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VINYL CHLORIDE MUTAGENESIS IN *DROSOPHILA MELANOGASTER*

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Summary

In inhalation experiments, *Drosophila* males were exposed to vinyl chloride at concentrations of 200, 850, 10,000, 30,000, or 50,000 ppm for 2 days, and to 30 or 850 ppm for 17 days. VCM was mutagenic in the recessive-lethal test both after short-term and long-term exposures. The lowest effective concentration (LEC) was 850 ppm after 2 day exposure, and this value could be lowered to 30 ppm by prolonging the exposure time to 17 days. With the concentration levels tested, the mutation frequency increased with concentrations and reached a plateau at 10,000 ppm. This indicates a substrate saturation effect.

In contrast with the recessive lethal assay, negative results were obtained when tests on dominant lethals, translocations, entire and partial sex-chromosome loss were carried out with VCM at 30,000 ppm for 2 days. This finding of a false negative seems a logical consequence of the observed saturation effect, and strengthens the concept that there exist two effective concentrations for point mutations vs the induction of chromosome breakage events. Vinyl chloride monomer provides another example to support our view that chromosome breakage is not a reliable measure of mutagenic activity.

Introduction

A 1975 survey of the top fifty chemical products [1] lists vinyl chloride monomer at position 23, thereby expressing its great economic importance. Over the past two years it has become clear that VCM can cause late toxic effects in experimental animals and man. Several reports indicate that VCM is

Abbreviations: ER, endoplasmic reticulum; PyDMT, 3,3-dimethyl-1-(3-pyridyl) triazene; PyODMT, 3,3-dimethyl-1-(3-pyridyl)-N-oxide triazene; VCM, vinyl chloride monomer.

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mutagenic in *Salmonella typhimurium* [2,3,13,16], *Escherichia coli* [7], and *Schizosaccharomyces pombe* [11]. Deelman et al. [5], Funes-Cravioto et al. [6], and Purchase et al. [15] have found chromosomal aberrations in workers exposed to VCM. A study of Infante et al. [8] on pregnancy among wives of workers exposed to vinyl chloride has indicated a significantly raised fetal loss, in comparison with controls. Systemic carcinogenic activity of VCM was observed in rats, mice and hamsters [14,19]. All these findings have raised serious concern about the genetic and carcinogenic risks of VCM to man.

Vinyl chloride as a chlorinated ethylene shows low reactivity, and its systemic action suggests that the biological effects of VCM are dependent on its biotransformation. This is in line with the failure of VCM to exert mutagenic activity in microorganisms in the absence of active microsomal enzymes [2,3,7,13,16]. Our interest in VCM stems from the observation that *Drosophila* can execute the essential activation steps needed to convert such pre-carcinogens into electrophilic species [24]. Its mode of action and its chemical properties made VCM a suitable material for further investigating the ability of the *Drosophila* system to detect different types of environmental mutagens. Preliminary data from our group [20] and the work by Magnusson and Ramel [12] demonstrate that *Drosophila* is capable of activating VCM.

In this study we report the mutagenicity of VCM in *Drosophila* at low and high concentrations, by comparing short-term vs long-term exposure, and by using different genetic end-points. It will be seen from the data that the detection of the mutagenic activity of VCM is dependent on the category of genetic damage used as a diagnostic criterion.

Materials and methods

Treatment procedure

VCM, produced by Matheson Gas Products (supplied by Hoek Loos, Amsterdam), was contaminated with acetylene (1–2 ppm), water (1 ppm) and phenol (82 ppm).

For exposure, groups of 50 males (2 days old) of our tester strain Berlin K were continuously exposed to VCM in 1-l bottles for a period of 2–17 days. Each bottle contained about 10 g of food (normal food with killed yeast) and was air-tight sealed with a rubber screw-cap. A known quantity of pure VCM gas was injected with a disposable syringe through this cap into the bottle.

A Packard gas chromatograph, model 417, with a stainless steel column (4 m, 3 mm, filled with poropack Q, 200 mesh) was used to exactly determine the initial quantity of VCM injected into each bottle. The temperature of the injection port, the column and the flame ionization detector was 200°C. N₂ with a flow of 1.5 l/h was used as the carrier gas. When low VCM concentrations were used, a known quantity of pure VCM gas was injected into a 35-ml bottle. From the resulting air-VCM mixture a sample was transferred into a new 1-l bottle, to obtain the desired concentration of VCM. The final concentration of VCM in the bottles was measured by gas chromatography analysis. This analysis shows that the deviation in VCM content obtained by this simple device was less than 10%, except for the lowest concentration of 30 ppm for which the error was larger, 30 ppm ± 10 ppm.

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The survival rate was 95% for males exposed to 200–50,000 ppm for 2 or 4 days. At the highest concentration of 50,000 ppm, the mortality rate was not enhanced, though the flies seemed slightly narcotized. No killing effect could be detected even in the long-term experiments of 17 days duration. The survival rate was equal (80–90%) for both the treated and the control groups. In these experiments the males were transferred to freshly prepared bottles every 3 or 4 days.

Mutagenicity assays

Sex-linked recessive lethals

Berlin K males, 2 days old, were exposed to increasing concentrations of VCM (30–50,000 ppm), for 2 or 4 days and then individually mated to 3-4 *Basc* (*In* (1) *sc*^{SH} *sc*^{RR} + *S*, *sc*¹ *sc*⁸ *u*¹ *B*) virgins [10]. The mutagenic response to VCM of successive stages of spermatogenesis was tested by brood pattern analysis, with breeding intervals of 2 or 3 days each and total breeding period of 12 days. All suspected recessive lethals were verified by re-tests.

Dominant lethals

The induction of dominant lethals by VCM at 30,000 ppm was tested for in mature sperm and spermatids by the technique developed by Sankaranarayanan [17]. To sample mature sperm, 2-day-old Berlin K males were exposed to VCM (30,000 ppm) for 2 days and then mated with one 4-day-old Berlin K virgin. The flies were allowed to oviposit in egg-laying units [17] for two 24-h periods. Since the data for both the control subjects and the treated groups were homogeneous, the flies were assumed to be from one 48-h brood.

A slightly modified technique was used for the analysis of spermatids. After exposure of 2-day-old Berlin K males to 30,000 ppm for 2 days, exposed (control) males were mass-mated to 3- to 6-day-old *Basc* virgins for 3 days, the ratio being 4 females : 1 male. This procedure was aimed at depleting from the males those germ cells that were already spermatozoa at the time of treatment. The males were then re-collected, individually mated to one Berlin K female and allowed to oviposit for two 24-h periods. Since the material was homogeneous within the control and the treated groups, the data were again summarized to one 48-h brood.

Sex-chromosome loss

Males of the genetic constitution *X*, *y/B*^S *Y* *y*⁺ were exposed to VCM (30,000 ppm) for two days and then mated with 3-4 *y ac In 49 v f mal su(f)* ♀♀ for a period of 3 days followed by a second 2-day brood. The F₁ progeny was scored for entire sex-chromosome loss, and for partial loss (*Y*^L; *Y*^S).

Translocations

Since the yield in recessive lethals was highest in spermatids, attention was focused on the induction of autosomal (2–3) translocations in this stage. In a storage experiment, 2-day-old Berlin K males were exposed to VCM (30,000 ppm) for 2 days. Exposed sperm were again depleted from the males by a procedure similar that used in the dominant-lethal experiments. The males

were then mated individually $(1) sc^{S1L} sc^{8R} + dl - 49, y sc^{S1} sc^8$ (*Inscy*); *bw*; *st*; virgins for 2 days. After 48 h, the males were discarded and the females allowed to lay eggs for a period of 5 consecutive 2- to 3-day broods, yielding for the last brood a total storage period of 14 days. The F_1 males were individually back-crossed to females of the maternal genotype, and the F_2 cultures were scored for the presence of autosomal translocations.

Results and discussion

Short-term experiments

When inhaled by *Drosophila* males for 2 days, vinyl chloride monomer was clearly efficient between 850 and 50,000 ppm (Table I). However, the incidence of recessive lethals was not a simple function of concentration: it increased from 850 to 10,000 ppm VCM and then remained constant at concentrations above 10,000 ppm. The weighted mean mutation frequencies were $2.19 \pm 0.25\%$ at 10,000 ppm, 2.22 ± 0.24 at 30,000 ppm, and 2.30 ± 0.26 at 50,000 ppm, indicating no further enhancement in the recessive lethal yield above 10,000 ppm VCM. Since VCM is believed to be a pre-carcinogen that must be oxidized to exert mutagenic activity, it is inferred that, above a certain concentration (which is between 850 and 10,000 ppm) the *Drosophila* enzymes are no longer capable of metabolizing additional substrate: this indicates that the detoxication (activation) mechanisms are saturated when too high doses are administered. This conclusion is also supported by the fact that an LD_{50} for mortality of flies could not be determined for VCM. A similar "saturation effect" to that described for VCM has been observed for other pre-carcinogens — PyODMT, PyDMT [22], cyclophosphamide, trofosfamide, and ifosfamide [23] — all of which are well-known compounds that need biotransformation to become biologically active. (For review see ref. [20].) The vinyl chloride data thus provide another instructive example of the need to study the effects over a range of concentrations as well as of problems involved in any attempt at an extrapolation from higher to lower concentrations.

With the mating procedures adopted, maximal sensitivity to the mutagenic action of VCM seemed to be in the second brood, about day 4 to day 5 after termination of treatment. In brood 2 the germ cell stages were sampled that were predominantly in the spermatid stage at the time of treatment. Though the differences in recessive lethal yield between that brood and the first and third was only slight, this tendency showed consistency through all experiments (series 4–8 in Table I), and the effect can be seen most clearly if the data from series 6, 7 and 8 are pooled (Table II). The data also show a considerable decline in the recessive lethal yield in broods 4 and 5. Broods 3, 4 and 5 all gave indications of a sterilizing effect. The mutational pattern described here for vinyl chloride coincides well with that observed for other pre-carcinogens, all indicating a typical, that is, a rather uniform, sensitivity pattern with a consistent peak activity in brood 2 or 3 [20,24]. Chandley and Bateman [4] have indicated that gametes in the spermatid stage during treatment become available for fertilization on the third to fifth day, whereas spermatocytes become available from the sixth to ninth day, and it is precisely these stages that have a highly developed endoplasmic reticulum (ER) [18]. In fact, in our

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TABLE I

INDUCTION OF SEX-LINKED RECESSIVE LETHALS IN DROSOPHILA MALES EXPOSED TO VINYL CHLORIDE FOR 2 OR 4 DAYS

Series	Concentration (ppm)	Exposure time (days)	Brood	Days after treatment	Chromosomes tested	Number of lethals	%
1	0	2	I	0-3	495	0	—
			II	3-5	414	1	0.24 ± 0.24
			III	5-7	324	0	—
			IV	7-10	258	0	—
			V	10-12	505	1	0.20 ± 0.20
			I-V	0-12	1996	2	0.10 ± 0.07
2	30	2	I	0-3	599	1	0.17 ± 0.17
			II	3-5	584	1	0.17 ± 0.17
			III	5-7	586	0	—
			IV	7-10	433	1	0.23 ± 0.23
			V	10-12	608	2	0.32 ± 0.23
			I-V	0-12	2810	5	0.18 ± 0.08
3	200	2	I	0-3	404	0	—
			II	3-5	612	0	—
			III	5-7	426	0	—
			IV	7-10	370	1	0.27 ± 0.27
			V	10-12	360	1	0.28 ± 0.28
			I-V	0-12	2172	2	0.09 ± 0.07
4	850	2	I	0-3	780	3	0.38 ± 0.22
			II	3-5	556	4	0.72 ± 0.36
			III	5-7	586	2	0.34 ± 0.24
			IV	7-10	324	1	0.31 ± 0.31
			V	10-12	304	0	—
			I-V	0-12	2550	10	0.39 ± 0.12
5	850 ^a	4	I	0-3	1884	8	0.43 ± 0.15
			II	3-5	1869	20	1.70 ± 0.24
			III	5-7	1420	9	0.63 ± 0.21
			IV	7-10	1048	2	0.19 ± 0.13
			V	10-12	1078	4	0.37 ± 0.19
			I-V	0-12	7299	43	0.59 ± 0.09
6	10,000 ^a	2	I	0-3	1071	17	1.59 ± 0.38
			II	3-5	1161	38	3.27 ± 0.52
			III	5-7	620	16	2.58 ± 0.64
			IV	7-10	228	0	—
			V	10-12	297	3	1.01 ± 0.58
			I-V	0-12	3377	74	2.19 ± 0.25
7	30,000 ^a	2	I	0-3	1469	34	2.31 ± 0.39
			II	3-5	1046	37	3.54 ± 0.57
			III	5-7	572	11	1.92 ± 0.57
			IV	7-10	255	1	0.39 ± 0.39
			V	10-12	527	3	0.57 ± 0.33
			I-V	0-12	3869	86	2.22 ± 0.24
8	50,000 ^a	2	I	0-3	1479	31	2.10 ± 0.37
			II	3-5	1146	35	3.05 ± 0.51
			III	5-7	287	7	2.44 ± 0.91
			IV	7-10	179	1	0.56 ± 0.56
			V	10-12	300	4	1.33 ± 0.66
			I-V	0-12	3391	78	2.30 ± 0.26

^a Pooled data from two experiments.

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TABLE II

THE WEIGHTED MEAN AVERAGES FROM 6 EXPERIMENTS^a ON THE INDUCTION OF RECESSIVE LETHALS IN SUCCESSIVE STAGES OF SPERMATOGENESIS

Brood	Days after treatment	Cell stages sampled ^b	Gametes tested	Number of lethals	Percentage lethals
I	0-3	sp, st	4019	82	2.04 ± 0.22
II	4-5	st	3353	110	3.28 ± 0.31
III	5-7	st, sc	1479	34	2.30 ± 0.39
IV	7-10	sc	662	2	0.30 ± 0.21
V	10-12	sg	1124	10	0.89 ± 0.28
I-V	0-12		10,637	238	2.24 ± 0.14

^a 3 series of two experiments each with VCM at 10,000, 30,000 and 50,000 ppm

^b sp, sperm; st, spermatids; sc, spermatocytes; sg, spermatogonia.

experiments, spermatocytes were found particularly sensitive to killing by pre-carcinogens. The drop in the number of lethals in broods 4 and 5, then, reflects the lower response to VCM of spermatogonia in which the ER is only poorly developed.

In the present study, it was considered useful to determine the ability of VCM to induce chromosome breakage. The results, presented in Table II, show that, in contrast with its substantial activity in the recessive lethal assay, VCM failed to produce dominant lethals to a noticeable extent in males that had been exposed to a high concentration of VCM (30,000 ppm) for 2 days. Although the size of the experiment was rather large, the survival rate in the treated groups did not differ significantly from that in controls. Moreover, one storage experiment for translocations was carried out, as a further check on the ability of VCM to break chromosomes in *Drosophila*. However, only 1 translocation was obtained amongst 1683 germ cells tested.

In tests on the incidence of loss of X or Y chromosomes or partial loss of the Y (Y^{+} , Y^{-}) no significant increase over control frequencies was found, despite the large numbers of gametes tested (Table IV). These findings are entirely in line with the negative results for dominant lethals and translocations. In the treated groups 6 mosaics were found, one of which was a double mosaic (not included in Table IV). Since no mosaics were observed in the control groups, a slight mutagenic effectiveness by VCM cannot be ruled out. What is obvious from these findings is that mutagenic effectiveness cannot be established for VCM on grounds of chromosome breakage phenomena, though

TABLE III

FAILURE OF VCM INDUCE DOMINANT LETHALS IN SPERM (BROOD 1) AND IN SPERMATIDS (BROOD 2) AFTER EXPOSURE TO 30,000 PPM FOR 2 DAYS

Brood	Days after treatment	Concentration (ppm)	Number of eggs, total	Hatchability %	Dominant lethals %
1	0-2	0	6950	84.6 ± 1.9	0
1	0-2	30,000	9889	82.9 ± 1.6	2.0 ± 2.9
2	3-5	0	8212	82.8 ± 3.1	0
2	3-5	30,000	11,300	81.8 ± 2.8	1.2 ± 5.1

TABLE IV

FREQUENCY OF RECESSIVE LETHALS IN SUCCESSIVE STAGES OF SPERMATOGENESIS

Brood	Days after treatment	Cell stages sampled	Gametes tested	Number of lethals	Percentage lethals
1	0-3	sp, st	4019	82	2.04 ± 0.22
2	4-5	st	3353	110	3.28 ± 0.31
3	5-7	st, sc	1479	34	2.30 ± 0.39
4	7-10	sc	662	2	0.30 ± 0.21
5	10-12	sg	1124	10	0.89 ± 0.28
6	0-12		10,637	238	2.24 ± 0.14

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FREQUENCIES OF ENTIRE CHROMOSOME LOSS AND PARTIAL LOSS IN SPERM (BROOD 1) AND SPERMATIDS (BROOD 2) EXPOSED TO VCM (30,000 PPM) FOR 2 DAYS

Brood	Concentration (ppm)	Regular progeny	Loss of X or Y		Loss of YL		Loss of Y ^S	
			n	%	n	%	n	%
1	0	7311	8	0.11	3	0.04	1	0.01
	30,000	13,987	8 ^a	0.06	2	0.01	1 ^a	0.01
2	0	6725	4	0.06	—	—	—	—
	30,000	8702	13 ^b	0.15	4	0.05	3	0.03
1 + 2	0	14,036	12	0.09 ± 0.02	3	0.02 ± 0.01	1	0.01 ± 0.01
	30,000	22,689	21	0.09 ± 0.02	6	0.03 ± 0.01	4	0.02 ± 0.01

^a One mosaic included.

^b Three mosaics included.

mutation induction provides evidence that there has been interaction with DNA under comparable experimental conditions. It has become clear over the past two years that there exists a significant difference between point mutations and breakage effects, in that the concentrations needed to increase the amount of the former are consistently lower than those required for the production of breaks [21,23,14]. The "two effective levels", together with the peculiar saturation effect, explain the failure of VCM to register positive in the *Drosophila* assays on chromosome breakage. Failure of VCM to produce dominant lethals has also been reported for mammalian test [15]. There was no significant increase in the number of early deaths per implantation after male exposure to such extraordinarily high levels as 3,000, 10,000 and 30,000 ppm. These findings have been interpreted as resulting from the inability of active metabolites of VCM to reach the testis [15]. On the basis of our findings, a negative result in the dominant-lethal test cannot be construed as a decisive one for demonstrating the absence of mutagenic effects.

