

Mutation Research, 67 (1979) 173-182
© Elsevier/North-Holland Biomedical Press

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

C. DREVON and T. KUROKI *

*Unit of Chemical Carcinogenesis, International Agency for Research on Cancer, 150 cours
Albert Thomas, 69372 Lyon Cédex 2 (France)*

(Received 5 June 1978)

(Revision received 2 January 1979)

(Accepted 16 January 1979)

Summary

The mutagenicity of vinyl chloride, vinylidene chloride (1,1-dichloroethylene) and chloroprene (2-chloro-1,3-butadiene) was tested in V79 Chinese hamster cells in the presence of a 15 000 X g liver supernatant from phenobarbitone-pre-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 produced 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 [15]. 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 [15]. Chloroprene has so far not been found to be carcinogenic in mice and rats, although some human

* Present address: Department of Cancer Cell Research, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108 (Japan).

Abbreviations: AZA^r, 8-azaguanine resistance; FCS, foetal calf serum; OUA^r, ouabain resistance; S15, 15 000 X g supernatant fraction; VCM, vinyl chloride (monomer); VDC, vinylidene chloride (1,1-dichloroethylene).

phenobarbitone (1 mg/ml; Sørensen phosphate buffer; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ per ml); 1 μmol glucose 6-phosphate; MEM and 0.25 ml FCS. (vitamins from Eagle MEM) ensure survival of the cells. Agar was added to modify.

The petri dishes were the vapour of the chemical in the desiccator, which had levels corresponding to 5 mm Hg. The pressure was adjusted by the addition of liquids which are liquids at low volumes of vapour (on the desiccator). The desiccator was at 37°C to allow the atmospheric pressure to be maintained. The foil, was then incubated for 24 hours. Extractions of the compounds were determined previously, using a standard period, the vapour was washed twice and the control cells were treated with the same chemicals or in reaction with glucose 6-phosphate. For this reason, controls for washing efficiency as compared to those for which the S15

The cells were then mutagenicity induced. 60 mm petri dish (4 dishes, 2×10^4 and 10^5 respectively) 8 dishes for drugs were added 1 mM ouabain. The media; for the 8-azaguanine non-essential amino acids stained with Giemsa at frequency was calculate plating efficiency being confirmed by repeated twice with chloroprene refer to the variability OUA^r colonies was determined which were cultured used for selection [8,19]

SL 066633

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 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ per ml); and 0.75 ml phosphate-buffered saline containing 12.5 μmol glucose 6-phosphate, 2 μmol 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 $\mu\text{g/ml}$ 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].

SL 066634

Results

Mutagenicity of VCM

To determine the optimal conditions for detecting the mutagenicity of these gaseous compounds, V79 cells were 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

caused a decrease in plating efficiency. Agar suspension caused no decrease in plating efficiency when added to the reaction mixture. Courses of mutation induction in liquid incubation: with controls were observed at 5 h and suspension, mutation frequency that microsomal enzyme Malaveille et al. [25]. In number of resistant colonies (OUA^r), obtained at 5 h. Consequently, incubation for the following experiments.

