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# Toxicity and Mutagenic Evaluation: Tetramethylammonium Borohydride

J. M. Holland  
J. L. Epler

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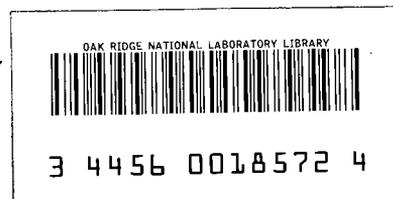
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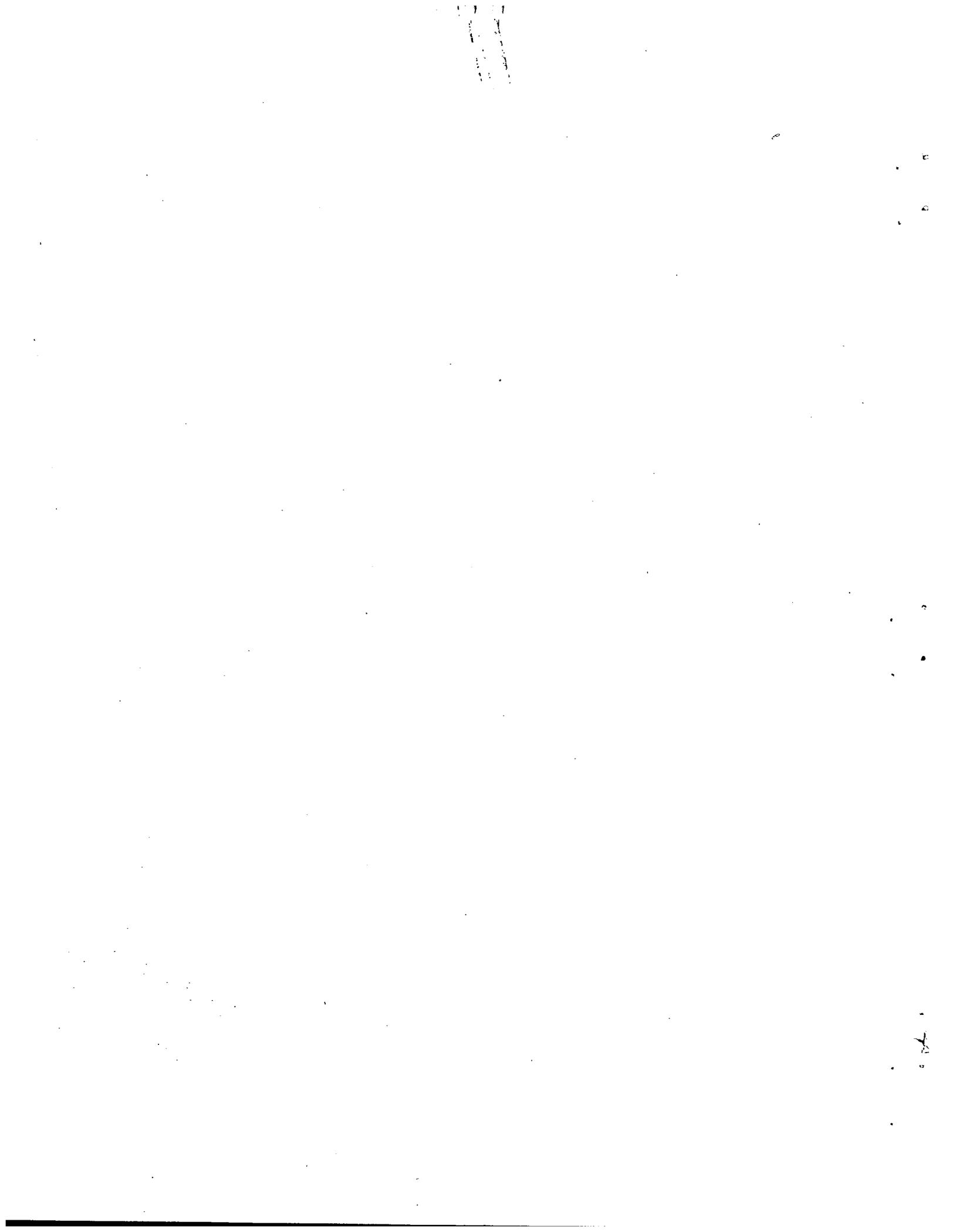
TOXICITY AND MUTAGENIC EVALUATION:  
TETRAMETHYLAMMONIUM BOROHYDRIDE

