MUTAGENIC AND CLASTOGENIC EFFECTS OF CHLOROFORM*

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ABSTRACT

Chloroform was shown to exhibit DNA damaging capacity. However, its DNA damaging capacity is not a consequence of its direct reactivity with DNA. Directly it did not effect base pair and frameshift mutations. But chloroform is metabolized to a frameshift mutagen in the male mouse. Thus, chloroform is a pro-mutagen although it is not a direct mutagen. It is not only a pro-mutagen but also a clastogen (chromosome breaking). Its clastogenicity and mutagenicity, however, is reduced in the presence of vitamin E.

INTRODUCTION

Chloroform, although no longer used as an anaesthetic, still finds widespread use as a solvent and as a catalyst in polymerization reactions. It is a very popular solvent in research and teaching laboratories. It is also a component of cough syrup, dentrifices, and mouth washes. Lately, the presence of chloroform in chlorinated water was reported.

It is therefore possible that specific population groups can be exposed to sub-toxic levels of chloroform. This led to our interest in studying the mutagenicity potential of this substance.

LITERATURE CITED

It has long been known that chloroform is hepato-toxic to rabbits (1) mice (2), rats (3), dogs (4), and man (5). Liver damage was evident not only at high but also at low doses. Liver damage, however, was prevented if oxygen was administered with chloroform (6). Protection was also offerred by methionine and cysteine (7).

Not only does chloroform cause damage in the liver but also in the kidney (8). This was also shown to be sex-specific. This was observed only in the male mouse.

It has also been reported that excessive inhalation of chloroform can result in hemorrhage of the gastro-intestinal tract and the pancreas (9), as well as necrosis of the placenta in pregnant females (10).

Chloroform exhibited mutagenic effects in *Drosophila melanogaster* (1) but it was not mutagenic in potatoes (12). While it was reported that chloro-

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form, caused liver cancer in the mouse, (13) there was a claim that it was beneficial to cancer patients (14).

MATERIALS AND METHODS

Materials:

Chloroform, a Mallinckrodt product, was redistilled before use.

Bacto-agar and Bacto tryptone were products of DIFCO Laboratories.

Yeast extract, Giemsa and May-Grunwald stains were obtained from Merck.

Fetal calf serum was obtained from Grand Island Biological Supply, New York, U.S.A.

Albino mice were obtained from Alabang Stock Farm.

Rec + and Rec – strains of *B. subtilis* were gifts from Dr, T. Kada, National Institute of Genetics, Mishima, Japan.

Mutant strains of *Salmonella typhimurium* were gifts from Dr. B.N. Ames, Department of Biochemistry, University of California, Berkeley, U.S.A.

METHODS:

Rec assay that was used to detect DNA damaging capacity was essentially the method of Kada (15). After the plates were incubated for 20 hours at 23°C, zones of inhibition were measured.

The pour-plate method for detecting direct mutagens was the method of Ames (16). After incubation for 48 hours revertant colonies were counted.

The host-mediated assay that was used to detect the pro-mutagenicity of the test substance was the method of Legator and Malling (17), with *Salmonella typhimurium* TA 1535 and TA 1537 as indicator organisms.

The micronucleus method of Dr. W. Schmid (18) was employed to study the clastogenic potential of the test substance. For each slide 1000 polychromatic erythrocytes were scored for the presence of micronuclei.

RESULTS AND DISCUSSION

The Rec assay data are presented in Table 1. The presence of an inhibition zone in the Rec – strain of *Bacillus subtilis* is an indication that the lethal effect of chloroform is a consequence of DNA damage. The Rec – strain is deficient in the recombinant repair system so that DNA damage remains for the most part unrepaired, leading to cell death. The wild type, Rec +, showed a small inhibition zone although this strain has capabilities for recombination repair. It suggests that its repair mechanism cannot cope with the damaging effects of chloroform used. There is no doubt that chloroform has DNA damaging interactions.

