†$p < 0.01$

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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	21
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[b]	77.28[b]
2	6.4	7.9	9.6	6.9	44.6[b]	31.5[b]
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] $p < 0.01$.

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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[a]	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] $p < 0.05$.

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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"	20
2	39	36	18	18	17	15	3	4	2"	23
3	45	37	18	16	15	11	4	1	7"	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

"$p < 0.001$.
'$p < 0.01$.

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"
2	0.69	0.75	0.56	0.71	4.04"
3	0.76	0.68	0.46	0.80	2.32"
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

"$p < 0.001$.

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"
2	5.5	6.9	4.4	5.7	41.4"
3	6.4	6.3	3.6	5.3	21.8"
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

"$p < 0.001$.

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

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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.

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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.

The authors would like to thank the Inhalation Section for exposing the males to VC and VDC, Mrs. S. Palmer for her technical assistance, and Mr. T. Weight and Mr. S. H. Ellis for their statistical evaluation of the data. All personnel mentioned are at this laboratory except Mr. S. H. Ellis, who is at ICI Pharmaceuticals Division, Alderley Park.

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GGC 000215

Popper about the reversibility of the phenomenon, particularly if caught early. If workers are exposed to moderate to low levels for relatively short periods of time, has there been any evidence that there is any irreversible change, or does the progression that you were documenting take time?

DR. POPPER: The only real experience we had was a case which Dr. Berk presented. In this case, after discontinuation of the exposure, fibrosis persisted, but other features were absent. The characteristic disturbing activation of the hepatocytes was no longer seen. I am not surprised. From experience with hepatic fibrosis in circumstances other than vinyl chloride, it is known that fibrous tissue may not disappear once present.

I suspect, but do not know, that the person may be left with innocent scars in the liver which possibly may not interfere with his life expectancy. One patient, however, is really no proof.

GGC 000216

PART II. CARCINOGENESIS ASSOCIATED WITH VINYL CHLORIDE

A. Experimental Studies

CARCINOGENICITY BIOASSAYS OF VINYL CHLORIDE: CURRENT RESULTS

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INTRODUCTION

Vinyl chloride (VC) has been produced in many countries for more than 30 years. It is the constituent of polyvinyl chloride (PVC), which nowadays is the plastic industrial material most used and diffused in the world. Moreover, it is used as a copolymer in Saran and other plastics, as a chemical intermediate, as a solvent, and as a propellant: in the past, for a short period, it was also used as an anesthetic. VC is at present produced at a rate of near 12 million tons per year.

The following population groups may be exposed to VC: (1) workers engaged in the production of PVC and of VC and in the use of VC for other industrial purposes; (2) workers manufacturing PVC, (3) residents in areas neighboring on factories producing PVC and VC; and to a much lesser extent (4) people using products containing VC, as some propellent sprays; (5) people having contact with resins made with VC and with materials containing these resins; and (6) populations consuming food contained in plastics made with VC. Bioassays on the long term effects of VC were not available until 1970.

This situation reflects the limited consideration which has been, up to the present, given to experimental bioassays in predicting the oncogenic effects on man of environmental and occupational agents. In part this is due to industrial and economical interests, in part to deficiencies in technical-scientific facilities, to the dilettantism of many experimental workers, and to the inadequacy of many experiments performed in the past.

What is now happening with vinyl chloride and the story of vinyl chloride carcinogenicity should bring about greater consideration of experimental bioassays and finally induce a new turn in the field of occupational and environmental carcinogenesis. In other words, experimental prediction on the pathogenetic potential of occupational and environmental agents before they are produced and released on a large scale into the human environment should preclude the need for later epidemiological evidence.

HISTORY

In 1970 Professor Viola presented, at the X International Cancer Congress (Houston), the results of a pilot experiment, which then were extensively published in 1971.[1] Professor Viola reported to have observed in 26 rats exposed by inhalatory route to 30,000 ppm of VC 4 h daily, 5 days weekly, for 10–12 months, surviving 10 months or more, 17 skin tumors, 7 pulmonary tumors, mainly carcinomas, and 5 osteochondromas.

In 1967 our Institute started a large investigation, among workers of several Italian industrial plants, to determine the effects of the exposure to different chemicals on respiratory epithelia. The pathological changes were studied by sputum cytology. In 1970 this research was extended also to workers in VC-PVC industries. A significant increase of cellular changes in bronchial epithelium, such as marked squamous metaplasia and squamous dysplasia, were found among the workers more heavily exposed to VC.

Immediately after the Houston Congress, we contacted Professor Viola who kindly put some slides of his experimental tumors at our disposal. According to our interpretation, the malignant tumors observed by Professor Viola in the skin were carcinomas arising from the Zymbal gland (a sebaceous gland of the exterior acoustic duct), which is responsive to a large number of carcinogens. The malignancies of the lung, in our opinion, were metastases from Zymbal gland tumors.

At that time (end of 1970) we planned a project of correlated investigations to further clarify the type and degree of the carcinogenic risk from VC. The project was encouraged and supported by Montedison (Italy), which later was joined by other major European companies, that is, ICI (U.K.), Solvay (Belgium), and Rhone-Progil (France).

The Head of the Health Services of Montedison supplied us with all the technical data dealing with occupational exposure: this made possible the reproduction in our experiments of the most important parameters of occupational conditions.

Planning, Materials, and Methods

This project included a series of experiments, which were started in sequence. They were designed to study the effects of VC, administered through different routes (inhalation, ingestion, and peritoneal and subcutaneous injection), at different concentrations, for varying periods of time, by continuous or intermittent treatment, on animals of different species (rats, mice, hamsters), strains (Sprague-Dawley and Wistar rats), sex and age (adults, newborns, embryos).

