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Mutation Research, 77 (1980) 175—179
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Short Communication

MUTAGENICITY OF VINYL CHLORIDE IN THE AMES TEST

POSSIBLE ARTIFACTS RELATED TO EXPERIMENTAL CONDITIONS

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(Received 11 June 1979)
(Revision received 11 September 1979)
(Accepted 17 September 1979)

The association between exposure to vinyl chloride (VCM) and the occurrence of cancer in man has stimulated numerous investigations on the possible mutagenicity of VCM in several test systems. Exposure to vinyl chloride vapour in the presence of an enzymatically active liver subcellular fraction has induced reverse mutations in *Salmonella typhimurium* [2,5,11,12] and *Escherichia coli* K12 [6], forward in mutations in *Schizosaccharomyces pombe* [9] and V79 Chinese hamster cells [4], and mitotic gene conversions in *Saccharomyces cerevisiae* [9].

However, some studies have indicated that vinyl chloride is able to induce mutations towards *S. typhimurium* strain TA1535 in the absence of a metabolic activating system. Some authors have reported that a much higher mutagenic response was obtained when liver post-mitochondrial fractions were added [2,12]. Another study [11], however, has shown that the reversion rates were only slightly different when the experiments were performed either in the presence or in the absence of a 9000 × *g* supernatant from the rat liver. Moreover, it has been reported that the addition of an NADPH-generating system to the liver extract was not required for vinyl chloride to induce mutations and that a mutagenic effect could even be observed when the liver extracts were previously heated [5].

These results are in disagreement with those reported by Bartsch et al. [2] which clearly show that the enzymatically mediated mutagenic activity of vinyl chloride is significantly enhanced in the presence of an NADPH-generating system and in assays where the liver supernatants are obtained from rats and mice that have been pretreated with phenobarbitone.

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TABLE 1

DIRECT MUTAGENIC ACTIVITY OF VCM TOWARDS TA 1530

Concentrations of VCM in the atmosphere (%)	2	7	12	20
Number of *his*$^+$ rev./plate	33 ± 6 (7)	48 ± 5 (10)	54 ± 9 (7)	101 ± 16 (10)

Mean values ± S.E.M. of 2 assays performed in triplicate. Spontaneous reversion rates are in parentheses.

TABLE 2

MUTAGENICITY OF VCM TOWARDS TA1530 AS OBSERVED IN SIMULTANEOUSLY INCUBATED PLATES CONTAINING EITHER BACTERIA ALONE OR BACTERIA AND THE S9 MIX OBTAINED EITHER FROM CONTROL MICE OR FROM MICE PRETREATED WITH AROCLOR 1254

The VCM concentration in the atmosphere was 2%.

Mice pretreatment	Control		Aroclor 1254	
Fortified S9 fraction	+	—	+	—
Number of *his*$^+$ rev./plate	206 ± 18 (14)	52 ± 9 (16)	562 ± 71 (12)	102 ± 8 (15)

Mean values ± S.E.M. of 2 assays performed in triplicate. Spontaneous reversion rates are in parentheses.

TABLE 3

MUTAGENIC ACTIVITY OF VCM TOWARDS TA1530

Plates containing either the bacteria alone or the S9 mix from control or pretreated mice alone were simultaneously incubated in an atmosphere of 2% VCM.

Mice pretreatment	Control	Aroclor 1254
Number of *his*$^+$ rev./plate	74 ± 8 (17)	261 ± 32 (18)

Mean values ± S.E.M. of 2 assays performed in triplicate. Spontaneous reversion rates are in parentheses.

TABLE 4

MUTAGENIC ACTIVITY OF VCM (2% IN THE ATMOSPHERE) TOWARDS TA1530 IN THE PRESENCE OF VARIOUS PROTEIN FRACTIONS

Protein fraction	Boiled S9 mice Aroclor 1254 no cofactors	S9 control mice no cofactors	BSA + cofactors
Number of *his*$^+$ rev./plate	40 ± 5 (19)	37 ± 5 (13)	18 ± 3 (14)

Mean values ± S.E.M. of 2 assays performed in triplicate. Spontaneous reversion rates are in parentheses.