J. M. Holland  
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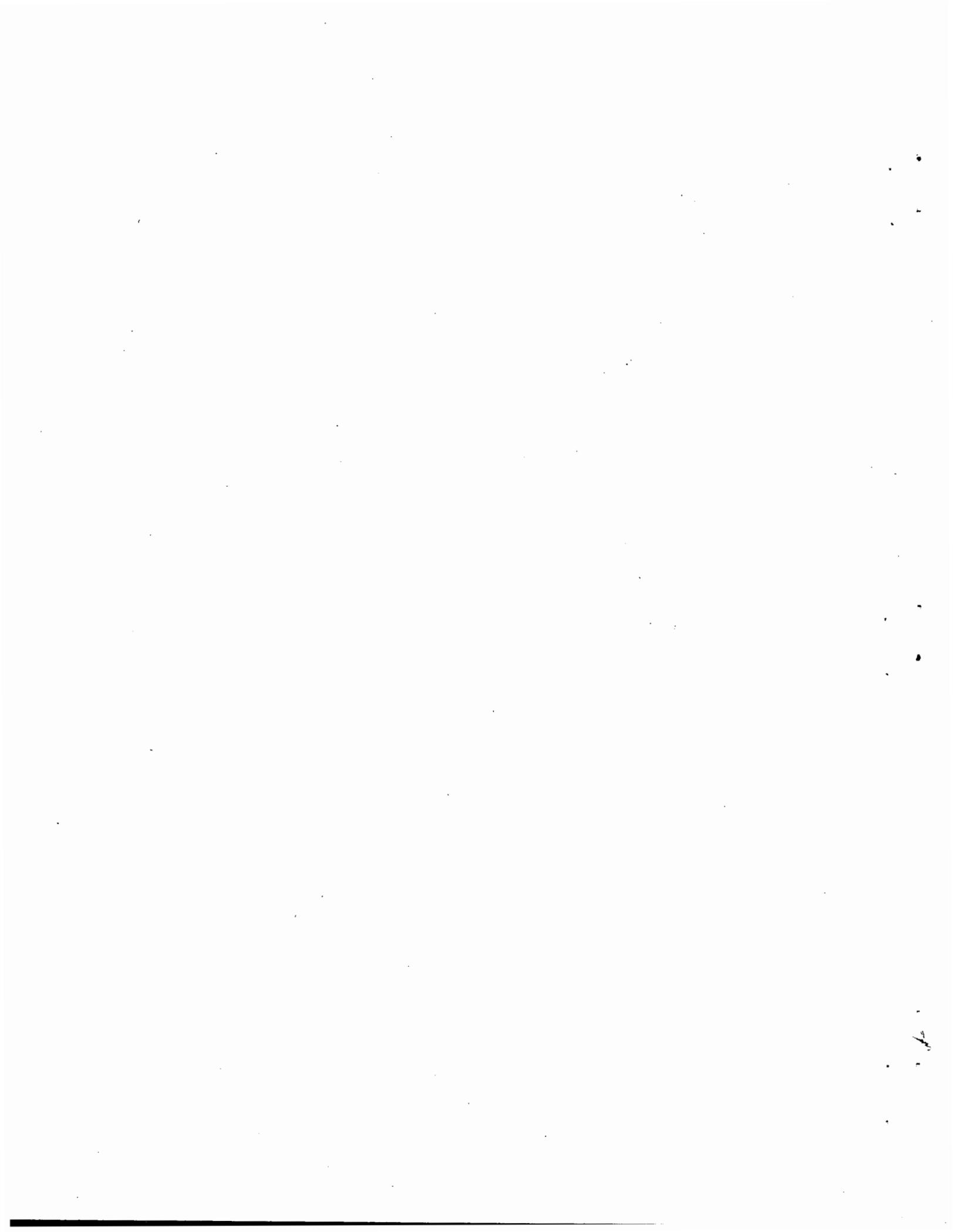