Long-term experiments

The lowest effective VCM concentration recorded in the short-term inhalation studies was 850 ppm (2-days exposure). In a second series of experiments we attempted to evaluate the mutagenic activity of VCM on *Drosophila* males at lower concentrations comparable to those to which rats, mice and hamsters were exposed in tests on carcinogenicity. Frequencies of recessive lethals obtained after exposure of males to 30 and 850 ppm for 17 days are given in Table V. For VCM at 850 ppm the estimated mean mutation frequency was $1.02 \pm 0.16\%$, this being significantly higher than the frequencies found in the controls. The experiments with 30 ppm consisted of three treated series, two of which had concomitant controls (series 1 and 2). The mutation frequencies seemed slightly enhanced in all three experiments with 30 ppm. Statistical analysis by the significance test of Kastenbaum and Bowman and the χ^2 test did not reveal any significant differences in the mutation rates between treated and control groups. The best result (on the verge of significance) was obtained when the data from broods 1 and 2 were pooled and compared with

TABLE V

INDUCTION OF SEX-LINKED RECESSIVE LETHALS IN DROSOPHILA MALES EXPOSED TO LOW LEVELS OF VCM FOR 17 DAYS

Series	Concentration VCM (ppm)	Brood	Days after treatment	Number of chromosomes tested	Number of lethals found	Percentage lethals
1	0	I	0-4	694	2	0.29 ± 0.20
		II	4-7	476	0	—
		III	7-10	527	2	0.38 ± 0.27
		I-III	0-10	1697	4	0.23 ± 0.12
1	30	I	0-4	1163	3	0.26 ± 0.15
		II	4-7	768	4	0.52 ± 0.26
		III	7-10	588	1	0.17 ± 0.17
		I-III	0-10	2519	8	0.32 ± 0.11
2	0	I	0-4	1091	1	0.09 ± 0.09
		II	4-7	724	1	0.14 ± 0.14
		III	7-10	609	2	0.33 ± 0.23
		I-III	0-10	2424	4	0.17 ± 0.08
2	30	I	0-4	1295	7	0.54 ± 0.20
		II	4-7	605	2	0.33 ± 0.23
		III	7-10	193	0	—
		I-III	0-10	2093	9	0.43 ± 0.14
3	30	I	0-4	1468	3	0.20 ± 0.12
		II	4-7	490	4	0.82 ± 0.41
		III	7-10	643	1	0.16 ± 0.16
		I-III	0-10	2601	8	0.31 ± 0.11
1-2	0	I	0-4	1785	3	0.17 ± 0.09
		II	4-7	1200	1	0.08 ± 0.08
		III	7-10	1136	4	0.35 ± 0.18
		I-III	0-10	4121	8	0.19 ± 0.07
1-3	30	I	0-4	3926	13	0.33 ± 0.09
		II	4-7	1863	10	0.54 ± 0.17
		III	7-10	1424	2	0.14 ± 0.10
		I-III	0-10	7213	25	0.35 ± 0.07
4 ^a	850	I	0-4	2034	28	1.38 ± 0.26
		II	4-7	1001	5	0.50 ± 0.22
		III	7-10	904	7	0.77 ± 0.29
		I-III	0-10	3939	40	1.02 ± 0.16

^a Mean values from two experiments.

the pooled data from the controls. However, since an increased mutation frequency was consistently found in all three treated series, the question of whether VCM at 30 ppm can induce mutations seems entirely a statistical problem, for with a larger material of about 20,000 gametes (instead of the 11,334 tested in our experiments) the differences would become statistically significant [25].

In summary, the results at the concentration of 30 ppm VCM in resolving

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In summary then, on the basis of the consistent effect in three large experiments, the conclusion seems justified that VCM still shows mutagenic activity at the very low concentration of 30 ppm. This constitutes a far lower concentration than those of 625–200,000 ppm used to demonstrate mutagenicity of VCM in other assays [2,3,7,11,13], and this further documents the high resolving power of the *Drosophila* recessive-lethal test.

Acknowledgement

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MUTAGENICITY OF VINYL CHLORIDE, VINYLIDENE CHLORIDE AND CHLOROPRENE IN V79 CHINESE HAMSTER CELLS

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Summary

The mutagenicity of vinyl chloride, vinylidene chloride (1,1-dichloroethyl-
 ene) and chloroprene (2-chloro-1,3-butadiene) was tested in V79 Chinese
 hamster cells in the presence of a 15 000 $\times$ g liver supernatant from pheno-
 barbitone-treated rats and mice. Mutations in terms of 8-azaguanine and
 ouabain resistance were induced in a dose-related fashion by exposure to vapour
 of vinyl chloride in the presence of liver supernatant from phenobarbitone-pre-
 treated rats. Vapours of vinylidene chloride and chloroprene induced a dose-
 related toxicity in the presence of liver supernatant from phenobarbitone-pre-
 treated rats, but these two compounds were not mutagenic in V79 Chinese
 hamster cells under the present assay conditions. The results are discussed with
 regard to the metabolic activation of the compounds and to the correlation
 with their carcinogenicity in man and experimental animals.

Chlorinated hydrocarbons, such as VCM, VDC and chloroprene, are pro-
 duced in large quantities and are widely used in the production of plastics and
 synthetic rubbers. There is unequivocal evidence that VCM is carcinogenic to
 man, as well as to a number of other animal species [1]. VDC, a structurally
 related compound and a copolymer of VCM, is carcinogenic to mice and rats,
 but no data are available on its carcinogenicity to man [1,5]. Chloroprene has so
 far not been found to be carcinogenic in mice and rats, although some human

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Abbreviations: AZAG, 8-azaguanine resistance; FCS, fetal calf serum; OUAf, ouabain resistance;
 S, 15 000 $\times$ g supernatant fraction; VCM, vinyl chloride (monomer); VDC, vinylidene chloride
 (1,1-dichloroethylene).

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VCM (purity 99.9%) was generously provided by Rhône-Progil (Lyon, France); it was contaminated with ethanol (30 ppm), water (20 ppm), methyl chloride (<20 ppm) and non-volatile substances (<5 ppm). VDC, containing 0.3% 4-methoxyphenol as antioxidant, was obtained from Merck-Schuchardt (Darmstadt, Federal Republic of Germany). Chloroprene (2-chloro-1,3-butadiene; purity 99%) was provided by Distugil (Le Pont de Claix, France); it was contaminated with 0.8% 1-chloro-1,3-butadiene, crotylchloride at <200 ppm and 2-chlorobutene-1 and 2-chlorobutene-2 at 80 ppm. 8-Azaguanine (Pfaltz and Bauer, Flushing, NY, U.S.A.), ouabain (Sigma Chemical Co., St. Louis, MO, U.S.A.) and agar (special agar Noble, Difco Lab., Detroit, MI, U.S.A.) were purchased. Autoclavable Eagle MEM was obtained from Flow Laboratories (Irvine, Ayrshire, Great Britain). FCS was purchased from Grand Island Biological Co. (Grand Island, NY, U.S.A.).

Mutations in terms of resistance to 8-azaguanine, a purine analogue, and to ouabain, a specific inhibitor of Na^+/K^+ -activated ATPase in cell membranes, were recorded in V79 Chinese hamster cells. Experimental procedures were a modification of the assay system for nitrosamines reported previously [8,19].

V79 Chinese hamster cells were plated at a concentration of 1.5×10^6 cells/60 mm petri dish and were cultured overnight in Eagle MEM, supplemented with 10% FCS. They were then incubated at 37°C for 5 h or more in 2.5 ml of the reaction mixture, either in liquid suspension or suspended in 0.3% agar. The reaction mixture consisted of 0.75 ml S15 post-mitochondrial fraction from the livers of BDVI male rats or OF-1 male mice, which had been treated with

phenobarbitone (1 mg/ml) in drinking water for 7 days; 0.75 ml modified Sørensen phosphate buffer (0.055 M, pH 7.4, containing 0.9% NaCl and 1.6 mg $MgCl_2 \cdot 6H_2O$ per ml); and 0.75 ml phosphate-buffered saline containing 12.5 μ mol glucose 6-phosphate, 2 μ mol $NADP^+$, amino acids, vitamins from Eagle MEM and 0.25 ml FCS. (Components of culture medium, such as amino acids, vitamins from Eagle MEM and FCS, were included in the reaction mixture to ensure survival of the cells for long incubation periods.) For agar suspensions, agar was added to modified Sørensen phosphate buffer at 1.0%.

The petri dishes were then placed in a desiccator (10–15 l) and exposed to the vapour of the chemicals being tested. For VCM, the gas was introduced into the desiccator, which had been evacuated, with the aid of a manometer, at levels corresponding to 5, 10, 20 and 30% in volume; after 20–30 min at 37°C the pressure was adjusted to atmospheric level. For VDC and chloroprene, which are liquids at low temperatures, the volume calculated to give a known volume of vapour (on the basis of Avogadro's law) was frozen and placed in the desiccator. The desiccator was partially evacuated and incubated for 20–30 min at 37°C to allow the compound to vaporize; air was then introduced until atmospheric pressure was obtained. The desiccator, covered with aluminium foil, was then incubated in a water-bath at 37°C for 5, 10 or 15 h. The concentrations of the compounds in aqueous phase under these conditions were determined previously, using gas-liquid chromatography [6,24]. After the exposure period, the vapour was removed under vacuum and replaced by air; the cells were washed twice and incubated for 2–3 h in fresh culture medium. The control cells were treated in the same way, either simply in the absence of the chemicals or in reaction mixture from which the S15 fraction or cofactors (glucose 6-phosphate and $NADP^+$) had been omitted. For some unknown reason, controls for which cofactors were omitted repeatedly yielded a lower plating efficiency as compared with cells incubated in the complete mixture or those for which the S15 fraction was omitted (see Table 2).

The cells were then plated for the determination of the cytotoxicity and mutagenicity induced. Cytotoxicity was determined by plating 100 cells/60 mm petri dish (4 dishes for each point) and cultured for 7 days. For mutagenesis, 2×10^4 and 10^5 cells/60 mm dish were plated for AZA^r and OUA^r , respectively (8 dishes for each point). After an expression period of 48 h, selection drugs were added to give final concentrations of 20 μ g/ml 8-azaguanine or 1 mM ouabain. The media containing the drugs were changed once, 5–7 days later; for the 8-azaguanine medium, FCS was replaced by dialysed FCS and non-essential amino acids were added to 0.1 mM. The cultures were fixed and stained with Giemsa at 12 days for AZA^r and at 14 days for OUA^r . Mutation frequency was calculated per 10^5 survivors, the number of cells plated and the plating efficiency being taken into account. Reproducibility of the results was confirmed by repeated experiments; 5 times with VCM, 4 times with VDC and twice with chloroprene. Means and standard errors in the figures and tables refer to the variability in a single experiment. The stability of the AZA^r and OUA^r colonies was demonstrated in 10 and 11 isolated colonies, respectively, which were cultured for more than one month in the absence of the drug used for selection [8,19].

Results

Mutagenicity of VCM

To determine the optimal conditions for detecting the mutagenicity of the various compounds, the reaction mixture was composed to 20% (v/v) VCM in air (for 5, 10 or 15 h in the presence of liver S15 from phenobarbitone-pre-treated rats); the cells were suspended either in liquid (left column of Fig. 1) or in 0.3% agar (right column of Fig. 1). Agar suspension was used, since VCM has been reported to induce mutations in *Salmonella typhimurium* only when tested in the agar-incorporation assay and not in the liquid-suspension assay [5]. Post-mitochondrial fractions from phenobarbitone-pre-treated rats or mice have been shown to increase VCM-induced mutagenicity in *S. typhimurium* over that obtained with fractions from untreated animals [5,24].

As seen in Fig. 1, the reaction mixture, in either suspension, was not by itself toxic to the V79 cells within 10-h incubation time; 15-h incubation in liquid

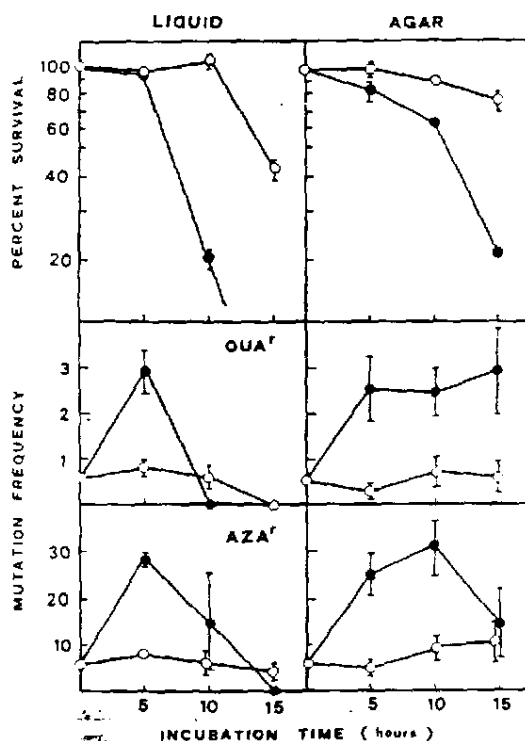


Fig. 1. The course of the induction of cytotoxicity and of mutations by exposure to 20% (v/v) VCM in air (●) as reaction mixture containing S15 liver fraction from phenobarbitone-pre-treated rats plus cofactors, in either liquid suspension (left column) or in 0.3% agar suspension (right column). Controls (○) were treated in the same way, but without VCM. Cytotoxicity was determined from the plating efficiency and is expressed as percentage of plating efficiency in controls. Mutation frequency is expressed as the number of resistant colonies per 10^6 survivors, taking into account the number of cells plated and the plating efficiency. Bars: SE.

caused a decrease in plating efficiency of about 50%, and 15-h incubation in agar suspension caused only slight toxicity. VCM induced cytotoxicity and mutagenicity when added in liquid suspension or in agar suspension. The time courses of mutation induction were much more gradual in agar suspension than in liquid incubation: with liquid incubation, the highest mutation frequencies were observed at 5 h and were followed by a sharp decline; whereas with agar suspension, mutation frequencies reached almost a plateau after 5 h, suggesting that microsomal enzymes are stabilized in this suspension, as reported by Malaveille et al. [25]. In both the agar and liquid incubations, the highest number of resistant colonies per 10^5 survivors was about 30 for AZA^r and 3 for OUA^r, obtained at 5 h in liquid incubation and at 10–15 h in agar incubation. Consequently, incubation for 5 h in a liquid reaction mixture was chosen for the following experiments.

VCM induced dose-related mutations and toxicity when given at concentrations of between 5 and 30% (v/v) in air (Fig. 2). With a concentration of 30% (v/v), mutation frequencies were 10–20 times that of the spontaneous back-

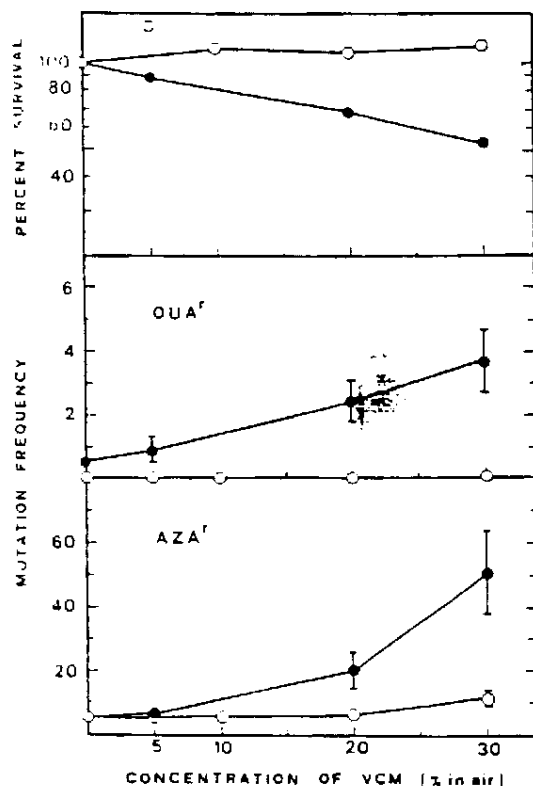


Fig. 2. VCM-induced cytotoxicity and mutagenicity in V79 cells as a function of concentration of VCM in air (% v/v). ●, complete reaction mixture containing S15 liver fraction from phenobarbitalone-pre-treated rats plus cofactors. ○, reaction mixture containing no S15. Cytotoxicity and mutation frequency are assessed as described in the legend to Fig. 1.

ground: 51 AZA^r and 4 OUA^r colonies were obtained per 10⁵ survivors. Neither toxicity nor mutation was induced when the S15 fraction was omitted from the reaction mixture (Fig. 2).

Mutagenicity of VDC

The mutagenicity of VDC in V79 Chinese hamster cells was tested by exposing them to 2.0 and 10.0% of the vapour in air for 5 h in reaction mixture containing S15 fraction of liver from phenobarbitone-pre-treated rats and mice. S15 fraction from mice was used because VDC was mutagenic in *S. typhimurium* only when tested with mouse-liver homogenate and was almost inactive when tested with liver homogenate from rats [6], although VDC has been shown to be carcinogenic in both species [15]. These results are summarized in Table 1. A dose-related toxicity was induced when cells were incubated in reaction mixture containing S15 fraction from rat liver plus cofactors, but VDC was not toxic with the S15 fraction from mouse liver; this suggests that a toxic metabolite(s) was formed by rat microsomal enzymes. However, no increase in the appearance of AZA^r and OUA^r colonies was observed after incubation in the presence of liver S15 fraction from either rats or mice.

Mutagenicity of chloroprene

V79 cells were exposed to 0.2, 1.0, 2.0 and 10% (v/v) chloroprene vapour in air for 5 h in reaction mixture containing S15 fraction of livers from phenobarbitone-pre-treated rats, plus cofactors. The results are summarized in Table 2. Chloroprene was toxic to the cells, in the absence of a metabolic activation system, at concentrations of more than 1%. This toxic effect could be a direct action of chloroprene or that of one of its enzymic (from the V79 cells) or non-enzymic breakdown products. An enhanced toxic response was observed when the cells were incubated with S15 fraction plus cofactors; this suggests

TABLE 1

CYTOTOXICITY AND MUTAGENICITY OF VDC IN V79 CHINESE HAMSTER CELLS IN THE PRESENCE OF POST-MITOCHONDRIAL FRACTION (S15) OF LIVER FROM PHENOBARBITONE-PRE-TREATED RATS AND MICE

Concentration in air (%)	Incubation	Cytotoxicity ^a	Mutagenicity ^b	
			AZA ^r	OUA ^r
0	complete, with rat S15	63.0	4.5	0.4
2.0		15.3	4.2	0
10.0		2.3	0	0
0	complete, with mouse S15	68.3	6.4	0.4
2.0		61.3	3.1	0
10.0		64.4	3.9	0.2
0	minus S15	62.8	1.0	0.4
2.0		49.5	3.8	0.3
10.0		60.3	2.1	0.6

^a Expressed as plating efficiency: the percentage of cells that formed colonies.

^b Mutation frequency is expressed as the number of resistant colonies per 10⁵ survivors, the number of cells plated and plating efficiency being taken into account.

TABLE 2
CYTOTOXICITY
IN THE PRESENCE OF
PHENOBARBITONE-PRE-TREATED
RAT LIVER S15 FRACTION

Concentration
in air (%)

0
0.2
1.0
2.0
10.0

0
0.2
1.0
2.0
10.0

^a The results are similar to those obtained with the S15 fraction from rat liver.
^b Expressed as the number of resistant colonies per 10⁵ survivors.
^c Mutagenicity expressed as the number of resistant colonies per 10⁵ survivors.

