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MUTAGENICITY OF SOME MUNITION WASTEWATER CHEMICALS AND CHLORINE TEST KIT REAGENTS

Final Report

VINCENT F. SIMMON, RONALD J. SPANGGORD,
SHARON ECKFORD, and VERNON McCLURG

May 1977

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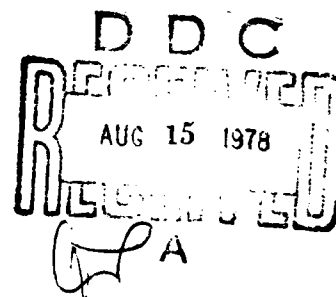
Dr. William F. Cowen, Project Officer
U.S. Army Medical Bioengineering
Research and Development Laboratory
Fort Detrick, Frederick, Maryland 21701

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)		
In this study, 20 compounds including munition wastewater chemicals, photolyzed wastewaters, and chlorine test kit reagents were evaluated for mutagenic activity at aqueous solubility levels before and after application of ozone or chlorine disinfection techniques.		

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assemble, and pack (LAP) wastewater: 7-50 LAP, 7-100 LAP, 9-100 LAP; condensate water, N,N-diethyl-p-phenylenediamine oxalate (DPO), N,N-dimethyl-p-phenylenediamine sulfate (DPS), syringaldazine, mutagenicity assays, Salmonella typhimurium, Saccharomyces cerevisiae.

20 ABSTRACT (Continued)

Before the application of disinfection techniques, 1,3,5-trinitrobenzene, pH7-50% photolyzed LAP (Load, Assemble, and Pack plant wastewater), pH7-100% photolyzed LAP, pH9-100% photolyzed LAP, 2,4,6-trinitrobenzonitrile, 2,4,6-trinitrobenzaldehyde, N,N-diethyl-p-phenylenediamine oxalate, and N,N-dimethyl-p-phenylenediamine sulfate were found to have mutagenic activity at their aqueous solubility concentrations. 1,3-Dinitrobenzene, 2,6-dinitrotoluene, 2,4-dinitrotoluene, 3,5-dinitrotoluene, trinitroglycerine, 2,4,6-trinitrotoluene, RDX, condensate water from TNT manufacture, syringaldazine, 2,4,6-trinitroresorcinol, PETN, and HMX did not show mutagenicity before chlorination or ozonation.

Under the chlorination conditions used, only five compounds (2,4,6-trinitroresorcinol, N,N-diethyl-p-phenylenediamine oxalate, N,N-dimethyl-p-phenylenediamine sulfate, syringaldazine, and 2,4,6-trinitrobenzaldehyde) underwent significant reaction. 2,4,6-Trinitroresorcinol and syringaldazine remained nonmutagenic after chlorination. The mutagenic activity of N,N-diethyl-p-phenylenediamine oxalate, N,N-dimethyl-p-phenylenediamine sulfate, and 2,4,6-trinitrobenzaldehyde increased slightly after chlorination.

Under the ozonation conditions, seven compounds (1,3,5-trinitrobenzene, 2,4,6-trinitrotoluene, 2,6-dinitrotoluene, 1,3-dinitrobenzene, 2,4,6-trinitrobenzaldehyde, 2,4,6-trinitroresorcinol, and 7-50 LAP) underwent significant reaction. Of these, only 1,3,5-trinitrobenzene, 7-50 LAP, and 2,4,6-trinitrobenzaldehyde were mutagenic before ozonation. 2,4,6-Trinitrobenzaldehyde was the only compound that appeared to have a slight increase in mutagenic activity as the result of ozonation. We did not observe any cases in which nonmutagenic compounds were converted into mutagens by either of the disinfection treatments. However, the concentrations tested were very low (upper limit was water solubility) and the possibility of false negative results should be considered.

None of the chemicals tested was mutagenic in assays with S. cerevisiae D3 under the assay conditions used in these experiments. It is possible that these compounds

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gave a negative response because they are not strongly mutagenic in this procedure, particularly in view of the relatively low concentrations of test compounds. Alternatively, these compounds may not be mutagens in S. cerevisiae D3 mitotic recombination assay. We conclude that this indicator microorganism is not useful in evaluating the mutagenicity of these compounds.

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EXECUTIVE SUMMARY

In this study, 20 compounds including munition wastewater chemicals, photolyzed wastewaters, and chlorine test kit reagents were evaluated for mutagenic activity at aqueous solubility levels before and after application of ozone or chlorine disinfection techniques.

Before the application of disinfection techniques, 1,3,5-trinitrobenzene, pH7-50% photolyzed LAP (Load, Assemble, and Pack plant wastewater), pH7-100% photolyzed LAP, pH9-100% photolyzed LAP, 2,4,6-trinitrobenzonitrile, 2,4,6-trinitrobenzaldehyde, N,N-diethyl-p-phenylenediamine oxalate, and N,N-dimethyl-p-phenylenediamine sulfate were found to have mutagenic activity at their aqueous solubility concentrations. 1,3-Dinitrobenzene, 2,6-dinitrotoluene, 2,4-dinitrotoluene, 3,5-dinitrotoluene, trinitroglycerine, 2,4,6-trinitrotoluene, RDX, condensate water from TNT manufacture, syringaldazine, 2,4,6-trinitroresorcinol, PETN, and HMX did not show mutagenicity before chlorination or ozonation.

Under the chlorination conditions used, only five compounds (2,4,6-trinitroresorcinol, N,N-diethyl-p-phenylenediamine oxalate, N,N-dimethyl-p-phenylenediamine sulfate, syringaldazine, and 2,4,6-trinitrobenzaldehyde) underwent significant reaction. 2,4,6-Trinitroresorcinol and syringaldazine remained nonmutagenic after chlorination. The mutagenic activity of N,N-diethyl-p-phenylenediamine oxalate, N,N-dimethyl-p-phenylenediamine sulfate, and 2,4,6-trinitrobenzaldehyde increased slightly after chlorination.

Under the ozonation conditions, seven compounds (1,3,5-trinitrobenzene, 2,4,6-trinitrotoluene, 2,6-dinitrotoluene, 1,3-dinitrobenzene, 2,4,6-trinitrobenzaldehyde, 2,4,6-trinitroresorcinol, and 7-50 LAP) underwent significant reaction. Of these, only 2,4,6-trinitrobenzene, 7-50 LAP, and 2,4,6-trinitrobenzaldehyde were mutagenic before ozonation. 2,4,6-Trinitrobenzaldehyde was the only compound that appeared to have a slight increase in mutagenic activity as the result of ozonation.

We did not observe any cases in which nonmutagenic compounds were converted into mutagens by either of the disinfection treatments. However, the concentrations tested were very low (upper limit was water solubility) and the possibility of false negative results should be considered.

None of the chemicals tested was mutagenic in assays with S. cerevisiae D3 under the assay conditions used in these experiments. It is possible that these compounds gave a negative response because they are not strongly mutagenic in this procedure, particularly in view of the relatively low concentrations of test compounds. Alternatively, these compounds may not be mutagens in S. cerevisiae D3 mitotic recombination assay. We conclude that this indicator microorganism is not useful in evaluating the mutagenicity of these compounds.

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INTRODUCTION

The use of chlorine in water purification as a disinfectant and oxidant has come under critical review in light of the findings of low levels of chlorinated organics in drinking water.¹ These newly formed organics may be detrimental to both man and the environment and should be investigated relative to their biological activity and potential health hazard.

The potential for formation of hazardous products is not unique to chlorine treatment of water since the potential exists with any treatment in which chemical transformation of organics is likely to occur. Therefore, purification methods in which ozone, chlorine dioxide, and halogens are used are subject to the same problems that are encountered with the use of chlorine as a disinfectant.

In this study, we evaluated the effects of ozonation or chlorination of 13 military-unique wastewater chemicals, 4 wastewater mixtures, and 3 reagents for chlorine test kits. The military chemicals tested were 1,3,5-trinitrobenzene, 1,3-dinitrobenzene, trinitroglycerine, 2,4,6-trinitrobenzaldehyde, pentaerythritol tetranitrate (PETN), 2,4,6-trinitrobenzonitrile, 1,3,5-trinitrohexahydro-1,3,5-triazine (RDX), 1,3,5,7-tetranitrooctahydro-1,3,5,7-tetrazocine (HMX), 2,6-dinitrotoluene, 2,4-dinitrotoluene, 3,5-dinitrotoluene, 2,4,6-trinitrotoluene (TNT), and 2,4,6-trinitroresorcinol.

The wastewater tested was load, assemble, and pack (LAP) wastewater obtained from Joliet Army Ammunition Plant (JAAP). This wastewater was adjusted to pH 7 and photolytically degraded (through Pyrex filters) in a laboratory reactor (Figure 1) to reduce the TNT concentration to 50% (7-50 LAP) and 100% (7-100 LAP) of the initial value. Another sample was adjusted to pH 9, and the TNT was 100% photolytically decomposed (9-100 LAP). Condensate water, which arises from the evaporative treatment of Sellite process waters at TNT production facilities, was obtained from JAAP and tested directly.

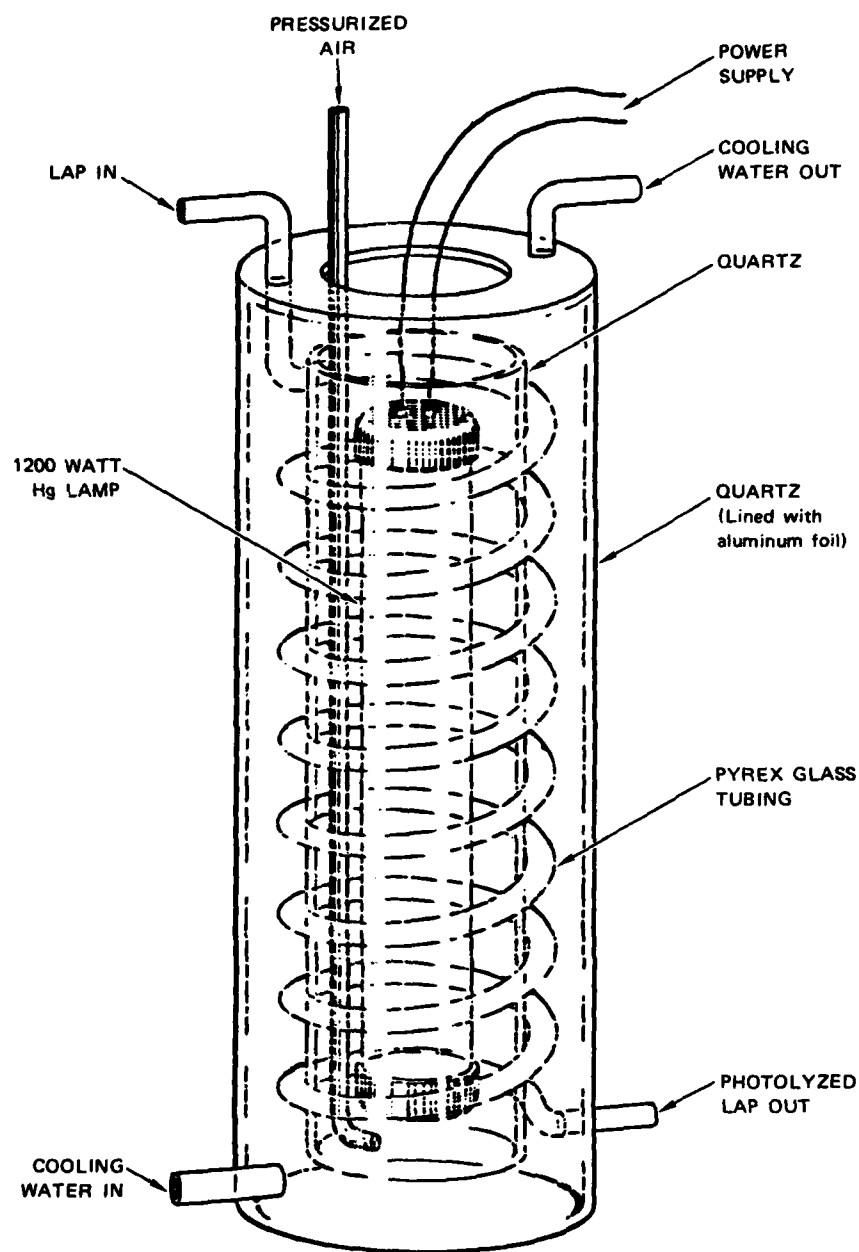


FIGURE 1 DIAGRAM OF FLOW-THROUGH PHOTOLYTIC REACTOR

The chlorine test reagent chemicals tested were N,N-diethyl-p-phenylenediamine oxalate (DPO) and syringaldazine. N,N-Dimethyl-p-phenylenediamine sulfate (DPS) was also supplied. It differs from DPO in counter-ion and has N-methyl groups instead of N-ethyl groups, but it is not used in chlorine test kits; it was tested for comparative results. DPO is the same chemical as DPD (diethylphenylenediamine), DPD being the more common abbreviation for this reagent, which is used for chlorine determinations. Syringaldazine is being considered as a substitute for DPD² in chlorine determinations.

One possible way of evaluating the safety of a particular water purification technique is through the use of rapid microbial bioassays, which serve as a prescreening method for assessing the mutagenic activity of organics resulting from the purification technique. The biological assays used to determine whether ozonation or chlorination produced biologically active (i.e., mutagenic) products were the Ames Salmonella/microsome assay (reversion to histidine independence in five strains of Salmonella typhimurium) and mitotic recombination in the yeast Saccharomyces cerevisiae D3. A rat-liver postmitochondrial supernatant fraction was incorporated into the assay procedure to provide mammalian metabolic pathways.