VCM induced dose-response curves of between 5 and 10% (v/v), mutation frequency

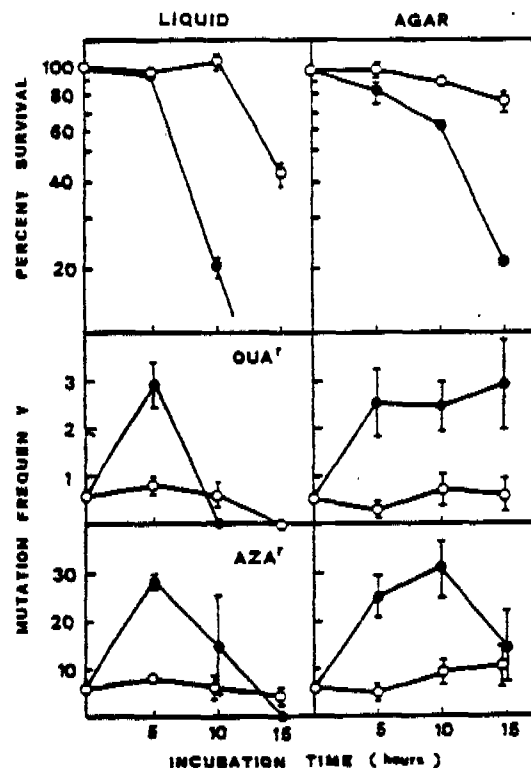


Fig. 1. Time courses of the induction of cytotoxicity and of mutations by exposure to 20% (v/v) VCM in air (●) in reaction mixture containing S15 liver fraction from phenobarbitone-pre-treated rats plus cofactors, in either liquid suspension (left column) or in 0.3% agar suspension (right column). Controls (○) 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 10^5 survivors, taking into account the number of cells plated and the plating efficiency. Bars: SE.

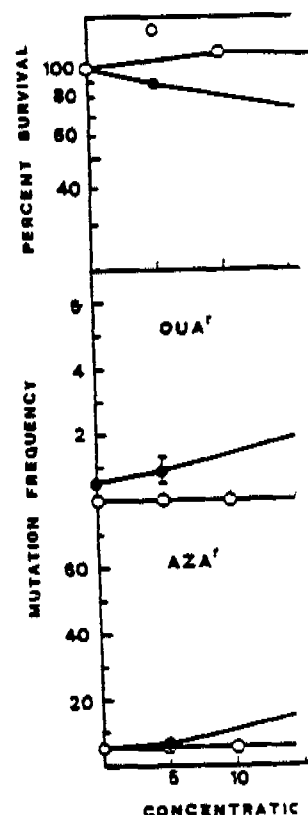


Fig. 2. VCM-induced cytotoxicity and mutations. ●, complete reaction mixture; ○, reaction mixture without VCM. Bars: SE.

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-

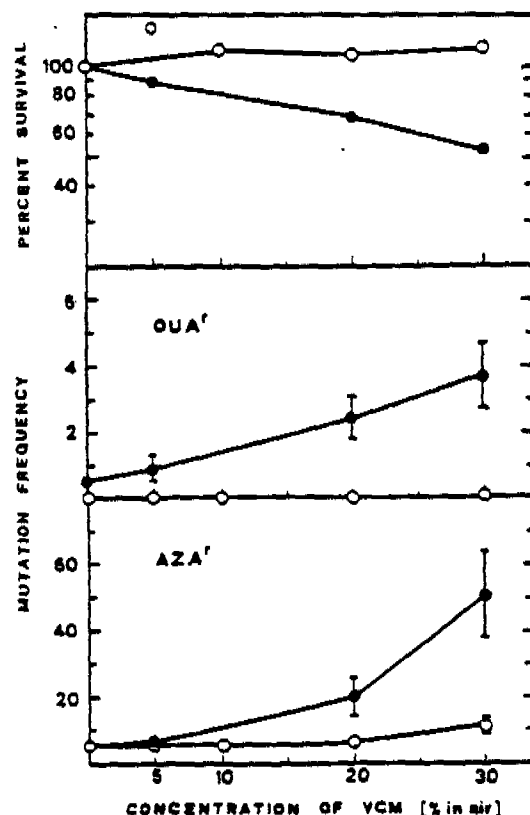


Fig. 2. VCM-induced cytotoxicity and mutagenicity in V79 cells as a function of concentration of VCM in air (% v/v). ●, complete reaction mixture containing S16 liver fraction from phenobarbitone-pre-treated rats plus cofactors. ○, reaction mixture containing no S16. Cytotoxicity and mutation frequency are expressed as described in the legend to Fig. 1.