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IC ACTIVITY OF VCM (2% IN THE ATMOSPHERE) TOWARDS TA1530 IN THE PRES-ARIOUS PROTEIN FRACTIONS

tion	Boiled S9 mice Aroclor 1254 no cofactors	S9 control mice no cofactors	BSA + cofactors
his$^+$ rev.[illegible]e	40 ± 5 (19)	37 ± 5 (13)	18 ± 3 (14)

± S.E.M. of 2 assays performed in triplicate. Spontaneous reversion rates are in parentheses.

Among the several possible me pounds, chloroethylene oxide a several test systems in the absen system [2,7,8,10,11] and have intermediates responsible for the of this report is to demonstrate used to evaluate the mutagenicit discrepancies observed between th

Plates of minimal glucose aga TA1530 (2–7 × 10^7 viable bacte described by Ames and cowork adult male NMRI mouse liver S9 conditions. The plates were expo ucts, Belgium) atmosphere in a trol device and its operation hav exposure in the VCM atmospher and further incubated in the dar had elapsed. The numbers of *his*$^+$

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The incorporation of the S9 reversion rate, and the number the S9 liver fraction was obtain (Table 2). These observations a ever, if one compares the result sion rate observed in the plates plates containing both the bac exposed to VCM in the same i enhanced reversion rates were o an S9 mix obtained from mice p

This observation prompted a taining the bacteria but no liver cator together with plates cont Comparison of the results from presence of plates containing genicity of VCM towards the enhancement was even more pr from mice that had been pretrea

Comparison of Tables 2 and 3

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Among the several possible metabolites of vinyl chloride, two volatile compounds, chloroethylene oxide and 2-chloroacetaldehyde, were mutagenic in several test systems in the absence of a liver microsomal metabolic activating system [2,7,8,10,11] and have therefore been considered as the metabolic intermediates responsible for the mutagenicity of vinyl chloride. The purpose of this report is to demonstrate that variations in the experimental conditions used to evaluate the mutagenicity of vinyl chloride could explain some of the discrepancies observed between the previously reported results.

Plates of minimal glucose agar were inoculated with *S. typhimurium* strain TA1530 ($2-7 \times 10^7$ viable bacteria/plate) in top agar according to the method described by Ames and coworkers [1]. S9 mix [1] (concentration, 150 μl adult male NMRI mouse liver S9/plate) was added to certain plates in various conditions. The plates were exposed to a controlled VCM (Matheson Gas Products, Belgium) atmosphere in a desiccator kept in the dark at 37°C. The control device and its operation have been previously described [3]. After a 16-h exposure in the VCM atmosphere, the plates were removed from the desiccator and further incubated in the dark at 37°C until a total incubation time of 48 h had elapsed. The numbers of *his*$^+$ revertants were counted.

In a preliminary step, the direct mutagenic activity of VCM was tested in the absence of any metabolic activating system (Table 1). The reversion rate of TA1530 increased proportionally to the concentration of VCM in the atmosphere.

In a subsequent experiment, two sets of Petri plates were simultaneously incubated in the desiccator, the atmosphere of which contained 2% VCM. Certain plates contained the bacteria without any metabolic activating system, while the remaining plates contained both the bacteria and a fortified liver post-mitochondrial fraction obtained from either control mice or from mice pretreated with Aroclor 1254. The plates containing liver fractions from control and pretreated animals were used in separate experiments.

The incorporation of the S9 mix into the plates significantly increased the reversion rate, and the number of *his*$^+$ revertants per plate was enhanced when the S9 liver fraction was obtained from animals pretreated with Aroclor 1254 (Table 2). These observations agree with previously reported data [2]. However, if one compares the results from Tables 1 and 2, one sees that the reversion rate observed in the plates without S9 mix was slightly increased when plates containing both the bacteria and an S9 mix from control mice were exposed to VCM in the same incubation desiccator. Even more significantly enhanced reversion rates were observed when those additional plates contained an S9 mix obtained from mice pretreated with Aroclor 1254.

This observation prompted a third set of experiments in which plates containing the bacteria but no liver fraction were exposed to 2% VCM in the desiccator together with plates containing the S9 mix but no bacteria (Table 3). Comparison of the results from Tables 1 and 3 indicates that the simultaneous presence of plates containing S9 mix in the desiccator enhanced the mutagenicity of VCM towards the bacteria inoculated in separate plates. The enhancement was even more pronounced when the S9 mix had been obtained from mice that had been pretreated with the inducing agent, Aroclor 1254.