The objective of this project was to evaluate the mutagenic activity of compounds produced by the reaction of chlorine or ozone with selected military-unique compounds. This effort is to assess potential health hazards resulting from discharges of pollutants into surface waters where ozone or chlorine may be used for water treatment before distribution from a water municipality.

To achieve this objective, the test chemicals were screened for mutagenic activity before ozone or chlorine treatment in water and then were evaluated for their reactivity with each reagent. The treated test chemical solutions were evaluated for enhanced mutagenicity relative to untreated test chemical solutions. Products formed were chemically evaluated to identify the products that might be responsible for increased mutagenic activity.

Preliminary results of this research were presented to the Society of Toxicology at its annual meeting in March, 1977. The abstract of this presentation is presented in Appendix C.

METHODS

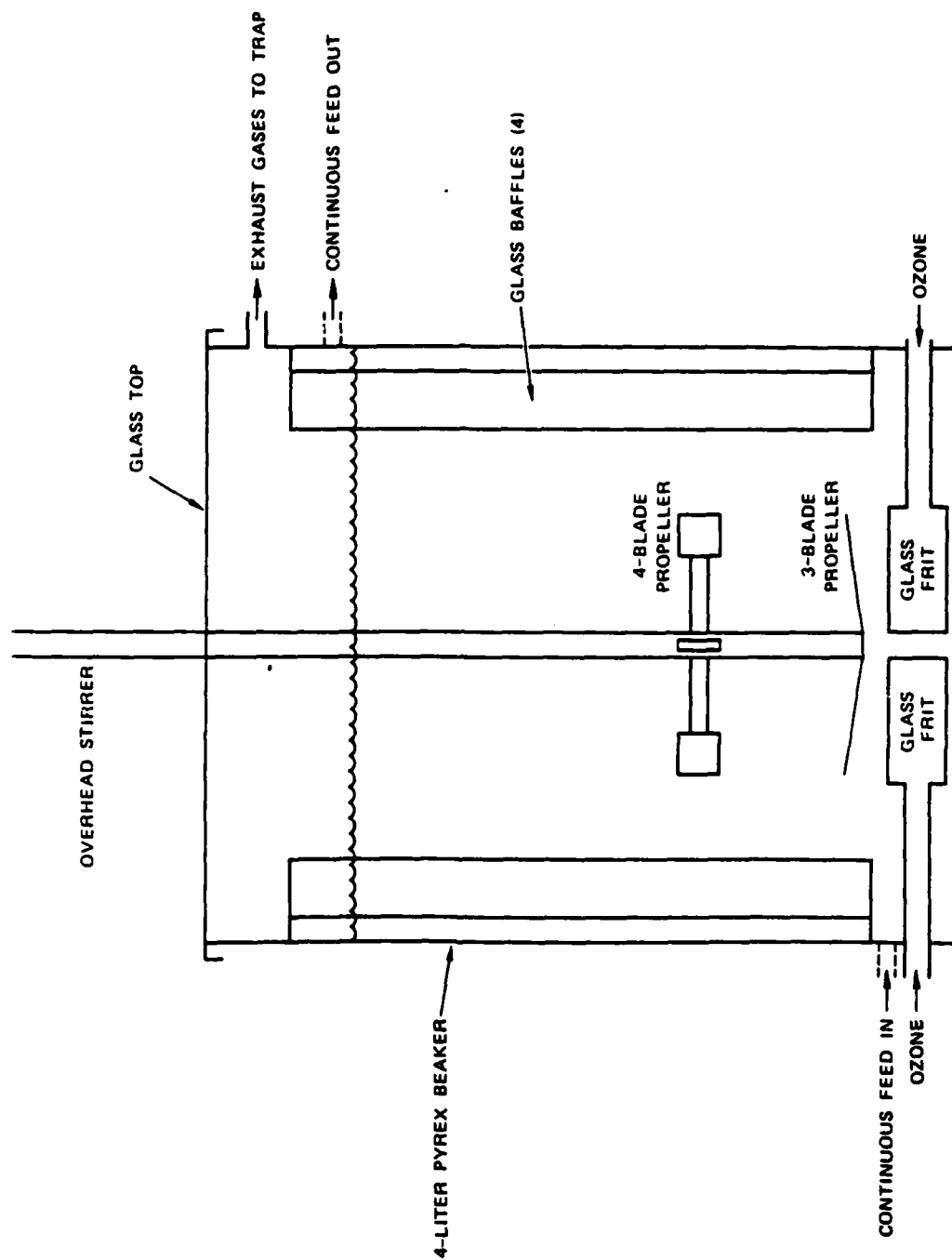
Chlorination

Solutions of hypochlorous acid (HOCl) were prepared by acidification of calcium hypochlorite (Fisher Chemical Co.) with hydrochloric acid in deionized water and buffered to pH 7 with commercial phosphate buffer (Micro Essential Laboratory). Initial stock chlorine concentrations were determined by standard iodometric titration.³ Final chlorine concentrations after reaction with the test chemical were adjusted to 0.1 to 0.2 mg/l with sodium thiosulfate and determined by the syringaldazine method⁴ before mutagenic screening.

Chlorinations were performed in Pyrex Erlenmeyer flasks that had been stoppered and covered with aluminum foil. Each solution was stirred with the aid of a Teflon-coated magnetic stir-bar throughout the contact time, which ranged from 30 minutes to more than 48 hour.

Ozonation

Ozonations were performed in an all-Pyrex glass reactor, pictured in Figure 2, equipped with an overhead stainless-steel stirrer. Ozone, generated from a Welsback Model T-408 ozone generator, was bubbled through the reactor so that 4 mg/l of ozone was introduced into the system. Aqueous solutions of the test compound in deionized water were buffered with phosphate at pH 7, and ozone contact times of 10 minutes were used. Ozone concentrations were monitored by standard iodometric titrations.³



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FIGURE 2 SCHEMATIC DIAGRAM OF OZONATION REACTION VESSEL

Microbiological Mutagenicity Assays

Salmonella typhimurium Strains TA1535, TA1537, TA1538, TA98, and TA100

The Salmonella typhimurium strains used at SRI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on a minimal media petri plate containing a trace of histidine, only those cells that revert to histidine independence (his⁺) are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; in many cases, this growth is essential for mutagenesis to occur. The his⁺ revertants are easily scored as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar the mutation frequency is increased 2- to 100-fold.

We obtained our S. typhimurium strains from Dr. Bruce Ames of the University of California at Berkeley.³⁻⁹ In addition to having mutations in the histidine operon, all the indicator strains have a mutation (rfa⁻) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of vitamin biotin (bio⁻) and in the repair of ultraviolet (uv)-induced DNA damage (uvrB⁻). The rfa⁻ mutation makes the strains more permeable to many large aromatic molecules, thereby increasing the mutagenic effect of these molecules. The uvrB⁻ mutation decreases repair of some types of chemically or physically damaged DNA and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to his⁺ by many mutagens that cause base-pair substitutions. TA100 is derived from TA1535 by the introduction of the resistance transfer factor plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations

for a given dose of most mutagens.⁹ In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of the plasmid in the cells. We have shown that TA100 can detect mutagens, such as benzyl chloride and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF2), that are not detected by strain TA1535. The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens (e.g., ICR-191, benzo(a)pyrene, aflatoxin B₁, and 7,12-dimethylbenz(a)anthracene). Strains TA1537 and TA1538 are reverted by many frameshift mutagens. TA1537 is more sensitive than TA1538 to mutation by some acridines and benzanthraces, but the difference is quantitative rather than qualitative. Strain TA98 is derived from TA1538 by the addition of the plasmid pKM101, which makes it more sensitive to some mutagenic agents.

All the indicator strains are routinely checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of the plasmid. Cultures are then stored in 10% sterile glycerol at -80°C. For each experiment, an inoculum from the stock cultures is grown overnight at 37° C in nutrient broth (Oxoid, CM67). After stationary overnight growth, the cultures are shaken for 3 to 4 hours to ensure optimal growth.

Aroclor 1254-Stimulated Metabolic Activation System

Some carcinogenic chemicals, either of the aromatic amino type or polycyclic hydrocarbon type, are inactive unless they are metabolized to active forms. In animals and man, an enzyme system in the liver or other organs (e.g., lung or kidney) is capable of metabolizing a large number of these chemicals to carcinogens.^{8,10-12} Some of these intermediate metabolites are very potent mutagens in the S. typhimurium test. Ames has described the liver metabolic activation system that we use.¹⁰ In brief, adult male rats (250 to 300 g) are given a single 500-mg/kg intraperitoneal injection of a polychlorinated biphenyl, Aroclor 1254. This treatment enhances the synthesis of enzymes involved in the metabolic conversion of chemicals. Four days after the injection, the animals' food is removed but drinking water is provided ad libitum. On the fifth day, the rats are killed and the liver homogenate is prepared as follows.

The livers are removed aseptically and placed in a preweighed sterile glass beaker. The organ weight is determined, and all subsequent operations are conducted in an ice bath. The livers are washed in an equal volume of cold, sterile 0.15 M KCl (1 ml/g of wet organ), minced with sterile surgical scissors in three volumes of 0.15 M KCl, and homogenized with a Potter-Elvehjem apparatus. The homogenate is centrifuged for 10 minutes at 9000 x g, and the supernatant, referred to as the S-9 fraction, is quickly frozen in dry ice and stored at -80° C.

The metabolic activation mixture for each experiment consists of, for 10 ml:

- 1.00 ml of S-9 fraction
- 0.20 ml of $MgCl_2$ (0.4 M) and KCl (1.65 M)
- 0.05 ml of glucose-6-phosphate (1 M)
- 0.40 ml of NADP (0.1 M)
- 5.00 ml of sodium phosphate (0.2 M, pH 7.4)
- 3.35 ml of H_2O .

Assays in Agar

To a sterile 13 x 100 mm test tube placed in a 43° C heating block, we add in the following order:

- (1) 2.00 ml of 0.6% agar*
- (2) 0.05 ml of indicator organisms
- (3) 0.50 ml of metabolic activation mixture (optional)
- (4) Up to 0.25 ml of a solution of the test chemical.

For negative controls, we use steps (1), (2), and (3) (optional). For positive controls, we test each culture by specific mutagens known to revert each strain, using steps (1), (2), (3) (optional), and (4).

* 0.6% agar contains 0.05 mM histidine and 0.05 mM biotin.

This mixture is stirred gently and then poured onto minimal agar plates.* After the top agar has set, the plates are incubated at 37° C for 2 days. The number of his⁺ revertant colonies is counted and recorded.

Saccharomyces cerevisiae D3

The yeast S. cerevisiae D3 is a diploid microorganism heterozygous for a mutation leading to a defective enzyme in the adenine-metabolizing pathway.¹³ When grown on a medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygotes by mitotic recombination. The frequency of this recombinational event may be increased by incubating the organisms with various mutagens. The degree of mutagenicity of a compound or of its metabolite is determined from the number of red-pigmented colonies appearing on the plates.¹⁴

The S. cerevisiae tester strain is stored at -80° C. For each experiment, the tester strain is inoculated in 1% tryptone and 0.5% yeast extract and grown overnight at 37° C with aeration.

The in vitro yeast mitotic recombination assay in suspension is conducted as follows. The overnight culture is centrifuged, and the cells are resuspended at a concentration of 10⁸ cells/ml in a .067 mM phosphate buffer (pH 7.4). To a sterile test tube are added:

- 1.0 ml of the organisms
- 0.5 ml of either the metabolic activation mixture or buffer
- 1.0 ml of the test chemical.

Several doses of the chemical (up to 5%, w/v or v/v, or maximum water solubility) are tested in each experiment, and appropriate controls are included.

* Minimal agar plates consist of, per liter, 15 g of agar, 50 g of glucose, 0.2 g of MgSO₄•7H₂O, 2 g of citric acid monohydrate, 10 g of K₂HPO₄, and 3.5 g of NaH₂PO₄•4H₂O.

The suspension mixture is incubated at 30° C for 4 hours on a roller drum. The sample is diluted serially in sterile physiological saline, and a volume of 0.2 ml of the 10^{-5} and 10^{-3} dilutions is spread on tryptone-yeast agar plates; five plates are used for the 10^{-3} dilution and three plates are used for the 10^{-5} dilution. The plates are incubated for 2 days at 30° C, followed by 2 days at 4° C to enhance the development of the red pigment indicative of adenine-deficient homozygosity. Plates of the 10^{-3} dilution are scanned with a dissecting microscope at 10 X magnification, and the number of red colonies or red sectors (mitotic recombinants) is recorded. The surviving fraction of organisms is determined from the number of colonies appearing on the plates of the 10^{-5} dilution.

The number of mitotic recombinants is calculated per 10^5 survivors. A positive response in this assay is indicated by a dose-related increase of more than threefold in the absolute number of mitotic recombinants per milliliter as well as in the relative number of mitotic recombinants per 10^5 survivors.

RESULTS AND DISCUSSION

Chlorination

The results for the chemicals subjected to chlorine water-treatment facility conditions (10 ppm chlorine, pH 7, 0.5 hr contact time) are given in Table 1. The only compounds that were found to undergo significant reaction were 2,4,6-trinitroresorcinol, DPO, DPS, syringaldazine, and 2,4,6-trinitrobenzaldehyde.

The recalcitrant nature of the majority of the compounds studied under these chlorination conditions is not surprising in light of the known pathways of chlorine transformation of organics, including substitution, addition, and oxidation reactions.¹⁵ Photochemical processes were eliminated by performing the reactions in flasks covered with aluminum foil.