The planning of the program and the preparation of suitable experimental conditions, particularly the building of an apparatus for inhalatory exposure, took us until May 1971. In July 1971 we started experimenting. The chambers of exposure are built basically of stainless steel and glass. They have been designed to deliver concentrations varying from 30,000 to 1 ppm of VC, and to treat simultaneously nearly 1,500 rodents. The VC concentration in the chambers is controlled by gas chromatography.

The VC is supplied by Montedison. Each stock is analyzed in the Montedison Research Unit. The impurities and their maximal levels in the VC employed have been as follows:

H_2O	100 ppm
Acetic aldehyde	5 ppm
Acetylene	2 ppm
Allene	5 ppm
Butane	8 ppm
1,3-Butadiene	10 ppm
Chlorophene	10 ppm
Diacetylene	4 ppm
Vinyl acetylene	10 ppm
Propine	3 ppm
Methyl chloride	100 ppm

The experiments utilized mainly Sprague-Dawley rats; Wistar rats, Swiss mice, and hamsters have also been used. All the animals, except the hamsters, are bred in our Institute for years and, whatever their use, are all examined at death by complete autopsy, giving us extensive information concerning their current pathology.

The animals have been weaned and classified by sex when 4–5 weeks old, at which time they have been numbered by ear punch and divided into groups by litter distribution. From weaning, the animals have been fed, *ad libitum*, an adequate commercial diet. During the period of treatment, the animals are housed in groups of 10 in stainless steel wire cages with a solid bottom of the same metal. After the period of experimental treatment, the animals are kept in groups of 5 in makrolon cages, with tops made of stainless steel wire. A shallow layer of white wood shavings serves as bedding. The animals are kept in a temperature-controlled laboratory at 19–20°C.

Fifteen experiments have been started in sequence. Some of them were programmed at the beginning; for some others the need appeared on the basis of the first experimental results. The plan of the experiments and the up-to-date situation is shown in Table 1.

Experiment 1 studies the effect of the atmospheric exposure to 10,000, 6,000, 2,500, 500, 250, 50 ppm of VC, 4 h daily, 5 days weekly, for 12 months. Two groups of animals were added as control: one untreated, as for all the following experiments, and one treated with vinyl acetate (VA) at 2,500 ppm, under the same conditions. This dose of VA appeared to be the maximum possible dose for a chronic exposure.

Experiment 2 was performed to investigate in a larger number of animals the effect of doses between 250 to 50, that is 200, 150 and 100, under the same conditions as in Experiment 1. It was started after it was clear that a dose of 250 ppm was still showing oncogenic effects.

Experiment 3 was planned to study the effects of a shorter period of exposure, keeping all the other conditions of Experiment 1.

Experiment 4 studies the effects of the same type of exposure as in Experiment 1 in another species (mice). The treatment lasted 7 months in consideration of the higher mortality and the shorter life span of the mice of our line as compared with rats.

Experiment 5 was undertaken as a pilot investigation on the possible effects of the atmospheric exposure, during pregnancy, on offspring.

Experiment 6 was planned to reproduce the same conditions as in Professor Viola's experiments.

Experiment 7 was planned to study the effects of the same type of exposure as in Experiment 1 in another strain of rats (Wistar).

Experiment 8 studies the effects of the same exposure as in Experiment 1, in a third species (hamsters) for the same period as in Experiment 4.

Experiment 9 was planned, before tumors were observed at 50 ppm in experiment BT1, to assess further whether inhalation of VC has any effect at a dose level of 50 ppm.

Experiment 10 studies whether a high, but limited and/or intermittent atmospheric exposure has any effect.

Experiment 11 was performed to evaluate the effects of VC by ingestion.

Experiment 12 studies the third general route of exposure.

Experiment 13 was started to assess whether VC acts on tissues directly or through metabolites.

Experiment 14 has been included to study the responsiveness of newborns.

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TABLE 1
PLAN OF THE EXPERIMENTS UP TO MAY 5, 1974