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Discussion

Table 1 shows that chloroprene was toxic to V79 cells and, in the absence of a metabolic activation system, at concentrations of more than 1%. This toxic effect could be a direct action of chloroprene or that of one of its enzymic (from the V79 cells) or non-enzymic breakdown products. An enhanced toxic response was observed when the cells were incubated with S15 fraction plus cofactors; this suggests

TABLE 2
SUMMARY OF
RESULTS OF
CYTOTOXICITY
TESTS

VCM
VDC
Chloroprene
^a Carcinogenicity

TABLE 2

CYTOTOXICITY AND MUTAGENICITY OF CHLOROPRENE IN V79 CHINESE HAMSTER CELLS IN THE PRESENCE OF POST-MITOCHONDRIAL FRACTION (S15) OF LIVER FROM PHENOBARBITONE-PTO-TREATED RATS ^a

Concentration in air (%)	Incubation	Cytotoxicity ^b	Mutagenicity ^c	
			AZA [†]	OUA [†]
0	complete	64.5	2.9	0.8
0.2		26.8	2.6	1.4
1.0		0	0	0
2.0		0	0	0
10.0		0	0	0
0	minus cofactors	31.3	2.1	0.8
0.2		23.8	7.9	1.0
1.0		0.0	7.7	0
2.0		8.8	0	0
10.0		0	0	0

^a The results of two independent experiments are combined: chloroprene was tested at 0, 0.2 and 1.0% in Expt. I and at 0, 2.0 and 10.0% in Expt. II. Values for controls (0%) are taken from Expt. I, although similar values were obtained in Expt. II.

^b Expressed as plating efficiency, the percentage of cells that formed colonies.

^c Mutation frequency is expressed as the number of resistant colonies per 10⁵ survivors, the number of cells plated and plating efficiency being taken into account.

enzymic formation of a toxic metabolite(s) from chloroprene. However, no mutations, in terms of AZA[†] and OUA[†], were induced by this compound in the presence or absence of metabolic activation systems.

Discussion

Table 3 summarizes the data on the carcinogenicity of VCM, VDC and chloroprene in man and experimental animals, taken from the IARC Monographs [15], and juxtaposes these with data on mutagenicity in *S. typhimurium* and, from the present study, with that in V79 Chinese hamster cells. VCM has been shown to be carcinogenic in man as well as in experimental animals: it produced tumours at different sites, including angiosarcomas of the liver, when tested by the oral route or inhalation exposure in rats, mice and hamsters.

TABLE 3

SUMMARY OF DATA ON CARCINOGENICITY AND MUTAGENICITY OF VCM, VDC AND CHLOROPRENE

Compound	Evidence of carcinogenicity ^a in		Evidence of mutagenicity in	
	Man	Animals	<i>S. typhimurium</i>	V79
VCM	+	+	+	+
VDC	?	±	+	—
Chloroprene	—	?	+	—

^a Carcinogenicity data are taken from IARC Monograph Vol. 19 [15]. See text for details.

Epidemiological studies have shown that exposure to VCM results in an increased carcinogenic risk to man, involving liver, brain, lung and the lymphato-haemopoietic system. The data in experimental animals, also indicative of a carcinogenic effect of VDC, are inadequate to allow an evaluation of the carcinogenicity of VDC or chloroprene. Occupational exposures to chloroprene have, however, been reported to be associated with a variety of toxicological effects, including chromosomal aberrations among exposed workers. In one study, an excess of lung and skin cancers was related to occupational exposure to chloroprene. These observations raise the possibility that chloroprene is a human carcinogen.

The present study confirms previous results on the mutagenicity of VCM in *S. typhimurium* [1,5,10,24,26,28], *Escherichia coli* K12 [11], *Schizosaccharomyces pombe* [22], *Saccharomyces cerevisiae* [22] and *Drosophila melanogaster* [23,30]. This subject has been reviewed by Bartsch and Montesano [7], Bartsch et al. [4], Fishbein [9] and the IARC Monograph [15]. Recently, Styles [29] reported VCM-induced transformation in BHK-21 cells in the presence of a microsome preparation. Although VCM induced mutations in *S. typhimurium* in the absence of a metabolic activation system, a much higher mutagenic response was observed when a 9000 $\times$ g supernatant from rat liver was added [1,5,26]. Pre-treatment of rats with phenobarbitone increased the mutation rate over that with a fraction from untreated rats [5,24]. Mutations have also been observed in the presence of liver homogenates from mice [5,10,22,24] and from human biopsies [5,24] as well as in the host-mediated assay in mice [22]. These results, together with those from the present study, indicate enzymic formation of a mutagenic metabolite(s) from VCM.

Among the possible metabolites that have been tested, chloroethylene oxide, an obligatory epoxide in metabolism by microsomal mixed-function oxidases, was the strongest mutagen in V79 Chinese hamster cells [12] as well as in microbial assays [14,21,24,27]; chloroacetaldehyde was mutagenic [5,12,21,24,26,27], but chloroacetic acid was not mutagenic, in such assays [5,12,24,26,27]. The above data strongly suggest that chloroethylene oxide is the metabolite of VCM that is principally responsible for the various adverse biological effects of the parent compound. VDC and chloroprene may follow a metabolic pathway similar to that of VCM [2,16].

VDC and chloroprene have been shown to be mutagenic in microbial assays: VDC produced reverse mutations in *S. typhimurium* and in *E. coli* in the presence of a metabolic activation system [6,11]; chloroprene induced reverse mutations in *S. typhimurium* in the absence of metabolic activation, but an increased mutagenic response was observed when liver homogenate from mice was added to such assays [6]. In *Drosophila*, recessive lethal mutations were induced by feeding male flies with chloroprene [31]. In V79 Chinese hamster cells, however, these two chemicals induced a dose-related toxicity in the presence of liver S15 fraction from phenobarbitone-pre-treated rats but were not mutagenic within the constraints of the present assay conditions. This suggests that there is at least enzymic formation of toxic metabolites from VDC and chloroprene by microsomal enzymes.

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POTENTIAL HALOGENATED INDUSTRIAL CARCINOGENIC AND MUTAGENIC CHEMICALS

I. HALOGENATED UNSATURATED HYDROCARBONS

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ABSTRACT

The halogenated aliphatic hydrocarbons represent one of the most important categories of industrial chemicals from a consideration of use categories, production volume, environmental and toxicological considerations, and hence most importantly, potential population risk.

The major unsaturated hydrocarbons reviewed, primarily in terms of their synthesis, utility, stability, distribution and levels of exposure as well as their reactivity, carcinogenicity, mutagenicity and metabolism, include: vinyl chloride, vinylidene chloride, trichloroethylene, perchloroethylene, chloroprene, *trans*-1,4-dichlorobutene, hexachlorobutadiene and allyl chloride.

HALOGENATED UNSATURATED HYDROCARBONS

The major objective of this series of reviews is to consider a number of the most industrially significant halogenated compounds: (a) unsaturated hydrocarbons; (b) saturated hydrocarbons; (c) alkanes, alkanols and ethers; (d) aryl derivatives; and (e) polyaromatic derivatives reported to be carcinogenic and/or mutagenic in order to better assess the nature of the *present* potential risk. Additionally, germane aspects (where known) of their synthesis, primarily in terms of the nature of the possible hazardous trace impurities, production volumes and use patterns (U.S.A., Western Europe and Japan), chemical and biological reactivity and stability, environmental occurrence, national permissible worker exposure levels (TLV's and MAC's) have been included for cohesiveness of treatment and hopefully for enhanced utility for a broader spectrum of scientists and public health officials.

In recent years, there has been recognized concern over the environmental and toxicological effects of a spectrum of halogenated hydrocarbons, primarily the organochlorine insecticides and related derivatives.

(1) Vinyl chloride

Vinyl chloride (chloroethylene; ethylene monochloride; chloroethene; VCM; $\text{CH}_2=\text{CHCl}$) is used in enormous quantities primarily (ca. 96-97%) for the production of homo-polymer (for PVC production) and co-polymer resins

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(e.g., Saran and other plastics). Lesser quantities of vinyl chloride are used in the production of 1,1,1-trichloroethane (methyl chloroform), as an additive in specialty coatings and as a component of certain propellant mixtures. It is important to note the VCM production processes because of the halocarbon precursors and intermediates as well as the nature of the potential carcinogenic and mutagenic trace impurities. VCM monomer production processes employ one of the following: (1) the acetylene plus hydrogen reaction; (2) the direct chlorination of ethylene and dehydrochlorination; and (3) the balanced direct and oxychlorination of ethylene and dehydrochlorination. The overall processes differ primarily in the manner in which the intermediate ethylene dichloride is produced. The bulk of VCM is produced by process (3) above [1-3] (over 95% of VCM produced in the U.S. was made from ethylene) [3]. A typical commercial product can contain the following impurities in mg/kg [2]: unsaturated hydrocarbons, 10; acetaldehyde, 2; dichloro compounds, 16; water, 15; HCl, 2; non-volatiles, 200; iron, 0.4; phenol as a stabilizer, 25-50; and trace amounts of organic impurities including: acetylene, 1,3-butadiene, methyl chloride, vinylidene chloride and vinylacetate [1]. VCM is generally supplied as a liquid under pressure and currently most VCM is not inhibited for shipping [3].

As will be discussed in more detail later, ethylene dichloride is mutagenic in *Drosophila* [1, 5] in *S. typhimurium* TA 1530, TA 1535 and TA 100 tester strains (without metabolic activation) [6, 7] and in *E. coli* (DNA polymerase deficient pol A⁻ strain [8]).

The growth patterns of VCM per se as well as that of its primary end-product polyvinylchloride (PVC) plastic resin have been well documented [1-3, 8-12]. The total world production of VCM in 1971 was 7,059 million kg and in various areas was estimated as follows (in millions of kg) [2]: Western Europe (2,497); Eastern Europe (817); U.S. (1,969); Japan (1,275); and other areas (499). VCM production in the U.S.A. in 1974 exceeded 2.6 billion kg (about one-third Western World's output) with the annual growth rate expected to exceed 10% per year through the 1980's [1]. (In 1976, nine U.S. companies reported the production of 2,580 million kg of VCM [9].)

Countries producing VCM, listed in decreasing order of estimated annual production in recent years and the numbers of producers in each year are as follows: Japan (15), the Federal Republic of Germany (5), Italy (3), France (4), Belgium (3), the United Kingdom (4), The Netherlands (2), Brazil (3), Spain (3), Turkey (3), Taiwan (2), Argentina (2), Sweden (1), Mexico (1), U.S.S.R. (2), South Korea (1), Finland (1), Czechoslovakia (1), Venezuela (1), Egypt (1), Thailand (1), Rumania (1), Chile (1), Greece (1) and Australia (1) [2, 13].

About 96% of the 2,271 million kg of VCM consumed in the U.S.A. in 1976 was used for the production of vinyl chloride homopolymer and copolymer resins [3]. The remainder was used (essentially by one company internally) as a co-monomer with vinylidene chloride in the production of resins, and in the production of methylchloroform [3]. The consumption patterns of vinyl chloride monomer in Western Europe and Japan are

believed to be similar to that in the U.S.A. [3]. The world production of PVC in 1975 is estimated to be 9-10 million tons.

The major market for PVC resins is in the production of plastic pipe and conduit. Other important uses include: in floor coverings, consumer goods, electrical applications, and in transportation [3].

The total world-wide employment in the VCM and PVC industries is over 70,000 workers. Those employed in industries using PVC as a basic element are believed to number in the millions [11].

PVC is produced (in U.S.) via 4 major processes (in % total production) as follows [1]: (1) suspension polymerization, 78; (2) emulsion polymerization, 12; (3) bulk polymerization, 6; and (4) solution, 4.

The current U.S. Occupational Safety and Health Administration (OSHA) health standards for exposure to air contaminants requires that an employee's exposure to vinyl chloride does not exceed an 8-h time weighted average of 1 ppm in the work-place air in any 8-h workshift of a 40-h work week. During any work shift, an employee's exposure may not exceed a ceiling concentration limit of 5 ppm averaged over any period of 15 min or less [3].

IARC [3] has summarized the work environment hygiene standards for exposure to vinyl chloride as reported by Bertram [14] in terms of time weighted averages for 8-hour (8-h) and 15-minute (15-min) (a ceiling concentration) time period unless otherwise stated for the following countries: Belgium - 200 ppm max; Canada - 10 ppm (8-h) and 25 ppm (15-min); Finland - 5 ppm (8-hr) and 10 ppm (10-min period); France - no limits; Federal Republic of Germany - 5 ppm max. technical standard, with the average concentration not exceeding 15 ppm over the period of 1 h; United Kingdom - 25 ppm (8-h) and 50 ppm (15-min); Italy - 50 ppm (8-h) (this is expected to change to 25 ppm (8-h) and 15 ppm (15-min)); Japan - expected to be 10 ppm; The Netherlands - 10 ppm (8-h); Norway - 1 ppm (8-h) and 5 ppm (15-min); Sweden - 1 ppm (8-h) and 5 ppm (15-min); Switzerland - 100 ppm (this is expected to change to 10 ppm (8-h)) and U.S.S.R. - 12 ppm.

The hazard of vinyl chloride was originally believed to primarily concern workers employed in the conversion of VCM to PVC who may receive a particularly high exposure of VCM in certain operations (e.g., cleaning of polymerization kettles) or a long-term exposure to relatively low concentrations in air of VCM at different factory sites. Much larger populations are now believed to be potentially at risk including: (1) producers of VCM; (2) people living in close proximity to VCM- or PVC-producing industries; (3) users of VCM as propellant in aerosol sprays; (4) persons in contact with resins made from VCM; (5) consumers of food and beverage products containing leachable amounts of unreacted VCM from PVC packaged materials; and (6) ingestion of water containing unreacted VCM leached from PVC pipes.

Gaseous vinyl chloride is emitted at both vinyl chloride and PVC resin plants and is distributed into the atmosphere surrounding the emissions source in patterns that depend on the amount of vinyl chloride released the nature of the plant area from which it is released and the meteorological

conditions [1]. It is estimated by the U.S. Environmental Protection Agency that the total vinyl chloride escaping to the atmosphere in the U.S.A. exceeded 100 million kg per year prior to 1975 [1]. Vinyl chloride loss from the average VC plant is estimated to be about 0.45 kg/100 kg of VCM produced [1]. Based on limited data, ambient concentrations of vinyl chloride exceeded 1 ppm ($2560 \mu\text{g}/\text{m}^3$) less than 10% of the time in residential areas located in the vicinity of plants producing VC or PVC. The maximum concentration of vinyl chloride found in ambient air was 33.0 ppm ($84,480 \mu\text{g}/\text{m}^3$) at a distance of 0.5 km from the center of the plant [1]. The average concentration of VCM in air around the PVC plants prior to 1975 was 17 ppb [15]. VCM has been determined in the air at levels of 3.1–1250 ppb in the Houston, Texas area (where an estimated 10% of the U.S. production capacity of PVC is located) [16].

The U.S. Environmental Protection Agency recently proposed new rules to reduce the national emission standard for vinyl chloride from 10 to 5 ppm in order to effect a reduction of vinyl chloride emissions by one-half within 3 years of the actual rulemaking. Based on new average-sized plants, this would result in hourly emissions of 5.1 kg from an ethylene dichloride-vinyl chloride plant instead of 10.3 kg; 9 kg from a dispersion PVC plant instead of 17.5 kg and 13.5 kg from a suspension PVC resin plant instead of 16 kg [17].

Early occupational exposure studies have revealed a wide range of VCM concentrations dependent on the manufacturing processes involved [18]. The air concentration of VCM in a polymerization reaction prior to ventilation has been reported [18] to be $7800 \text{ mg}/\text{m}^3$ (3000 ppm) and range from 1560 – $2600 \text{ mg}/\text{m}^3$ (600–1000 ppm) in a polymerization reactor after washing [19].

Concentrations of VCM in the working atmospheres in some plants producing PVC have been reported in the ranges of 100 – $800 \text{ mg}/\text{m}^3$ (40–312 ppm) with peaks up to $87,300 \text{ mg}/\text{m}^3$ (34,000 ppm) [20].

Additional concentrations of vinyl chloride have been reported for workplace air including: (1) 0.15 to 0.35 ppm in air in three English cable factories [21]; (2) greater than 75 ppm in air in a Yugoslavian PVC manufacturing plant [22]; and (3) greater than $113.6 \text{ mg}/\text{m}^3$ in air in a Russian synthetic leather plant [23].

In 1974, Heath [24] estimated that 20,000 U.S. workers in the past and to 1974 had been exposed to VCM in manufacturing plants. Baretta [25] in 1969 reported that on a time-weighted average, the concentrations of VCM to which coagulator operators are exposed ranges from 130–650 mg/m^3 (50–250 ppm). A more recent survey by NIOSH of 3 VCM plants reported that the time-weighted-average exposure to VCM ranged from 0.07–26.46 ppm [3, 26].

The concentration of residual VCM monomer in PVC powder that is fabricated into final products is also an important determinant of VCM in the ppm range. The entrapped concentration is dependent upon the production process and can range from 0.1 to 5.8 thousand ppm, which can be liberated during fabrication, particularly when heated [1]. PVC leaving

certain plants may contain 200–400 ppm VCM, on delivery to the customer, the level of VCM is about 250 ppm, and after processing, levels of 0.5–20 ppm are reached, depending on the method of fabrication [27]. However, new processing methods developed since 1974, leave as little as 1 to 2 ppm residual VCM in VCM resins [28]. Residual VCM in commercial food grade resins have been reduced by special techniques to 115 ppb (wt/wt) for resin and less than 0.048 ppb (wt/wt) for compound and sheet PVC [29].

VCM has been detected in effluents discharged by chemical and latex manufacturing plants and in raw water in the U.S.A. [30]. The U.S. Environmental Protection Agency estimated in 1974 that about 12.3 kg/day of VCM were discharged in the wastewater effluent from two VCM plants in the Long Beach, California area [3].

Vinyl chloride has been found in municipal water supplies in the U.S.A. [1, 14] in representative samples of the nation's community drinking water supplies that chlorinate their water and represent a wide variety of raw water sources, treatment techniques and geographical locations. The highest concentration of vinyl chloride detected in finished drinking water in the U.S.A. was 10.0 $\mu\text{g/l}$ [31]. The sources of the vinyl chloride found in the Miami, Florida and Philadelphia water supplies (5.6 and 0.27 $\mu\text{g/l}$, respectively) have not been identified [1].

Available results indicate that migration of vinyl chloride from rigid PVC water pipes does occur, and that it is a linear function of the residual vinyl chloride level in the pipe itself [1]. Only limited data are available on vinyl chloride emissions from the incineration of plastics. The quantities of vinyl chloride and combustion products varied as a function of temperature as well as with the type of plastics and their polymers [1].