Of the above pathways, oxidative transformation appeared to be the most probable mode of decomposition for the munition compounds, and attempts were made to increase rates of transformation by varying chlorine concentrations, pH, temperature, and contact time. It was hoped that stressed conditions would produce significant concentration levels of degradation products for mutagenic screening.

Using TNT as a prototype compound to study reaction conditions, a 100-ppm solution of TNT at pH 7 was allowed to stir with 1000 ppm of hypochlorous acid. Samples were analyzed for TNT at intervals of 1, 2, and 24 hours, and essentially all the starting material was recovered. In a similar study performed at pH of 2, 4, 7, and 8 (variable hypochlorous acid concentrations), no TNT decomposition was noted over a 24-hour period. Finally, temperatures were varied, and TNT decomposition occurred at 75° C and above.

Table 1

REACTIVITY OF TEST MATERIALS SUBJECTED TO 10 ppm
CHLORINE FOR 30 MINUTES AT pH 7 (Buffered with
phosphate)

Compound	Initial Concentration (ppm)	Percentage Reacted
3,5-Dinitrotoluene	127	0
2,4,6-Trinitroresorcinol	518	4.8
2,4,6-Trinitrotoluene	139	0
2,4-Dinitrotoluene	212	0
Condensate water	67*	0
N,N-Diethyl-p-phenylenediamine oxalate	226	20
N,N-Dimethyl-p-phenylenediamine sulfate	139	27
Syringaldazine	122	34
7-50 LAP	17†	0
RDX	47	0
HMX	1	0
2,6-Dinitrotoluene	179	0
Pentaerythritol tetranitrate	1	0
1,3,5-Trinitrobenzene	117	0
2,4,6-Trinitrobenzaldehyde	59	25
Trinitroglycerine	800	0
1,3-Dinitrotoluene	126	0
1,3-Dinitrobenzene	126	0

* Based on 2,4-dinitrotoluene concentration.

† Based on 2,4,6-trinitrotoluene concentration.

‡ -- No parameter used to measure reactivity.

A second series of experiments was designed to detect mutagenicity under stressed chlorination conditions. The pH of a 1000-ppm solution of chlorine was adjusted to 4 with hydrochloric acid (no buffer), and the solution was allowed to react with the munition compound for 2 hours at 70 to 80° after standing at room temperature for 48 hours. Under these conditions, slight reactions of the munition compounds were observed. These data are summarized in Table 2. No reaction products were observed in either high-pressure liquid chromatographic (hplc) or gas chromatographic (gc) profiles of these solutions.

Ozonation

The results for chemicals subjected to ozone treatment are presented in Table 3. The nitroaromatics react readily with ozone in aqueous solution. The "Percentage Reacted" in Table 3 should not be construed as an order of reactivity since in each case ozone is the limiting reagent and it is possible that intermediate products (such as nitrobenzaldehydes from the nitrotoluenes) consume ozone at a faster rate than does the parent compound. To better understand reactivity, the extent of reaction was calculated by first computing the maximum percentage of reaction (defined as the moles of ozone divided by the moles of substrate) and dividing this value into the observed "Percentage Reacted" as shown below:

Moles ozone/moles substrate = maximum % of reaction

$$\frac{\% \text{ reacted}}{\text{max \% of reaction}} = \text{extent of reaction.}$$

The extent of reaction values can be used to predict reactivity between structurally related compounds (such as related nitrotoluenes and related nitrobenzenes) and indicate autocatalytic effects or the participation of oxygen in the oxidation reactions (2,4,6-trinitroresorcinol).

Several compounds yielded products that were identified by gas chromatography/mass spectroscopy (gc/ms). From TNT, trinitrobenzene was obtained; this suggests alkyl oxidation rather than electrophilic substitution on the deactivated aromatic ring (Eq. 1). Alkyl oxidation

Table 2

AQUEOUS CHLORINATIONS OF SELECTED TEST MATERIALS: 1000 ppm Cl₂ AT VARIOUS TIME INTERVALS

Compound	Initial Concentration (ppm)	Contact Time and Temperature	Percentage Reacted	pH	
				Initial	Final
3,5-Dinitrotoluene	72	2 hr, 70°	2	4.0	4.6
2,4,6-Trinitrotoluene	106	2 hr, 70°	3	4.0	5.3
2,4-Dinitrotoluene	138	2 hr, 70°	5	4.0	5.3
1,3-Dinitrobenzene	251	2 hr, 70°	0	4.0	3.5
RDX	20	2 hr, 70°	0	4.0	3.6
7-50 LAP	21.3*	2 hr, 70°	33	4.0	3.0
Condensate water	51	2 hr, 70°	12	4.0	3.3
HMX	3	2 hr, 70°	8	4.0	4.1
2,4,6-Trinitroresorcinol	160	2 hr, rt	91	4.0	3.0
N,N-Diethyl-p-phenylenediamine oxalate	4377	2 hr, 70°	100	4.0	2.2
N,N-Dimethyl-p-phenylenediamine sulfate	2195	2 hr, 70°	100	4.0	2.3

* Based on 2,4,6-trinitrotoluene concentration.

Table 3

REACTIVITY OF MUNITION MATERIALS WHEN SUBJECTED TO
4 mg/l OZONE FOR 30 MINUTES AT BUFFERED pH 7

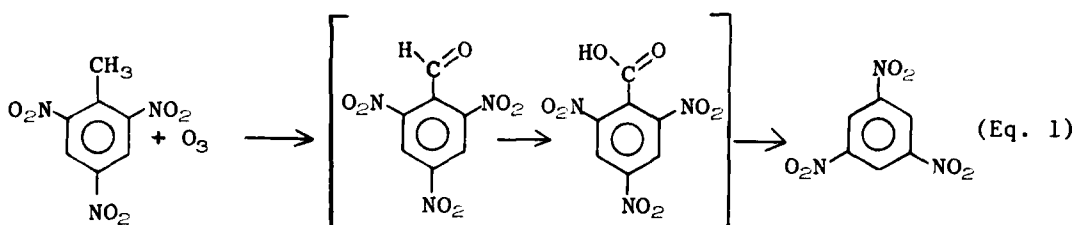
Compound	Initial Concentration (ppm)	Percentage Reacted	Max. Reaction (%)	Extent of Reaction (%)
Trinitrobenzene	362	2.4	4.0	60
2,4,6-Trinitrotoluene	30	15.5	59	26
2,6-Dinitrotoluene	139	20	27	74
1,3-Dinitrobenzene	52	25	26	96
2,4,6-Trinitroresorcinol	308	12	5.4	225
2,4,6-Trinitrobenzaldehyde	71	11	28	39
7-50 LAP	32*	3	59	5
Pentaerythritol tetranitrate	1	0	0	0
2,4,6-Trinitrobenzonitrile	ND†	NT‡	NT	NT
7-100 LAP	0*	NT NA§	NT NA	NT NA

* Based on 2,4,6-TNT concentration.

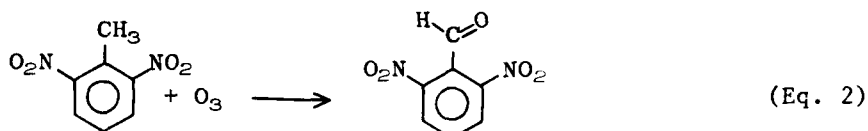
† ND, not determined; chemical hydrolyses in water.

‡ NT, not tested.

§ NA, not applicable because of complete photolysis of 2,4,6-TNT in 7-100 LAP.



also occurred with 2,6-dinitrotoluene, and 2,6-dinitrobenzaldehyde was identified as a reaction product (Eq. 2).



In general, very few products resulting from ozonation of the munitions compounds possessed sufficient volatility for gc/ms analysis or sufficient absorbance at 254 nm for detection in hplc profiles. These phenomena would be expected when the aromatic ring is ruptured.

Microbiological Mutagenicity

All 20 compounds were subjected to chlorine water treatment facility conditions (10 ppm chlorine, pH 7, 0.5 hr contact time). Also, 15 of the compounds were chlorinated with 1000 ppm chlorine, pH 4, 48 hr contact time at 85° C. Mutagenic assays of the compounds showed no significant differences between the two chlorination processes. The concentrations for each chemical are listed in Tables 1 and 2.

Table 4 summarizes the results of mutagenic screening tests on the 20 compounds. It should be noted that, in general, the concentrations tested were low. This was necessitated by the requirement that the compound be dissolved in water. Usually, dimethylsulfoxide (DMSO) would be used to achieve higher concentrations for testing. Thus, the absence of mutagenic activity in these assays should not be construed as proof that a compound is not mutagenic. Rather, it can only be stated that at the highest concentrations tested, the compound was not mutagenic. (1,3-Dinitrobenzene, 2,4-dinitrotoluene,

2,6-dinitrotoluene, 3,5-dinitrotoluene, and 2,4,6-trinitrotoluene have been found to be mutagenic when tested at doses higher than those reported in this study.¹⁶

Moreover, certain classes of carcinogens are not detected as mutagens by the Salmonella/microsome procedure (e.g., inorganics, hormones, polychlorinated hydrocarbons such as chloroform, carbon tetrachloride, dieldrin, and DDT).

1,3,5-Trinitrobenzene was mutagenic on S. typhimurium strains TA98 and TA100 before and after ozonation and chlorination, with and without metabolic activation. The mutagenic activity was not significantly altered by disinfection treatment. In most cases, there was much less mutagenic activity with the metabolic activation system. Trinitrobenzene was not mutagenic on S. cerevisiae. (Tables A-1 through A-5 and B-1 through B-4).

1,3-Dinitrobenzene was not mutagenic in these assays before or after chlorination or ozonation (Tables A-6 through A-11 and B-5 through B-8).

Trinitroglycerine was not mutagenic before or after chlorination in these assays (Tables A-12 through A-14 and B-9 and B-10).

Pentaerythritol tetranitrate (PETN) was not mutagenic on S. typhimurium or S. cerevisiae before or after chlorination or ozonation (Tables A-15 to A-18 and B-11 through B-14).

Condensate water was not mutagenic on S. typhimurium or S. cerevisiae before or after chlorination. (Tables A-19 and A-20 and B-15 and B-16).

Syringaldazine was not mutagenic on S. typhimurium and S. cerevisiae before or after chlorination or ozonation (Tables A-21 and A-22 and B-17 and B-18).

HMX and RDX were not mutagenic before or after chlorination in these assays (Tables A-23 through A-28 and B-19 through B-22).

Table 4

IN VITRO MICROBIOLOGICAL ASSAYS WITH MNITON WASTEWATER CHEMICALS AND CHLORINE TEST KIT REAGENTS

Absence of Mutagenic Activity (-); Presence of Mutagenic Activity (+)

Compound	Salmonella typhimurium			Saccharomyces cerevisiae			Reactivity	
	Chlorination	Ozonation		Chlorination	Ozonation		Chlorination	Ozonation
1,3,5-Trinitrobenzene	+	+	+	-	-	-	-	+
1,3-Dinitrobenzene	-	-	-	-	-	-	-	+
Trinitroglycerine	-	-	-	-	-	-	-	NT*
Pentaerythritol tetranitrate	-	-	-	-	-	-	-	-
Condensate water	-	-	-	-	-	-	-	NT
Syringaldazine	-	-	-	-	-	+	+	NT
HMX	-	-	-	-	-	-	-	NT
RDX	-	-	-	-	-	-	-	NT
N,N-Diethyl-p-phenylenediamine oxalate	+	+	+	-	-	+	+	NT
N,N-Dimethyl-p-phenylenediamine sulfate	+	+	+	-	-	+	+	NT
7-50 LAP	+	++	+	-	-	+	+	NT
7-100 LAP	+	++	+	-	-	ND*	ND*	+
2,6-Dinitrotoluene	+	++	+	-	-	ND	ND	ND
2,4-Dinitrotoluene	-	-	-	-	-	-	-	+
3,5-Dinitrotoluene	-	-	-	-	-	-	-	NT
2,4,6-Trinitrotoluene	-	-	-	-	-	-	-	NT
2,4,6-Trinitroresorcinol	-	-	-	-	-	-	-	+
2,4,6-Trinitrobenzonitrile	+	+	+	-	-	-	-	ND
2,4,6-Trinitrobenzaldehyde	+	++	+	-	-	+	+	+

* ND, not determinable; NT, not tested.

+ +Post-chlorination samples show an increase in mutagenic activity over the pre-chlorination samples.

+Post-chlorination samples show a decrease in mutagenic activity over the pre-chlorination samples.

DPO and DPS were mutagenic before and after chlorination. However, chlorination markedly decreased the mutagenicity of DPS. With both compounds, metabolic activation was required for mutagenicity, although, in some instances, DPS appeared to be weakly mutagenic without metabolic activation.

Both DPO and DPS form purple solutions when they are added to water, and the color darkens with time. Although the data are not presented, we observed that solution of freshly prepared DPS was less mutagenic if the distilled water in which it was prepared was bubbled with nitrogen (presumably this would remove oxygen). After 1 day of standing at room temperature, the mutagenic activity of both nitrogen-treated and untreated DPS had increased approximately 10-fold.

Of the two compounds, DPS was the more mutagenic (approximately 3-fold). Mutagenic activity was observed primarily in strains TA1538 and TA98. DPO was weakly mutagenic in TA100 and DPS was weakly mutagenic in TA1537 and TA100.

Our results suggest that both compounds are frameshift mutagens. Preliminary results suggest that the mutagenic activity may be attributable to some oxidation product(s) of these compounds. It seems likely that the difference in mutagenic activity between the two compounds is due to the methyl and ethyl groups rather than to the salts (oxalate and sulfate). Tables A-29 through A-34 and B-23 through B-28 present the results from assays on DPO and DPS.