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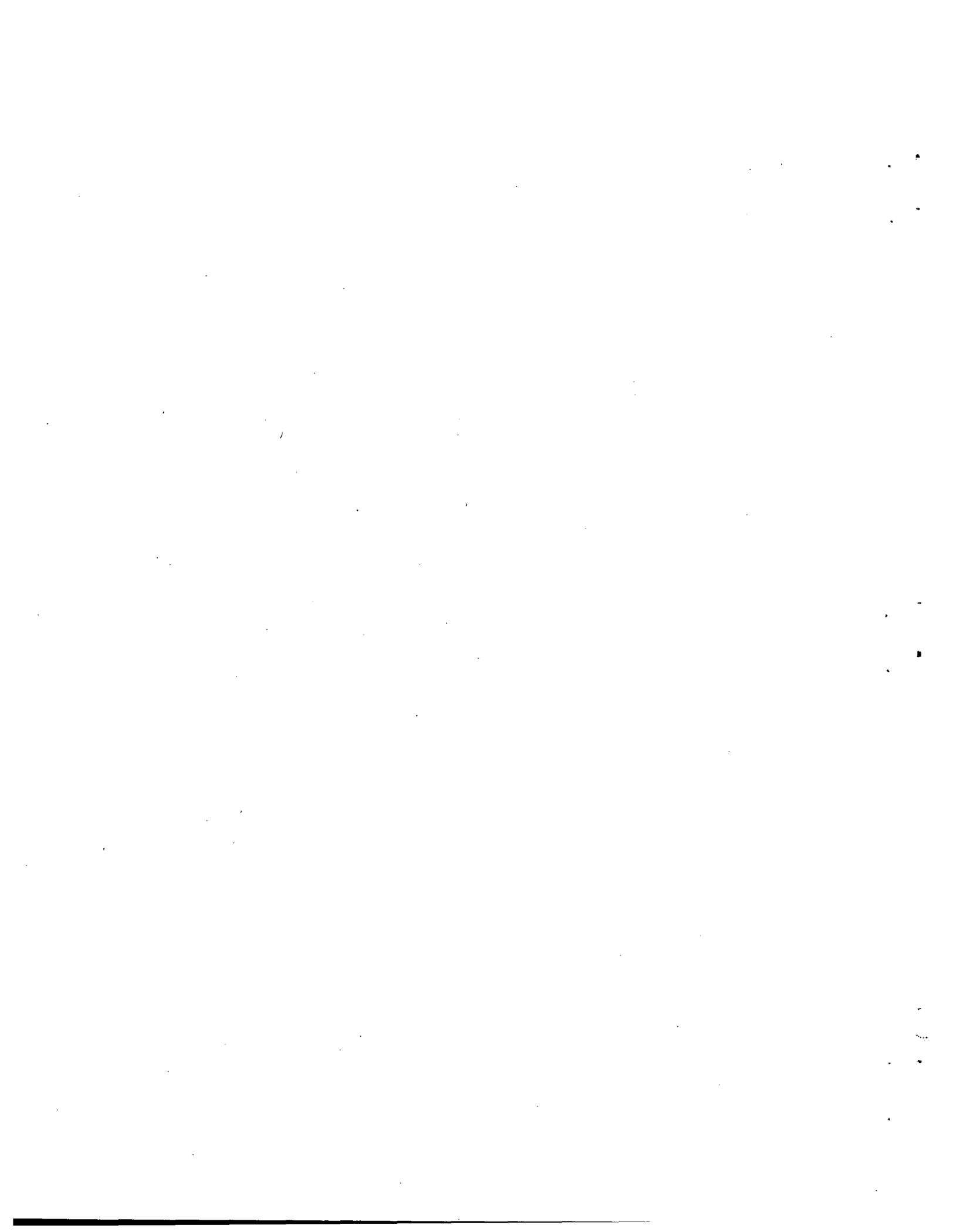
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ABSTRACT