It is believed that vinyl chloride should disappear significantly in its transport over long distances, however, in the immediate vicinity of emission sources, vinyl chloride can be considered a stable pollutant [1]. While no mechanism is presently known for the removal of vinyl chloride from the air at night, biological sinks such as microbial removal in soil may be of significance in depletion of vinyl chloride over a long time period. However, such sinks would not be expected to be important in terms of urban scale transport of vinyl chloride [1].

In studies performed in a laboratory model ecosystem, it was reported that vinyl chloride, despite its lipophilicity, is so volatile (vapor pressure in 2660 mm at 25°C) that it does not bioaccumulate or transfer appreciably through food chains, at least at ordinary temperatures [32].

Vinyl chloride has been found in a relatively small number of products packaged in PVC containers (e.g., in edible oils in a concentration of 0.05 to 2 ppm [33] and in butter and margarine [34]).

The U.S. Treasury Department in 1973 banned the use of PVC for the packaging of alcoholic beverages as a result of FDA reports indicating the presence of VCM at levels up to 20 mg/kg in some alcoholic beverages packaged in this material [35].

Vinyl chloride has been found in domestic and foreign cigarettes and little cigars in concentrations of 15–17 nanograms/cigarette [36].

New automobile interiors have been found to contain vinyl chloride in concentrations of 0.4 to 1.2 ppm [3, 37].

Experimental as well as human evidence of the carcinogenicity of vinyl chloride has been reviewed by Bartsch and Montesano [10] and IARC [2, 3]. The results to date show that vinyl chloride administered by inhalation is carcinogenic in rats, mice and hamster, producing angiosarcoma of the liver as well as of other tissues in all three species. In addition in rats, vinyl chloride induces tumors of the Zymbal glands and of the skin, nephroblastomas, hepatomas and neuroblastomas, in mice tumors of the lung and skin were also observed [38-48]. The lowest reported effective dose which is carcinogenic in rats and mice is 50 ppm [10, 44-48]. Two angiosarcomas of the subcutaneous tissue and a carcinoma of the Zymbal glands were observed in the offspring of mothers exposed during pregnancy to inhalation of vinyl chloride at 10,000 and 5000 ppm [3, 10]. It is noteworthy that these experiments not only indicate the carcinogenicity of vinyl chloride but also demonstrate that the type of tumor induced, angiosarcoma of the liver, was the same as that observed in workers exposed to VCM [2, 3, 10].

The relationship between exposure to VCM and development of angiosarcoma of the liver was first reported by Creech and Johnson [49] who cited three cases among workers employed in a single vinyl polymerization facility in the U.S.A. These men had cleaned reactor vessels as part of their employment. At present, a total of 17 cases of angiosarcoma of the liver have been reported among workers exposed to VCM in the U.S.A. Extensive world wide epidemiological studies to date have indicated about 50 cases of angiosarcomas of the liver associated with VCM exposure among workers employed in the manufacture of PVC resins (Table 1) [2, 3, 10, 12, 19-52]. Most of the tumors were observed in workers employed in the cleaning of autoclaves where the concentration of VCM is high (e.g., ranging from 500 to 2000 or more ppm in the air). It should be noted however that angiosarcomas of the liver were also found in people exposed to much lower concentrations of VCM (e.g., in a worker in the production of PVC bags who had been exposed to VCM for a total of 3 years and in an accountant employed for 10 years in a vinyl fabric factory) [10]. It has also been suggested that at least two other types of cancer, carcinoma of the brain and of the lung, appear with increased frequency in workers exposed to VCM [10, 54, 55].

Infante et al. [52] cited a significant excess of mortality from cancer of the lung and brain in addition to cancer of the liver, among workers occupationally exposed to VCM. The risk of dying from cancer of the lymphatic and hematopoietic system also appears to increase with an increase in latency. A study of cancer mortality among populations residing proximate to VCM polymerization facilities also demonstrated an increased risk of dying from CNS and lymphatic cancer [52]. However, it was noted by Infante et al. [52] that although these findings raise cause for concern about out-plant emissions of VCM, without further study these cancers cannot be unequivocally interpreted as being related to out-plant exposure to VCM.

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TABLE 1

CASES OF ANGIOSARCOMA OF THE LIVER AMONG WORKERS EXPOSED TO
VCM OR PVC[10]

Country	Number of cases	Age at diagnosis	Years from first exposure to	Total exposure in years	Occupation
U.S.A.	15	48(36-61) ^a	20(12-30) ^a	17(4-30) ^a	Polymerization workers
U.K.	2	71	26	20	Polymerization workers
		37	8	3.5	Polymerization workers
Norway	1	56	22	21	Polymerization workers
Sweden	1	43	19	18	Polymerization workers
Federal Republic of Germany	5	40	14	12	Polymerization workers
		38	12	12	Polymerization workers
		44	17	17	Polymerization workers
		49	17	11	Polymerization workers
France	2	43	19	19	Polymerization workers
		54	15	12	Polymerization workers
		43	13	12	Polymerization workers
Italy	2	43	15	6	Polymerization workers
		55	22	21	Polymerization workers
Rumania	1 ^b	27	11	5.5	Polymerization workers
Czechoslovakia	2	37	14	14	Polymerization workers
		45	15	15	Polymerization workers
Canada	9		no data available		Polymerization workers
Yugoslavia	4		no data available		Polymerization workers
Japan	1		no data available		Polymerization workers
U.K.	1	55	24	11	Pouring PVC oil onto fabric base
Sweden	1	61	27	23	VCM production worker
Federal Republic of Germany	1	43	14	14	Filling pesticide cans with VCM as propellant
Italy	1	36	6	3	Production of PVC bags

^a Average with, in parenthesis, minimal and maximal values.

^b This case was diagnosed as hepatocarcinoma associated with cholangiosarcoma.

The risk of cancer mortality among residents 45 years of age and older in three communities having vinyl chloride polymerization facilities was studied by Infante [56]. Among males the death rate from CNS tumor was higher than that for the state as a whole. No excess mortality from leukemia and aleukemia or from lymphoma was found.

A four- to five-fold excess of cancer of the liver and pancreas was found among 771 workers employed in a Swedish vinyl chloride/PVC plant since its start in the early 1940's [57]. Although the risk of cancer of the brain and of the lung was increased among workers exposed to vinyl chloride, it was reported by Byren et al. [57] not to be statistically significant.

The cancer mortality among 7561 males who were at some time between 1940 and 1974 exposed to vinyl chloride in the manufacture of PVC in the United Kingdom was investigated by Fox and Collier [58]. While an excess mortality from liver cancer was reported for each group of workers whether exposure was thought to be high, medium or low was reported, no evidence was found to support an excess of mortality from cancer other than of the liver. Relatively few persons had long term exposure to VCM and even in cases in which men had completed 20 years of employment, the follow-up period was considered too short to permit an evaluation of the carcinogenic effect of vinyl chloride [3,58].

While no excess mortality from lung cancer was demonstrated among individuals currently employed 15 years after initial occupational exposure to vinyl chloride, a 56% excess of lung cancer was observed among those individuals who had terminated employment prior to 15 years since initial VCM exposure. Among this later group, the excess lung cancer mortality was found for each duration of exposure [3, 59].

An excess of deaths due to cancer of four organ systems (e.g., brain and CNS, respiratory system, hepatic system and lymphatic and hematopoietic systems) was found by Waxweiler et al. [60] among 1294 individuals who had 5 or more years of employment and 10 years since onset of employment in departments or jobs directly related to VCM exposure at one of four VCM/PVC production plants in the U.S.A.

The cancer risk among a cohort of males who had at least one year of occupational exposure to VCM was studied by Tabershaw and Gaffey [55]. When compared to the U.S. white male population, excess of cancer of the digestive system (primarily angiosarcoma of the liver), respiratory system, brain and of unknown sites, as well as lymphomas was observed in those members of the study cohort with the greatest estimated exposure to vinyl chloride. Vital statistics was undetermined for 15% of the study cohort and only 50% of the study cohort had 15 or more years since onset of exposure to VCM [3].

A number of cytogenic studies have revealed high incidences of chromosome aberrations among workers occupationally exposed to vinyl chloride in the U.S.A. [61-63], the United Kingdom [64], Sweden [65], Belgium [66], Norway [67] and Hungary [68]. In most cases, these aberrations included "unstable" chromosome changes (e.g., fragments, dicentric and rings) and simple aberrations (e.g., breaks and gaps).

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Infante et al. [69] reported a significant excess of fetal deaths in wives whose husbands were exposed to vinyl chloride, e.g., 15.8% 23 fetal deaths in 139 pregnancies as compared to 8.8% (24 out of 273) in the age-adjusted control group.

The mutagenicity of vinyl chloride has been reviewed by Bartsch and Montesano [10], Bartsch et al. [70], Fishbein [71] and IARC [13].

Vinyl chloride vapor induced reverse mutations of the base-pair substitution type in *S. typhimurium* G-46, TA 1530, TA 1535 and TA 100 in the presence of 9000 g supernatant from rat liver [72-77], mouse liver [72, 75, 76] and human biopsies [72, 75]. Although vinyl chloride induced mutations in the absence of a metabolic activation system, a much higher mutagenic effect was noted when a 9000 g supernatant from liver was added [72, 74, 77].

Whereas vinyl chloride in aqueous solution was not mutagenic in the *Salmonella* test system [72, 73], it produced reverse mutations in *E. coli* K-12 [78], gene conversions in *S. cerevisiae* in the presence of 9000 g supernatant from mouse liver. Vinyl chloride also induced forward mutations in *S. pombe* in the host-mediated assay in mice [79, 84].

Vinyl chloride as a vapor or in ethanolic solution was non-mutagenic in *Neurospora crassa* in the presence or absence of metabolic activation [81].

While vinyl chloride was mutagenic in inhalation experiments in *Drosophila melanogaster* [82, 83], it was non-mutagenic in the tests for dominant lethals, translocations and sex-chromosome loss [83].

Anderson et al. [84] reported no dominant lethals in male CD-1 mice after exposure by inhalation to 3000, 10,000 and 30,000 ppm vinyl chloride for 6 h/day for 5 days.

Forward mutations (in terms of 8-azaguanine and ouabain resistance) were induced in V-79 Chinese hamster cells following their exposure to vinyl chloride vapor in the presence of 15,000 g supernatant from rat liver [85].

The mutagenicity of possible metabolites of vinyl chloride (e.g. chloroethylene oxide, chloroacetaldehyde, chloroethanol and chloroacetic acid) has been extensively studied. Chloroethylene oxide was reported to be the strongest mutagen among those tested in *S. typhimurium* G-46, TA 1530 and TA 1535 strains [72, 73, 75], *E. coli* [86], *S. cerevisiae* [79], *S. pombe* [79], and V-79 Chinese hamster cells [87].

Chloroacetaldehyde was mutagenic in *S. typhimurium* [72-75] and V-79 Chinese hamster cells [87]. Chloroethanol was weakly mutagenic in *S. typhimurium* [72-75, 88], while chloroacetic acid was not mutagenic in *S. typhimurium* [72-75].

Elmore et al. [89] screened without exogenous activation seven potential metabolites of vinyl chloride in their pure forms as well as the related epichlorohydrin in tester strains of *Bacillus* and *Salmonella*. Chlorooxirane (chloroethylene oxide), chloroacetaldehyde, chloroacetaldehyde monomer hydrate, chloroacetaldehyde dimer hydrate, chloroacetaldehyde trimer and epichlorohydrin produced significant mutagenic activity in *S. typhimurium* strains sensitive to base pair mutation. A recombination repair deficient strain of *B. subtilis* was inhibited in growth by these compounds, whereas

excision repair deficient and wild type strains of *B. subtilis* were relatively unaffected.

Vinyl chloride at a concentration of 0.0103 M (723 ppm) in nutrient broth was negative in both the *Salmonella* and *Bacillus* cultures. (High concentrations of VCM (20% v/v in air - 200,000 ppm) produced mutagenic action in previous assays with *Salmonella* tester strains [72, 73].)

Chloroethanol and chloroacetic acid were non-mutagenic at 1 mM concentrations in the above mutagenicity assays of Elmore et al. [89]. Among the compounds tested by Elmore et al. [89], chloroacetaldehyde and chlorooxirane were the most mutagenic with the lowest toxic side-effects. Hence, it was suggested that they may be the active carcinogenic derivatives of vinyl chloride [89]. However, chloroacetaldehyde monomer hydrate was considered a more realistic choice as the *ultimate* carcinogen than the monomer compound which reacts immediately with water. The lower mutagenic activity of chlorooxirane compared to chloroacetaldehyde monomer hydrate may reflect the unstable nature of chlorooxirane as an α -chloroether [73]. One mode of action of chlorooxirane is a rearrangement to chloroacetaldehyde [90] via the NIH shift [91]. Another is a homolytic ring cleavage to yield a stabilized diradical intermediate $\text{ClCH}-\text{CH}_2\text{O}$ with both being capable of reacting with DNA to account for the mutagenicity of chlorooxirane [73].

Bartsch [92] recently reported on a collaborative study involving the biological activity of vinyl chloride, three of its identified mammalian metabolites (chloroethylene oxide, chloroacetaldehyde and chloroacetic acid) and a putative metabolic intermediate (2-chloroethanol) which were assayed in several prescreening tests for carcinogens, e.g., (a) assay for electrophilic reactivity; (b) tissue-mediated mutagenicity tests with *S. typhimurium* or with (c) *S. pombe* and *S. cerevisiae*; (d) host-mediated assay in mice with *S. pombe*; (e) *in vitro* mutagenicity in Chinese hamster V79 cells using ouabain or 8-azaguanine resistance; (f) induction of DNA repair synthesis ("unscheduled" incorporation of [^3H] thymidine) in cultured human fibroblasts; and (g) tests for recessive lethal mutations in *Drosophila melanogaster*. Chloroethylene oxide, which was the most active compound in the assay systems (a) to (f), induced local tumors in mice upon repeated subcutaneous administration. It was stated by Bartsch [92] that these results strongly support the hypothesis that chloroethylene oxide is one of the principal mutagenic and/or carcinogenic intermediates formed from vinyl chloride by mammalian metabolism.

Negative results were reported by Mattern et al. [93] in their attempts to use the mutagenicity testing of urine from 20 workers in vinyl chloride producing plants in Holland, and vinyl chloride-treated rats using the *Salmonella* strains TA 100 in the former case and strains TA 1535, TA 98 and TA 100 in the latter case. Urine samples were assayed in the presence or absence of liver homogenates from Aroclor 1254-treated rats, with or without β -glucuronidase in the plates. Whether a different treatment of the urine samples would reveal mutagenic activity, or whether no mutagenic metabolites of vinyl chloride are excreted in the urine remains to be determined [93].

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While the predominant risk has been at the level of occupation health due to the exposures in vinyl chloride and PVC plants, it is also of importance to determine whether other products in the process could also be carcinogenic and/or mutagenic. The synthesis of vinyl chloride either from acetylene or ethylene or from a mixture of these substances, results in a tar-like by-product called EDC-tar. This name originates from one of the main components, ethylene dichloride (or 1,2-dichloroethane) [94, 95]. EDC-tar, however, is a complex mixture, consisting chiefly of chlorinated aliphatic hydrocarbons [94] (e.g., approximately 33% ethylene dichloride; 1,1,2-trichloroethane and about 0.06% vinyl chloride monomer). The composition of EDC-tar varies not only from factory to factory, but also from time to time from the same factory [96].

Recent mutagenicity studies of a Swedish sample of EDC-tar utilizing *Salmonella*/mammalian microsome test (TA 1535) showed that this sample contained direct as well as indirect mutagenic compounds [96]. The mutagenic effect observed was not due to any significant extent to one of the main components (ethylene dichloride).

The *in vivo* and *in vitro* metabolism of vinyl chloride has been extensively studied resulting in a diversity of results and conclusions [10, 75, 78, 97-107]. The metabolism of vinyl chloride has recently been reviewed by Pflugge and Safe [108] and IARC [3]. The major metabolic pathway for the formation of the major urinary metabolites (chloroacetate; S-carboxymethyl cysteine; S-(2-hydroxyethyl)cysteine; N-acetyl-S-(2-hydroxyethyl)-cysteine and thioldiglycollate) is shown in Fig. 1 [108]. Vinyl chloride is metabolized by the liver mixed function oxidase system (MFOS) (predominantly through the cytochrome P-450 system) [109-112] to the oxirane, chloroethylene oxide and chloroacetaldehyde) which then can either bind directly or enzymatically via glutathione-epoxide-transferase [113] or other glutathione transferases [114] to form S-formyl methyl glutathione. Chloroacetaldehyde can also be directly oxidized to chloroacetaldehyde which is

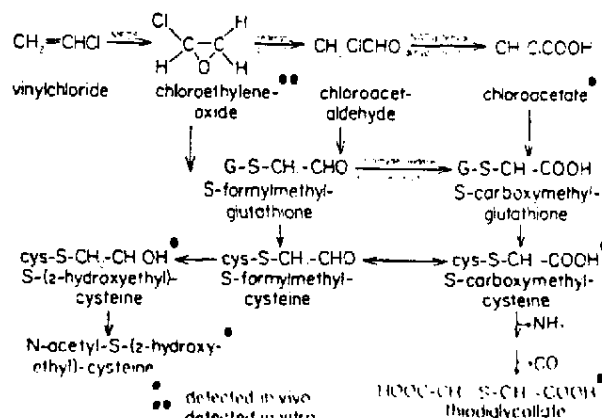


Fig. 1. Major metabolic pathway of vinylchloride for the formation of the major urinary metabolites.

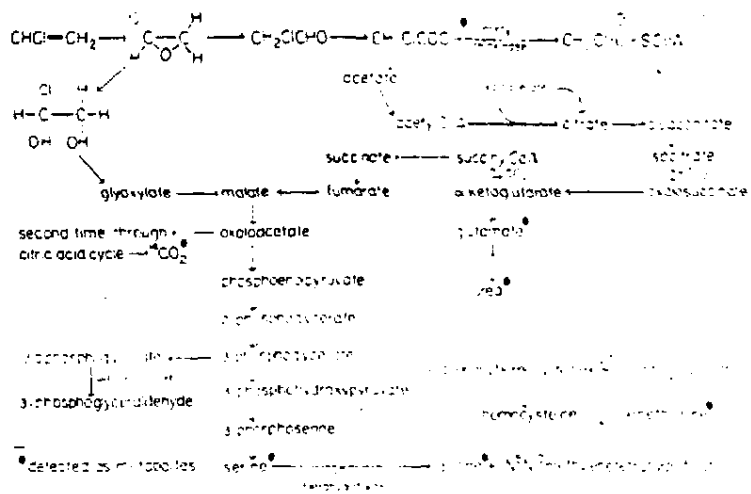


Fig. 2. Metabolism of vinylchloride via chloroacetaldehyde.

then either excreted or bound to GSII to form carboxymethyl glutathione which can also be formed from S-formylmethyl glutathione.