7-50 LAP was mutagenic on S. typhimurium strains TA1537, TA1538, TA98, and TA100 before and after chlorination and ozonation. Somewhat more mutagenic activity was observed after chlorination than before treatment. This compound was more mutagenic without metabolic activation than with metabolic activation. No mutagenicity was observed with S. cerevisiae (Tables A-35 through A-40 and B-29 through B-33).

7-100 LAP, like 7-50 LAP, was mutagenic on S. typhimurium strains TA1537, TA1538, TA98, and TA100 before and after chlorination and ozonation, particularly without metabolic activation. Unlike 7-50 LAP,

there was a slight reduction in mutagenic activity after chlorination. No mutagenicity was observed with S. cerevisiae (Tables A-41 through A-45 and B-34 through B-37).

9-100 LAP was mutagenic on S. typhimurium strains TA1538, TA98, and TA100 before and after chlorination. There was somewhat more activity without metabolic activation. 9-100 LAP was not mutagenic in assays with S. cerevisiae (Tables A-46 and A-47 and B-38).

2,6-Dinitrotoluene was tested twice on S. typhimurium before and after chlorination. In one test at 2.4 ppm, it showed slight mutagenicity after chlorination; in a second test at 58.7 ppm, it showed a mutagenic dose response after chlorination. It was not mutagenic on S. typhimurium either before or after ozonation, nor was it mutagenic on S. cerevisiae either before or after chlorination or ozonation (Tables A-48 through A-52 and B-39 through B-42).

2,4-Dinitrotoluene was not mutagenic either before or after chlorination, nor was it mutagenic in any of the S. cerevisiae assays (Tables A-53 through A-55 and B-43 and B-44).

3,5-Dinitrotoluene was not mutagenic either before or after chlorination on S. typhimurium or on S. cerevisiae (Tables A-56 and A-57 and B-45 and B-46).

2,4,6-Trinitrotoluene was not mutagenic either before or after chlorination or ozonation in any of the assays performed. In two experiments (#18 and 28), it appeared to be weakly mutagenic (< 2-fold increase in revertants) on TA100 without metabolic activation (Tables A-58 through A-62 and B-57 through B-50).

2,4,6-Trinitroresorcinol was not mutagenic on S. typhimurium either before or after ozonation or chlorination, nor was it mutagenic in any of the S. cerevisiae assays (Tables A-63 through A-67 and B-51 through B-54).

2,4,6-Trinitrobenzonitrile was mutagenic on S. typhimurium strains TA98 and TA100 before and after ozonation and chlorination. In general, it was slightly less mutagenic after chlorination than before, and it

was more than twice as mutagenic after ozonation than before. Metabolic activation was not required for mutagenicity. Trinitrobenzonitrile was not mutagenic on S. cerevisiae before or after ozonation or chlorination (Tables A-68 through A-73 and B-55 through B-58).

2,4,6-Trinitrobenzaldehyde was mutagenic on S. typhimurium strains TA1537, TA1538, TA98, and TA100 both before and after chlorination and ozonation. Neither treatment appeared to significantly alter the mutagenic response, although significant (5 to 30%) chemical reaction appears to have occurred. Metabolic activation decreased mutagenicity. This compound was not mutagenic in any of the assays with S. cerevisiae. Tables A-74 through A-80 and B-59 through B-62 present the results.

Amino-dinitrotoluenes. Additionally, we investigated three amino-dinitrotoluenes as potentially reactive components in condensate water. The compounds (5-amino-2,4-dinitrotoluene; 4-amino-3,5-dinitrotoluene; and 4-amino-2,6-dinitrotoluene) were allowed to react with chlorine under the conditions previously described. As shown in Table 5, the two 4-amino compounds showed significant reactivity (75%) with chlorine, but the reactivity was only 12% for the 5-amino compound. No new products were evident in gc profiles of the reacted solutions. Also, there was no indication of mutagenic activity associated with the reacted solutions.

Table 5
EFFECT OF CHLORINATION ON AMINO-DINITROTOLUENES

Compound	Initial Concentration (ppm)	Percentage Reacted	Mutagenicity*			
			Pre		Post	
			Bacteria	Yeast	Bacteria	Yeast
5-Amino-2,4-dinitrotoluene	3	12	--	--	--	--
4-Amino-3,5-dinitrotoluene	18	68	--	--	--	--
4-Amino-2,6-dinitrotoluene	20	81	--	--	--	--

* -- No indication of mutagenic activity.

CONCLUSIONS

Eight of the compounds assayed were mutagenic at aqueous solubility before disinfection treatment. The mutagenic compounds were 1,3,5-trinitrobenzene, 2,4,6-trinitrobenzonitrile, 2,4,6-trinitrobenzaldehyde (munition wastewater chemicals), 7-50 LAP, 7-100 LAP, and 9-100 LAP (photolyzed wastewaters), N,N-diethyl-p-phenylenediamine oxalate, and N,N-dimethyl-p-phenylenediamine sulfate (chlorine test kit reagents).

In other studies we have conducted under contract to the U.S. Army Bioengineering Research and Development Laboratory, we have found that 1,3-dinitrobenzene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, 3,5-dinitrotoluene, and 2,4,6-trinitrotoluene are mutagenic in the Salmonella/microsome assay when tested at higher doses. We are not aware of mutagenicity assays at higher doses on other munition wastewater chemicals (e.g., trinitroglycerine, pentaerythritol tetranitrate, HMX, RDX, etc.).

The results of these experiments suggest that although the nitroaromatics react readily with ozone, any mutagenic activity that they showed was not greatly altered by ozonation. Only a few of the compounds reacted with chlorine. Based on these experiments, it would appear that disinfection treatment does not greatly alter mutagenic activity. For compounds that were mutagenic before treatment, both slight increases and slight decreases in mutagenic activity were observed after treatment. We did not observe any cases in which nonmutagenic compounds were converted into mutagens by either of the disinfection treatments.

None of the chemicals tested was mutagenic in assays with S. cerevisiae D3 under the assay conditions used in these experiments. It is possible that these compounds gave a negative response

because they are not strongly mutagenic in this procedure, particularly in view of the relatively low concentrations of test compounds. Alternatively, these compounds may not be mutagens in S. cerevisiae D3 mitotic recombination assay. We conclude that this indicator microorganism is not useful in evaluating the mutagenicity of these compounds.

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APPENDIX A

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

APPENDIX A--IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

A-1	1,3,5-Trinitrobenzene--Experiment 5	41
A-2	1,3,5-Trinitrobenzene--Experiment 25.	43
A-3	1,3,5-Trinitrobenzene--Experiment 26.	44
A-4	1,3,5-Trinitrobenzene--Experiment 35.	45
A-5	1,3,5-Trinitrobenzene--Experiment 44.	46
A-6	1,3-Dinitrobenzene--Experiment 30	47
A-7	1,3-Dinitrobenzene--Experiment 39	48
A-8	1,3-Dinitrobenzene--Experiment 42	49
A-9	1,3-Dinitrobenzene--Experiment 27	50
A-10	1,3-Dinitrobenzene--Experiment 36	51
A-11	1,3-Dinitrobenzene--Experiment 40	52
A-12	Trinitroglycerine--Experiment 25.	53
A-13	Trinitroglycerine--Experiment 34.	54
A-14	Trinitroglycerine--Experiment 40.	55
A-15	PETN--Experiment 24	56
A-16	PETN--Experiment 39	57
A-17	PETN--Experiment 26	58
A-18	PETN--Experiment 35	59
A-19	Condensate Water--Experiment 29	60
A-20	Condensate Water--Experiment 44	61
A-21	Syringaldazine--Experiment 20	62
A-22	Syringaldazine--Experiment 33	63
A-23	HMX--Experiment 21.	64
A-24	HMX--Experiment 30.	65
A-25	HMX--Experiment 37.	66
A-26	RDX--Experiment 21.	67
A-27	RDX--Experiment 29.	68
A-28	RDX--Experiment 40.	69
A-29	DPO--Experiment 24.	70
A-30	DPO--Experiment 33.	71

A-31	DPS--Experiment 19.	73
A-32	DPS--Experiment 24.	74
A-33	DPS--Experiment 33.	75
A-34	DPS--Experiment 45.	77
A-35	7-50 LAP--Experiment 29	79
A-36	7-50 LAP--Experiment 38	80
A-37	7-50 LAP--Experiment 44	82
A-38	7-50 LAP--Experiment 34	84
A-39	7-50 LAP--Experiment 37	85
A-40	7-50 LAP--Experiment 44	86
A-41	7-100 LAP--Experiment 20.	87
A-42	7-100 LAP--Experiment 22.	88
A-43	7-100 LAP--Experiment 44.	89
A-44	7-100 LAP--Experiment 34.	91
A-45	7-100 LAP--Experiment 40.	92
A-46	9-100 LAP--Experiment 22.	94
A-47	9-100 LAP--Experiment 44.	95
A-48	2,6-Dinitrotoluene--Experiment 23	97
A-49	2,6-Dinitrotoluene--Experiment 31	98
A-50	2,6-Dinitrotoluene--Experiment 44	99
A-51	2,6-Dinitrotoluene--Experiment 27	100
A-52	2,6-Dinitrotoluene--Experiment 34	101
A-53	2,4-Dinitrotoluene--Experiment 18	102
A-54	2,4-Dinitrotoluene--Experiment 28	103
A-55	2,4-Dinitrotoluene--Experiment 39	104
A-56	3,5-Dinitrotoluene--Experiment 17	106
A-57	3,5-Dinitrotoluene--Experiment 18	107
A-58	2,4,6-Trinitrotoluene--Experiment 18.	108
A-59	2,4,6-Trinitrotoluene--Experiment 28.	109
A-60	2,4,6-Trinitrotoluene--Experiment 27.	110
A-61	2,4,6-Trinitrotoluene--Experiment 36.	111
A-62	2,4,6-Trinitrotoluene--Experiment 40.	112
A-63	2,4,6-Trinitroresorcinol--Experiment 17	113
A-64	2,4,6-Trinitroresorcinol--Experiment 28	114

A-65	2,4,6-Trinitroresorcinol--Experiment 27	115
A-66	2,4,6-Trinitroresorcinol--Experiment 35	116
A-67	2,4,6-Trinitroresorcinol--Experiment 40	117
A-68	2,4,6-Trinitrobenzonitrile--Experiment 26	118
A-69	2,4,6-Trinitrobenzonitrile--Experiment 31	119
A-70	2,4,6-Trinitrobenzonitrile--Experiment 38	120
A-71	2,4,6-Trinitrobenzonitrile--Experiment 26	122
A-72	2,4,6-Trinitrobenzonitrile--Experiment 36	123
A-73	2,4,6-Trinitrobenzonitrile--Experiment 40	125
A-74	2,4,6-Trinitrobenzaldehyde--Experiment 27	127
A-75	2,4,6-Trinitrobenzaldehyde--Experiment 31	128
A-76	2,4,6-Trinitrobenzaldehyde--Experiment 37	129
A-77	2,4,6-Trinitrobenzaldehyde--Experiment 45	130
A-78	2,4,6-Trinitrobenzaldehyde--Experiment 27	131
A-79	2,4,6-Trinitrobenzaldehyde--Experiment 36	132
A-80	2,4,6-Trinitrobenzaldehyde--Experiment 40	133

Table A-1
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3,5-TRINITROBENZENE
EXPERIMENT 5

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TAl535	TAl537	TAl538	TAl98
Negative control	-		67	11	22	32
	+		102	13	16	28
Positive controls						
	-	50	157			
	-	100		1252		
	-	50			9	
	-	0.1				218
2-Nitrofluorene AF2	-					554
2-Anthramine	+	20	381	79	777	1632
Pre-chlorination 1,3,5-Trinitrobenzene	-	4.3	93	6	18	23
	-	10.8	116	9	10	33
	-	21.6	86	15	17	35
	-	43.0	92	12	22	67
	-	107.0	74	15	23	85
	+	4.3	109	17	21	32
	+	10.8	127	10	17	26
	+	21.6	126	14	20	44
	+	43.0	115	7	35	34
	+	107.0	77	13	38	52
						217

Table A-1 (concluded)

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Post-chlorination 1,3,5-Trinitrobenzene	-	4.3	55	13	14	29	93
	-	10.8	62	14	13	36	119
	-	21.6	73	14	20	74	132
	-	43.0	93	23	24	40	191
	-	107.0	130	17	33	107	285
	+	4.3	136	11	27	25	104
	+	10.8	137	13	22	34	112
	+	21.6	156	18	20	33	129
	+	43.0	125	14	35	54	160
	+	107.0	147	20	25	70	193

Table A-2
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3,5-TRINITROBENZENE
EXPERIMENT 25

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TAL535	TAL537	TAL538	TA98 TA100
Negative control	-		38	14	15	24
	+		24	16	33	31
Positive controls			1500	700		
8-Propiolactone	-	50				
9-Aminoacridine	-	100				
2-Nitrofluorene	-	50			300	
AF2	-	0.1				0
2-Anthramine	+	20	600	600	2000	1200
						2200
Pre-chlorination	-	44	49	14	122	121
1,3,5-Trinitrobenzene	+	44	13	7	19	72
						354
						167
Post-chlorination	-	0.9	32	9	18	26
1,3,5-Trinitrobenzene	-	1.8	32	15	15	31
	-	4.5	31	16	23	37
	-	9.0	30	17	33	79
	-	18.0	27	16	47	161
	-	44.0	33	34	129	115
						347
	+	0.9	11	15	22	31
	+	1.8	15	9	21	39
	+	4.5	14	16	17	44
	+	9.0	12	9	26	31
	+	18.0	21	12	23	45
	+	44.0	25	12	28	81
						103
						103
						126
						139
						148
						163