Table 1. DNA damaging capacity of chloroform as revealed by Rec Assay using **Bacillus subtilis.**

Strain of B. subtilis	Length of Inhibition Zone (mm)
H 17 (rec +)	4.0 ± 0.4
H 45 (rec –)	14.7 ± 0.9

Values represent readings from 5 trials

To have an insight into the type of DNA damaging interactions of chloroform, *Salmonella typhimurium* mutants TA 1535 and TA 1537 were used in the pour plate method. TA 1537 can be used for detecting frameshift mutagens while TA 1535 can detect base-pair mutagens. The results shown in Table 2 indicate that chloroform is not a direct mutagen. It was not a direct base-pair nor a frameshift mutagen.

Table 2. A summary of the results gathered from the pour plate test. Tester strains are histidine-requiring mutants of **Salmonella typhimurium.**

Tester strain	no. of revertants $CHCl_3$	control[a]
TA1535	17 ± 6*	16 ± 2
TA1537	10 ± 3**	7 ± 3
TA98	27 ± 3***	28 ± 2

* Using 0.3% $CHCl_3$ (v/v) in DMSO, average of 25 plates.
** Using 0.05% $CHCl_3$ (v/v) in DMSO, average of 25 plates.
*** Using 0.02% $CHCl_3$ (v/v) in DMSO, average of 25 plates.
[a]Each entry is an average of 25 plates.

Although not a direct mutagen, chlorofom was shown to be a pro-mutagen (Table 3 and Table 4). It is metabolized to a frameshift mutagen in the male mouse only. This could be a consequence of the enhancing in the transformation of chloroform to a mutagenic metabolite. Liver damaging effects of chloroform were shown to be male specific (8).

Since chloroform was shown to be transformed to free radicals (8) it is possible that one of the DNA damaging species could be a free radical. Dichloromethane was also reported as one of the metabolites of chloroform. This is a possible alkylating agent. Alkylation on some minor alkylation site maybe responsible for its tendency to induce frameshift mutations after metabolic activation. Attack by free radicals on some bases or on the backbone of DNA may result in base deletions leading to frameshift mutations.

Table 3. Mutation frequency of chloroform in the host-mediated assay using **S. typhimurium** *TA 1535 as the indicator organism.*

	Mutation Frequency Mf_t / MF_c
Control (Female)	1.00
Control (Male)	1.00
Experimental (Female)	0.12
Experimental (Male)	0.61

Groups of 5 mice each were used. Mf_t/MF_c was computed with respect to the control for each group.

Table 4. Mutation frequency of chloroform in the host-mediated assay using **S. typhimurium** *TA 1537 as the indicator organism.*

	Mutation Frequency Mf_t/MF_c
Control (Female)	1.00
Control (Male)	1.00
Experimental (Female)	2.30
Experimental (Male)	36.75

Groups of five mice each were used. Mf_t/MF_c was computed with respect to the control for each group.

To confirm the presence of mutagenic metabolites, urinary metabolites from mice given chloroform were examined (Table 5). Results reveal the presence of mutagenic metabolites from the male mice.

To find out whether the pro-mutagenic effects of chloroform can be reflected on the chromosomal level, data on micronuclei formation was examined (Table 6). The clastogenic effect (chromosome breaking effects) is suggested by the appreciable amount of formation of micronucleated polychromatic erythrocytes from the bone narrow of the mouse. This was shown to be an effect of chloroform on DNA, not on the spindle apparatus, because there was a constant turnover of normocytes throughout the dose range employed. That chloroform was first metabolized before it became clastogenic is indicated in Figure 1. For a compound directly clastogenic (without metabolic activation) a straight line is expected.

Vitamin E, a free radical scavenger and a biological antioxidant, when administered an hour after, reduced the number of micronucleated polychromatic erythrocytes.

Table 5. Mutagenicity of urine concentrates towards TA1537 using the Pour Plate Test (spot testing).