The generation of $^{14}\text{CO}_2$ following the administration of ^{14}C -vinyl chloride in inhalation [105], oral [106], intragastric [101,102] and intraperitoneal [101,102] was postulated by Plugge and Safe [108] to occur via two possible pathways: (1) addition/transfer of a chloroacetyl group to CoA followed by metabolism in the TCA cycle (Fig. 2) or (2) the formation of glycolate followed by its oxidation to glyoxylate (Fig. 3) after which it enters the C_2 and C_1 pools.

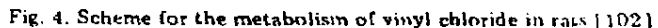
The main eliminative route for ^{14}C -vinyl chloride after oral, i.v. or i.p. administration to rats is pulmonary: both unchanged vinyl chloride and $^{14}\text{CO}_2$ are excreted by that route and other ^{14}C -metabolites via the kidneys. After intragastric administration, pulmonary output of unchanged vinyl chloride is proportional to the logarithm of reciprocal dose. Pulmonary excretion of unchanged vinyl chloride after oral dosing is complete with 3–4 h; but pulmonary elimination of CO_2 and excretion of metabolites occupies 3 days. In comparison, 99% of a small i.v. dose is excreted unchanged within 1 h of injection; 80% after 2 min. The rate of elimination of single oral doses of ^{14}C -vinyl chloride is uninfluenced by up to 60 days chronic dosing with the unlabeled substance [101,102].

A scheme for the metabolism of vinyl chloride in rats (Fig. 4) was proposed by Green and Hathway [102] in which the urinary metabolites S-(2-chloroethyl)cysteine (a) and N-acetyl-S-(2-chloroethyl)cysteine (b) are formed only through direct interaction of glutathione derived cysteine with vinyl chloride *per se*.

There is a strong supposition that in rats chloroethylene oxide (Fig. 5) [101] is formed from vinyl chloride and transformed spontaneously

ethylene oxide
| spontaneously

Vinylidene chloride (1,1-dichloroethylene; asym. dichloroethylene; 1,1-dichloroethene; DCE; VDC; $\text{CH}_2=\text{CCl}_2$) is produced commercially in the U.S.A. and Japan by the dehydrochlorination of 1,1,1-trichloroethane



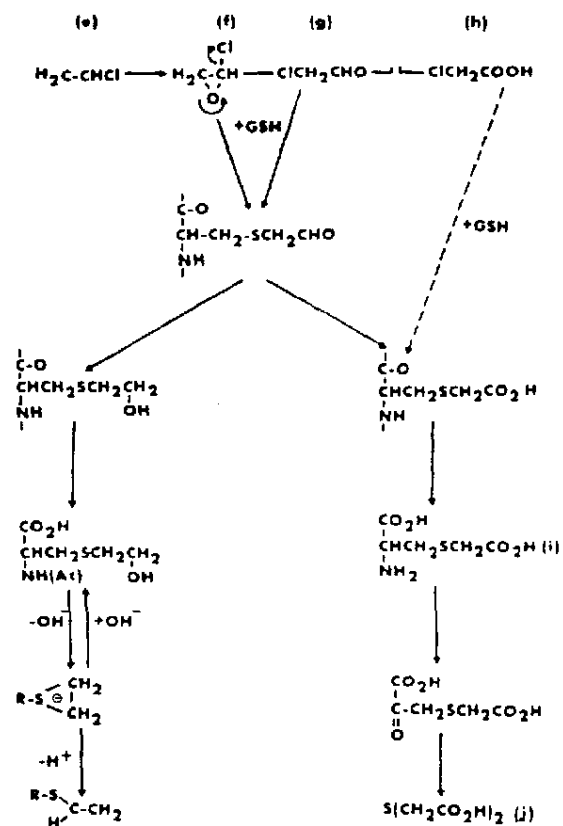


Fig. 5. Scheme for the biogenesis of S-containing vinyl chloride metabolites [101].

(methyl chloroform) or 1,1,2-trichloroethane, both of which are derived from ethylene dichloride [116,117].

Specific impurities in vinylidene chloride monomer depend upon its method of manufacture and isolation. A typical commercial grade of vinylidene chloride, 99.7% by weight as prepared by dehydrochlorination of 1,1,2-trichloroethane with lime or caustic [117] contains the following impurities (in ppm): vinyl chloride, 850; *cis*-1,2-dichloroethylene, 500; *trans*-1,2-dichloroethylene, 1500; 1,1-dichloroethane, 10. Phenol at levels of 0.6--0.8% or 200 ppm or monomethyl ether or hydroquinone (MEHQ) are added to prevent polymerization during shipment and storage. A typical analysis of vinylidene chloride monomer (unstabilized) largely produced in the U.S.A. [118] includes as impurities (in ppm): vinyl chloride, 23; vinyl bromide; *trans*-1,2-dichloroethylene, 1000; and *cis*-1,2-dichloroethylene, 410.

It was estimated that two U.S. companies at three sites (Gulf Coast) in 1976 produced a combined total of 70 million kg of vinylidene chloride and

that one of these companies manufactured an additional 50 million kg of vinylidene chloride for captive use as an unisolated intermediate in the production of 1,1,1-trichloroethane [119].

It was postulated that in the U.S.A., the growth rate during the 1974-1979 period in the production of vinylidene-chloride will be based on 1,1,2-trichloroethane which is used as a feedstock in one vinylidene chloride production method. It was also estimated that the consumption of 1,1,1-trichloroethane used in a second vinylidene chloride will not change over this time period [120].

In 1976, three Japanese companies produced a combined total of 28.2 million kg of vinylidene chloride [119]. No information is available concerning the production of vinylidene chloride elsewhere.

Excluding the amount of vinylidene chloride used as an unisolated intermediate in the production of 1,1,1-trichloroethane, more than 90% of the vinylidene chloride produced in the U.S.A. and Japan is used in the production of copolymers of high vinylidene chloride content, the other major monomer usually being vinylidene chloride [119]. The remaining 10% or less of vinylidene chloride produced is used for the manufacture of modacrylic fibers which are largely based on acrylonitrile with small amounts of vinylidene chloride and other monomers [119]. A number of copolymers of vinylidene chloride (in latex, fiber, film and resin forms) are referred to as "Saran" and have wide utility for film wraps for food. Saran production is estimated at about 150 million pounds per year [120].

The copolymers presently of commercial interest in the U.S.A. include: vinylidene chloride-vinyl chloride (Saran B); vinylidene chloride-alkyl-acrylate (Saran C) and vinylidene chloride-acrylonitrile (Saran F) [119]. U.S. production of vinylidene chloride copolymers in 1977 [121] has been estimated at 68 million kg while in 1976, four Japanese companies produced an estimated 31.9 million kg of vinylidene chloride-vinyl chloride copolymers, 2.7 million kg of which was fibers and the remainder was latex and film [119].

The number of workers engaged in the production of vinylidene chloride monomer *per se* in the U.S.A. (compared to vinyl chloride monomer) appears to be small e.g., 75 and 12-15 at two major vinylidene chloride production facilities [122]. Vinylidene chloride is polymerized to plastic resins at 12 facilities and the resins are then fabricated into plastics at 60 to 75 plants throughout the country [120]. Estimates of the number of workers engaged in the preparation of polymers and co-polymers of vinylidene chloride and vinyl chloride (e.g., the preparation of Saran wrap) are not available, nor are data available at present on workers exposed to only vinylidene chloride during their working lifetimes.

The American Conference of Governmental Industrial Hygienists (ACGIH) recommends that an employee's exposure to vinylidene chloride does not exceed an eight-hour time weighted average of 10 ppm (10 mg/m³) in the workplace air in any eight-hour work shift of a forty-hour work week. During any fifteen-minute period, the ACGIH proposes an absolute ceiling concentration limit of 29 ppm (80 mg/m³) provided the daily threshold

limit value (in terms of 8-hour time weighted average values) is not exceeded [123].

There is a paucity of data concerning worker exposure to vinylidene chloride. Worker exposure has generally not been monitored in the past according to EPA [120]. Tests have shown that 20,000 ppm can easily be reached in the proximity of a spill in some cases past worker exposure to vinylidene chloride may have exceeded those of vinyl chloride which were measured at 300–1000 ppm before OSHA limits were imposed.

Workers involved in manufacturing facilities using vinylidene chloride in polymerization processes such as the production of PVC have been reported to be exposed to vinylidene chloride in amounts of less than 5 ppm and most frequently to trace levels [124,125]. Levels of 2 ppm of vinylidene chloride have also been detected as a contaminant of submarine and spacecraft atmospheres [119,126].

A substantial amount of vinylidene chloride appears to be vented to the atmosphere during its production, polymerization and fabrication. Emissions of vinylidene chloride in the U.S.A. in 1974 have been estimated at 1.5 million kg from monomer synthesis operations. This was reduced to 277 thousand kg by new control technology in late 1975. Vinylidene chloride losses from polymer synthesis operations and polymer fabrication operations were 308 thousand kg and 13.8 thousand kg, respectively [127].

To estimate the population at risk due to vinylidene chloride emissions, EPA [120] assumed that the populations of the cities and countries which the production and use of vinylidene chloride exist are at risk. Using a total U.S. population figure of 212 million, the population at risk due to these producers and major users of vinylidene chloride (>\$1000 annually or >1000 lbs annually) was estimated to be about 4.7% to the U.S. population or approximately 10 million people. Other sources of exposure to the population include the other user facilities located throughout the country and those involved in transportation. Based on solubility data, it is estimated by EPA that all vinylidene chloride in wastewater is released to the atmosphere and thus probably little exposure occurs through the use of water [120].

Vinylidene chloride has been detected in effluent discharged by chemical and latex manufacturing plants in the U.S.A. [119] and in effluent discharged from chemical manufacturing plants in the Netherlands at a concentration of 32 $\mu\text{g/l}$ [128]. Vinylidene chloride has also been identified in the U.S.A. in well, river, and raw water [129]. It has also been found in finished drinking water in the U.S.A. where the highest reported concentration was 0.1 $\mu\text{g/l}$ [130].

Vinylidene chloride has also been found as an impurity in vinyl chloride monomer [131], and trichloroethylene [132] and at a level of 0.011% in commercial chloroprene [133].

As much as 25% of the vinylidene chloride used in any given Saran production run has been estimated to be disposed of in landfill (primarily in polymerized form) although there are no estimates of the levels of unreacted monomer [120].

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Commercial household and industrial Saran wrap have been analyzed for residual vinylidene chloride monomer. Six rolls of household film had monomer concentrations ranging from 6.5 to 10.4 ppm with an average of 8.8 ppm with no significant differences found in samples taken from the beginning (outside) or the end (inside) of each roll. Levels of monomer ranging from 10.8 to 26.2 ppm were found in the industrial film with levels increasing from the beginning to the end of the roll [119,134].

Although the widespread use of vinylidene polymers as food wraps could result in the release of unreacted monomer into the food chain [121,135], and vinylidene chloride copolymers containing a minimum of 85% vinylidene chloride have been approved for use with irradiated foods [136], information is scant as to the migration of unreacted monomers from these sources either into food or via disposal of the polymeric material *per se*. One report states that no more than 10 ppm of unreacted vinylidene chloride is contained in Dow's product Saran Wrap and that within detectable limits, no more than 10 ppb could get into food, even under severe conditions of use [137].

An investigation of the cancer risk among a cohort of 138 workers exposed to vinylidene chloride (where vinyl chloride was not used as a copolymer) revealed no findings statistically related or individually attributed to vinylidene chloride exposure [124]. Fifty-five people had less than 15 years since first exposure and only 5 deaths were observed; 27 workers were lost to follow up but were considered alive in the analysis [124].

The health effects of vinylidene chloride have been reviewed by Hadey [138] and IARC [119] and the U.S. Environmental Protection Agency [139].

Aspects of the reported carcinogenicity of vinylidene chloride appear conflicting and indicate sex, species, and strain specificity. Twenty-four of 150 Swiss male mice exposed to 25 ppm of vinylidene chloride in air for 4 hours daily, 4-5 weeks for 52 weeks, developed adenocarcinoma of the kidney (compared to 1 out of 150 females). No such tumors occurred in mice exposed to 10 ppm for 52 weeks or in controls [140,141], nor in BALB/C, C56BL or C₃H mice or Sprague-Dawley rats and hamsters similarly exposed to vinylidene chloride [142].

An increased incidence of mammary fibroadenomas and carcinomas was reported in female Sprague-Dawley rats exposed to 10, 25, 50, 100 and 150 ppm vinylidene chloride in air for 4 h/day, 4-5 days a week for 52 weeks and observed for up to 82 weeks. No dose-response relation was found and in one rat treated with 100 ppm, vinylidene chloride, one Zymbal gland carcinoma was observed [141]. Viola [143] reported that male and female Wistar rats exposed to 100 ppm of vinylidene chloride by inhalation developed abdominal lymphomas and subcutaneous fibromas.

Two-year studies at Dow Chemical Co., involving both vinylidene chloride administered in the drinking water (60, 100 and 120 ppm) and repeated inhalation (10 or 40 ppm 6 h/day; 5 days/week; after 5 weeks, 75 ppm for up to 18 months) to male and female Sprague-Dawley rats have been carried out [145,146] and indicated no dose-related clinical differences or

cumulative mortality differences or findings of neoplasia. Reproduction studies with vinylidene chloride administered to Sprague-Dawley rats by inhalation or ingestion in the drinking water showed the compound to be neither a teratogen or mutagen or one adversely affecting reproductivity [145]. The vinylidene chloride (99.5%) tested in Dow studies contained trace amounts (ppm) of the following impurities: vinyl bromide, 4; vinyl chloride, 3-50; *trans*-1,2-dichloroethylene, 138-1300; *cis*-1,2-dichloroethylene, 0.013-0.16%; 1,1,1-trichloroethane, 0.03; and 1,1,2-trichloroethane [145].

Winston et al. [147] and Lee [148] reported the only tumor in CD-rats exposed to 55 ppm vinylidene chloride for 9 months was a subcutaneous hemangiosarcoma of the skin in one of the rats tested. Hemangiosarcoma of the liver and lung in CD rats exposed for 9-12 months to 250 or 1000 ppm of vinylidene chloride have been reported. In addition, there were no lesions in the testes or accessory organs indicative of a treatment related effect on reproductive performance [66].

In a preliminary report by Maltoni et al. [141] of an ongoing study involving Sprague-Dawley rats administered 5, 10 or 20 mg/kg body weight vinylidene chloride by stomach tube once daily, 4-5 days/week for 52 weeks, 1 carcinoma of the Zymbal gland was observed in a rat treated with a 10 mg/kg dose. At the time of reporting, the rats had been observed for 93 weeks after the start of treatment.

In most recent studies at IARC [119], vinylidene chloride when given by the oral route, induced malignant and benign liver tumors in C57B1 mice of both sexes and gastric tumors in female mice [119] and or oral cavity tumors and of meningiomas in male BDIV rats.

Maltoni et al. [141] reported in a study still in progress that no tumors had occurred at 74 weeks among a group of 30 male and 30 female Chinese hamsters, 28 weeks of age exposed to 25 ppm vinylidene chloride in air for 4 h/day, 4-5 days/week for 52 weeks.

Vinylidene chloride in air (2% and 20%) produced reverse mutations in *S. typhimurium* TA 1530 and TA 100 in the presence of 9000 g supernatant from mouse and rat liver, lung and kidney [149]. The lower mutagenic response observed with a concentration of 20% vinylidene chloride may have resulted from an inhibitory action of vinylidene chloride and/or its metabolite(s) on the microsomal enzymes responsible for its metabolic activation. It was postulated by Bartsch et al. [149] that 1,1-dichloroethylene oxide (in analogy with chloroethylene oxide the suggested primary metabolite of vinyl chloride) may be a primary reactive metabolite of vinylidene chloride. It is also considered possible that partial dechlorination of vinylidene chloride by microsomal enzymes results in vinyl chloride and its metabolic products [149].

Vinylidene chloride in solution induced reverse mutations in *E. coli* K12 in the presence of 9000 g supernatant from mouse liver [78].

Vinylidene chloride was not mutagenic in the dominant lethal test in male CD-1 mice exposed by inhalation to 10, 30, 50 [150] and 55 [151] ppm for 6 h/day for 5 days/week.

U.S.A. and resultant legislation restricted its use, it is expected that during the next five years consumption of trichloroethylene for metal cleaning will decline at an average rate of 3% and be most probably replaced by 1,1,1-trichloroethane and perchloroethylene [157].

Six percent (25 million pounds) of the trichloroethylene production is used as a chain terminator for polyvinyl chloride production [153,154]. Additional areas of utility include: as an extract in food processing (e.g., for decaffeinated coffee), as a chemical intermediate; as a solvent in the textile industry and research laboratories; as an ingredient in printing inks, lacquers, varnishes and adhesives, and in the dry-cleaning of fabrics. A pharmaceutical grade of trichloroethylene is used as a general anesthetic in surgical, dental and obstetrical procedures.

Largely because of its solvent properties, trichloroethylene is incorporated in a number of consumer products (e.g., cleansers for automobiles, buffing solution, spot remover, rug cleaner, disinfectant and deodorant) [158].

The threshold limit value for trichloroethylene in the U.S.A. is 0.535 mg/m^3 (100 ppm). OSHA is currently in the process of approving the Criteria Document for trichloroethylene which recommends that a TLV expressed as a time-weighted average exposure for an 8-h workday continue at 100 ppm and that the present ceiling be reduced from 1.07 mg/l (200 ppm) to 0.80 mg/l (150 ppm) [159,160]. The maximum allowable concentration in the U.S.S.R. is 10 mg/m^3 and the MAC in several European countries has been set at 0.273 mg/l (50 ppm) or even lower [161].

The number of U.S. workers exposed to trichloroethylene has been estimated to be about 283 thousand [158] (Table 2). Levels of $1076\text{--}43,000 \text{ mg/m}^3$ (200–8000 ppm) of trichloroethylene have been reported in a small U.S. factory [162].

Emissions of commercial organic solvent vapors into the atmosphere have been increasing dramatically in the last decade [163]. The loss of trichloroethylene and perchloroethylene to the global environment in 1973 was estimated to be each over 1 million tons [163]. Trichloroethylene emissions can occur principally from three sources, production, transportation and consumption. Estimated emissions from trichloroethylene production are 57.0 lbs emitted/ton produced [154,164]. The quantity of trichloroethylene discharged from domestic transport is very difficult to evaluate but it is believed that emissions occur almost inevitably from loading and transfer operations as well as accidental spills [165].

The major sources of emissions resulting from trichloroethylene consumption can be attributed to its use as a solvent in open top vapor degreasers [154]. The average emission rate for an open top vapor degreaser in the U.S.A. is 110 tons/year [166]. Assuming that 55% of the vapor degreasing operations in 1974 used trichloroethylene, the total national emissions would have been approximately 121,000 tons or roughly 70% of the total amount of trichloroethylene used in metal cleaning operations [154,167].