Table A-3
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3,5-TRINITROBENZENE
EXPERIMENT 26

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		50	20	41	28 144
	+		19	35	43	54 137
Positive controls						
8-Propiolactone	-	50	1500			
9-Aminoacridine	-	100		440		
2-Nitrofluorene	-	50			324	
AF2	-	0.1				
2-Anthramine	+	20				
			217	500	3000	600 1500 1000 6000
Pre-ozonation	-	94	42	23	38	60 176
1,3,5-Trinitrobenzene	+	94	31	28	33	34 179
Post-ozonation	-	1.8	17	25	19	24 112
1,3,5-Trinitrobenzene	-	3.6	13	29	24	36 130
	-	9.0	17	30	14	37 137
	-	18.0	18	23	18	34 156
	-	36.0	21	11	23	48 128
	-	90.0	48	16	28	60 143
	+	1.8	30	27	44	44 120
	+	3.6	17	17	39	44 158
	+	9.0	19	15	48	52 126
	+	18.0	28	27	44	43 142
	+	36.0	18	17	30	45 150
	+	90.0	18	25	34	40 133

Table A-4
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3,5-TRINITROBENZENE

EXPERIMENT 35

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TAL535	TAL537	TAL538	TAL98 TAL100
Negative control	-		30	8	13	22 73
	+		18	12	19	39 71
Positive controls						
β-Propiolactone	-	50	1050			
9-Aminoacridine	-	100		218		
2-Nitrofluorene	-	50			2000	
AF2	-	0.1				24 73
2-Anthracene	+	20	500	1270	2300	2540 1875
Pre-ozonation	-	91	6	52	854	C* 0
1,3,5-Trinitrobenzene	+	91	40	13	65	C 237
Post-ozonation	-	18	42	15	177	337 301
1,3,5-Trinitrobenzene	-	35	36	75	494	877 520
	-	53	2	C	787	764 609
	-	71	3	147	503	1124 702
	-	88	28	8	370	163 250
	+	18	20	26	22	63 139
	+	35	21	9	36	100 140
	+	53	11	12	35	130 164
	+	71	C	12	54	100 231
	+	88	26	10	C	27 276

* C, contaminated.

Table A-5

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3,5-TRINITROBENZENE

EXPERIMENT 44

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		13	4	6	133
	+		9	5	11	139
Positive controls						
Sodium azide	-	1.0	238			
9-Aminoacridine	-	100		1152		
2-Nitrofluorene	-	50			1608	
AF2	-	0.1				1080
2-Anthramine	+	2.5	104	67	726	998
Pre-ozonation	-	91	0	131	0	15K*
1,3,5-Trinitrobenzene	+	91	18	13	68	361
Post-ozonation	-	18	20	16	160	435
1,3,5-Trinitrobenzene	-	35	15	67	682	783
	-	53	0	172	1338	1223
	-	71	0	223	1016	1248
	-	88	0	203	365	OK
	+	18	9	8	14	186
	+	35	15	5	16	279
	+	53	13	8	24	290
	+	71	19	17	52	372
	+	88	20	25	59	436

* K, killing.

Table A-6
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3-DINITROBENZENE
EXPERIMENT 30

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	
Negative control	-		*	13	12	27	
	+		22	9	21	34	
Positive controls							
8-Propiolactone	-	50	*				
9-Aminoacridine	-	100		910			
2-Nitrofluorene	-	50			230	320	
AF2	-	0.1				142	
2-Anthræmine	+	20	100	325	330		
Pre-chlorination	-	46.00	54	*	26	*	
1,3-Dinitrobenzene	+	46.00	18	20	*	41	
Post-chlorination	-	0.92	44	15	16	26	
1,3,-Dinitrobenzene	-	1.84	53	16	28	29	
	-	4.60	59	18	22	24	
	-	9.20	40	*	27	42	
	-	18.40	39	*	19	41	
	-	46.00	53	*	21	*	
	+	0.92	14	*	19	*	
	+	1.84	14	21	20	*	
	+	4.60	18	37	21	*	
	+	9.20	12	22	16	42	
	+	18.40	26	31	*	44	
	+	46.00	24	23	*	32	

* Problem with plates and media.

Table A-7
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3,-DINITROBENZENE
EXPERIMENT 39

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		67	11	22	32 92
	+		102	13	16	29 114
Positive controls						
β-Propiolactone	-	50	157	1252		
9-Aminoacridine	-	100				
2-Nitrofluorene	-	50			9	
AF2	-	0.1				218 554
2-Anthramine	+	20	381	79	777	1632 2138
Pre-chlorination	-	41.0	70	12	40	61 89
1,3-Dinitrobenzene	+	41.0	93	15	19	33 83
Post-chlorination	-	1.6	74	5	16	22 89
1,3,-Dinitrobenzene	-	4.0	51	11	15	36 108
	-	8.0	72	8	19	31 75
	-	16.0	76	17	22	40 96
	-	41.0	68	15	25	62 94
	+	1.6	85	16	25	31 103
	+	4.0	101	16	24	38 85
	+	8.0	86	12	23	38 100
	+	16.0	125	9	15	37 115
	+	41.0	104	15	23	44 99

Table A-8
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3-DINITROBENZENE
EXPERIMENT 42

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		26	6	14	17 127
	+		13	12	20	30 96
Positive controls						
β-Propiolactone	-	50	42			
9-Aminoacridine	-	100		536		
2-Nitrofluorene	-	50			1633	
AF2	-	0.1				293 997
2-Anthramine	-	2.5	18	5	14	134
	+	2.5	87	27	437	634
Pre-chlorination	-	41	21	2	28	27 125
1,3-Dinitrobenzene	+	41	12	7	17	21 120
Post-chlorination	-	8	26	4	17	13 110
1,3-Dinitrobenzene	-	16	17	4	16	17 138
	-	24	24	5	15	20 116
	-	32	22	4	19	30 138
	-	41	14	3	17	29 126
	+	8	8	5	18	30 112
	+	16	12	5	21	30 138
	+	24	11	5	15	17 110
	+	32	11	9	16	28 132
	+	41	9	5	17	17 137

Table A-9
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3-DINITROBENZENE
EXPERIMENT 27

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		34	7	12	82
	+		14	6	24	96
Positive controls						
8-Propiolactone	-	50	547	400	700	
9-Aminoacridine	-	100				
2-Nitrofluorene	-	50				
AF2	-	0.1				
2-Anthramine	+	20	108	11	54	300
						1200
Pre-ozonation	-	13.0	23	2	24	107
1,3-Dinitrobenzene	+	13.0	11	10	36	99
Post-ozonation	-	0.2	24		17	99
1,3-Dinitrobenzene	-	0.4	27	11	21	92
	-	1.0	28	5	12	76
	-	1.9	29	7	12	87
	-	3.9	34	6	13	77
	-	9.7	35	8	29	86
	+	0.2	10	11	36	103
	+	0.4	11	9	39	86
	+	1.0	17	7	39	82
	+	1.9	19	8	33	88
	+	3.9	21	7	39	72
	+	9.7	21	9	45	85

Table A-10
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3-DINITROBENZENE
EXPERIMENT 36

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98
Negative control	-		28	5	9	22
	+		14	2	15	21
Positive controls						
8-Propiolactone	-	50	224			
9-Aminoacridine	-	100		900	1300	
2-Nitrofluorene	-	50				451
AF2	-	0.1				2200
2-Anthramine	+	20	416	322	1955	
Pre-ozonation	-	27.0	15	6	18	23
1,3-Dinitrobenzene	+	27.0	7	3	8	20
Post-ozonation	-	4.6	25	4	11	18
1,3-Dinitrobenzene	-	9.2	33	5	20	23
	-	13.8	21	8	12	25
	-	18.4	27	9	25	24
	-	23.0	17	5	21	43
	+	4.6	15	3	7	22
	+	9.2	20	3	6	15
	+	13.8	7	5	10	9
	+	18.4	5	5	7	19
	+	23.0	6	2	4	7

Table A-11

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3-DINITROBENZENE

EXPERIMENT 40

<u>Compound</u>	<u>Metabolic Activation</u>	<u>Micrograms of Compound Added per Plate</u>	<u>Histidine Revertants per Plate</u>	
			<u>TAL00</u>	
Negative control	-		115	
	+		119	
Positive controls AF2	-	0.1	446	
	+	10	1755	
2-Anthramine				
Pre-ozonation 1,3-Dinitrobenzene	-	12.8	137	
	+	12.8	175	
Post-ozonation 1,3-Dinitrobenzene	-	2.6	156	
	-	5.1	144	
	-	7.7	140	
	-	10.2	149	
	-	12.8	145	
	+	2.6	122	
	+	5.1	139	
	+	7.7	123	
	+	10.2	143	
	+	12.8	134	

Table A-12
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
TRINITROGLYCERINE*
EXPERIMENT 25

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		38	14	15	24	86
	+		24	16	33	31	126
Positive controls							
β-Propiolactone	-	50 μg	1500				
9-Aminoacridine	-	100		700			
2-Nitrofluorene	-	50			300		
AF2	-	0.1				0	0
2-Anthramine	+	20	600	600	2000	1200	2200
Pre-chlorination	-	0.25 ml	35	15	16	26	119
Trinitroglycerine	+	0.25	18	17	13	25	80
Post-chlorination	-	0.05 ml	26	18	16	23	110
Trinitroglycerine	-	0.01	31	9	23	19	83
	-	0.025	40	10	16	21	90
	-	0.05	36	15	16	20	101
	-	0.1	38	10	13	34	105
	-	0.25	26	14	23	24	126
	+	0.05	18	9	15	30	115
	+	0.01	15	14	17	25	93
	+	0.025	19	11	14	32	89
	+	0.05	17	7	15	31	93
	+	0.1	13	9	17	34	73
	+	0.25	23	10	21	18	84

* Saturated solution.

Table A-13
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
TRINITROGLYCERINE*
EXPERIMENT 34

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TAL535	TAL537	TAL538	TA98
Negative control	-		52	16	13	38
	+		87	17	9	57
Positive controls 8-Propiolactone 9-Aminoacridine 2-Nitrofluorene AF2 2-Anthramine	-	50 µg	1590			
	-	100		475		
	-	50			2054	
	-	0.1				46
	+	20	516	760	2800	3000
Pre-chlorination Trinitrolycerine	-	0.25 ml	52	17	17	30
	+	0.25	78	9	17	25
Post-chlorination Trinitrolycerine	-	0.05 ml	50	12	18	33
	-	0.1	69	8	14	30
	-	0.15	42	16	15	31
	-	0.2	41	8	18	26
	-	0.25	60	18	12	21
	+	0.05	53	24	23	42
	+	0.1	55	20	C†	40
	+	0.15	55	18	22	41
	+	0.2	72	18	16	25
	+	0.25	70	14	17	33

* Saturated solution.
† C, contaminated.

Table A-14

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
TRINITROGLYCERINE*

EXPERIMENT 40

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate	
			Tal00	
Negative control	-		115	
	+		119	
Positive controls AF2	-	0.1 µg	446	
	+	10	1755	
Pre-chlorination Trinitrolycerine	-	0.25 ml	105	
	+	0.25	130	
Post-chlorination Trinitrolycerine	-	0.05 ml	127	
	-	0.1	146	
	-	0.15	138	
	-	0.2	123	
	-	0.25	120	
	+	0.05	118	
	+	0.1	123	
	+	0.15	93	
	+	0.2	139	
	+	0.25	115	

* Saturated solution.

Table A-15
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
PENTAERYTHRITOL TETRANITRATE*

EXPERIMENT 24

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		35	16	29	38	100
	+		24	18	33	30	133
Positive controls							
3-Propiolactone	-	50 µg	1740				
9-Aminoacridine	-	100		168			
2-Nitrofluorene	-	50			200		
AF2	-	0.1					1200
2-Anthramine	+	20	T†	432	~ 6000	236	~ 8000
Pre-chlorination PETN	-	0.25 ml	36	16	15	21	108
	+	0.25	20	17	35	38	106
Post-chlorination PETN	-	0.005 ml	27	11	24	24	109
	-	0.01	32	13	17	30	103
	-	0.025	36	C#	24	21	105
	-	0.05	40	15	17	20	100
	-	0.1	31	15	9	16	108
	-	0.25	33	17	13	31	95
	+	0.005	16	10	29	40	84
	+	0.01	14	16	37	38	106
	+	0.025	20	9	26	37	87
	+	0.05	21	10	31	56	87
	+	0.1	15	12	16	37	106
	+	0.25	23	9	27	39	120

* Saturated solution.

† T, toxic.

C, contaminated.

Table A-16
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
PENTAERYTHRITOL TETRANITRATE*
EXPERIMENT 39

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		67	11	22	32 92
	+		102	13	16	28 114
Positive controls						
β-Propiolactone	-	50 µg	157			
9-Aminoacridine	-	100		1252		
2-Nitrofluorene	-	50			9	
AF2	-	0.1				218 554
2-Anthramine	+	20	381	79	777	1632 2138
Pre-chlorination	-	0.25 ml	85	16	11	31 77
PETN	+	0.25 ml	128	15	14	44 95
Post-chlorination	-	0.01	75	11	14	23 94
PETN	-	0.025	111	13	10	21 79
	-	0.05	95	19	9	24 82
	-	0.1	86	18	13	25 90
	-	0.25	93	18	11	24 83
	+	0.01	118	14	15	27 104
	+	0.025	126	13	9	33 93
	+	0.05	127	15	15	42 111
	+	0.1	141	18	15	39 107
	+	0.25	156	10	13	32 128

* Saturated solution.