Sample	no. of revertants	zone of inhibition, mm.
control	10 ± 2	none
9-aminoacridine	TNTC*	15 ± 3
ether extract I	302 ± 66	29 ± 4
ether extract II	101 ± 20	32 ± 4

The urine of 10 adult male mice which had received $CHCl_3$ at a dose of 700 mg/kg was pooled and then subjected to an extraction procedure described in Methods. The ether extracts I and II obtained were subsequently plated using five plates per extract.

*Too numerous to count.

Table 6. Micronucleus formation of $CHCl_3$ in mouse bone marrow as a function of dosage.

dose, mg/kg	no. of micronucleated polychromatic erythrocytes per thousand polychromatic erythrocytes
0 (control)	4 ± 1
100	3 ± 1
200	5 ± 1
400	5 ± 1
600	9 ± 2
700	17 ± 4
800	9 ± 2
900	10 ± 2

The value for the number of micronucleated polychromatic erythrocytes at the dose of 700 mg/kg is an average of 27 staining procedures. The rest are average values of 9 staining procedures.

Figure 1. A plot showing the effect of varied doses of $CHCl_3$ on the number of micronucleated polychromatic erythrocytes in the mouse bone marrow.

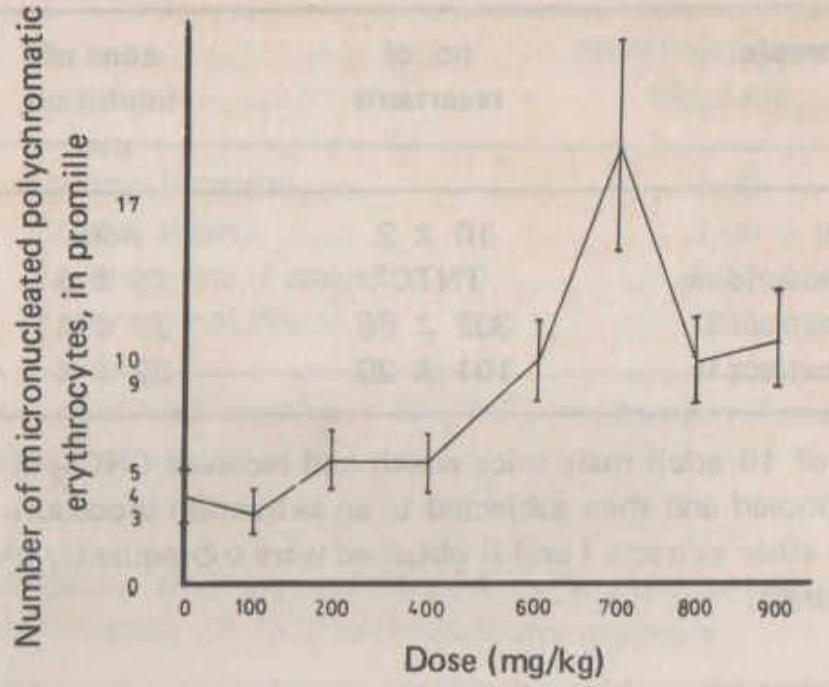

Table 7. The effect of vitamin E (125 IU/kg) on the number of micronucleated polychromatic erythrocytes under $CHCl_3$ dosing of 700 mg/kg.

	no. of micronucleated polychromatic erythrocytes per thousand polychromatic erythrocytes
Vitamin E given 1 hour ahead of $CHCl_3$	8 ± 3*
Vitamin E given 1 hour after $CHCl_3$	4 ± 2*
$CHCl_3$	17 ± 4**
control	4 ± 1***

* Average of 15 staining procedures
** Average of 27 staining procedures
*** Average of 9 staining procedures

SUMMARY

Chloroform possesses DNA damaging interactions. However, it is not a direct mutagen. It becomes mutagenic only after metabolic activation in the male mouse. It is not only a pro-mutagen but also a clastogen. Its chromosome breaking effect, however, is reduced when vitamin E was given after an hour.

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