Exp No	Treatment			Animals							Length of the Experiments at the Present Date (weeks)
	Route	Doses of VC	Length	Species	Strain	Age (weeks)	No ♀	No ♂	No Total	No Per group	
BT1	Inhalation	10,000, 6,000, 2,500, 500, 250, 50 ppm Untreated controls Treated controls: VA 2,500 ppm	4 h daily, 5 days weekly, 52 wk	Rat	Sprague-Dawley	13	268	309	577	64-96	135 (Ended)
BT2	Inhalation	200, 150, 100 ppm Untreated controls	4 h daily, 5 days weekly, 52 wk	Rat	Sprague-Dawley	13	280	265	545	120-185	43
BT3	Inhalation	10,000, 6,000, 2,500, 500, 250, 50 ppm Untreated controls	4 h daily, 5 days weekly, 17 wk	Rat	Sprague-Dawley	21	262	288	550	60-190	68
BT4	Inhalation	10,000, 6,000 2,500, 500, 250, 50 ppm Untreated controls	4 h daily, 5 days weekly, weekly, 30 wk	Mouse	Swiss	11	250	260	510	60-150	43
BT5	Transplacental	10,000, 6,000 ppm	4 h daily, 7 days (from 12th to 18th day of pregnancy)	Rat	Sprague-Dawley	19 (breeders) 12 days (embryos)	110	36	146	30-54	77
BT6	Inhalation	30 000 ppm	4 h daily, 5 days weekly, 52 wk	Rat	Sprague-Dawley	17	30	30	60	60	43
BT7	Inhalation	10 000, 6,000, 2,500, 500, 250, 50 ppm Untreated controls	4 h daily, 5 days weekly, 52 wk	Rat	Wistar	11	—	220	220	30-40	43
BT8	Inhalation	10,000, 6,000, 2,500, 500, 250, 50 ppm Untreated controls	4 h daily, 5 days weekly, 30 wk	Hamster	Golden	11	—	268	268	32-70	30
BT9	Inhalation	50 ppm Untreated controls	4 h daily, 5 days weekly, 52 wk	Rat	Sprague-Dawley	11	200	200	400	100 (c) 300 (t)	15
BT10	Inhalation	10,000, 6,000 ppm Untreated controls	4 h daily, 5 days weekly, 5 wk; 4 h daily, 1 day weekly, 25 wk, 1 h daily, 4 days weekly, 25 wk	Rat	Sprague-Dawley	11	420	420	840	120	15
BT11	Ingestion	16 6, 33 2, 50 mg/kg body weight in olive oil Controls, olive oil	5 times weekly, 52 wk	Rat	Sprague-Dawley	13	160	160	320	80	15
BT12	Endoperitoneal injection	4 25 mg in 1 0 cc olive oil Controls, 1.0 cc olive oil	4, 3, 2, umes by 2 months and once	Rat	Sprague-Dawley	13	150	150	300	60	15
BT13	Subcutaneous injection	4 25 mg in 1 0 cc olive oil Controls: 1 0 cc olive oil	1 injection	Rat	Sprague-Dawley	21	80	70	150	75	15
BT14	Inhalation	10,000, 6,000 ppm	4 h daily, 5 days weekly, 5 wk	Rat	Sprague-Dawley	1 day	45	44	89	43-46	10
BT15	Inhalation	25, 10, 5 ppm Untreated controls	4 h daily, 5 days weekly, 52 wk	Rat	Sprague-Dawley	13	240	240	480	120	Just started

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TABLE 2
EXPERIMENT BT1: RESULTS AFTER 135 WEEKS (END OF THE EXPERIMENT)

Groups and Treatment	Animals (Sprague-Dawley Rats)		Animals with Tumors															
			Zymbal gland carcinomas[b]			Nephroblastomas[c]			Angiosarcomas									
									Liver[d]			Other sites	Subcutaneous angiomas	Skin carcinomas	Hepatomas	Brain neuroblastomas	Other type and/or site[e]	Total[f]
	Total	Corrected No.	No	%	Average latency time (wk)	No.	%	Average latency time (wk)	No.	%	Average latency time (wk)							
I: VA, 2,500 ppm	96	49																
II: VC, 10,000 ppm	69	61	16	26	50	5	8	59	9	15	64	3[g]	4	3	1	7	7[h]	38
III: VC, 6,000 ppm	72	60	7	12	62	4	7	65	13	22	70	3[i]	3	1	1	3	8[j]	31
IV: VC, 2,500 ppm	74	59	2	3	33	6	10	74	13	22	78	3[k]	3	1	2	5	4[l]	32
V: VC, 500 ppm	67	59	4	7	79	4	7	83	7	12	81	2[m]	1	1	3		4[n]	22
VI: VC, 250 ppm	67	59				6	10	80	4	7	79	2[o]		4			3[p]	16
VII: VC, 50 ppm	64	59				1	2	135	1	2	135	1[q]	1	1			9[r]	10
VIII: No treatment	68	58															10[s]	6
Total	577	461	29			26			47			14	12	11	7	15	45	155

[a] Alive animals after 26 weeks, when the first tumor (a Zymbal gland carcinoma) was observed. The percentages are referred to the corrected number.
[b] Metastases to lung
[c] Metastases to liver, lung, spleen, and brain.
[d] Metastases to lung.
[e] Several cases of breast fibroadenomas, adrenal and pituitary tumors (generally adenomas) have not been considered, since their distribution in the different groups does not vary.
[f] Several animals with 2 or more tumors.
[g] One angiosarcoma of the lips; 1 angiosarcoma of the nose; 1 intraabdominal angiosarcoma (next to liver).
[h] Two Zymbal gland adenomas, 3 mammary carcinomas; 1 neurilemmioma; 1 ovarian cystoadenocarcinoma.
[i] One angiosarcoma in subcutaneous fibrosing angioma; 1 ossifying parauricular angiosarcoma; 1 intraabdominal angiosarcoma (next to liver).
[j] Four Zymbal gland adenomas; 1 salivary gland adenocarcinoma; 2 hepatic and 1 peritoneal angiomas.
[k] Two intraabdominal angiosarcomas (1 next to spleen and 1 next to ovary); 1 ossifying angiosarcoma of neck.
[l] One Zymbal gland adenoma; 1 mammary carcinoma; 2 ependymomas.
[m] One pulmonary angiosarcoma, 1 angiosarcoma of uterus.
[n] One mammary carcinoma; 2 lymphomas; 1 pulmonary fibrosarcoma.
[o] One intraabdominal angiosarcoma (next to spleen); 1 intrathoracic ossifying angiosarcoma.
[p] One Zymbal gland adenoma; 1 mammary carcinoma; 1 lymphoma.
[q] One intraabdominal diffused angiosarcoma.
[r] Three Zymbal gland adenomas; 2 mammary carcinomas; 1 subcutaneous angiopericytoma; 3 uterine adenocarcinomas (1 with sarcomatous component)
[s] One invasive acanthoma of Zymbal gland; 1 subcutaneous fibrosarcoma; 2 peritoneal fibroangiomas; 2 uterine adenocarcinomas (1 with sarcomatous component); 1 uterine leiomyosarcoma; 1 ovarian fibrosarcoma; 1 pulmonary rhabdomyosarcoma; 1 lymphoma.