Table A-17

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
PENTAERYTHRITOL TETRANITRATE*
 EXPERIMENT 26

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	- +		50 19	20 35	41 43	28 54 144 137
Positive controls						
8-Propiolactone	-	50 µg	1500			
9-Aminoacridine	-	100		440		
2-Nitrofluorene	-	50			324	
AF2	-	0.1				600 1500
2-Anthramine	+	20	217	500	3000	1000 6000
Pre-ozonation TETN	- +	0.25 ml 0.25 ml	39 27	13 15	24 24	30 54 144 132
Post-ozonation PETN	-	0.005 ml	47	14	17	39 126
	-	0.01	66	15	22	40 132
	-	0.025	54	21	22	27 144
	-	0.05	44	18	19	40 134
	-	0.1	52	8	27	32 137
	-	0.25	68	15	21	40 121
	+	0.005	16	25	37	41 131
	+	0.01	22	19	33	46 146
	+	0.025	15	18	40	60 142
	+	0.05	21	31	36	51 134
	+	0.1	19	13	32	52 156
	+	0.25	26	23	28	34 149

* Saturated solution.

Table A-18
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
PENTAERYTHRITOL TETRANITRATE*

EXPERIMENT 35

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		30	8	13	22	73
	+		18	12	19	39	71
Positive controls							
β-Propiolactone	-	50 μg	1050				
9-Aminoacridine	-	100		218	2000		
2-Nitrofluorene	-	50					
AF ₂	-	0.1					73
2-Anthramine	+	20	500	1270	2300	2540	1875
Pre-ozonation PETN	-	0.25 ml	15	2	11	14	78
	+	0.25	11	6	17	23	85
Post-ozonation PETN	-	0.05 ml	15	10	14	26	62
	-	0.1	22	2	6	35	85
	-	0.15	C†	5	13	29	72
	-	0.2	20	9	3	18	68
	-	0.25	C	4	15	26	74
	+	0.05	15	10	25	20	58
	+	0.1	18	12	15	17	89
	+	0.15	16	5	13	29	78
	+	0.2	11	9	24	29	70
	+	0.25	8	8	24	33	71

* Saturated solution.
† C, contaminated.

Table A-19

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
CONDENSATE WATER

EXPERIMENT 29

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate		
			TA1535	TA1538	TA98
Negative control	-		35	12	16
	+		5	14	40
Positive controls					
β-Propiolactone	-	50	1000		
2-Nitrofluorene	-	50		1200	
AF2	-	0.1			28
2-Anthramine	+	20	325	480	1400
Pre-chlorination	-	17	17	38	37
Condensate water	+	17	26	27	43
Post-chlorination	-	3.2	31	22	27
Condensate water	-	6.4	13	23	35
	-	9.6	30	55	35
	-	12.8	26	49	73
	-	16	25	77	67
	+	3.2	25	20	30
	+	6.4	10	20	25
	+	9.6	10	26	51
	+	12.8	7	32	44
	+	16	14	27	55

Table A-20

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
CONDENSATE WATER
 EXPERIMENT 44

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate	
			TA98	TA100
Negative control	-		22	133
	+		26	139
Positive controls				
AF2	-	0.1	494	1080
2-Anthramine	+	2.5	705	998
Pre-chlorination	-	16.8	24	148
Condensate water	+	16.8	26	163
Post-chlorination	-	3.4	14	132
Condensate water	-	6.7	18	144
	-	10.1	14	137
	-	13.5	25	160
	-	16.8	24	134
	+	3.4	17	123
	+	6.7	25	135
	+	10.1	24	149
	+	13.5	27	134
	+	16.8	25	145

Table A-21
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
SYRINGALDAZINE
EXPERIMENT 20

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		35	15	16	52
	+		30	15	50	104
Positive controls						
8-Propiolactone	-	50	800			
9-Aminoacridine	-	100		1500		
2-Nitrofluorene	-	50			500	
AF2	-	0.1				1500
2-Anthramine	+	20	850	500	3000	450
						3000
Pre-chlorination	-	30	37	10	12	54
Syringaldazine	+	30	43	19	20	43
						128
Post-chlorination	-	0.003	31	9	25	62
Syringaldazine	-	0.006	32	13	20	42
	-	0.016	43	11	24	34
	-	0.03	32	7	20	54
	-	0.06	34	9	25	16
	-	0.16	43	17	18	21
						124
	+	0.003	100	6	30	45
	+	0.006	35	13	40	45
	+	0.016	30	11	35	54
	+	0.03	28	24	23	44
	+	0.06	43	K*	22	33
	+	0.16	50	14	26	18
						102

* K, killing.

Table A-22
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
SYRINGALDAZINE
EXPERIMENT 33

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		37	6	5	21	98
	+		17	7	16	26	106
Positive controls							
8-Propiolactone	-	50	950				
9-Aminoacridine	-	100		239			
2-Nitrofluorene	-	50			1260		
AF2	-	0.1					137
2-Anthramine	+	20	324	14	585	2400	1530
Pre-chlorination	-	31	46	9	13	14	87
Syringaldazine	+	31	44	5	16	21	91
Post-chlorination	-	4	44	2	3	19	100
Syringaldazine	-	8	51	4	9	21	95
	-	12	46	3	6	14	96
	-	16	33	5	10	18	94
	-	20	46	4	8	15	77
	+	4	31	6	14	20	97
	+	8	37	10	14	23	80
	+	12	32	5	15	24	72
	+	16	28	4	16	20	81
	+	20	42	4	11	16	71

Table A-23

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM*
HMX*

EXPERIMENT 21

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate		
			TA1535	TA1537	TA100
Negative control	-		77	14	147
	+		53	29	166
Positive controls					
β-Propiolactone	-	50 μg	2400	2115	188
9-Aminoacridine	-	100			~6000
AF2	-	0.1			
2-Anthramine	+	20	253	1305	
Pre-chlorination HMX	-	0.25 ml	72	14	151
	+	0.25	35	29	153
Post-chlorination HMX	-	0.005 ml	88	14	165
	-	0.01	87	20	158
	-	0.025	86	34	195
	-	0.05	91	27	144
	-	0.1	93	+	+
	-	0.25	+	+	+
	+	0.005	35	26	151
	+	0.01	45	28	147
	+	0.025	76	34	135
	+	0.05	78	+	145
	+	0.1	43	+	161
	+	0.25	+	+	+

* Saturated solution.

+ Problem with plates or media.

Table A-24

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

HX*

EXPERIMENT 30

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TA1537	TA1538	TA98	TA100
Negative control	-		13	12	27	131
	+		9	21	34	84
Positive controls						
9-Aminoacridine	-	100 µg	910			
2-Nitrofluorene	-	50		230		
AF2	-	0.1			320	800
2-Anthramine	+	20	325	330	142	710
Pre-chlorination	-	0.25 ml	+	11	39	94
HMX	+	0.25	21	6	38	86
Post-chlorination	-	0.005 ml	10	11	27	150
HMX	-	0.01	18	14	28	142
	-	0.025	13	12	27	138
	-	0.05	+	16	30	165
	-	0.1	+	16	42	144
	-	0.25	+	11	39	102
	+	0.005	22	11	33	+
	+	0.01	20	12	44	+
	+	0.025	21	11	48	+
	+	0.05	21	17	47	86
	+	0.1	24	C†	51	121
	+	0.25	34	8	48	91

* Saturated solution.

† Problem with media or plates.

‡ C, contaminated.

Table A-25
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
HMX*

EXPERIMENT 37

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		7	10	11	14	91
	+		5	7	9	19	77
Positive controls							
β-Propiolactone	-	50 µg	262				
9-Aminoacridine	-	100		1405			
2-Nitrofluorene	-	50			1130		
AF2	-	0.1				400	1176
2-Anthramine	+	20	330	247	1825	1836	1290
Pre-chlorination HMX	-	0.25 ml	9	3	7	11	70
	+	0.25	5	8	5	13	74
Post-chlorination HMX	-	0.05 ml	6	2	5	17	63
	-	0.1	11	2	5	10	84
	-	0.15	6	6	5	11	65
	-	0.2	10	6	9	21	74
	-	0.25	9	3	6	10	68
	+	0.05	7	6	5	20	85
	+	0.1	5	2	5	25	82
	+	0.15	2	3	12	9	94
	+	0.2	5	2	7	16	97
	+	0.25	8	3	7	11	79

* Saturated solution.

Table A-26
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
RDX

EXPERIMENT 21

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		77	14	42	64	147
	+		53	29	41	81	166
Positive controls							
β-Propiolactone	-	50	2460				
9-Aminoacridine	-	100		2115	361		
2-Nitrofluorene	-	50				77	188
AF2	-	0.1				~5000	~6000
2-Anthramine	+	20	253	1305	6870		
Pre-chlorination	-	12	71	15	33	56	149
RDX	+	12	37	7	37	C*	136
Post-chlorination	-	0.24	66	9	44	70	127
RDX	-	0.48	40	17	38	48	128
	-	1.20	51	14	42	64	148
	-	2.40	67	16	41	58	162
	-	4.80	68	15	37	57	133
	-	12.00	63	16	39	56	167
	+	0.24	23	15	42	54	126
	+	0.48	49	18	47	57	139
	+	1.20	72	16	45	55	136
	+	2.40	44	22	49	62	178
	+	4.80	46	16	43	60	174
	+	12.00	43	24	47	43	133

* C, contaminated.

Table A-27

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM*
RDX

EXPERIMENT 29

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate		
			TA1535	TA1538	TA98
Negative control	-		35	12	16
	+		5	14	40
Positive controls					
8-Propiolactone	-	50	1000		
2-Nitrofluorene	-	50		1200	28
AF2	-	0.1			1400
2-Anthramine	+	20	325	480	
Pre-chlorination	-	14.0	12	11	28
RDX	+	14.0	20	10	16
Post-chlorination	-	2.9	20	15	33
RDX	-	5.8	23	10	12
	-	8.7	28	13	30
	-	11.6	15	18	16
	-	14.0	16	10	23
	+	2.9	14	12	15
	+	5.8	13	15	16
	+	8.7	22	14	20
	+	11.6	8	8	36
	+	14.0	8	17	19

Table A-28

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
RDX

EXPERIMENT 40

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA98	TA100
Negative control	-		20	15	19	115
	+		9	14	28	119
Positive controls						
β-Propiolactone	-	50	456			
9-Aminoacridine	-	100		546	98	446
AF2	-	0.1				
2-Anthramine	+	10	604	444	2320	1755
Pre-chlorination	-	4.1	13	9	23	106
RDX	+	4.1	20	17	23	120
Post-chlorination	-	0.8	28	16	13	99
RDX	-	1.6	19	15	14	106
	-	2.5	19	22	27	107
	-	3.3	23	16	18	133
	-	4.1	17	16	26	104
	+	0.8	13	17	22	126
	+	1.6	8	14	25	127
	+	2.5	12	12	30	105
	+	3.3	5	7	25	121
	+	4.1	14	8	28	120

Table A-29

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
N,N-DITHYL-P-PHENYLENEDIAMINE OXALATE*

EXPERIMENT 24

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		35	16	29	38	100
	+		24	18	33	30	133
Positive controls							
8-Propiolactone	-	50 µg	1740				
9-Aminoacridine	-	100		168			
2-Nitrofluorene	-	50			200	236	1200
AF2	-	0.1				840	8000
2-Anthramine	+	20	T†	432	6000		
Pre-chlorination DPO	-	0.25 ml	35	13	18	32	162
	+	0.25	20	14	55	66	143
Post-chlorination DPO	-	0.005 ml	39	17	20	C‡	145
	-	0.01	31	17	12	27	119
	-	0.025	37	18	27	34	102
	-	0.05	34	15	27	26	141
	-	0.1	32	20	15	29	128
	-	0.25	41	16	24	29	126
	+	0.005	21	16	61	23	126
	+	0.01	19	16	64	C	159
	+	0.025	15	15	80	107	116
	+	0.05	18	14	75	85	133
	+	0.1	17	17	59	92	127
	+	0.25	28	16	71	129	165

* Photolyzed.

† T, toxic.

‡ C, contaminated.

Table A-30

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
N,N-DIETHYL-p-PHENYLENEDIAMINE OXALATE

EXPERIMENT 33

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		37	6	5	21	98
	+		17	7	16	26	106
Positive controls							
3-Propiolactone	-	50	950				
9-Aminoacridine	-	100		239			
2-Nitrofluorene	-	50			1260		
AF2	-	0.1				21	137
2-Anthramine	+	20	324	14	585	2400	1530
Pre-chlorination							
DPO	-	2.3	37	2	9	14	105
	-	5.7	37	6	8	19	94
	-	11.5	36	9	9	21	96
	-	23	34	7	12	10	112
	-	57	44	6	12	61	97
	+	2.3	20	4	29	31	83
	+	5.7	20	5	56	64	88
	+	11.5	28	7	135	122	95
	+	23	14	7	232	223	96
	+	57	11	12	489	441	141

Table A-30 (concluded)

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		37	6	5	21 98
	+		17	7	16	26 106
Positive controls						
8-Propiolactone	-	50	950	239	1260	
9-Aminoacridine	-	100				
2-Nitrofluorene	-	50				
AF2	-	0.1				21 137
2-Anthramine	+	20	324	14	585	2400 1530
Post-chlorination						
DPO	-	1.8	43	2	11	24 102
	-	4.5	34	7	5	20 98
	-	9.0	41	10	6	23 75
	-	18.0	48	5	5	27 115
	-	45.1	25	5	6	57 120
	+	1.8	14	2	38	35 92
	+	4.5	10	9	75	64 76
	+	9.0	7	11	114	134 98
	+	18.0	18	9	290	294 102
	+	45.1	15	16	395	312 78

Table A-31
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
N,N-DIMETHYL-P-PHENYLENEDIAMINE SULFATE*
EXPERIMENT 19

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		25	9	29	53
	+		26	9	30	68
Positive controls						
β-Propiolactone	-	50 µg	900			
9-Aminoacridine	-	100		500		
2-Nitrofluorene	-	50			310	
AF2	-	0.1				1000
2-Anthramine	+	20	700	28	8920	450
					3000	4000
Pre-chlorination	-	0.25 ml	39	22	32	66
DPS	+	0.25	26	19	41	96
Post-chlorination	-	0.005 ml	40	15	36	43
DPS	-	0.01	45	16	21	68
	-	0.025	51	16	29	61
	-	0.05	34	13	30	72
	-	0.1	39	14	29	71
	-	0.25	30	21	26	66
	+	0.005	17	20	29	73
	+	0.01	25	14	24	71
	+	0.025	20	11	19	72
	+	0.05	20	21	45	71
	+	0.1	32	28	53	94
	+	0.25	25	36	66	142
						250

* Photolyzed.