SL 066636

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	3.6	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 AND MUTAGENICITY IN THE PRESENCE OF POST-MITOBARBITONE-PRE-TREATED RATS

Concentration in air (%)	Incubation
0	complete
0.2	
1.0	
2.0	
10.0	
0	minus cofa.
0.2	
1.0	
2.0	
10.0	

^a The results of two independent Expt. 1 and at 0, 2.0 and 10.0 similar values were obtained in

^b Expressed as plating efficiency: 1

^c Mutation frequency is expressed cells plated and plating efficien

enzymic formation of a mutations, in terms of A⁷ presence or absence of m

Discussion

Table 3 summarizes chloroprene in man and graphs [15], and juxtapose and, from the present st been shown to be carcin produced tumours at diffe tested by the oral rout

TABLE 3

SUMMARY OF DATA ON CARCINOGENICITY OF CHLOROPRENE

Compound	Evidence of carcinogen
	Man
VCM	+
VDC	?
Chloroprene	-

^a Carcinogenicity data are taken fr

SL 066637

TABLE 2

CYTOTOXICITY AND MUTAGENICITY OF CHLOROPRENE IN V79 CHINESE HAMSTER CELLS IN THE PRESENCE OF POST-MITOCHONDRIAL FRACTION (S15) OF LIVER FROM PHENOBARBITONE-PRE-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		9.0	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 [5,11]; 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.

Acknowledgements

The authors thank Mr. [Name obscured] with gaseous compounds for editorial assistance; the critical reading of t NCI Contract 1CP-55630

References

- Andrews, A.W., E.S. Zawisto vinyl chloride and methyl chloride by various chemicals. Toxicology, Elsevier/North-Holland, 32 (1976) 267-3.
- Barbin, A., H. Br  sil, A. C. microsome-mediated formaldehyde and 2-chlorobutadiene.
- Bartsch, H., T. Kuroki, C. Rainaldi, E. Vogel and A. schistosomai drug in bacteria.
- Bartsch, H., C. Malaveille, metabolism of vinyl chloride.
- Bartsch, H., C. Malaveille a vinyl chloride in *S. typhimurium*.
- Bartsch, H., C. Malaveille, R. chloride and 2-chlorobutadiene.
- Bartsch, H., and R. Montesano, 32 (1975) 93-114.
- Drevon, C., T. Kuroki and line by various chemicals. Toxicology, Elsevier/North-Holland, 32 (1976) 267-3.
- Fishbein, L., Industrial mutation Res., 32 (1976) 267-3.
- Gatto, A.J., J.B. Guttanplac extracts and free radicals, Mu.
- Graham, H., G. Boone, A. R. carcinogenicity of chloroacetaldehyde, 24 (1975) 201.
- Huberman, E., H. Bartsch ar vinyl chloride metabolites, ch 639-644.
- Huberman, E., and L. Sac gen. Int. J. Cancer, 13 (1974) 1-11.
- Huassan, S., and S. Osterman metabolites, Chem.-Biol. Inter., 15 (1975) 1-11.
- International Agency for genic Risk of Chemicals to Lyon, 1979.
- Jones, B.K., and D.E. Hath act., 20 (1978) 27-41.
- Krahn, D.F., and C. Heid cells by polycyclic hydrocarbons.
- Kuroki, T., and Fluorene in V79.
- Kuroki, T., C. Drevon cells by various nitroaromatic compounds.
- Langenbach, R., H.J. F. with liver carcinogens, Proc. Natl. Acad. Sci. USA, 75 (1978) 1-11.
- Loprieno, N., R. Barale, S. Neri, C. Leporini, D. Rose vinyl chloride metabolites.

Acknowledgements

The authors thank Mr. C. Malaveille for his guidance concerning treatment with gaseous compounds; Miss J. Mitchell for secretarial aid; Mrs. E. Heseltine for editorial assistance; and Drs. L. Tomatis, R. Montesano and H. Bartsch for the critical reading of this manuscript. This work was supported in part by US NCI Contract 1CP-55630 and CEC contract No. 190-77-1 ENVF.