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Experiment 15 was started as soon as we found 1 liver angiosarcoma, 1 extrahepatic angiosarcoma, and 1 nephroblastoma in 3 animals of the first experiment, exposed to 50 ppm of VC for 1 year, and surviving 135 weeks from the beginning of the treatment.

The animals are controlled weekly and weighed every 2 weeks during the period of treatment and monthly after the treatment is over. All the detectable gross pathological changes are recorded during the control. All the animals are kept under observation until spontaneous death. The animals, when moribund, are isolated, in order to avoid cannibalism.

A complete autopsy is made on each animal. Histological examinations are performed on Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, liver, kidneys, spleen, stomach, different segments of the intestine, bladder, brain, bones of the legs and feet, and any other organ with pathological lesions.

All the animals exposed to the highest doses (30,000 and 10,000 ppm), with or without tumors, are examined radiologically, during treatment and/or at death; moreover, radiological examinations are made on all animals bearing tumors, although exposed to the lower doses.

Results

The current results of several experiments are shown in the Tables 2–9. Some others are still not available.

Tumors of different types may be present in the same animals.

Zymbal gland carcinomas may be bilateral. Histologically they may reproduce, to some extent, the structure of the normal Zymbal gland (Figure 1), but often they present squamous, glandular, solid, anaplastic, and polymorphous patterns. Several of these different pictures may be observed in the same tumor. Metastases of these tumors are sometimes found in the lung (Figure 2).

Nephroblastomas are often bilateral. Their histological picture reminds us of an embryonal kidney (Figure 3). They metastasize to the liver, spleen, lung, and brain. Hyperplasia of nephroblastoma-like tissue is often observed in the kidney of treated rats, with or without nephroblastoma.

Angiosarcomas and angiomas may be found at more than one site in the same animal. In liver they are often polycentric. Hepatic and extrahepatic angiosarcomas are more or less differentiated (Figures 4–9). They are morphologically

Table 3
Experiment BT6: Results After 41 Weeks

Groups and Treatment	Animals (Sprague-Dawley Rats)			Animals with Tumors							
				Zymbal gland carcinomas			Nephroblastomas	Angiosarcomas		Other type and/or site	Total
	Total	Corrected no.* (a)	Survivors	No.	%	Average latency time (wk)		Liver	Other sites		
I: VC, 30,000 ppm	60	60	48	12	20	35					12

* Animals alive after 24 weeks, when the first tumor (a Zymbal gland carcinoma) was observed. The percentages are referred to the corrected number.

Table 4
Experiment BT2: Results After 41 Weeks

Groups and Treatment	Animals (Sprague-Dawley Rats)		Animals with Tumors			
	Total	Survivors	Zymbal gland carcinomas	Nephroblastomas	Angiosarcomas	Other type and/or site
I: VC, 200 ppm	120	115				1*
II: VC, 150 ppm	120	109				
III: VC, 100 ppm	120	112				
IV: No treatment	185	171				
Total	545	507				1

* One subcutaneous fibroangioma

GGC 000220

similar in rats, mice, and hamsters. Angiosarcomas frequently metastasize; as far as the liver angiosarcomas are concerned, the lung is the most frequent site of distant metastases.

Blood vessels ectasias, endothelial hyperplasia, associated or not associated with cellular atypia, are often observed in the liver and in other organs and tissues in treated animals, with or without local or distant angiosarcomas or angiomas. Therefore, the effect of VC on blood vessels and endothelia should be considered systematic.

Angioblastic proliferation, angiomas, and angiosarcomas in the liver and in other sites may or may not be associated with fibroplasia. From the histological observations, the sequence of local changes in the genesis of malignant vascular tumors appears to be as follows: endothelial hyperplasia, angiomas, atypias of endothelial cells in hyperplasia (dysplasia) and angiomas, angiosarcomas.

Fibroangioblastic proliferation undergoing fibrosis is frequently observed in the spleen of the treated rats and mice.

Skin carcinomas in treated rats appear frequently to arise from sebaceous glands, of which they may reproduce in some extent the morphological characters (Figure 10).

The hepatomas observed in treated rats are usually well differentiated. Metastases have been observed in the lung.

Brain neuroblastomas are very similar to the human medulloblastomas (Figure 11). Since they may be quite small and therefore not easily macroscopically detectable, we are now making serial sections of the brains of the treated rats, to obtain more precise information on their real incidence.

The lung adenomas in treated mice are usually multicentric; their number is higher in animals treated with heavier doses. Many of these tumors appear to undergo early malignant transformation.

Mammary carcinomas may be solitary or multiple. They are usually well differentiated adenocarcinomas, with monomorphous or polymorphous arrangement. The most distinct feature of these tumors is the frequency with which they present areas of squamous metaplasia (Figure 12).

Up to the present no acroosteolytic lesion has been observed on our treated animals.