Toxicity of Tetramethylammonium Borohydride

Acute oral LD <sub>50</sub> :	67 ± 3 mg/kg, mice 77 ± 7 mg/kg, rats
Primary skin irritation:	Grade 0 without abrasion Grade 1 with abrasion at 1% w/v Grade 3 with abrasion at 10% w/v
Eye irritation:	Positive, grade 1
Skin sensitization:	Negative
Dermal toxicity:	Approximately 200 mg in aqueous solution lethal to rabbits within 12 hr

Mutagenicity of Tetramethylammonium Borohydride

Negative in the Ames test and negative in the human leukocyte chromatid aberration assay.

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INTRODUCTION

The purpose of this study was to determine the mammalian toxicity of the compound tetramethylammonium borohydride (TMAB) and assess its genotoxic activity by use of microbial assays.

MATERIAL

TMAB was supplied by the Ventron Company. The material used was from a representative lot of the product. TMAB consists of 52.8–54.0% carbon, 15.6–15.7% nitrogen, 18.0–18.1% hydrogen, and 12.0–12.1% boron. The

theoretical hydridic hydrogen content is 4.53%; the material will average between 98.0 and 99.5% of this value. Except for hydrolysis products, the only significant impurities are sodium (0.2 to 0.5%), chlorine (up to 600 ppm), and potassium (about 100 ppm).

TMAB is soluble in water to the extent of approximately 48 g/100 g water at 20°C. However, the compound hydrolyzes slowly to produce tetramethylammonium pentaborate  $[(\text{CH}_3)_4\text{NB}_5\text{O}_6(\text{OH})_4]$ , trimethylamine, methane, and hydrogen. Below pH 8 conditions are conducive to an increasing rate of hydrolysis.

The compound is a white powder having a real density of 0.843 g/cc and a bulk density of  $\sim 0.46$  g/cc. Solubility in ethanol is  $\sim 0.5$  g/100 g solvent at 25°C, and solubility in dimethyl sulfoxide (DMSO) is 1.87 g per 100 g solvent at 25°C.

## DISCUSSION AND RESULTS

The computer data bases of both the environmental mutagenesis and the toxicology information centers were searched, and no citations were found for TMAB. There were, however, a few citations for related quaternary ammonium compounds, including some limited mutagenesis testing in microbial systems.

### Toxicity Tests

#### Acute Oral LD<sub>50</sub>

The acute toxic oral dose was determined in both 25 g, 8- to 10-week-old nonfasting male C3H/He mice and 300-400 g, 8- to 12-week-old nonfasting male Sprague-Dawley rats. The compound was dissolved in distilled water immediately before treatment and administered in a total volume of 0.1 ml for mice and 0.5 ml for rats. Based on linear least-squares lines fitted to all available data (excluding doses that resulted in 0 mortality) for both species (Fig. 1), the following relationships between dose and response could be expected:

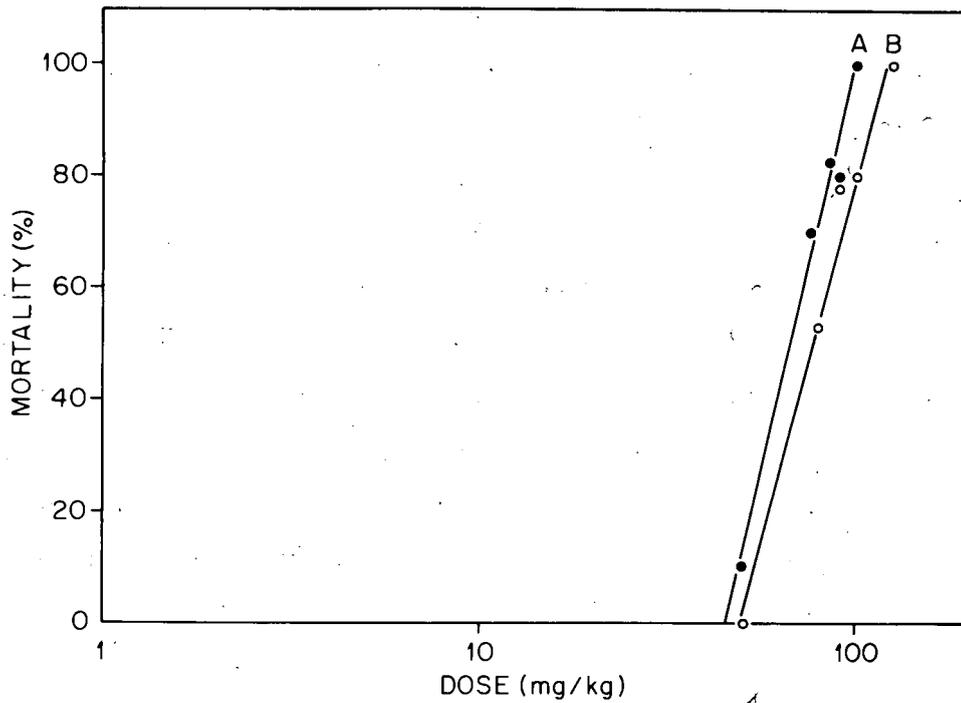


Fig. 1. Linear regression of % mortality on log dose.