Comparison of Tables 2 and 3 indicates that VCM metabolites were preferen-

tially trapped by bacteria that were in close contact with the S9 mix. Thus, when plates contain bacteria plus S9 mix, the amount of reactive metabolites which can migrate to the neighbouring plate appears to be lower than when bacteria are omitted.

Finally, assays were performed to investigate the possible effects of the proteins of the S9 mix on the mutagenicity of VCM. To plates of bacteria were added various protein fractions: S9 from mice pretreated with Aroclor 1254, heated in boiling water for 5 min (150 μl/plate); S9 from control mice (150 μl/plate); and bovine serum albumin (±3.8 mg (~150 μl S9)/plate) supplemented with salts and cofactors. These plates were successively incubated in an atmosphere of 2% VCM (Table 4). The results indicate that, under the present conditions, addition of proteins to the plates had no significant effect on the mutagenicity of VCM.

In conclusion, and in agreement with previously reported studies [2,11,12], VCM has been shown to exert a direct mutagenic activity towards *S. typhimurium* TA1530 which is related to its concentration in the atmosphere. The origin of the direct mutagenic effect remains unclear.

The mutagenicity of VCM is strongly enhanced in the presence of a fortified metabolizing liver extract. However, co-incubation of Petri plates containing either bacteria alone or only S9 subcellular fractions from liver tissue in an atmosphere of VCM enhances the mutation frequency as compared with frequencies observed when *S. typhimurium* alone is exposed to the same atmosphere. The increase is more pronounced when the liver homogenates are derived from animals that have been pretreated with a metabolizing enzyme inducer. However, it must be pointed out that the results presented here could be affected by numerous experimental conditions, e.g. the amount of volatile metabolites, the volume of the desiccator, the number of plates, the composition of plates.

The results suggest that the observed enhancement of the mutagenicity of VCM is correlated with the formation, under the influence of the liver-metabolizing enzymes, of proximate mutagens such as chloroethylene oxide and 2-chloroacetaldehyde. These two compounds are volatile intermediates and are, therefore, able to exert their direct mutagenic action towards the neighbouring bacteria.

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The obtained results reveal dose dependence for both lethality and teratogenic outcome. Types of teratomas indicate that most of them are chondropathic and affect skeleton causing different malformations, evisceration and runting in somatic development. Further experimentation with some compounds derived from erythromycin point to the fact that carbamide induces skeletal malformations and isoniazide leads to evisceration.

ABSTRACT #133

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I.L. Hansteen, L. Hillestad and E. Thiis-Evensen, St. Joseph Hospital, Porsgrunn (Norway)

Chromosome studies in workers exposed to vinyl-chloride

Chromosome analysis have been done on 48-h lymphocyte cultures from 39 workers in the PVC factory in Norway, and on 16 control persons matched to sex and age. Bone marrow samples were studied in four cases.

Measurements of exposure are unfortunately not available before 1974.

Hundred cells per individual were scored for breaks, gaps rings, dicentrics, fragments, chromatide exchanges, and "stable" rearrangements. Breaks and gaps were the predominant aberrations in the cells both in the controls and in the workers. "Stable" rearrangements were found in more cells than was expected. Gaps are not included in the results.

The results are presented as number of cells with aberrations, and also considered as total breaking events per 100 cells. The number of cells with aberrations and total breaking events are not greatly increased for the workers compared with the controls. The results were tested statistically by a Wilcoxon two-sample test, which showed that workers scored significantly higher on a 2.5% level. The results are discussed.

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Gh. Deknudt and A. Leonard, Dept. of Radiobiology, S.C.K.-C.E.N., Mol (Belgium)

Cytogenetic investigations on leucocytes of workers from a cadmium plant

Chromosome analysis has been performed on workers of a cadmium plant, who were exposed to fumes and dust of cadmium and lead. The 35 workers were classified, according to the type and duration of exposure, into a group (cadmium service) of 23 people exposed to high levels of lead and cadmium in the absence of zinc and into a group of 12 rolling-mill workers subjected mostly to zinc but also to lower levels of lead and cadmium. A higher yield of severe chromosome anomalies (chromatid exchange, disturbance of spiralisation, chromosome translocation, ring and dicentric chromosomes) together with a total lower number of structural aberrations was observed in the cadmium workers when compared to the rolling-mill group.

These data will be discussed together with the results obtained in a previ-

ous study f

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