Table A-32
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
N,N-DIMETHYL-P-PHENYLENEDIAMINE SULFATE*
EXPERIMENT 24

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		35	16	29	38	100
	+		24	18	33	30	133
Positive controls							
β-Propiolactone	-	50 µg	1740				
9-Aminoacridine	-	100		168			
2-Nitrofluorene	-	50			200		
AF2	-	0.1					
2-Anthramine	+	20	T†	432	~6000	236 840T	1200 ~8000
Pre-chlorination DPS	-	0.25 ml	31	12	23	49	118
	+	0.25	26	111	102	123	215
Post-chlorination DPS	-	0.005 ml	34	18	19	25	86
	-	0.01	53	25	24	31	87
	-	0.025	41	17	23	20	105
	-	0.05	37	15	21	35	128
	-	0.1	34	12	26	42	144
	-	0.25	24	14	19	44	317
	+	0.005	17	24	29	41	140
	+	0.01	21	21	36	48	127
	+	0.025	14	35	59	70	141
	+	0.05	19	40	98	158	123
	+	0.1	23	71	116	146	172
	+	0.25	22	119	142	171	171

* Photolyzed.

† T, Toxic.

Table A-33
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
N,N-DIMETHYL-p-PHENYLEDIAMINE SULFATE
 EXPERIMENT 33

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		37	6	5	21 98
	+		17	7	16	26 106
Positive controls						
β-Propiolactone	-	50	950			
9-Aminoacridine	-	100		239		
2-Nitrofluorene	-	50			1260	
AF2	-	0.1				21 137
2-Anthramine	+	20	324	14	585 2400	1530
Pre-chlorination	-	1.4	29	1	11	18 109
DPS	-	3.5	35	3	15	19 92
	-	7	30	7	11	36 89
	-	14	26	11	12	45 82
	-	35	34	28	15	15 93
	+	1.4	10	10	87	87 110
	+	3.5	21	15	268	391 93
	+	7	14	20	457	531 117
	+	14	10	14	625	805 127
	+	35	8	42	901	958 147

Table A-33 (Concluded)

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		37	6	5	21 98
	+		17	7	16	26 106
Positive controls						
8-Propiolactone	-	50	950			
9-Aminoacridine	-	100		239		
2-Nitrofluorene	-	50			1260	
AF2	-	0.1				21 137
2-Anthramine	+	20	324	14	585	2400 1530
Post-chlorination						
DPS	-	1	41	2	12	23 109
	-	2.5	29	2	7	21 96
	-	5	37	7	7	9 100
	-	10	27	6	11	29 85
	-	25	31	5	14	38 113
	+	1	13	9	18	27 102
	+	2.5	20	7	19	15 73
	+	5	13	6	13	17 83
	+	10	11	5	13	9 88
	+	25	13	6	11	12 93

Table A-34
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
N,N-DIMETHYL-P-PHENYLENEDIAMINE SULFATE
EXPERIMENT 45

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1537	TA1538	TA98	TA100
Negative control	-		15	16	32	147
	+		18	28	42	141
Positive controls 9-Aminoacridine 2-Nitrofluorene AF2 2-Anthramine	-	100	3141			
	-	50		1475		
	-	0.1			139	885
	-	2.5	26	37	41	193
	+	2.5	277	2384	877	2334
Pre-chlorination DPS	-	6.7	14	25	27	164
	-	13.4	11	15	27	152
	-	20.1	23	23	24	158
	-	26.8	14	9	20	152
	-	33.5	14	13	23	157
	+	6.7	32	104	92	186
	+	13.4	21	182	168	167
	+	20.1	24	285	206	183
	+	26.8	26	308	282	185
	+	33.5	22	341	243	147

Table A-34 (concluded)

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Post-chlorination DPS	-	5.7		13	22	38	135
	-	11.4		10	17	35	151
	-	17.1		11	14	39	144
	-	22.8		17	14	29	148
	-	28.5		18	20	33	171
	+	5.7		63	1410	1297	221
	+	11.4		78	2562	1952	156
	+	17.1		68	2574	2659	224
	+	22.8		99	3186	3055	301
	+	28.5		148	3021	3266	311

Table A-35
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
7-50 LAP
EXPERIMENT 29

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate		
			TA1537	TA1538	TA98
Negative control	-		35	12	16
	+		5	14	40
Positive controls					
β-Propiolactone	-	50	1000		
2-Nitrofluorene	-	50		1200	
AF2	-	0.1			28
2-Anthramine	+	20	325	480	1400
Pre-chlorination 7-50 LAP	-	9	22	104	C*
	+	9	11	41	46
Post-chlorination 7-50 LAP	-	0.18	25	13	30
	-	0.36	19	14	41
	-	0.9	14	27	53
	-	1.8	28	58	78
	-	3.6	26	99	167
	-	9.0	30	233	308
	+	0.18	12	10	41
	+	0.36	11	62	31
	+	0.9	27	20	33
	+	1.8	11	31	37
	+	3.6	17	34	43
	+	9.	26	76	88

* C, Contaminated.

Table A-36
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
7-50 LAP

EXPERIMENT 38

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		95	11	13	23
	+		130	12	19	29
Positive controls						
β-Propiolactone	-	50	298			
9-Aminoacridine	-	100		1165		
2-Nitrofluorene	-	50			882	
AF2	-	0.1				840
2-Anthramine	+	20	427	99	1866	2910
Pre-chlorination						
7-50 LAP	-	0.41	71	10	41	55
	-	0.82	112	16	80	65
	-	2.05	133	48	138	165
	-	4.1	127	38	236	249
	-	8.2	128	85	480	414
	-	21	161	158	775	920
	+	0.41	232	12	514	40
	+	0.82	213	14	40	C*
	+	2.05	192	24	323	C*
	+	4.1	236	16	248	C*
	+	8.2	199	28	55	59
	+	21	216	50	111	106

* C, Contaminated.

Table A-36 (concluded)

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Post-chlorination 7-50 LAP	-	0.41	70	10	59	55	107
	-	0.82	86	11	104	80	110
	-	2.05	96	32	299	196	115
	-	4.1	96	54	429	285	124
	-	8.2	92	104	741	564	163
	-	21	65	160	968	1116	265
	+	0.41	130	8	23	30	53
	+	0.82	173	7	25	31	90
	+	2.05	150	5	33	31	69
	+	4.1	147	9	33	40	82
	+	8.2	144	8	47	65	87
	+	21	125	K*	105	153	192

* K, Killing.

Table A-37
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIMUM
7-50 LAP
EXPERIMENT 44

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		13	4	6	22
	+		9	5	11	26
Positive controls						
Sodium azide	-	1	238	1152		
9-Aminoacridine	-	100			1608	
2-Nitrofluorene	-	50				1080
AF2	-	0.1				998
2-Anthramine	+	2.5	104	67	726	
Pre-chlorination	-	0.85	C*	C	157	94
7-50 LAP	-	1.69	33	19	225	211
	-	2.54	29	35	308	224
	-	3.38	22	41	406	336
	-	4.23	19	64	340	405
	+	0.85	19	12	28	36
	+	1.69	17	15	40	41
	+	2.54	11	12	38 C	49
	+	3.38	15	13	46	55
	+	4.23	15 C	14	74	73
						0

* C, Contaminated.

Table A-37 (concluded)

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TAL535	TAL537	TAL538	TA98	TAL100
Negative control	-		13	4	6	22	133
	+		9	5	11	26	139
Positive controls							
Sodium azide	-	1	238	1152			
9-Aminoacridine	-	100			1608		
2-Nitrofluorene	-	50				494	1080
AF2	-	0.1				705	998
2-Anthramine	+	2.5	104	67	726		
Post-chlorination							
7-50 LAP	-	0.85	24	22	65	81	181
	-	1.69	30	27C	126	107	161
	-	2.54	20	29	172	154	203
	-	3.38	27	39	208	170	284
	-	4.23	22	58	224	215	326
	+	0.85	C	10	28	26	151
	+	1.69	20	14	15	25	175
	+	2.54	13	11	42	41	169
	+	3.38	13	8	49	53	185
	+	4.23	24	15	51	64	214

Table A-38
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
7-50 LAP
EXPERIMENT 34

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Reverants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		52	16	13	38	333
	+		87	71	9	57	340
Positive controls							
β-Propiolactone	-	50	1590				
9-Aminoacridine	-	100		475			
2-Nitrofluorene	-	50			2054		
AF2	-	0.1				46	275
2-Anthramine	+	20	516	760	2800	3000	C*
Pre-ozonation 7-50 LAP	-	9.8	36	45	163	217	355
	+	9.8	28	18	60	71	345
Post-ozonation 7-50 LAP	-	0.1	56	15	10	30	304
	-	0.3	57	13	11	41	307
	-	0.7	56	16	25	39	292
	-	1.4	54	22	33	48	304
	-	2.8	56	17	71	103	320
	-	7.1	46	26	145	185	328
	+	0.1	24	18	13	28	351
	+	0.3	27	12	21	39	383
	+	0.7	28	17	14	33	352
	+	1.4	32	14	23	43	326
	+	2.8	36	17	35	54	330
	+	7.1	25	14	64	74	353

* C, Contaminated.

Table A-39
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
7-50 LAP
EXPERIMENT 37

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		7	10	11	14	92
	+		5	7	9	19	77
Positive controls							
β-Propiolactone	-	50	262				
9-Aminoacridine	-	100		1405			
2-Nitrofluorene	-	50			1130	400	1176
AF2	-	0.1				1836	1290
2-Anthramine	+	20	330	247	1825		
Pre-ozonation	-	1.6	21	7	46	55	110
7-50 LAP	-	3.2	9	22	80	85	120
	-	4.8	11	37	144	130	123
	-	6.4	16	43	204	205	152
	-	8	21	68	290	269	196
	+	1.6	10	3	4	21	104
	+	3.2	12	2	7	21	95
	+	4.8	7	5	8	15	89
	+	6.4	5	1	9	17	86
	+	8	7	3	10	17	102

Post-ozonation 7-50 LAP contaminated.

Table A-40
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
7-50 LAP
EXPERIMENT 44

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		13	4	6	22 133
	+		9	5	11	26 139
Positive controls						
Sodium azide	-	1	238			
9-Aminoacridine	-	100		1152		
2-Nitrofluorene	-	50			1608	
AF2	-	0.1				
2-Anthramine	+	2.5	104	67	726	494 1080
Pre-ozonation	-					
7-50 LAP	+	7.9	21	38	185	295 340
		7.9	20	16	37	45 267
Post-ozonation	-					
7-50 LAP	-	1.5	21	11	106	90 190
	-	3.1	18	37	144	146 237
	-	4.6	28	29	197	174 301
	-	6.2	24	46	200	202 333
	-	7.7	20	51	290	198 408
	+	1.5	12	5	15	29 201
	+	3.1	19	10	23	47 204
	+	4.6	14	12	30	37 253
	+	6.2	15	13	39	52 271
	+	7.7	27	16	61	59 255

Table A-41
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
7-100 LAP*
EXPERIMENT 20

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate	
			TA98	
Negative control	-		52	
	+		52	
Positive controls				
AF2	-		450	
2-Anthramine	+	0.1 µg 20	3000	
Pre-chlorination	-		750	
7-100 LAP	+	0.25 ml 0.25	70	
Post-chlorination	-		58	
7-100 LAP	-	0.005 ml 0.01	42	
	-	0.025	98	
	-	0.05	173	
	-	0.1	368	
	-	0.25	484	
	+	0.005	42	
	+	0.01	46	
	+	0.025	42	
	+	0.05	37	
	+	0.1	65	
	+	0.25	87	

* 100% Photolysis.

Table A-42
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

7-100 LAP*

EXPERIMENT 22

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		42	22	45	185
	+		32	22	60	246
Positive controls						
β-Propiolactone	-	50 μg	1830			
9-Aminoacridine	-	100		1500		
2-Nitrofluorene	-	50			1065	
AF2	-	0.1				3000
2-Anthramine	+	20	2010	1515	6000	7000
Pre-chlorination†						
7-100 LAP	-	0.005 ml	72	11	25	286
	-	0.01	60	18	30	270
	-	0.025	62	15	41	295
	-	0.05	43	17	67	244
	-	0.1	36	13	79	262
	-	0.25	52	29	184	353
	+	0.005	36	18	47	254
	+	0.01	25	19	31	280
	+	0.025	28	18	36	250
	+	0.05	25	8	29	288
	+	0.1	27	20	40	314
	+	0.25	30	18	77	230

* 100% Photolysis.