Several of the tumors produced by VC are quite rare or exceptional. To our knowledge liver angiosarcomas, nephroblastomas, and neuroblastomas have

TABLE 5
EXPERIMENT BT3: RESULTS AFTER 66 WEEKS*

Groups and Treatment	Animals (Sprague Dawley Rats)		Animals with Tumors						
	Total	Survivors	Zymbal gland carcinomas	Nephroblastomas	Angiosarcomas: Liver	Angiosarcomas: Other sites	Brain neuroblastomas	Other type and/or site	Total
I: VC, 10,000 ppm	60	29	4 (15)	— (4)	— (6)	— (2)	3 (6)	1† (12)	8 (35)
II: VC, 6,000 ppm	60	42	2 (3)	— (2)	— (6)	— (2)	— (3)	2‡ (3)	4 (17)
III: VC, 2,500 ppm	60	51	— (2)	— (4)	— (5)	— (1)	— (1)	— (3)	1 (14)
IV: VC, 500 ppm	60	54	— (1)		— (1)			1§ (2)	1 (4)
V: VC, 250 ppm	60	39		1	— (2)			— (1)	1 (3)
VI: VC, 50 ppm	60	41							
VII: No treatment	190	166						1¶	1
Total	550	422	7 (21)	1 (10)	— (20)	— (5)	3 (10)	5 (21)	16 (73)

* In parentheses are recorded the tumors found in experiment BT1 after 66 weeks.
† One fibroepithelial papilloma of the skin.
‡ One squamous carcinoma of the skin, 1 subcutaneous angioma.
§ One lymphoma.
¶ One lymphoma.

TABLE 6
EXPERIMENT BT7: RESULTS AFTER 41 WEEKS

Groups and Treatment	Animals (Wistar Rats)		Animals with Tumors*				
	Total	Survivors	Zymbal gland carcinomas	Nephroblastomas	Angiosarcomas: Liver	Angiosarcomas: Other sites	Other type and/or site	Total
I: VC, 10,000 ppm	30	27	— (1)			— (1)	— (1)	— (2)
II: VC, 6,000 ppm	30	26						— (1)
III: VC, 2,500 ppm	30	23	— (1)					— (1)
IV: VC, 500 ppm	30	27	— (1)				1†	— (1)
V: VC, 250 ppm	30	26						1
VI: VC, 50 ppm	30	28						
VII: No treatment	40	38						
Total	220	195	— (3)			— (1)	1 (1)	1 (5)

* In parentheses are recorded the tumors found in male Sprague-Dawley rats of experiment BT1, after 41 weeks.
† One angioma of the cecum.

TABLE 7
EXPERIMENT BT5: RESULTS AFTER 75 WEEKS

Groups and Treatment	Animals (Sprague-Dawley Rats)		Animals with Tumors				
	Total	Survivors	Zymbal gland carcinomas	Nephroblastomas	Angiosarcomas: Liver	Angiosarcomas: Other sites	Other type and/or site	Total
I: VC, 10,000 ppm (breeders)	30	21						
II: VC, 6,000 ppm (breeders)	30	24						
III: VC, 10,000 ppm (offsprings)	54	48				1*		1
IV: VC, 6,000 ppm (offsprings)	32	30				1†		1
Total	146	123				2		2

* Subcutaneous angiosarcoma on a 34-week-old male.
† Subcutaneous angiosarcoma on a 32-week-old female.

never been described as spontaneously occurring in rats. Liver and extrahepatic angiosarcomas have been induced only in a few instances in experimental rodents (rats, hamsters, and mice) (for references see Maltoni and Lefemine). As far as we know this is the first time that nephroblastomas and neuroblastomas have been experimentally reproduced.

CONCLUSIONS

1. Under our experimental conditions, VC produced tumors in the three animal species studied: rats, mice, and hamsters (as far as the last species is concerned, the data should still be considered as preliminary).

GGC 000221

TABLE 8
EXPERIMENT BT1. RESULTS AFTER 41 WEEKS

Groups and Treatment	Animals (SAS-Mice)									Animals with Tumors										
	Total			Corrected number[a]			Survivors			Pulmonary tumors[b]			Mammary carcinomas[c]			Liver angiosarcomas	Vascular tumors of other type and/or site	Epithelial tumors of the skin	Other type and/or site	Total[d]
	♂	♀	♂♀	♂	♀	♂♀	♂	♀	♂♀	No	%	Average latency time (wk)	No	%	Average latency time (wk)					
I: VC, 10,000 ppm	30	30	60	22	28	50	1	7	8	27	54	31	9	18	28	1	7[e]	2[f]		28
II: VC, 6,000 ppm	30	30	60	26	28	54	4	12	16	22	40	33	8	14	33	2	1[g]	3[h]	1[i]	27
III: VC, 2,500 ppm	30	30	60	23	30	53	9	15	24	12	22	35	4	7	32	4	1[j]	2[k]		13
IV: VC, 500 ppm	30	30	60	29	29	58	7	21	28	16	27	34	2	3	33	4	1[l]	1[m]		17
V: VC, 250 ppm	30	30	60	29	29	58	19	11	33	11	19	34	6	10	30	3	1[n]			15
VI: VC, 50 ppm	30	30	60	27	30	57	11	18	29				7	12	35		4[o]			8
VII: No treatment	80	70	150	71	67	141	51	57	108	1	0.7	39								1
Total	260	250	510	230	241	471	102	141	246	89			36			17	15	8	1	109

[a] Alive animals after 16 weeks, when the first tumor (a mammary carcinoma) was observed. The percentages are referred to the corrected number.
[b] Adenomas, some of which undergoing malignant transformation.
[c] In females.
[d] Several animals with 2 or more tumors.
[e] Three subcutaneous angiomas, 2 liver fibroangiomas, 1 heart fibroangioma, 1 ossifying interscapular angioma.
[f] One squamous carcinoma and 1 invasive acanthoma of the skin.
[g] One hepatic angioma.
[h] One squamous carcinoma and 1 acanthoma of the skin, 1 squamous carcinoma of the nose.
[i] One lymphoma.
[j] One renal angiosarcoma.
[k] One squamous carcinoma and 1 acanthoma of the skin.
[l] One subcutaneous angioma.
[m] One acanthoma of the skin.
[n] One hepatic angioma.
[o] Two subcutaneous angiomas, 2 hepatic fibroangiomas.