in mice, when

<u><math>\bar{y}</math>, Mortality (%)</u>	<u><math>\bar{x}</math>, Dose of TMAB (mg/kg)</u>
0	45
10	49
50	67
100	100

in rats, when

<u><math>\bar{y}</math>, Mortality (%)</u>	<u><math>\bar{x}</math>, Dose of TMAB (mg/kg)</u>
0	44
10	48
50	73
100	122

Slopes were not significantly different for the two species; however, the lateral displacement of the lines appears to be real and suggests that the mouse is

somewhat more sensitive than the rat. The median LD<sub>50</sub> dose [determined by Finney's method (1)] for mice was 67 mg/kg with a standard error of 3 mg/kg, and for rats 77 mg/kg with a standard error of 7 mg/kg.

In both species, signs of toxicity were evident within 15 min. Animals became lethargic and later nonresponsive. Respiration was rapid and shallow with coarse tremors of the extremities, head, and neck. Shortly thereafter death occurred, usually within 1 hr after treatment but occasionally later on the same day of exposure. Animals surviving the acute depressive phase showed no clinical evidence of toxicity the next day. Gross necropsy of both rats and mice revealed severe gaseous distention of the stomach and upper gastrointestinal tract. It is possible that intestinal distention led to circulatory and respiratory collapse and therefore was directly responsible for death.

#### Skin Irritation and Sensitization

Skin irritation and sensitization was evaluated in adult guinea pigs (2). Aqueous solutions (1%) were nonirritating unless the skin was abraded. With abrasion, mild erythema without edema was observed at 24 hr but had disappeared by 48 hr. No intensification of the skin reaction was observed after repeated exposure to 0.5 ml of a 1% aqueous solution with abrasion. At a concentration of 10% and with abrasion, severe erythema and edema were observed that eventually led to skin necrosis and scarring. It was concluded, therefore, that if the material does not penetrate the intact skin there is little skin irritation, but if penetration occurs a severe reaction can result, the extent of which is limited only by concentration and contact time.

#### Percutaneous Toxicity

Adult New Zealand white rabbits were shaved, and 0.5 ml of aqueous solution of TMAB was absorbed onto Band-Aid pads and placed upon the skin of the back. Two areas were exposed simultaneously, on each side of the thorax. One site was abraded and the other left undisturbed. At a 40% (w/v) concentration (200 mg),

three of three rabbits were dead within 12 hr of exposure. Two more rabbits were exposed to a 20% solution (100 mg); 6 hr later one rabbit appeared comatose and shortly thereafter, died. The other rabbit exhibited decreased activity and awareness but eventually recovered. Specific gross or microscopic lesions were not observed. The heart was pale and in systole; the area of skin in contact with TMAB in the surviving rabbit was necrotic and had a primary irritation index of 4 based on erythema and edema at 24 and 72 hr. It was concluded that in aqueous solution TMAB is potentially toxic via direct skin absorption if contact is prolonged.

### Eye Irritation

Eye irritation tests were conducted according to standard protocols and were supervised and scored by a consulting clinical ophthalmologist (3). Rabbits were restrained without anesthesia for administration of the compound and examination of the eye with a slit lamp. The dry chemical (0.1 ml; ~24 mg) was placed into the lower conjunctival sac. Lachrymation occurred immediately. Froth was observed, indicating that the compound had decomposed. At 24 hr after exposure there was moderate injection of the bulbar and palpebral conjunctiva with slight edema and focal hemorrhage (Grade 1). The cornea exhibited multiple superficial erosions but was translucent (Grade 1). The iris appeared normal (Grade 0). By 48 hr the initial acute reaction had subsided but superficial erosion was still evident. After 72 hr the acute conjunctival reaction had completely resolved; superficial corneal erosions were present but gave no indication of intensification. The eyes were examined 2 weeks later. There was still evidence of superficial corneal erosion but some indication of healing. It was concluded that the corneal damage would heal without complication.