Cold cleaners are another type of metal degreaser which can contribute to trichloroethylene emissions. The average emission rate for a cold cleaner is

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TABLE 2
OCCUPATIONAL EXPOSURE ESTIMATED NUMBER OF WORKERS EXPOSED TO
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Industry	Estimated No. exposed*
Agricultural services	121
Oil and gas extraction	267
Ordinance	57
Food products	2,502
Textile mill products	1,014
Apparel/textile products	858
Lumber products	72
Furniture mfg.	162
Paper products mfg.	2,230
Printing trades	2,876
Chemical mfg.	9,352
Plastics products	713
Rubber/plastics mfg.	4,985
Leather products	725
Stone/clay products	2,685
Primary steel mfg.	11,672
Metall fabrication	11,709
Machinery mfg.	7,481
Electrical equipment	66,727
Transportation equipment	54,174
Instrument mfg.	4,815
Miscellaneous mfg.	1,516
Trucking/warehousing	642
Air transportation	23
Communication	5,560
Wholesale trade	3,327
Automotive dealer	223
Furniture stores	597
Ranking	2,391
Personal services	383
Misc. business services	27,759
Auto repair	5,246
Misc. repair	17,198
Amusement services	7,987
Marine services	20,053
Misc. unclassified	4,138
Estimated total	282,653

*Projections based on preliminary data obtained from the National Occupational Hazard Survey, Hazard Surveillance Branch, Office of Occupational Health Surveillance and Research, NIOSH (Does not include anesthetic use or use in trademark products).

about 0.33 tons/year [166] and compared to vapor degreasers, a minor amount of trichloroethylene is used in this type of operation.

Concentrations of trichloroethylene vapor in degreasing units have been reported to range from 20-500 ppm (at breast height above the bath) with the highest levels being over the baths which relied on manual removal of

articles [168], and between 150 and 250 ppm in degreasing rooms *per se* [169]. Concentrations of vapor in a dial assembly workshop in a Japanese factory ranged from below 135 mg/m³ (25 ppm) to over 538 mg/m³ (100 ppm) [169].

Concentrations of anesthetics (including trichloroethylene) in operating rooms to which surgeons and nurses were exposed varied from 1.6-554 mg/m³ (0.3 to 103 ppm) [170]. It is estimated that about 5000 medical, dental, and hospital personnel are routinely exposed to trichloroethylene [158].

Typical concentrations of trichloroethylene at 8 locations in 5 U.S. states in 1974 ranged from 970 ng/m³ (180 ppt) in urban areas to less than 110 ng/m³ (20 ppt) in rural areas [171]. Less than 30 ng/m³ (5 ppt) were found in air samples in rural Pullman, Washington, from December, 1971 to February, 1975 [172]. Trichloroethylene has been recently detected in air over 3 New Jersey industrialized cities [173]. Concentrations of trichloroethylene in air samples taken at 5 land stations ranged from 2-28 ng/m³ (0.5-5 ppt) at 11 sea stations from 1-22 ng/m³ (0.2-4 ppt) and over the northeast Atlantic Ocean from 5-11 ng/m³ (1.2 ppt) [174].

Slightly enhanced levels of trichloroethylene following chlorination of water at sewage treatment plants have been found in the U.S.A. [175,176]. Trichloroethylene has been found in the organic constituents of Mississippi River water (before and after treatment) and in the organic constituents of commercial deionized charcoal filtered water [177]. Trichloroethylene concentrations of 54 kg/day of 1.2 ng/l in average raw wastewater flow have resulted from a decaffeination process used in the manufacture of soluble (instant) coffee in California [178].

Trichloroethylene has been found in foodstuffs such as dairy products, meats, oils and fats, beverages and fruits and vegetables in levels ranging from 0.02 µg/kg in wine to 60 µg/kg in packaged tea [163]. Trace levels of trichloroethylene have also been found in edible oils after extraction [179]. The use of trichloroethylene for caffeine has recently been discontinued in the U.S.A. and the FDA in 1977 announced that it intends to ban the use of trichloroethylene in foods, drugs and cosmetics. In the latter category, it had been used as a topical anesthetic in some cosmetics [180,181].

The National Cancer Institute (NCI) in the U.S.A. has recently issued a "state of concern" alert, warning producers, users, and regulatory agencies that trichloroethylene administered by gastric intubation to B6C3F₁ mice induced predominantly hepatocellular carcinomas with some metastases to the lungs, e.g., 30 of 98 (30.6%) of the mice given the low dose (1200 mg/kg and 900 mg/kg for male and female respectively) and 41 of 95 (43.2%) of the mice given the higher dose (2400 mg/kg and 1800 mg/kg for male and female, respectively). Only one of 40 (2.5%) control mice developed these carcinomas [158,182].

No hepatocellular carcinomas were observed in both sexes of Osborne-Mendel rats administered trichloroethylene at levels of 1.0 or 0.5 g/kg by gastric intubation 5 times weekly for an unspecified period [158].

No liver lesions or hepatomas were found in N1C⁺ mice given oral doses by

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Henschler et al. [184] recently postulated that the carcinogenic effect of the technical sample of trichloroethylene used in the bioassay experiments [185] is most probably predominantly, if not exclusively, due to the epoxides [186] which are added to some but by no means to all brands of trichloroethylene. Technical grade trichloroethylene contains several impurities which must be stabilized for use as a degreasing agent, by antioxidants [153,184] (e.g., amines at levels of 0.001 to 0.2%) or combinations of epoxides and esters (0.2 to 2% total) [153]. Gas chromatographic - mass spectrometric analysis of the trichloroethylene sample performed by Henschler et al. [184] indicated a proportion of identified contaminants amounting to 0.65% including strong monofunctional alkylating agents of known mutagenicity and/or carcinogenicity (e.g., epichlorohydrin and 1,2-epoxybutane).

Trichloroethylene (3.3 mM) in the presence of a metabolic activating microsomal system induced reverse mutations in *E. coli* strain K12 [78]. It has also been shown to induce frameshift as well as base substitution mutation in *S. cerevisiae* strain XV185-1⁴C in the presence of mice liver homogenate [187].

Trichloroethylene exhibited dose-dependent mutagenicity at concentrations of 0.01, 0.1 and 1 mg/ml when tested in *S. typhimurium* strains TA 1535 and 1538 without metabolic activation [188]. In the host-mediated assay (with female ICR mice) using strains TA 1950, TA 1951 and TA 1952, there was a significant increase in the number of revertants at trichloroethylene and perchloroethylene doses on the level of LD₅₀ and 1/2LD₅₀.

Cytogenetic analysis of mice bone marrow cells performed after single and repeated i.p. applications (5 applications in one-day intervals) after 6, 24 and 48 h following the last application showed no significant increase in chromosomal aberrations following treatment by either trichloroethylene or perchloroethylene [188].

No adverse effects on embryonal or fetal development were observed following exposure of pregnant mice and rats to levels of 0.34 mg/l (65 ppm) or 1.605 mg/l (300 ppm) of trichloroethylene [189].

The metabolism of trichloroethylene has been reviewed by IARC [153], Kelley [190], Henschler [191], Bonse and Henschler [192] and Van Duuren [193].

There are three major metabolic transformations: (1) oxidation to chloral hydrate which takes place in the microsomal fraction of liver cells; (2) reduction to trichloroethanol and trichloroethylene is metabolized to trichloroethanol and trichloroacetic acid in rats [194] and dogs [195]. The earlier suggestion of Powell [196] in 1945 that formation of these metabolites implies rearrangement of the transient trichloroethylene oxide intermediate into chloral has been confirmed by a number of findings including: (1) the identification of chloral as a trichloroethylene metabolite *in vitro* [197,198] and (2) by study of the rearrangement of the oxides belonging to

a series of chlorinated ethylenes [191,192]. The formation of trichloroethylene oxide (2,2,3-trichloro-oxirane) from trichloroethylene in aerobic incubates of liver microsomes and NADPH was confirmed by spectral investigation of the cytochrome P-450 complex [199].

Epoxides are now recognized as obligatory intermediates in the metabolism of olefins by hepatic microsomal mixed-function oxidases [200]. The metabolism of trichloroethylene through the oxirane includes different reactive molecular species. The participation of radical intermediates during opening of the oxirane ring and chlorine migration is possible [192].

Trichloropropane oxide has recently been reported to augment the microsomal dependent covalent binding of trichloroethylene to DNA or protein, to a greater degree in male than female mice. A correlation between such binding and liver tumor induction by trichloroethylene in B6C3F1 mice (but not in Osborne-Mendel rats) was suggested [193,201,202]. The irreversible binding of ^{14}C -labeled trichloroethylene to mice hepatic protein *in vivo* and *in vitro* has also recently been reported [203].

(4) Tetrachloroethylene

Tetrachloroethylene (perchloroethylene; $\text{Cl}_2\text{C}=\text{CCl}_2$) is prepared primarily via two processes [203]: (1) the Huels method whereby direct chlorination of ethylene yields 70% perchloroethylene, 20% carbon tetrachloride and 10% other chlorinated products and (2) hydrocarbons such as methane, ethane or propane are simultaneously chlorinated and pyrolyzed to yield over 95% perchloroethylene plus CCl_4 and HCl .

The world-production of perchloroethylene in 1972 was 680 million kg; its growth rate estimated at 7%/year with a total world production estimated at 1100 million kg for 1980 [204]. The consumption pattern for perchloroethylene in the U.S.A. in 1974 is estimated to have been as follows: textile and dry cleaning industries, 69%; metal cleaning, 16%; chemical intermediate (e.g., preparation of trichloroacetic acid in some fluorocarbons), 12%; and miscellaneous 3%. Perchloroethylene is used as a solvent in the manufacture of rubber solutions, paint removers, printing inks, and solvent soaps, as a solvent for fats, oils, silicones and sulfur and as a heat-transfer medium [203].

Some 500,000 workers currently are at risk of exposure to perchloroethylene according to NIOSH. It was also noted that over 20,000 dry-cleaning establishments and a large number of other industries manufacture or use perchloroethylene [205].

The current TLV for perchloroethylene in the U.S.A. is 670 mg/m^3 (100 ppm). OSHA is attempting to reduce this standard to 50 ppm [206].

Work environment hygiene standards (all in terms of 8-hour time weighted averages) for perchloroethylene reported by Winell [207] are as follows (mg/m^3): Federal Republic of Germany, 670; the German Democratic Republic, 300; Sweden, 200; Czechoslovakia, 250. The MAC for perchloroethylene in the U.S.S.R. is 10 mg/m^3 .

Depending on its source strength, meteorological dilution, sunlight intensity, and the presence of other trace constituents, perchloroethylene or its

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predominant product, phosgene, accordingly may or may not be observed in non-urban areas [171]. It was estimated that an ambient concentration of 10 ppb perchloroethylene observed in New York City should lead to the formation of 12 ppb phosgene [171] (TLV = 100 ppb). In 1974, at 8 locations, in 5 industrial states, the concentrations of perchloroethylene ranged from 1.2 ppb in the urban area to less than 0.02 ppb in rural areas. Perchloroethylene was measured at concentrations exceeding 0.06 ppb at least 50% of the time at all locations [171]. Pearson and McConnell [208] reported that city atmospheres contain from less than 0.1 to up to 10 ppb (<0.68 – $68 \mu\text{g}/\text{m}^3$) of perchloroethylene. The air in the center of Munich was found to be contaminated with 0.88 ppb ($6 \mu\text{g}/\text{m}^3$) of perchloroethylene in 1975, while the suburbs of Munich had an ambient concentration of 0.59 ppb ($4 \mu\text{g}/\text{m}^3$) [209]. Populations living in urban atmospheres polluted by $6 \mu\text{g}/\text{m}^3$ of perchloroethylene were calculated to breathe in daily levels of about 90 μg [210].

Persons consuming three liters of tap water contaminated by 28.3 ppb of the solvent as described by Giger and Molnar [211], would ingest 85 μg perchloroethylene/day [210].

Chlorination at sewage treatment plants has resulted in slightly enhanced levels of perchloroethylene in water [212,176]. Similarly to trichloroethylene, perchloroethylene (5 $\mu\text{g}/\text{l}$) has been found in the organic constituents of Mississippi River water and in the organic constituents of commercial deionized charcoal-filtered water [177].

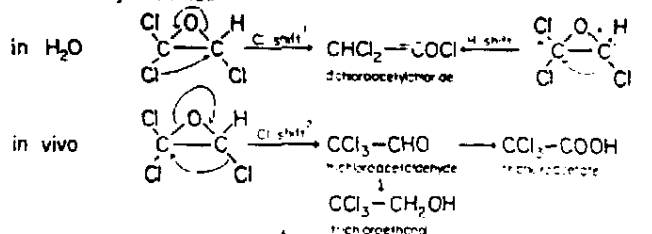
Pearson and McConnell [208] reported the concentration of perchloroethylene in animals to range from 0.5 to 50 ppb. The levels in tissues of animals at the upper end of food chains were elevated 100 times the environmental concentrations at most. This was considered a weak bioaccumulation potential as compared to other halogen compounds. The accumulation coefficient in the quotient from the uptake rate and clearance. Neeley et al. [213] found an accumulation ratio of perchloroethylene of 39.6 ± 5.5 . The respective value of hexachlorobenzene (HCB) is 7880.

Perchloroethylene has very recently reported to be carcinogenic in NCI studies, producing liver hepatocellular tumors in B6C3F1 hybrid male and female mice when tested at MTD and 1/2MTD dose levels in corn oil solution by gavage [214,415]. No carcinogenic activity was observed in analogously treated Osborne-Mendel rats of both sexes [214].

Perchloroethylene has not been found to be carcinogenic in inhalation studies with rabbits, mice [216], rats, guinea pigs and monkeys. Perchloroethylene showed dose-dependent mutagenicity at concentrations of 0.01, 0.1 and 1 mg/ml in *S. typhimurium* TA 100 without metabolic activation [188].

Perchloroethylene (0.6 mM) as well as the *cis*- and *trans*-isomers of 1,2-dichloroethylene were found to be non-mutagenic when tested in the metabolizing *in vitro* system with *E. coli* K12 [78,217]. The mutagenicity of vinyl chloride, vinylidene chloride, trichloroethylene, in the above test system was attributed to their initially forming unstable oxiranes, whereas halocarbons such as perchloroethylene and *cis* and *trans* 1,2-dichloroethylene which form much more stable oxiranes were non-mutagenic [78,192,217].

Trichloroethyleneoxide



Chloroethyleneoxide

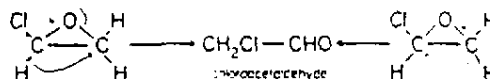


Fig. 7. Rearrangements of trichloroethyleneoxide and chloroethyleneoxide.

Figure 7 illustrates the metabolic pathways of tetrachloroethylene as proposed by Honse and Henschler [192]. The metabolic formation of trichloroacetic acid can be explained by the primary formation of the oxirane and subsequent rearrangement to trichloroacetyl chloride and its subsequent hydrolysis.

In a recent review, Henschler [271] contrasted the metabolism and mutagenicity of chlorinated olefins. In this class of molecules, the electron withdrawal effect dominates over the mesomeric donor effect of the involved carbon atom hence decreasing the electron density in the double bond which in turn results in a chemical stabilization against electrophilic attack [192]. It is optimal in perchloroethylene as has been demonstrated by the reactivity of chlorinated ethylenes with ozone [218].

As has been previously cited [108,192,217], the chlorinated ethylenes may undergo a variety of reactions, e.g., (1) reaction with nucleophilic cellular macromolecules under alkylation; (2) conjugation with low molecular nucleophiles (mainly glutathione) both enzymatically and non-enzymatically; (3) hydrolysis to diols, with and without the catalytic action of enzymes such as epoxide hydase; and (4) intramolecular rearrangement. The latter reaction according to Henschler [217] represents a deactivation mechanism and is of considerable importance for the potential of acute toxicity as well as of carcinogenicity and mutagenicity of the different members of the series of chlorinated ethylenes.

(5) Chloroprene

Chloroprene (2-chlorobutadiene; 2-chloro-1,3-butadiene; beta-chloroprene; $CH_2=C(Cl)-CH=CH_2$) is the monomer for neoprene, the specialty rubber.

It is prepared by two major routes: (1) the dimerization of acetylene to monovinylacetylene and addition of hydrogen chloride and (2) the chlorination of butadiene to a mixture of dichlorobutenes, from which 2,4-dichloro-1-butene is isolated and then is subjected to dehydrochlorination

[219, 220]. The latter method is believed to be the basis of the current U.S. production of chloroprene. In this procedure, butadiene is first reacted with chlorine to yield a mixture of dichlorobutene isomers from which the 3,4-dichlorobutene-1 isomer is isolated and then reacted with caustic to form chloroprene. 1,1-Dichlorobutene-2, the other isomer, can either be isomerized to 3,4-dichlorobutene-1 for additional chloroprene production or it can be utilized for in the production of adiponitrile [220].

A typical specification for chloroprene made from butadiene is as follows: chloroprene, 98.5% min., 1-chlorobutadiene, 1.0% max., aldehydes (as acetaldehydes), 0.2% max., 3,4-dichlorobutene-1, 0.01% max., dimers, 0.01% max., peroxides, 1 ppm max., and no detectable amount of vinyl acetylene [221].

In 1976, two U.S. companies produced an estimated 164 million kg of chloroprene, while three Japanese companies produced a total of 80 million kg (70% of chloroprene is based on acetylene and 30% on butadiene). The total Western European production in 1977 amounted to an estimated 100 million kg of chloroprene. The total world production of chloroprene in 1977 is estimated to have been 300 million kg [220].

Chloroprene is extremely reactive, e.g., it can polymerize spontaneously at room temperatures, the process being catalyzed by light, peroxides, and other free radical initiators. It can also react with oxygen to form polymeric peroxides and because of its instability, flammability and toxicity, chloroprene has no end product uses. It is used exclusively and without isolation in the production of neoprene elastomers [222].

Neoprene as obtained by emulsion polymerization of chloroprene consists mainly of *trans*-polychloroprene. There are two main classes, the sulfur modified type and the non-sulfur modified type, indicating the differences in polymerization techniques [222, 223].

About 100 million kg of neoprene were consumed in the U.S.A. in 1976 with the following consumption pattern: the production of industrial and automotive rubber goods, 63%; wire and cable applications, 13%; construction applications, 10%; adhesives applications, 8% and miscellaneous uses, 6% [220].

An estimated 2500 workers are currently exposed to chloroprene in the U.S.A. [222, 224].

The TLV for chloroprene in the U.S.A. is 25 ppm (90 mg/m³). In August, 1977, NIOSH recommended that occupational exposure to chloroprene be limited to a maximum concentration of 1 ppm (3.6 mg/m³) in air determined as a ceiling for a 15-min. period during a 40-h work week [225].

Work environment hygiene standards (all in terms of 8-hour time weighted averages) for chloroprene reported by Winell [207] are as follows (in mg/m³): Federal Republic of Germany, 90; the German Democratic Republic, 10; Sweden, 90 and Czechoslovakia, 50. The MAC of chloroprene in the U.S.S.R. is 2 mg/m³.

Chloroprene has been detected as an impurity in commercial vinyl chloride in Italy [226], Japan [227] and in acrylonitrile in the U.S.S.R. [228].