References

- 1 Andrews, A.W., E.S. Zawistowski and C.E. Valentine. A comparison of the mutagenic properties of vinyl chloride and methyl chloride. *Mutation Res.* 40 (1976) 273-276.
- 2 Barbin, A., H. Brétil, A. Croisy, P. Jacquignon, C. Malaveille, R. Montesano and H. Bartsch. Liver microsome-mediated formation of alkylating agents from vinyl bromide and vinyl chloride. *Biochem. Biophys. Res. Commun.* 67 (1975) 596-603.
- 3 Bartsch, H., T. Kuroki, C. Malaveille, N. Lopriano, R. Barale, A. Abbondandolo, S. Bonatti, G. Rainaldi, E. Vogel and A. Davis. Absence of mutagenicity of praziquantel, a new, effective anti-schistosomal drug in bacteria, yeasts, insects and mammalian cells. *Mutation Res.* 58 (1978) 133-142.
- 4 Bartsch, H., C. Malaveille, A. Barbin, H. Brétil, L. Tomatis and R. Montesano. Mutagenicity and metabolism of vinyl chloride and related compounds. *Environ. Health Persp.* 17 (1976) 193-198.
- 5 Bartsch, H., C. Malaveille and R. Montesano. Human, rat and mouse liver mediated mutagenicity of vinyl chloride in *S. typhimurium* strains. *Int. J. Cancer* 15 (1975) 429-437.
- 6 Bartsch, H., C. Malaveille, R. Montesano and L. Tomatis. Tissue-mediated mutagenicity of vinylidene chloride and 2-chlorobutadiene in *Salmonella typhimurium*. *Nature (London)* 255 (1975) 641-643.
- 7 Bartsch, H., and R. Montesano. Mutagenic and carcinogenic effects of vinyl chloride. *Mutation Res.* 32 (1975) 93-114.
- 8 Drevon, C., T. Kuroki and R. Montesano. Microsome-mediated mutagenesis of a Chinese hamster cell line by various chemicals. in: D. Scott, B.A. Bridges and F.H. Sobels (Eds.), *Progress in Genetic Toxicology*. Elsevier/North-Holland, Amsterdam, 1977, pp. 207-213.
- 9 Fishbein, L., Industrial mutagens and potential mutagens. I. Halogenated aliphatic derivatives. *Mutation Res.* 32 (1976) 267-308.
- 10 Garro, A.J., J.B. Gustenplan and P. Milvy. Vinyl chloride dependent mutagenesis: effects of liver extracts and free radicals. *Mutation Res.* 38 (1976) 81-88.
- 11 Green, H., G. Borne, A. Radwan, D. Reichart and D. Henschler. Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation. *Biochem. Pharmacol.* 24 (1975) 2013-2017.
- 12 Huberman, E., H. Bartsch and L. Sachs. Mutation induction in Chinese hamster V79 cells by two vinyl chloride metabolites, chloroethylene oxide and 2-chloroacetaldehyde. *Int. J. Cancer* 16 (1975) 639-644.
- 13 Huberman, E., and L. Sachs. Cell-mediated mutagenesis of mammalian cells with chemical carcinogens. *Int. J. Cancer* 13 (1974) 326-333.
- 14 Humain, S., and S. Osterman-Golkar. Comment on the mutagenic effectiveness of vinyl chloride metabolites. *Chem.-Biol. Interact.* 12 (1976) 245-247.
- 15 International Agency for Research on Cancer, IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Vol. 19, Some monomers, plastics and synthetic elastomers, acrolein. Lyon, 1979.
- 16 Jones, B.E., and D.E. Hathway. The biological fate of vinylidene chloride in rats. *Chem.-Biol. Interact.* 20 (1978) 27-41.
- 17 Krahn, D.F., and C. Heidelberger. Liver homogenate-mediated mutagenesis in Chinese hamster V79 cells by polycyclic hydrocarbons and aflatoxins. *Mutation Res.* 46 (1977) 27-44.
- 18 Kuroki, T., and H. Bartsch. Mutagenicity of some N- and O-acyl derivatives of N-hydroxy-2-amino-fluorene in V79 Chinese hamster cells. *Cancer Lett.* 6 (1979) 67-72.
- 19 Kuroki, T., C. Drevon and R. Montesano. Microsome-mediated mutagenesis in V79 Chinese hamster cells by various nitroamines. *Cancer Res.* 37 (1977) 1044-1050.
- 20 Langenbach, R., H.J. Freed and E. Huberman. Liver cell-mediated mutagenesis of mammalian cells with liver carcinogens. *Proc. Natl. Acad. Sci. (U.S.A.)* 75 (1978) 2864-2867.
- 21 Lopriano, N., R. Barale, S. Baroncelli, H. Bartsch, G. Bronzetti, A. Cammellini, C. Corsi, D. Frezza, R. Mieri, C. Leperini, D. Rosellini and A.M. Rossi. Induction of gene mutations and gene conversions by vinyl chloride metabolites in yeast. *Cancer Res.* 37 (1977) 253-257.