### Pulmonary Toxicity

Attempts were made to estimate the toxicity of the material via the respiratory route by direct intratracheal or intranasal instillation of aqueous solutions. These efforts were frustrated by the tendency of the animal to cough up and swallow the

material, which led to death following gastrointestinal distention. Further attempts to evaluate toxicity via the respiratory route were also unsatisfactory. Aqueous solutions of the compound decomposed with sufficient speed that generation of an aerosol was impractical. Nor was it possible to make an aerosol of the dry powder due to a tendency of the material to agglomerate.

To obtain an estimate of the pulmonary toxicity of TMAB, fasting 500-g male rats were given TMAB by direct intratracheal instillation. To prevent hydrolysis the TMAB was dissolved in DMSO at a concentration of 18 mg/ml. Under these conditions the direct intratracheal LD<sub>50</sub> was estimated to be 31 mg/kg.

### Mutagenicity Testing

#### Methodology

##### Indicator Strains

The Salmonella strains used in the various assays are listed below. All strains were obtained through the courtesy of Dr. Bruce Ames, Berkeley, California.

##### Salmonella typhimurium Strains

TA1535 hisG46, uvrB, rfa (missense)

TA1537 hisC3076, uvrB, rfa (frameshift)

TA98 hisD3052, uvrB, rfa (frameshift plus R factor)

TA100 hisG46, uvrB, rfa (missense plus R factor)

#### Methods

Microbial protocol. In the screening of test material, the four strains (TA1535, TA1537, TA98, and TA100) were employed. Standard experimental procedures have been given by Ames et al. (4). Briefly, the strain to be treated with the potential mutagen(s) is added to soft agar containing a low level of histidine and biotin along with varying amounts of the test substance. The suspension containing approximately  $2 \times 10^8$  bacteria is overlaid on minimal agar plates. The bacteria undergo several divisions with the reduced level of histidine, thus forming

a light lawn of background growth on the plate and allowing the mutagen to act. Revertants to the wild-type state appear as obvious large colonies on the plate. The assay can be quantitated with respect to dose (added amount) of mutagen and modified to include "on-the-plate" treatment with the liver homogenate required to metabolically activate many compounds.

Material and/or control compounds to be tested were suspended in DMSO (supplied sterile, spectrophotometric grade from Schwarz/Mann) to concentrations in the range of 10–20 mg solids. The potential mutagen was assayed for general toxicity (bacterial survival) with strain TA1537. Generally, the fraction was tested with the plate assay over at least a 1000-fold concentration range with the tester strains. Revertant colonies were counted after a 48 hr incubation. Data were recorded and plotted versus added concentration, and the approximate slope of the induction curve was determined. It is assumed that the slope of the linear dose-response range reflects the mutagenic activity. Positive or questionable results were clarified for a narrower range of concentrations. All studies were carried out with parallel series of plates plus and minus the liver enzyme preparation for metabolic activation. The background lawn of bacterial growth was routinely examined so that any effects attributed to massive cell death and subsequent growth of the few surviving bacteria (availability of more histidine) could be differentiated from mutation induction. Routine controls demonstrating the sterility of samples, enzyme or S-9 preparations, and reagents were included, as were positive controls with known mutagens to recheck strain response and enzyme preparations.

Leukocyte protocol. Chromosomal preparations of leukocytes cultured from human peripheral blood (5) were used to score induced aberrations and were compared with untreated controls. Blood was drawn with 2% heparin (1000 units/ml) in a sterile syringe and transferred to a sterile centrifuge tube. After centrifugation at 1000 rpm for 10 min, the buffy coat was drawn off and inoculated into RPMI medium containing 27% human serum and 3% phytohemagglutinin at pH 7.0. The culture was incubated at 37°C until mutagen treatment. After treatment the cells were rinsed twice with Hanks' balanced salt solution, and new medium was added.