Chloroprene concentrations of 14.5–53.4 mg/m³ have been reported in

the air inside a Russian neoprene rubber plant: 0.2-1.57 mg/m³ 500 meter from the plant and 0.12-0.38 mg/m³ 7000 meters from the plant [229]. In another neoprene rubber plant in the U.S.S.R., the chloroprene concentration in air in the immediate vicinity was 28.45 mg/m³; 0.727 mg/m³ 500 meters from the plant, and 0.199 mg/m³ 7000 meters away [230]. Workers in a Russian shoe factory have been reported to be often exposed to chloroprene concentrations of 20-25 mg/m³ [231].

Chloroprene which has been used since 1930 in the manufacture of synthetic rubber has recently been suggested to be responsible for the increased incidence of skin and lung cancer in exposed workers in the U.S.S.R. [232, 233]. During the period 1956-1970, epidemiological studies of industrial workers in the Yerevan region revealed 137 cases of skin cancer among approximately 25,000 workers over 25 years of age. Each of the 25,000 workers was classified as belonging to one of the following groups: (I) Never worked in industrial plants; (II) persons working in non-chemical industries; (III) persons working in chemical industries but not exposed to chloroprene or its derivatives; (IV) persons working in industries using chloroprene derivatives; and (V) persons with extended work experience in chloroprene production. The incidence of skin cancer within these groups in order from (I) to (V) revealed a striking gradient, e.g., 0.12%, 0.40%, 0.66%, 1.60% and 3.0%. The study also indicated a gradient in the average age of the skin cancer cases and the average duration of employment, with workers exposed to chloroprene or the derivatives showing the lowest values. The chloroprene workers who developed skin cancer had an average age of 59.6 years and an average duration of employment of 9.5 years [233].

During the same period, 87 cases of lung cancer were identified among 19,979 workers in the same region. The groups exposed to chloroprene or its derivatives had the highest incidence of lung cancer (1.16%). These workers' average age was 44.5 years with an average duration of employment of 8.7 years. Of the 34 cases of lung cancer in this group, 18 were among persons having a direct and prolonged exposure to chloroprene monomer, the remaining 16 involved individuals exposed to chloroprene latexes [232]. The frequency ratio of primary lung cancer occurrence among the comparison or control groups compared to the chloroprene groups was: 2.67 times lower in workers with chemicals unrelated to chloroprene; 6.3 times lower in workers in non-chemical industries and 17.5 times lower in workers in cultural and civic institutions.

The limitations of the above two studies [232, 233] have been recently cited by IARC [220] and include: failure to distinguish prevalent from incident cases, to document completeness of case ascertainment among the exposure group, to adjust for effect of age and sex, to measure the extent of exposure, to control for the potential confounding effect of smoking and to furnish pathology information on cell type (particularly important in the study of reported skin cancer).

A study of cancer mortality among two cohorts of males engaged in the production and polymerization of chloroprene in the U.S.A. was recently

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reported by Pell [234]. One cohort consisted of 270 men first exposed between 1931--1948 and the other of 1576 men first exposed between 1942--1957. The number of lung cancer deaths in each cohort (3 in the first and 16 in the second) were about the same as was expected on the basis of U.S. or company wide rates. However, the risk of digestive cancer (19 versus 13.3) and of lymphatic and hematopoietic cancer (7 versus 4.5) were slightly elevated when contrasted with company wide experience.

Among maintenance mechanics in the study/cohort of 1576, there were 8 lung cancer cases (4 living and 4 deceased) which accounted for approximately 37% of the lung cancers found in the total study cohort. In contrast, only 17% of the total cohort was composed of maintenance mechanics.

It must be noted that these mechanics whose tasks include the general maintenance in the reactor areas, installation of equipment and the replacement of leaking pipe fittings would be expected to have a potential for exposure (perhaps to high levels) to chloroprene [220]. A number of limitations to the above Pell study [234] were enumerated [220]. These include: no data were presented on any potential confounding variables such as smoking history and other occupational exposure; no specific exposure information based on chemical measurements was provided; no data presented on cell type analysis of the malignancies; and methodological shortcomings of combining of workers engaged in chloroprene monomer production and those in polymerization. There may also have been selective removal of high risk, high exposure workers from the cohort examined, in that retirees, disabled workers and former chloroprene individuals exposed in job categories not actively involving chloroprene were not included in the inception cohort. Additionally, the major limitations in interpreting this study were cited by IARC [220] to be: (a) the period of follow-up for the cohort is still quite incomplete for an adequate latent period and thus there may eventually be demonstration of statistically significant excesses and (b) the power of this study is limited due to the small number of person-years of exposure.

No carcinogenic effects of chloroprene have been noted to date in animal studies [220] involving oral [235] and intratracheal [235] administration to rats and dermal application [235,236] and subcutaneous injection [235,236] of chloroprene to mice and rats. A number of additional studies are in progress to investigate the carcinogenicity by oral administrations to rats and by inhalation exposure in rats and hamsters [237-239].

Exposure of *S. typhimurium* TA 100 and TA 1538 strain to 0.5-8% of chloroprene vapor in air in the absence of any metabolic activation system caused a linear increasing mutagenic response (reaching 3 times the spontaneous mutations rate at a concentration of 8%) [149]. Exposure to a higher concentration (20%) caused a strong toxicity in the bacteria. This mutagenic and/or toxic effect could be caused by a direct action of chloroprene or more likely, by one of its enzymic (bacteria) or non-enzymic breakdown products. Up to a 3-fold increased mutagenic response was found when a fortified 9000 g liver supernatant from either phenobarbitone treated

or untreated mice was added to such assays supporting an enzymic formation of mutagenic metabolite(s) from chloroprene [149] (probably an oxirane (epoxide) in analogy with vinyl chloride, vinylidene chloride, and trichloroethylene) [10, 149]. Liver supernatant from some human biopsies also enhanced mutagenicity of chloroprene [10].

Treatment of male *Drosophila* for 3 days with 5.7 mM and 11.4 mM chloroprene resulted in an increase of X-linked recessive lethal mutations from $0.8 \pm 0.04\%$ in the control to $0.58 \pm 0.3\%$ and $1.0 \pm 0.4\%$, respectively [240].

Vapor of chloroprene (0.04–1.0 ppm) [241–244] induced dominant lethal mutations in sperm and chromosome aberrations in bone marrow cells of rats and of mice exposed to 1.83–3.5 mg/m³ [243]. Mixtures of chloroprene and methyl methacrylate [24,246] chloroprene, dodecylmercaptan and ammonia [245] also induced chromosomal aberrations in bone-marrow cells of rats.

An increase of chromosomal aberrations have been reported in cultured peripheral lymphocytes from workers occupationally exposed to chloroprene [243, 244, 247] or chloroprene and methyl methacrylate [245]. For example, Katosova [247] noted a significant rise in the number of chromosome aberrations in blood cultures of workers exposed to an average chloroprene concentration of 18 ppm for 2 to more than 10 years. In addition, decreases in motility and number of sperm were noted in exposed male workers as well as a three-fold excess of miscarriages in the wives of chloroprene workers have also been noted [224].

Testicular atrophy and reduction in the numbers and mobility of sperm in rats with non-atrophoid testicles have been noted to an exposure level of chloroprene down to 0.06 ppm [241,244], while spermatogenesis in C57B1/6 mice was affected after 2 months exposure to 0.32 ppm chloroprene [243,248].

Sanotiskii [243] reported that the threshold for chronic effects of chloroprene on animals based upon the indicators of general systemic effect is 1.69 ± 0.987 mg/m³, about the same as the maximum permissible concentration (MPC) in the U.S.S.R. formerly adopted. The threshold concentration based on specific indicators (e.g., embryotropic, gonadotropic and mutagenic effects) was 0.15 ± 0.0059 mg/m³ (e.g., one order of magnitude below the former MPC). Neither embryotoxic nor teratological effects have been noted after exposure of pregnant rats to 90.5 mg/m³ (25 ppm) of chloroprene 4 h/day from day 1 until day 12 and day 3 until day 20 of gestation [249]. The biotransformation of chloroprene has been postulated by Haley [250] to occur in an analogous fashion to vinyl chloride and vinylidene chloride, e.g., via the formation of the epoxide by the action of mixed function oxidases. The intermediate epoxide would give rise to the aldehyde or combine with glutathione and subsequently form a mercapturic acid derivative. The known oxidation of chloroprene in positions 1 and 2 as well as the decreased tissue-SH content would appear to lend some support to this postulated biotransformation.

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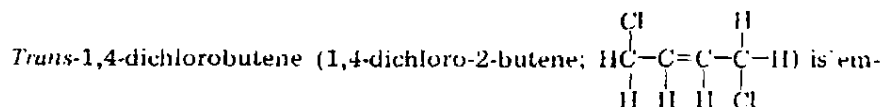
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(6) *Trans-1,4-dichlorobutene*



ployed in the U.S.A. mainly as an intermediate in the manufacture of hexamethylenediamine and chloroprene. Hexamethylenediamine is further used as a chemical intermediate in the production of nylon 66 and 612 polyamide resins, while chloroprene is used in the production of polychloroprene rubber. While the U.S. production of hexamethylenediamine and polychloroprene rubber in 1975 was 340 and 143.9 million kg respectively, the percentage originally derived from *trans-1,4-dichlorobutene* is not known [251].

Trans-1,4-dichlorobutene has been shown to be weakly carcinogenic by subcutaneous and intraperitoneal administration in ICR/HA Swiss mice but not carcinogenic in mice via skin application [252].

Trans-1,4-dichlorobutene produced mutations in *S. typhimurium* TA 100 strains [253] with the mutagenic effect enhanced by liver microsomal fractions from mouse or humans. It has also been reported mutagenic in *E. coli* [254] and *S. cerevisiae* [255].

It has been suggested that *trans-1,4-dichlorobutene-2* could conceivably be metabolized to an epoxide intermediate which is analogous in structure to open-chain β -chloroethers [252].

(7) *Hexachlorobutadiene*

Hexachlorobutadiene (1,1,2,3,4,4-hexachlorobutadiene; HCBd; per-
 $\text{Cl}_2-\text{C}=\text{C}(\text{Cl})-\text{C}(\text{Cl})=\text{CCl}_2$) is normally obtained in commercial quantities as a by-product in some chlorinated hydrocarbon processes (e.g., perchloroethylene production). It is found in the tarry wastes (HEX wastes) along with hexachlorobenzene, hexachloroethane and other chlorinated by-products [256]. In 1974, although no HCBd was produced in the U.S.A., 200,000 to 500,000 pounds were reported imported in the same year [256]. The production of perchloroethylene, trichloroethylene, carbon tetrachloride and chlorine in the U.S.A. in 1972 produced HCBd (thousands of pounds) as follows: 8670; 3000; 2790; and 70 [255]. The production of perchloroethylene, trichloroethylene, and carbon tetrachloride accounts for 99% of the HCBd in the U.S.A. [256].

Approximately 10 million pounds of HCBd and 5 million pounds of hexachlorobenzene (HCB) are generated annually as hex waste in the U.S.A. [257].

The largest use for HCBd in the U.S.A. is for the recovery of "snift" or chlorine-containing gas in chlorine plants. HCBd is also used as a chemical intermediate to produce lubricants, as a solvent and in heat transfer and hydraulic fluids.

HCBD (analogous to HCB) is highly resistant to chemical, biological and physical degradation and hence is a stable environmental pollutant.

HCBD in the ppb range has been found in water, soil, selected aquatic organisms [258] and food [257,259] (fish, eggs, milk, vegetables) samples taken along the lower Mississippi River in Louisiana.

There is a paucity of carcinogenicity and mutagenicity information on hexachlorobutadiene. In one limited study, no tumors were found in rats after 6 months administration of HCBD at levels of 2-7 mg/kg in the diet [260].

Recent chronic toxicological studies at Dow Chemical Co. in the U.S.A. [261,262] suggest the possibility of a threshold level for HCBD. For example, in a study where male and female Sprague-Dawley rats were maintained on diets containing 20, 2.0, 0.2 mg/kg/day of HCBD for two years, the lowest dosage caused no observed adverse effects. Ingestion of the intermediate dose level of 2.0 mg/kg/day caused some degree of toxicity, affecting primarily the kidney in which increased renal tubular epithelial hyperplasia as well as an increase of urinary excretion of coproporphyrin was noted. Ingestion of the highest dose level (20 mg/kg/day) resulted in renal tubular adenomas and adenocarcinomas, some of which metastasized to the lung [261].

A review of health data on Dow employees working in areas where HCBD has been found, revealed no abnormalities which could be attributed to the chemical. It was also noted that wastes containing the material are being recycled or incinerated in specially designed facilities [262].

HCBD has been found mutagenic (with and without activation) in *Salmonella typhimurium* TA 1535 and TA 100 [263]. Schwetz et al. [264] described the results of a reproduction study in Sprague-Dawley fed diets containing 0.2, 2.0, or 20 mg/kg/day for 90 days prior to mating, 15 days during mating, and subsequently throughout gestation and lactation. Signs of toxicity among the adult rats were observed at the two higher dose levels and included decreased weight gain and food consumption as well as alterations in kidney structure. There was no effect on pregnancy or neonatal survival and development. No toxic effects were observed among the adults at a dose level of 0.2 mg/kg/day or among the neonates at dose levels of 0.2 or 2.0 mg/kg/day [264].

8. Allyl chloride

Allyl chloride (3-chloro-1-propene; 3-chloro-propylene; chlorallylene; $\text{CH}_2=\text{CHCH}_2\text{Cl}$) is the most important of all commercial allyl compounds. It is reactive, both as an organic halide and as an olefin. In contrast to the vinyl halides which are characteristically inert in either $\text{S}_\text{N}1$ or $\text{S}_\text{N}2$ nucleophilic substitution reactions, allyl halides are very reactive, much more than corresponding saturated compounds in both $\text{S}_\text{N}1$ and $\text{S}_\text{N}2$ reactions. The double bond facilitates breaking the bond to the functional group in displacement and substitution reactions. In addition, the allyl group when introduced into other molecules, is usually reactive hence permitting many syntheses of potential commercial interest [265].

Although other reactions for the preparation of allyl chloride are known,

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the high-temperature substitutive chlorination of propylene is believed to be the only route used commercially at present [265]. Allyl chloride is mainly used as a monomer in the production of various plastics and resins that are used *per se* or incorporated into surface coatings adhesives, etc. A number of useful specialty resins are derived from allyl esters and polyesters that may be made directly from allyl chloride. Resin uses also include a number of copolymers and inter-polymers of allyl chloride with acrylonitrile, vinylidene cyanide, styrene and diallyl esters developed to provide special properties, while allyl chloride also serves as a catalyst or modifier in the production of other resins.

A number of commercially important compounds are made directly from allyl chloride, e.g., "first-generation derivatives", glycerol, epichlorohydrin and allyl alcohol. Medicinal derivatives of allyl chloride include allyl-substituted barbiturates, and mercury diuretics derived from allylamine, as well as the anesthetic, cyclopropane [265].

The production of allyl chloride in the U.S.A. in 1973 was estimated to be about 300 million pounds most of it produced by two manufacturers [266]. No data are available on the amounts of allyl chloride produced elsewhere.

NIOSH estimates that approximately 5000 workers are potentially exposed to allyl chloride during its manufacture or use. NIOSH has submitted to OSHA a criteria document recommending adherence to the present Federal standard of 1 ppm of allyl chloride as a time-weighted average for up to a 10-h workday, 10-h work week and proposed the addition of a 3 ppm ceiling concentration for any 15-min period [266].

The MAC [267] for allyl chloride in the U.S.S.R. is 3 mg/m³.

Data are sparse concerning environmental exposure levels of allyl chloride. In one study of an allyl chloride production facility in the U.S.S.R., the concentration of allyl chloride in air of production-working areas varied between 6.4–140 mg/m³. Workers in this facility showed early renal function impairment, higher glomerular filtration of creatinine (40%) and urea (50%) considerable hypernatremia, moderate kaliemia, higher blood Cl⁻ (80%) and dysproteinemia [267].

There is a paucity of information regarding the chronic effects of allyl chloride. In a limited study, allyl chloride did not induce tumors in rats, guinea pigs and rabbits exposed to 3 ppm of the agent by inhalation for up to six months [268].

In the standard *Salmonella* mutagenicity assay in which the bacteria as well as the test agent are incorporated in the agar overlay [269], allyl chloride did not exhibit any significant mutagenic activity [270]. This initial activity of allyl chloride under the above conditions can be ascribed to its volatility. When steps are taken to minimize dissipation of allyl chloride vapors into the atmosphere (e.g., via impregnation of filter discs with the test material then placing them on the surface of the agar plates containing the microorganisms, and the plates then sealed in separate plastic bags) mutagenic activity of allyl chloride (0.1, 1 and 10 μ l/plate) was then demonstrable for *S. typhimurium* TA 100 and TA 1535, but not TA 1538. Mutagenic activity was not significantly enhanced by microsome preparations derived from the livers of rats induced with Aroclor [269]. Hence these findings

suggest that allyl chloride acts as a direct acting base-substitution mutagen since it affects only those strains TA 1535 and to some extent TA 100 capable of detecting such mutations [270].

Allyl chloride (at 10 μ l) is mutagenic in the *E. coli* DNA polymerase deficient (*E. coli* pol A⁺/pol A⁻) disc test [271,272] procedure, preferentially inhibiting the growth of the pol A⁻ strain which is indicative of DNA modifying activity [270]. Allyl chloride (at levels of 18.4, 24.5 and 30.7 $\times 10^{-5}$ M) induces gene conversion in *S. cerevisiae* D4 [270].

Aspects of the biotransformation of allyl halides have been described by Kaye et al. [273]. Allyl mercapturic acid, its sulphoxide, and 3-hydroxypropylmercapturic acid were identified as urinary metabolites following subcutaneous administration of allyl chloride, bromide and iodide to rats [273]. S-allyl glutathione and S-allyl-2-cysteine were also detected in the bile of a rat dosed with allyl chloride. The study of Kaye et al. [272] did not establish with certainty the pathway or pathways whereby allyl halides give rise to the formation of 3-hydroxypropylmercapturic acid. There are a number of pathways by which this may occur because the allyl halide can undergo reaction either at the double bond or at the site of the halogen atom. Although it is not known whether metabolic formation of a 3-halogenopropanol ($\text{HOCH}_2\text{CH}_2\text{CH}_2\text{X}$) from an allyl halide can occur, if such a reaction did take place it would probably be followed by the formation of 3-hydroxypropylmercapturic acid since the excretion of this mercapturic acid has been reported following the administration of 3-chloropropanol to rats [274]. The conversion of allyl chloride to S-allyl glutathione and the conversion of the latter compound to allyl mercapturic acid was demonstrated by the work of Kaye et al. [272]. Additional potentially possible metabolic pathways for allyl chloride could involve the epoxidation of the double bond to form epichlorohydrin (e.g., $\text{CH}_2=\text{CHCH}_2\text{Cl} \rightarrow \text{CH}_2-\text{CHCH}_2\text{Cl}$) a known carcinogen and mutagen. The sub-

sequent oxidation products of epichlorohydrin are glycidol ($\text{H}_2\text{C}-\text{CH}-$

CH_2OH) and glycidaldehyde ($\text{H}_2\text{C}-\text{CHCHO}$) both are mutagenic and the

latter is carcinogenic as well. The second metabolic pathway involves conversion to allyl alcohol, then acrolein, then acrylic acid, viz., $\text{H}_2\text{C}=\text{CH}_2\text{CH}_2\text{Cl} \rightarrow \text{H}_2\text{C}=\text{CHCH}_2\text{OH} \rightarrow \text{CH}_2=\text{CHCHO} \rightarrow \text{CH}_2=\text{CHCOOH}$. Acrolein (vinyl aldehyde) is mutagenic in *Drosophila* and *S. typhimurium* (strains TA 1538 and TA 98) [275, 276] and non-mutagenic in the dominant lethal assay in ICR/HA Swiss mice when tested at 1.5 and 2.2 mg/kg [277].