EXPERIMENT BT6. RESULTS AFTER [illegible]

Groups and Treatment	Animals (Golden Hamsters)		Animals with Tumors				
	Total	Survivors	Liver angiosarcomas	Skin trichoepitheliomas	Lymphomas	Melanomas	Total
I: VC, 10,000 ppm	35	19		1			1
II: VC, 6,000 ppm	32	21		1	1	1	1
III: VC, 2,500 ppm	33	19					
IV: VC, 500 ppm	33	23	—†		1		1
V: VC, 250 ppm	32	18					
VI: VC, 50 ppm	33	23			1		1
VII: No treatment	70	49					
Total	268	172		2	3	1	4

* One animal with 3 tumors
† At the date (September 1974) on which the final draft of this report was submitted for publication, 1 liver angiosarcoma was observed in this group.



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FIGURE 1. Well differentiated Zymbal gland carcinoma in rat. H&E, × 190.



FIGURE 2. Pulmonary metastasis of well differentiated Zymbal gland carcinoma in rat. H&E, × 190.

2. The range of induced tumors varies to some extent from species to species. When given by inhalation VC produced: in rats, Zymbal gland carcinomas, nephroblastomas, angiosarcomas, and angiomas of the liver and other sites, skin carcinomas, hepatomas, and brain neuroblastomas; in mice, lung adenomas (frequently undergoing malignant transformation), mammary carcinomas, of a peculiar type, angiosarcomas and angiomas of the liver and of other sites, skin epithelial tumors; in hamsters, liver angiosarcomas and, as early evidence seems to suggest, skin trichoepitheliomas and lymphomas. Incidentally, liver angiosarcomas have been observed in all three animal species.

3. In the BT1 and BT4 experiments, VC shows a carcinogenic effect at 50 ppm.

4. From the BT1 experiment (the only one completed) a dose-response relationship clearly emerges, as far as angiosarcomas and nephroblastomas are concerned, in the lower dose ranges: from 500 to 50 ppm for angiosarcomas and from 250 to 50 ppm for nephroblastomas.



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FIGURE 3. Nephroblastoma in rat. H&E, × 470.

5. A comparison of the results available at the present moment in rats exposed for 12 and 4 months (BT1 and BT3 experiments) shows that the neoplastic response, as far as angiosarcomas and nephroblastomas are concerned, is affected by the length of exposure to VC.

6. The comparison of the results obtained in rats of two different strains, i.e. Sprague-Dawley (BT1) and Wistar (BT7), at the present moment, seems to suggest that the strain factor considerably affects the neoplastic response.

7. The onset of two subcutaneous angiosarcomas in the offspring of breeders exposed during pregnancy for 7 days appears to indicate a transplacental effect of VC.



FIGURE 4. Liver angiosarcoma in rat. H&E, × 190.

8 The observation of a few cases of ossifying angiosarcomas suggests a new orientation in the pathogenetic interpretation of acroosteolysis in workers exposed to VC.

The results presented in this report should be considered both preliminary and incomplete: preliminary since most of the experiments are still in progress, and some at the initial stages; incomplete because a more profound and systematic microscopical examination, which is now under way, of further serial histological sections, may disclose new initial, minute, undetected tumors. For the latter reason previous reports have been even more incomplete. The release of the preliminary and incomplete results has been made necessary because of the importance

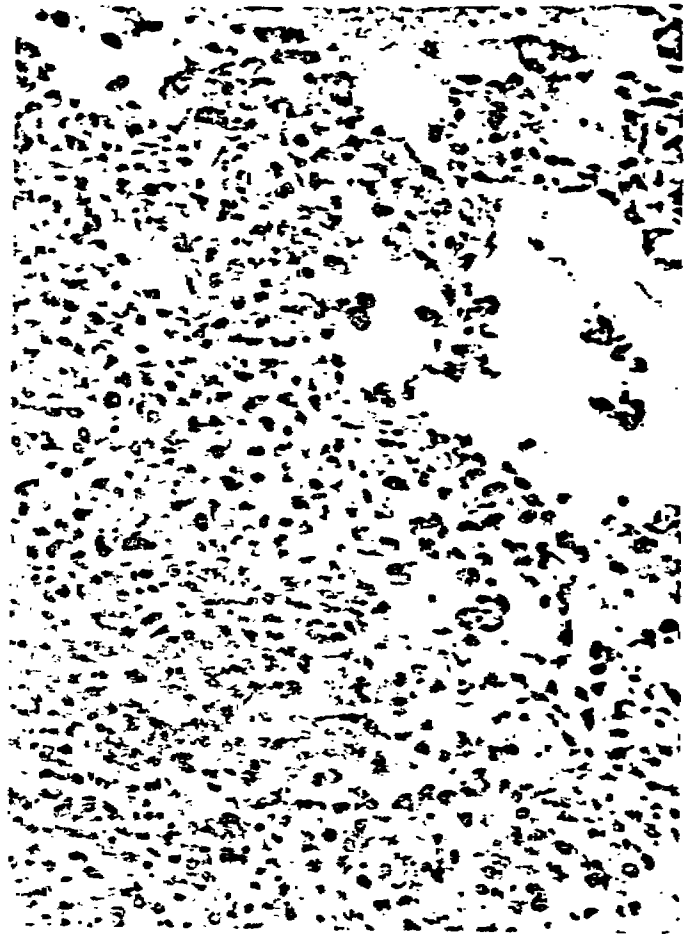


FIGURE 5. Liver angiosarcoma in rat. H&E, × 190.

of the topic, the pressure of the events and the requests. Since there have been more than 100,000 slides up to now, I am sure that those engaged in pathological screening of experimental material are aware of our difficult task.