Chromosome preparations were made in the usual manner. The cells were collected after a 52- to 54-hr incubation, including a pretreatment of  $2 \times 10^{-7}$  M colchicine for 2.5 hr. They were centrifuged for 5 min., the medium was decanted, and the cells were resuspended with 4 ml of Hanks' solution. The resuspended cells were centrifuged for 5 min, and the Hanks' solution was decanted down to a total volume of 1 ml. The cells were resuspended with 3 ml of warm water (37°C) and incubated at 37°C for 15 min with constant agitation. The suspension was again centrifuged, and the supernatant was removed. The cells were fixed with one part glacial acetic acid and three parts methyl alcohol for 20 min with two changes of fixative. Air-dried preparations were made and stained with 2.5% Giemsa-water solution, mounted with Euparal, and covered.

### Results

Table 1 summarizes the relative responses of the Salmonella strains after treatment with the test material. The preliminary finding with the Salmonella histidine reversion (Ames) assay is that TMAB is negative. Five separate examinations were carried out. Attention was paid to the possible aqueous instability of the compound, and fresh solutions were added for comparisons with solutions prepared a few hours before bacterial treatment. A slight increase (10-20 colonies) over background with strain TA98 was occasionally observed but was not found to be reproducible. No other strain showed an increase. The TMAB was toxic in the assay beyond the 250- $\mu$ g level of addition.

Table 2 gives results for positive controls.

TMAB is also negative in the human leukocyte chromatid aberration assay over the range of  $10^{-3}$ - $10^{-5}$  M.

Table 1. Salmonella histidine reversion (Ames) test with TMAB — Summary

Added concentration (µg)	Metabolic activation <sup>a</sup>	Response over background for strain			
		TA1535	TA1537	TA98	TA100
0	(+)	— <sup>b</sup>	—	—	—
	(-)	—	—	—	—
1	(+)	—	—	—	—
	(-)	—	—	—	—
5	(+)	—	—	—	—
	(-)	—	—	—	—
10	(+)	—	—	—	—
	(-)	—	—	—	—
25	(+)	—	—	—	—
	(-)	—	—	—	—
50	(+)	—	—	—	—
	(-)	—	—	—	—
100	(+)	—	—	—	—
	(-)	—	—	—	—
250	(+)	—	—	—	—
	(-)	—	—	—	—

<sup>a</sup> Assays without (-) and with (+) metabolic activation with an enzyme preparation from rat livers (uninduced, induced with 0.1% sodium phenobarbital, or induced with Aroclor 1254).

<sup>b</sup> — signifies no significant response over background or negative control level (see Table 2);  $2 \times 10^8$  bacteria per plate with 5 experimental conditions: (1) aqueous "solution," (2) DMSO solution, (3) aqueous "solution" at 1 hr, (4) DMSO solution at 1 hr, and (5) DMSO solution, immediate treatment.

Table 2. Salmonella histidine reversion (Ames) test— Positive controls

Compound	Dose ( $\mu$ g)	Revertants per plate			
		TA1535	TA1537	TA98	TA100
8-Aminoquinoline	20 <sup>a</sup>	22	1614	132	424
4-Nitroquinoline-N-oxide	1	120	35	550	720
Ethyl methanesulfonate	0.5 <sup>b</sup>	10,000	6	37	250
Benzo(a)pyrene	15 <sup>c</sup>	22	293	763	420
Acetylaminofluorene	2.4 <sup>a</sup>	13	64	2000	810
None	0	17	9	38	235

<sup>a</sup> Activated with phenobarbital-induced rat liver preparation.

<sup>b</sup> Measured as  $\mu$ l.

<sup>c</sup> Activated with Aroclor 1254 rat liver preparation.

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## APPENDIX. Toxicity of Some Related Compounds

The 1975 Edition of the Registry of Toxic Effects of Chemical Substances, PB-246 557 (National Institute for Occupational Safety and Health) provides some information on the toxicity of compounds related to TMAB.

Tetramethylammonium chloride. The sequence number in this listing is BS 77000; the Chemical Abstracts Registry No. is 000075570. Toxic dose data given are as follows:

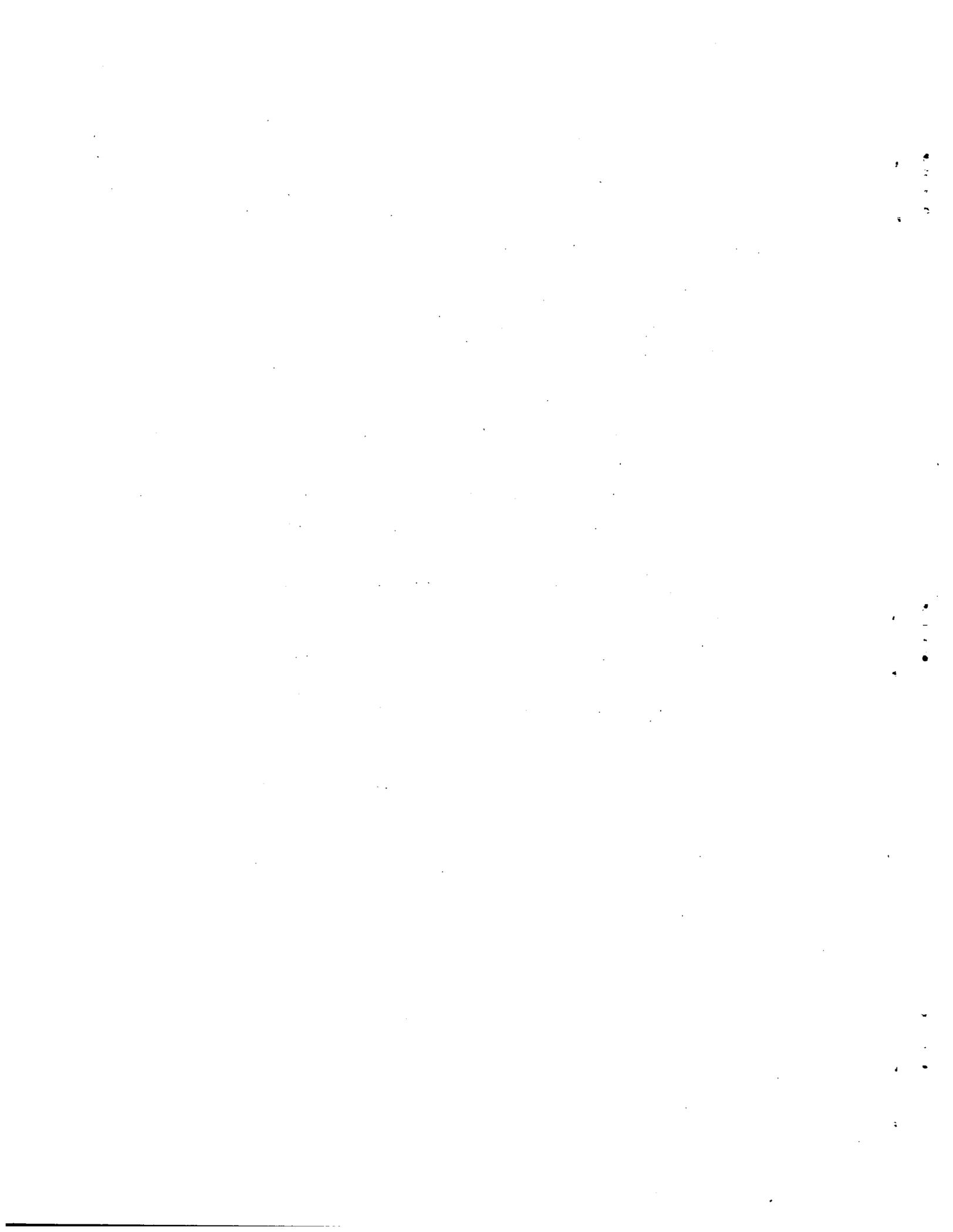
Subcutaneous — mouse	LD <sub>50</sub> : 40 mg/kg
Intraperitoneal — mouse	LD <sub>50</sub> : 25 mg/kg
Unreported — mouse	LD <sub>Lo</sub> : 20 mg/kg
Subcutaneous — rabbit	LD <sub>Lo</sub> : 6 mg/kg
Unreported — guinea pig	LD <sub>Lo</sub> : 20 mg/kg

Tetramethylammonium hydroxide (Sequence No. PA 08750, CAS No. 000075592):

Subcutaneous — mouse	LD <sub>Lo</sub> : 20 mg/kg
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Trimethylamine compounded with borane [(CH<sub>3</sub>)<sub>3</sub>N·BH<sub>3</sub>] (Sequence No. PA 05250, CAS No. 000075229):

Intraperitoneal — rat	LD <sub>Lo</sub> : 970 mg/kg
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