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Note added in proof

The present update of the NIOSH register provided details on 64 cases of angiosarcoma (world-wide) among vinyl chloride polymerization workers reported as of October, 1977 [278]. Of this total, 23 cases of angiosarcoma have now been reported in the U.S.A., 10 in Canada, 9 in the Federal Republic of Germany, 8 in France, 3 in Sweden, 2 each in the U.K., Italy, Czechoslovakia and Yugoslavia and 1 each in Belgium, Japan and Norway. The 23 cases identified among U.S. polymerization workers represents approximately 10% of all liver angiosarcomas identified in the U.S.A. since 1964 [278]. The ages at diagnosis for the 64 cases ranged from 37 to 71 years with a median of 49 years, while the latency periods (years from first exposure to diagnosis) ranged from 9 to 38 years with a median of 21 years. The total years exposed ranged from 4 to 31 years with a median of 18 years [278].

Fleig [278] reported an increased incidence of chromosomal aberrations in lymphocyte cultures from workers occupationally exposed to both estimated and monitored concentrations (5000 ppm and 600 mg/kg) of vinyl chloride only in those subjects showing symptoms of VC illness or in an angiosarcoma case. In 200 metaphases from exposed subjects, the rate of aberrant metaphases including gaps and isogaps was 16.6% compared to a control group with a rate of 5.5%. The frequency of structural anomalies, inclusive and exclusive of gaps was also increased compared to controls in bone marrow cells of Chinese hamsters exposed to inhalation of 2500 and 5000 ppm VC for 6 h/day at 24 h intervals over 5 days, or after i.p. injection of 300 and 600 mg VC/kg/day for 5 days.

Watanabe et al. [280] studied the covalent binding to hepatic macromolecules in rats exposed to ^{14}C -labeled vinyl chloride to determine if VC-induced carcinogenesis may be related to electrophilic alkylation of macromolecules in vivo. At exposures of VC > 50 ppm, the amount of ^{14}C bound to macromolecules in the liver correlated with induction of hepatic angiosarcoma, there was no detectable binding of radioactivity to either DNA or RNA in the liver. The hepatic glutathione content was depressed only at exposure concentrations > 100 ppm.

Recent studies by Suzuki [281] on the induction of pulmonary tumors in CD1 Charles River white strain male mice suggests that the mouse lung may be an extremely sensitive indicator of the oncogenicity of vinyl chloride. The

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pulmonary tumors corresponded to "alveologenic tumors or alveologenic cancer". It was suggested that the neoplastic cells were transformed from type II alveolar epithelium via its hyperplastic form. It was also noted that various carcinogens such as polycyclic aromatic hydrocarbons, nitrogen mustard, and chromate compounds are known to induce alveologenic tumors in mice and to also be associated with excess broncheogenic carcinoma among workers exposed to the carcinogens [282].

Vinyl bromide (bromoethene) is used in small amounts as a flame retardant co-monomer with acrylonitrile and other vinyl monomers in modacrylic fibers. These fibers are used in fabrics and fabric blends with polyesters for children's sleepwear and other apparel, home furnishings and in industrial applications [283]. Vinyl bromide has also been detected as an impurity in commercial vinyl chloride [284, 285]. Vinyl bromide has been recently disclosed to be a possible carcinogen in a preliminary 18-month study submitted to the U.S. Environmental Protection Agency [286]. After 18 months of a 24-month chronic inhalation study, primary angiosarcomas of the liver were found at sacrifice in male and female rats exposed to vinyl bromide at 50, 250 and 1250 ppm. There also appeared to be carcinogenic effect in Zymbal's gland in rats exposed to 250 and 1250 ppm during this period. In addition, two primary lung neoplasms were found as well as some malignant mammary neoplasms in female rats. Van Duuren [193] reported that skin application of vinyl bromide in acetone solution and polyvinyl-bromide in commercial aqueous latex suspension using female ICR/11a Swiss mice did not result in any skin tumors in either group. When tested as initiators with phorbol myristate acetate (PMA) as promoter (in two stage carcinogenesis) the test group (vinylbromide followed by PMA) did not show any significant increase in tumors induced compared with the control group (PMA alone).

Exposure of *S. typhimurium* TA 1530 [287] or TA 100 [288, 289] to gaseous mixtures of vinylbromide in air caused mutagenic effects which were enhanced by the addition of liver supernatant fractions from phenobarbitone pretreated mice or from human liver biopsies [287]. In addition, the epoxide formation from vinyl bromide (as well as vinyl chloride and chloroprene) was suggested by passing a gaseous mixture of the test compound and air through a mouse liver microsomal system and trapping volatile alkylating metabolites by reaction with excess 4-(4-nitrobenzyl)pyridine (NBP) [3, 115].

The species sensitivity and specificity to both vinyl chloride and vinylidene chloride has been further elaborated by Lee et al. [289]. Rats were more resistant than mice to the carcinogenic effects of VC or VDC. Hepatic hemangio sarcomas were observed in rats exposed to 250 or 1000 ppm of VC starting in the ninth month. In contrast to mice, many of the rats with hepatic hemangiosarcomas also developed hemangiosarcomas in the lung; VC did not cause any other tumors in the rat. A small number of rats exposed to 55 ppm of VDC developed hemangiosarcomas in the mesenteric lymphnode or subcutaneous tissue [289]. Rats were also found to be more resistant than mice to the acute or other chronic effects of VC or VDC [46, 289].

Comparative studies suggest clues in the differences between rats and mice in the processing of vinylidene chloride [152, 290, 291]. In mice, for example, the production of thiodiglycolic acid is considerably reduced and the formation of the N-acetyl-S-cysteinylacetyl metabolite is increased. The higher beta-thionase activity in mice than in rats accounts for the greater conversion of thioglycolic acid into dithioglycolic acid via thioglycolic acid in the former species. In mice, the metabolic pathway from chloroacetic acid to thiodiglycolic acid seems to be readily saturable, possibly on account of an inadequacy in the reaction catalyzed by glutathione S-acyl transferase [290]. Hence, under these circumstances, detoxification of 1,1-dichloroethylene oxide by glutathione S-epoxide transferase and the modification of DNA by 1,1-dichloroethylene oxide or chloroacetyl chloride would be expected to be more significant in mice than in rats [152, 290, 291]. According to Hathaway [291], vinylidene chloride is an agent of low, perhaps very low, oncogenic potential, which can be damaging only in a special set of biological circumstances.

McKenna et al. [292] recently elaborated the metabolism and pharmacokinetic profile of vinylidene chloride in rats following oral administration. The fate of VDC following oral administration to rats is dependent upon both the dose administered and the nutritional status of the animal. The diminished ability of fasted animals to metabolize the high dose (50 mg/kg) of VDC correlated well with the previously reported enhancement of VDC-induced hepatotoxicity in fasted animals [293, 294]. Both the hepatotoxic response to vinylidene chloride, and the extent of its detoxification appear to be dependent on the concentration of glutathione (GSH) in the liver. When hepatic GSH is depleted (e.g., in fasted animals or at higher doses of VDC) a toxic response to VDC is elicited. After a single oral dose of 1 mg/kg of ^{14}C -VDC, 78% of the dose was metabolized and excreted in urine and feces as non-volatile metabolites of VDC the remainder was exhaled as $^{14}\text{CO}_2$ (21%) and unchanged ^{14}C -VDC (1-3%). After a single 50 mg/kg dose of ^{14}C -VDC, excretion of the parent compound via the lungs was 19 and 29% of the dose in fed and fasted rats, respectively. Two of the major urinary metabolites of ^{14}C -VDC were identified as S-(2-hydroxyethyl)-N-acetylcysteine and thiodiglycolic acid. The identification of these metabolites substantiates the hypothesis that detoxification of VDC occurs mainly via conjugation with glutathione. While GSH may act as a site of detoxification, other tissue nucleophiles may be sites of attack or toxicity. This may arise via covalent binding of the reactive metabolite of VDC to macromolecules in the liver cells, subsequently causing liver injury [292].

A cytogenic investigation on 28 individuals employed in degreasing operations with trichloroethylene has been recently reported by Konietzko et al. [295]. On the basis of chromosome studies on lymphocyte cultures, 9 of these cases showed pathological rates of hypodiploid cells, but otherwise normal karyotypes. In these 9 cases, the trichloroethylene load, but not the exposure periods in the working place, was significantly higher than in the control group.

In a study of the metabolism of trichloroethylene (TCE) and tetrachloroethylene in human subjects, Ikeda [296] reported a linear correlation

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between the TCE concentration in the work environments and the level of total trichloro compounds in the urine of the workers, as expressed by the equation: $Y = 7.25X + 5.5$ where Y is TCE in air (ppm) and X is total trichloro compounds in urine (mg/l). Trichloroethanol level is also linearly related to TCE concentration, while trichloroacetic acid level deviates from the linear relation when the TCE level exceeds 50 ppm. For tetrachloroethylene exposure, both trichloroethanol and trichloroacetic acid levels, and consequently the total trichloro compound level, reach a plateau at tetrachloroethylene level well below 100 ppm. The mean urinary biological half-lives for TCE and tetrachloroethylene are 41 and 144 hrs respectively. The respiratory half-life is shorter than the urinary half-life for both TCE and tetrachloroethylene [288].

Axelson et al. [297] recently described a fairly small cohort study comprising 518 men with rather low levels of trichloroethylene exposure as estimated through trichloroacetic acid in the urine. This study did not reveal any excess cancer mortality. Requiring 10 years of latency time, the sub-cohort with trichloroacetic acid in urine above 100 mg/l (e.g., exposure supposed to be above 30 ppm) showed a close agreement between expected and observed number of cancer cases as did the subcohort with lower exposure (548 and 3643 person-years of observation, respectively). The authors cautioned that the cancer risk to man from trichloroethylene can by no means be ruled out from this study, particularly with regard to uncommon malignancies such as liver cancer. However, they also concluded that "there is probably no serious cancer hazard at low exposures" [297].

Trichloroethylene was toxic but not genetically active without micro-somal activation when tested for its ability to induce both point mutation and mitotic gene conversion in diploid strain of *S. cerevisiae* (strain D7) of yeast. When a mouse liver 10,000 kg supernatant was included in the suspension tests, dose-related increases in both mutation and gene conversion were noted at survival levels of greater than 50%. In the host-mediated assay, TCE induced both point mutation and gene conversion in D4 when recovered from the liver and kidneys after both acute and subacute dosing [298].

Preliminary data submitted to OSHA and NIOSH on two rat studies suggests that TCE does not cause abnormalities in animal embryos or genetic changes in germinal cells [299]. In the first study, pregnant female rats inhaled air containing 300 ppm of TCE for 6 h/day. There were no deaths or behavior reactions among the pregnant rats and no abnormalities in their offspring. In the second study, male rats were exposed to the same concentration of TCE for 9 months, then mated. No genetic changes were found in the offspring [299].

Additional recent studies by Culik et al. [300] suggest that concentrations of 25 ppm chloroprene do not adversely affect male rat reproduction capability and are not embryotoxic or teratogenic. These results are at variance with previous studies from the U.S.S.R. which reported that chloroprene was embryotoxic, teratogenic and mutagenic at atmospheric concentrations below the existing U.S.S.R. maximum allowable concentration (MAC) of 1 ppm [301, 302].

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~~ONE-YEAR TIME~~ SEQUENCE INHALATION TOXICITY STUDY OF
VINYL CHLORIDE IN RATS. I. GROWTH, MORTALITY, HAEMATO-
LOGY, CLINICAL CHEMISTRY AND ORGAN WEIGHTS*

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SUMMARY

Wistar rats were exposed to atmospheres containing 0 (control) or 5000 ppm vinyl chloride monomer (VCM), 7 h/day, 5 days/week, for a period of 52 weeks. After 4, 13, 26 and 52 weeks each time 10 rats/sex/group were killed and subjected to extensive examinations. The present paper deals with growth, mortality, haematology, clinical chemistry and organ weights.

Slight growth retardation throughout the experimental period and high mortality in the second half of the study were observed in VCM-exposed animals. Some of the haematological parameters and biochemical blood parameters were slightly influenced by VCM after an experimental period of 52 weeks only. Blood clotting time was generally slightly shorter in VCM-exposed rats than in controls. There were minor indications of increased potassium contents of the blood serum in VCM-exposed animals during the first half of the test period. The kidneys were adversely affected by VCM as appeared from increased blood urea nitrogen levels and relative kidney weights. After 52 weeks increased weights of heart and spleen, and slight signs of anaemia were noticed in VCM-exposed rats.

The present study did not produce obviously suitable parameters for early diagnosing "VCM-disease" in man.

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INTRODUCTION

Industrial exposure to vinyl chloride monomer (VCM) has been associated with several disorders such as acro-osteolysis, non-neoplastic liver lesions, angiosarcoma of the liver and other types of tumours at various sites [2,8,16,17]. Moreover, experimental exposure to VCM by inhalation has been found to result in degenerative and neoplastic changes of the liver and other organs in several species [1,10,14,18,19].

From the point of industrial safety it is important to recognize VCM-effects in plant workers as early as possible [3,4,5,9,11,12,13,15]. Therefore, experiments aimed at detecting the earliest changes attributable to VCM-exposure as well as at studying their further development and consequences, were deemed desirable. Against this background a 1-year inhalation study with a serial killing design was conducted in rats repeatedly exposed to VCM at a level as high as 5000 ppm. The results of this investigation are presented in 3 papers of which this is the first. It deals with the design of the study, growth, mortality, haematology, clinical chemistry and organ weights. The 2 other papers describe the morphological changes found in several organs [6,7].

MATERIALS AND METHODS

VCM was obtained from Akzo Zout Chemie, Rotterdam, The Netherlands, in stainless steel pressure bottles of about 50 litres.

124 male and 124 female, newly weaned, rats (Cpb : WU; Wistar random) were evenly distributed according to sex and body weight over 2 inhalation chambers. In one of the chambers the rats were exposed to 5000 ppm VCM 7 h/day, 5 days/week, for a period of 52 weeks; in the other chamber the controls were housed, where they were exposed to fresh air.

The institute's stock diet and tap water were constantly available to the animals.

Individual body weights were frequently recorded, and at weeks 4, 13, 26 and 52 haematological examinations, serum and urine analyses and organ function tests were conducted. Haematological examinations involved determinations of haemoglobin concentrations, packed cell volume, blood clotting time and counts of erythrocytes, thrombocytes and of total and differential leucocytes. Serum analyses involved measurements of total protein, albumin, globulins, α -feto-protein and the activity of glutamic-oxalacetic and glutamic-pyruvic transaminases, alkaline phosphatase and lactic dehydrogenase and of electrolytes, viz. sodium, potassium, calcium, magnesium and chlorine. Measurements were made of fasting blood glucose and blood urea nitrogen some days prior to each autopsy. Urine examinations included volume, specific gravity, uric acid, activity of glutamic-oxalacetic transaminase, pH, protein, glucose, ketones, occult blood and microscopic constituents. Liver function tests involved bromsulphophthalein (BSP) and sleeping time. The kidney function was measured by the phenol-red excretion test.

After 4, 13, 26 and 52 weeks, 10 rats/sex/group were killed and autopsied, and the weights of heart, kidneys, liver, spleen, brain, gonads, thymus, pituitary, thyroid, adrenals and lungs were recorded.

RESULTS

In week 33 the first rat died (a male of the test group). Thereafter, mortality among VCM-exposed rats gradually increased, and in week 52 only 9 males and 10 females of the test group were still alive. The controls remained in good shape, except for one lethargic animal which was killed in week 50.

Mean body weights in the test group were significantly lower than those of the controls throughout the experimental period.

Haemoglobin content, packed cell volume and the number of erythrocytes were slightly decreased and the total number of leucocytes were somewhat increased in VCM-exposed males and females at week 52. Blood clotting time in test animals was lower than in controls at all intervals examined.

Blood urea nitrogen levels were slightly increased in the test group in females at week 26 and in males and females at week 52. Albumin and γ -globulin levels were slightly decreased in the test group in both sexes at week 52 only. Alpha-feto-protein levels were slightly increased in the test group after 26 and 52 weeks, the differences with the controls being statistically significant after 52 weeks only. A markedly elevated level of this protein indicating the presence of a α_1 -feto-protein-producing tumour, was not found in any of the animals. The potassium content of the blood serum was somewhat higher in test animals than in controls during the first half of the experimental period.

Specific gravity values and uric acid levels were decreased, whereas the volume of the urine produced and UGOT-values were increased in both males and females of the test group at week 52 only. However, the UGOT-values are within the normal range and well below those considered pathological.

BSP-retention was decreased in the test group at weeks 13, 26 and 52, but slightly increased in females at week 4.

In the test group, the relative weights of the liver and kidneys were nearly always significantly increased in both sexes at weeks 13, 26 and 52 and those of the spleen at weeks 26 and 52. The relative weights of the heart and lungs were slightly increased in the test group at week 52.

DISCUSSION*

An attempt was made to indicate the earliest VCM-related changes detected in the present study. Comparison of toxicity data on VCM from the literature with those obtained in the study described here, and being aware of

* The reader is referred to the unabridged paper for a detailed discussion on all VCM-effects observed.

the fact that "a statistically significant difference between the control and test group" is not always synonymous with "a VCM-effect", the following changes might represent early VCM-effects, i.e. growth retardation, shortened blood clotting time and increased potassium content of the blood serum. From the results published in a subsequent paper [7] it appears that another early VCM-effect is the occurrence of swollen and malformed mitochondria in hepatocytes. VCM-effects appearing at a later stage were increased urea nitrogen content of the blood and enlargement of kidneys, liver and spleen. Foci of cellular alterations in the liver and increased amount of smooth endoplasmic reticulum in the parenchymal cells of the liver were also found at this later stage [7]. Other VCM-effects became visible only at the final stage when tumours had already emerged in many VCM-exposed animals.

Because of the nature or the scantiness of the early VCM-effects observed in the present study, it seems unlikely that any of the parameters affected by VCM at an early stage are obviously suitable as tools for a timely diagnosis of "VCM-disease" in humans. On the other hand, these and other observations made in the experiment described here may indicate the direction in which such an "early diagnostic tool" should be looked for.

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