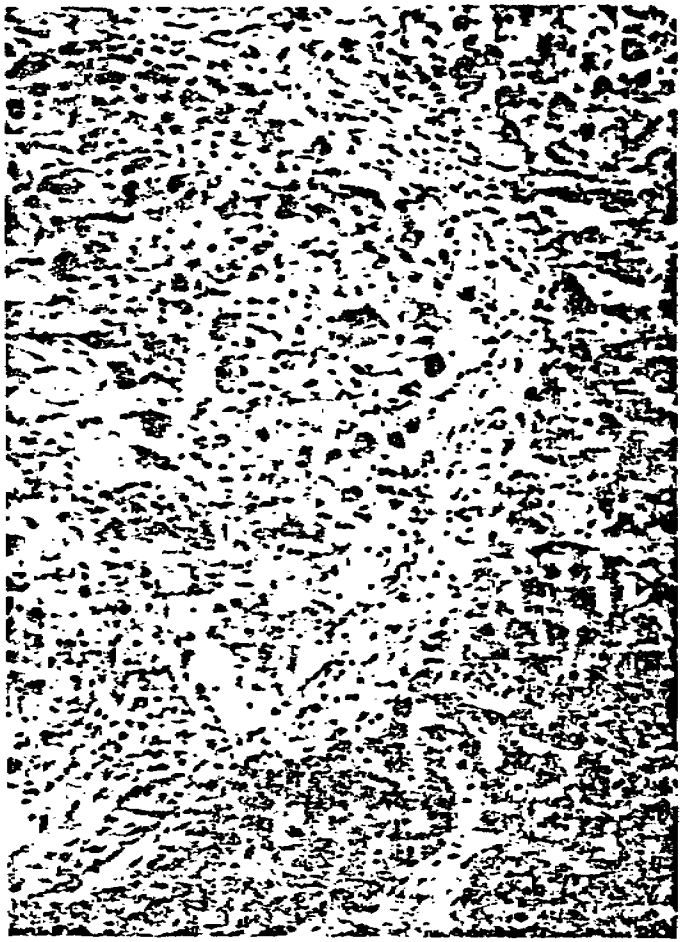
Our data have been periodically transmitted to the European Companies which have supported our research and are available to all interested parties.

Before the end of 1972, a short time after it was known that VC was inducing in rats not only Zymbal gland tumors, but also nephroblastomas and liver angiosarcomas, these results were also made known to the MCA (U.S.A.). This information promoted clinical observations and epidemiological investigations, which in December 1973 led to the first discovery of a liver angiosarcoma, as an occupational disease, in a worker of a U.S.A. factory producing VC-PVC.

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FIGURE 6. Pulmonary metastasis of liver angiosarcoma in rat. H&E, × 190.



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FIGURE 7. Liver angiosarcoma in mouse associated with fibrosis. H&E, × 190.

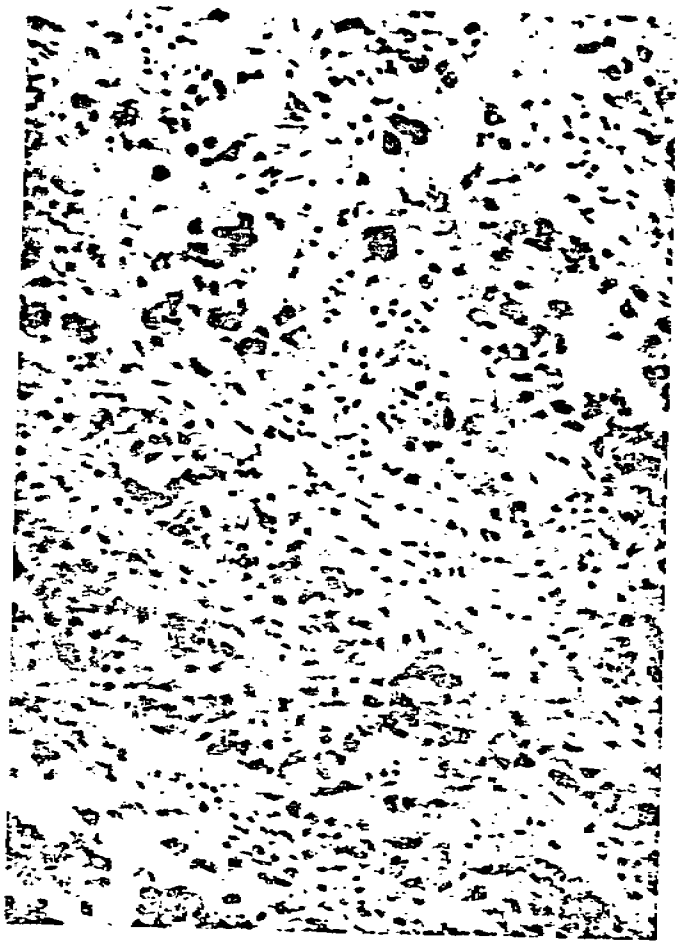


FIGURE 8. Liver angiosarcoma in mouse associated with fibrosis. H&E. × 190.