SL 066640

- 22 Lepriano, N., R. Barale, S. Baroneselli, C. Bauer, G. Bronzetti, A. Cammellini, G. Carrignani, C. Corni, G. Gervasi, C. Leporini, R. Nieri, A.M. Rossi, G. Stretti and G. Turchi. Evaluation of the genetic effects induced by vinyl chloride monomer (VCM) under mammalian metabolic activation: studies in vitro and in vivo. *Mutation Res.*, 40 (1976) 85-96.
- 23 Magnuson, J., and C. Ramel. Mutagenic effects of vinyl chloride in *Drosophila melanogaster*. *Mutation Res.*, 38 (1976) 115.
- 24 Malaveille, C., H. Bartsch, A. Barbin, A.M. Camus and R. Montesano. Mutagenicity of vinyl chloride, chloroethylene oxide, chloroacetaldehyde and chloroethanol. *Biochem. Biophys. Res. Commun.*, 63 (1975) 363-370.
- 25 Malaveille, C., G. Planche and H. Bartsch. Factors for efficiency of the *Salmonella*/microsome mutagenicity assay. *Chem.-Biol. Interact.*, 17 (1977) 129-136.
- 26 McCann, J., V. Simmon, D. Straitwaser and B.N. Ames. Mutagenicity of chloroacetaldehyde, a possible metabolic product of 1,2-dichloroethane (ethylene dichloride), chloroethanol (ethylene chlorohydrin), vinyl chloride and cyclophosphamide. *Proc. Natl. Acad. Sci. (U.S.A.)*, 72 (1975) 3190-3193.
- 27 Rannug, U., R. Göthe and C.A. Wachtmeister. The mutagenicity of chloroethylene oxide, chloroacetaldehyde, 2-chloroethanol and chloroacetic acid, conceivable metabolites of vinyl chloride. *Chem.-Biol. Interact.*, 12 (1976) 251-263.
- 28 Rannug, U., A. Johansson, C. Ramel and C.A. Wachtmeister. The mutagenicity of vinyl chloride after metabolic activation. *Ambio*, 3 (1974) 194-197.
- 29 Styles, J.A.. A method for detecting carcinogenic organic chemicals using mammalian cells in culture. *Br. J. Cancer*, 36 (1977) 558-563.
- 30 Verburgt, F.G., and E. Vogel. Vinyl chloride mutagenesis in *Drosophila melanogaster*. *Mutation Res.*, 48 (1977) 327-336.
- 31 Vogel, E.. Mutagenicity of carcinogens in *Drosophila* as a function of genotype-controlled metabolism, in: F.J. de Serres, J.R. Fouts, J.R. Bend and R.M. Philpot (Eds.), *In vitro Metabolic Activation in Mutagenesis Testing*, Elsevier/North-Holland, Amsterdam, 1976, pp. 63-79.

Mutation Research, 67 (1979)
© Elsevier/North-Holland

Short Communication

CHEMICALS WHICH R *typhimurium* TESTER S

RICHARD H. MCKEE, JAM

Litron Laboratories, Ltd.

(Received 8 August 1978)
(Revision received 16 January 1979)
(Accepted 2 February 1979)

Previous uses of aryl
their photosensitive p
been synthesized for su
4-fluoro-3-nitrophenyl
inactivation of enzym
amino acid transport
azides can also photo
the mechanism of ba
(DNPA) were tested
test system. The Ame
bacteria which contain
the mutations carried
frameshift mutations,
tagenic event mediated
chemicals have been s
which induce reversion
Ames strains could prov
experiments within the
ferent laboratories. V
DNPA induce the rev
strains (TA98, TA100,

Materials and method

The *Salmonella typhimurium* strains obtained from Dr. Bruce testing with these strains for plate-incorporation procedure is that Vogel concentration as in the

SL 066641