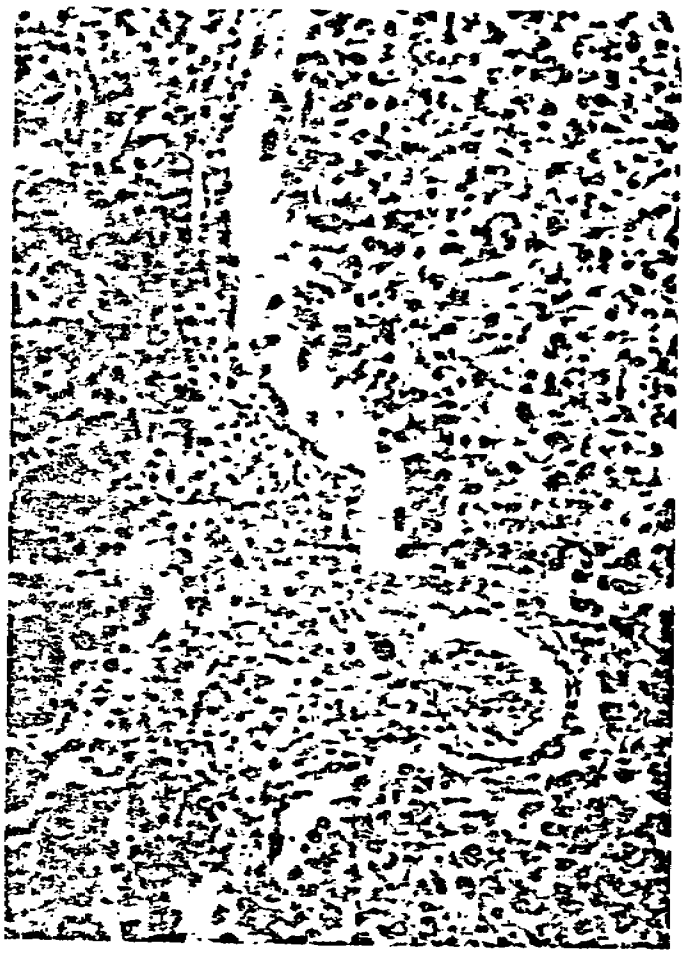


FIGURE 9. Kidney angiosarcoma in mouse. H&E. × 190.

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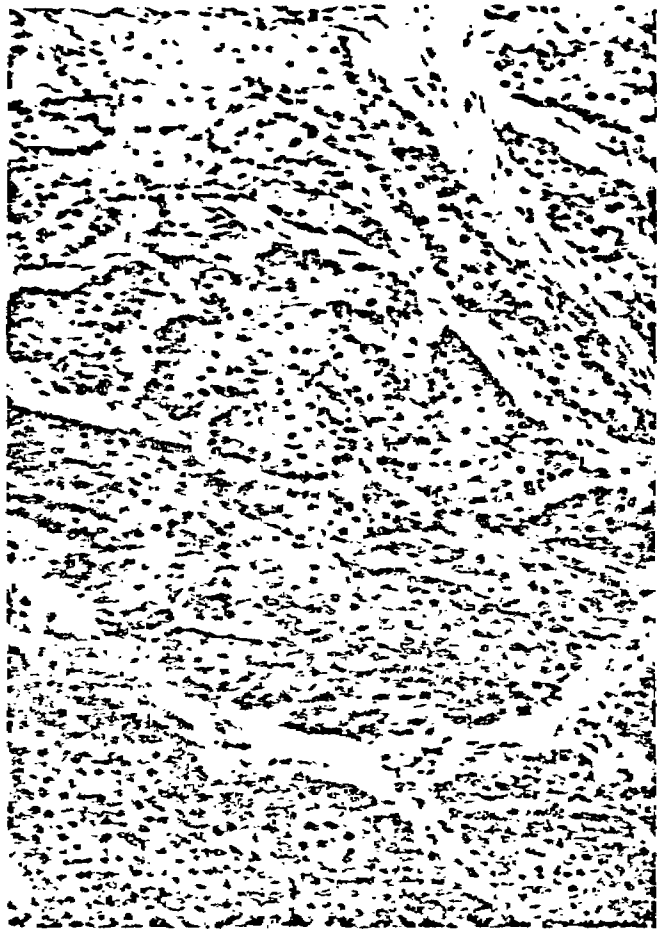


FIGURE 10. Skin sebaceous, well differentiated, carcinoma in rat. H&E, × 190.



FIGURE 11. Brain neuroblastoma with typical rosettes in rat. H&E, × 470.

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FIGURE 12. Mammary adenocarcinoma with squamous metaplasia in mouse. H&E. × 190.

May I recommend again that the VC carcinogenesis may serve as a convincing example for urgently promoting experimental bioassays for all new industrial compounds and for those compounds that are already widespread but whose effect we still ignore, to avoid occupational cancers.

Our Institute is now undertaking projects of carcinogenicity bioassays of other monomers employed in the plastic industry.

REFERENCES

1. VIOLA, P. L., A. BIGOTTI & A. CAPUTO. 1971. Oncogenic response of rat skin, lungs and bones to vinyl chloride. Cancer Res. 31: 516–519.
2. MALTONI, C. & G. LEFEMINE. 1974. Carcinogenicity to bioassays of vinyl chloride. I. Research plan and early results. Environ. Res. 7: 387–405.

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M. L. Keplin,

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The following data are p.
Bio-Test Laboratories, Inc.
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In this study which was
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INTERIM SUMMARY OF

Exposure Group	No. of Mortalities Total	Mal
Control†	0	–
50 ppm‡	4	3
200 ppm‡	15	8
2500 ppm‡	30	1

* Preliminary findings
Laboratory, April, 1974
† Seven male and 2 fema
had tumors in spleen, liver,
‡ Only mice with gross