+ No post-chlorination was done.

Table A-43
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
7-100 LAP*
EXPERIMENT 44

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		13	4	6	22	133
	+		9	5	11	26	139
Positive controls							
Sodium azide	-	1 µg	238				
9-Aminoacridine	-	100		1152			
2-Nitrofluorene	-	50			1608		
AF2	-	0.1				494	1080
2-Anthramine	+	2.5	104	67	726	705	998
Pre-chlorination	-	0.05 ml	29	30	149	131	150
7-100 LAP	-	0.1	28	30	228	191	216
	-	0.15	20	38	306	294	217
	-	0.2	14	49	388	381	257
	-	0.25	25	63	526	425	288
	+	0.05	8	12	22	29	150
	+	0.1	14	14	37	36	147
	+	0.15	13	10	45	55	141
	+	0.2	13	14	64	63	162
	+	0.25	17	17	66	48	175

* 100% Photolized.

Table A-43 (concluded)

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		13	4	6	22	133
	+		9	5	11	26	139
Positive controls							
Sodium azide	-	1 µg	238				
9-Aminoacridine	-	100		1152			
2-Nitrofluorene	-	50			1608		
AF2	-	0.1				494	1080
2-Anthramine	+	2.5	104	67	726	705	998
Post-chlorination							
7-100 LAP	-	0.05 ml	25	13	94	86	167
	-	0.1	29	19	170	142	171
	-	0.15	18	25	238	189	181
	-	0.2	26	39	336	263	237
	-	0.25	15	66	375	305	233
	+	0.05	19	11	20	22	167
	+	0.1	11	9	29	43	155
	+	0.15	12	9	57	45	162
	+	0.2	13	9	41	48	127
	+	0.25	16	14	75	60	158

Table A-44
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
7-100 LAP*
EXPERIMENT 34

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		52	16	13	333
	+		87	17	9	340
Positive controls						
β-Propiolactone	-	50 μg	1590			
9-Aminoacridine	-	100		475		
2-Nitrofluorene	-	50			2054	
AF2	-	0.1				46
2-Anthramine	+	20	516	760	2800	3000
						275 C+
Pre-ozonation	-	0.25 ml	26	68	316	300
7-100 LAP	+	0.25	63	23	80	76
						323 359
Post-ozonation	-	0.005 ml	40	4	11	28
7-100 LAP	-	0.01	66	11	18	32
	-	0.025	42	16	38	31
	-	0.05	49	19	60	78
	-	0.1	60	20	75	86
	-	0.25	52	50	152	220
						270 256
	+	0.005	30	13	11	37
	+	0.01	C	12	16	37
	+	0.025	60	22	16	40
	+	0.05	60	13	32	37
	+	0.1	79	30	50	64
	+	0.25	90	34	95	83
						282

* 100 Photolyzed.
+ C, Contaminated.

Table A-45
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
7-100 LAP*
EXPERIMENT 40

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		20	15	9	19	115
	+		9	14	23	28	119
Positive controls							
8-Propiolactone	-	50 µg	456				
9-Aminoacridine	-	100		546			
2-Nitrofluorene	-	50			1295		
AF2	-	0.1				98	446
2-Anthramine	+	10	604	444	2560	2320	1755
Pre-ozonation							
7-100 LAP	-	0.05 ml	14	27	171	120	159
	-	0.1	27	29	283	209	204
	-	0.15	28	50	440	303	260
	-	0.2	23	66	483	354	292
	-	0.25	12	70	624	417	365
	+	0.05	14	13	28	38	127
	+	0.1	12	11	36	56	101
	+	0.15	27	6	66	50	93
	+	0.2	19	14	62	60	132
	+	0.25	11	8	107	79	170

* 100% Photolysis.

Table A-45 (concluded)

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Post-ozonation 7-100 LAP	-	0.05 ml	45	29	280	187	190
	-	0.1	68	71	491	297	279
	-	0.15	106	82	587	423	350
	-	0.2	105	83	950	493	357
	-	0.25	133	122	897	552	430
	+	0.05	35	19	48	46	143
	+	0.1	44	34	103	71	155
	+	0.15	56	40	122	88	155
	+	0.2	58	35	163	90	193
	+	0.25	56	104	288	120	231

Table A-46
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
9-100 LAP*
EXPERIMENT 22

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		42	22	45	185
	+		32	22	60	246
Positive controls						
8-Propiolactone	-	50 µg	1830			
9-Aminoacridine	-	100		1500		
2-Nitrofluorene	-	50			1065	
AF2	-	0.1				3000
2-Anthramine	+	2.5	2010	1515	6000	7000
Pre-chlorination	-	0.005 ml	55	20	33	271
9-100 LAP	-	0.01	54	20	30	230
	-	0.025	67	16	38	250
	-	0.05	70	19	16	300
	-	0.1	61	22	33	276
	-	0.25	56	23	41	276
	+	0.005	21	15	24	334
	+	0.01	31	21	25	250
	+	0.025	36	34	30	300
	+	0.05	16	17	32	450
	+	0.1	35	22	32	270
	+	0.25	23	19	46	270

* Solution photolyzed.

Table A-47
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
9-100 LAP*
EXPERIMENT 44

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		13	4	6	22 133
	+		9	5	11	26 139
Positive controls						
Sodium azide	-	1 µg	238			
9-Aminoacridine	-	100		1152		
2-Nitrofluorene	-	50			1608	
AF2	-	0.1				
2-Anthramine	+	2.5	104	67	726	494 1080 705 998
Pre-chlorination	-	0.05 ml	20	11	34	38 208
9-100 LAP	-	0.1	38	19	44	40 278
	-	0.15	35	18	83	61 304
	-	0.2	28	36	83	89 291
	-	0.25	22	27	102	92 429
	+	0.05	16	15	15	17 182
	+	0.1	16	8	26	30 170
	+	0.15	14	21	31	28 189
	+	0.2	19	15	30	47 244
	+	0.25	20	16	36	33 255

* Solution photolyzed.

Table A-47 (concluded)

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		13	4	6	22
	+		9	5	11	26
Positive controls						
Sodium azide	-	1 μ g	238			
9-Aminoacridine	-	100		1152		
2-Nitrofluorene	-	50			1608	
AF2	-	0.1				494
2-Anthramine	+	2.5	104	67	726	705
Post-chlorination	-	0.05 ml	22	16	19	28
9-100 LAP	-	0.1	27	8	31	42
	-	0.15	31	24	42	47
	-	0.2	27	20	55	71
	-	0.25	25	28	70	93
	+	0.05	18	5	13	31
	+	0.1	18	9	25	37
	+	0.15	15	15	29	27
	+	0.2	17	12	39	43
	+	0.25	27	15	55	35

Table A-48

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM2,6-DINITROTOLUENE

EXPERIMENT 23

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		29	15	34	49	164
	+		17	24	38	33	187
Positive controls							
8-Propiolactone	-	50	1100				
9-Aminoacridine	-	100		500			
2-Nitrofluorene	-	50			850		
AF2	-	0.1				74	650
2-Anthramine	+	20	570	860	~1300	~1100	~1400
Pre-chlorination	-	45	31	22	22	33	201
2,6-Dinitrotoluene	+	45	26	13	31	46	161
Post-chlorination	-	0.9	31	21	13	37	193
2,6-Dinitrotoluene	-	1.8	36	32	C*	36	181
	-	4.5	45	19	27	41	211
	-	9	38	21	31	29	192
	-	18	34	17	C	C	C
	-	45	C	C	C	C	C
	+	0.9	19	22	32	52	177
	+	1.8	21	13	43	46	209
	+	4.5	23	19	35	53	151
	+	9	29	15	31	58	182
	+	18	24	12	C	C	C
	+	45	C	C	C	C	C

* C, Contaminated.

Table A-49
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,6-DINITROTOLUENE
EXPERIMENT 31

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		17	11	14	14
	+		8	9	15	20
Positive controls						164
β-Propiolactone	-	50				147
9-Aminoacridine	-	100				
2-Nitrofluorene	-	50	650			
AF2	-	0.1		250		
2-Anthramine	+	20			291	
Pre-chlorination						
2,6-Dinitrotoluene	-	15	10	430	1600	21
	+	15	23	10	16	94
			8	6	11	12
						16
						195
						171
Post-chlorination						
2,6-Dinitrotoluene	-	0.3	24	11	7	24
	-	0.6	27	15	4	12
	-	1.5	22	20	11	12
	-	3	57	38	30	32
	-	6	84	64	65	186
	-	15	204	147	230	233
						235
						418
	+	0.3	17	11	15	24
	+	0.6	18	14	33	24
	+	1.5	28	25	26	18
	+	3	47	24	50	32
	+	6	47	60	64	47
	+	15	111	130	110	70
						170
						369

* C, Contaminated.

Table A-50
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,6-DINITROTOLUENE
EXPERIMENT 44

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		13	4	6	22	133
	+		9	5	11	26	139
Positive controls							
Sodium azide	-	1	238				
9-Aminoacridine	-	100		1152			
2-Nitrofluorene	-	50			1608		
AF2	-	0.1					1080
2-Anthramine	+	2.5	104	67	726	494	998
Pre-chlorination	-	0.6	16	7	9	26	127
2,6-Dinitrotoluene	+	0.6	16	5	17	23	166
Post-chlorination	-	0.12	15	4	8	13	140
2,6-Dinitrotoluene	-	0.24	23	6	7	12	128
	-	0.36	20	9	3	18	134
	-	0.48	22	6	6	16	124
	-	0.6	24	4	12	15	115
	+	0.12	13	4	14	17	137
	+	0.24	14	10	14	15	124
	+	0.36	23	6	18	22	169
	+	0.48	26	3	18	22	153
	+	0.6	26	7	26	19	155

Table A-51
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,6-DINITROTOLUENE
EXPERIMENT 27

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		37	28	23	29
	+		12	40	21	44
Positive controls						
β-Propiolactone	-	50	550			
9-Aminoacridine	-	100		430		
2-Nitrofluorene	-	50			65	
AF2	-	0.1				324
2-Anthramine	+	20	332	850	1800	1200
						1900
Pre-ozonation						
2,6-Dinitrotoluene	-	5	23	23	22	23
	+	5	14	22	34	59
Post-ozonation						
2,6-Dinitrotoluene	-	0.04	21	21	13	30
	-	0.08	20	19	13	33
	-	0.2	26	33	21	25
	-	0.4	38	26	14	26
	-	0.8	36	18	13	29
	-	2	37	24	15	33
						102
	+	0.04	11	34	18	31
	+	0.08	20	27	26	37
	+	0.2	20	21	28	24
	+	0.4	15	27	23	43
	+	0.8	17	29	22	38
	+	2	16	25	27	52
						119

Table A-52
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,6-DINITROTOLUENE
EXPERIMENT 34

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative Control	-		52	16	13	38
	+		87	17	9	57
Positive controls						
β-Propiolactone	-	50	1590			
9-Aminoacridine	-	100		475		
2-Nitrofluorene	-	50			2054	
AF2	-	0.1				
2-Anthramine	+	20	516	760	2800	46
						3000
						275
						C*
Pre-Ozonation	-	35	64	12	9	33
2,6-Dinitrotoluene	+	35	C	12	14	35
						320
						340
Post-Ozonation	-	5.6	73	8	11	33
2,6-Dinitrotoluene	-	11.2	57	15	11	29
	-	16.8	64	4	13	27
	-	22.4	55	13	15	32
	-	28	87	12	9	32
						335
	+	5.6	52	20	8	32
	+	11.2	65	18	23	32
	+	16.8	102	13	25	38
	+	22.4	66	13	14	40
	+	28	70	6	26	28

* C, Contaminated.

Table A-53
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4-DINITROTOLUENE
EXPERIMENT 18

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		72	28	34	78	116
	+		24	54	41	90	129
Positive controls							
8-Propiolactone	-	50	~800	C*			
9-Aminoacridine	-	100					
2-Nitrofluorene	-	50			C		
AF2	-	0.1				~ 600	~1500
2-Anthramine	+	20	~600	~550	~1500	~1000	~2000
Pre-chlorination	-	53	61	34	28	72	125
2,4-Dinitrotoluene	+	53	C	75	C	117	C
Post-chlorination	-	1	88	37	32	83	120
2,4-Dinitrotoluene	-	2	63	24	27	75	137
	-	5	57	27	24	97	106
	-	10	74	C	36	89	161
	-	20	57	C	C	C	166
	-	50	C	C	C	C	C
	+	1	C	70	44	112	119
	+	2	C	96	45	86	99
	+	5	C	C	43	103	106
	+	10	C	C	37	106	103
	+	20	C	C	C	135	162
	+	50	C	C	C	C	158
					43	C	119

* C, Contaminated.

Table A-54
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4-DINITROTOLUENE
EXPERIMENT 28

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		20	15	9	85	117
	+		21	10	22	74	121
Positive controls							
	-	50	2400				
	-	100		1000			
	-	50			1500		500
	-	0.1				260	2000
2-Anthramine	+	20	336	81	270	1200	
Pre-chlorination 2,4-Dinitrotoluene	-	35	24	19	21	91	132
	+	35	25	18	27	102	174
Post-chlorination 2,4-Dinitrotoluene	-	0.67	38	14	12	102	180
	-	1.34	27	8	24	96	132
	-	3.35	31	17	21	90	136
	-	6.7	16	15	15	94	141
	-	13.4	13	17	14	68	157
	-	33.5	15	11	16	93	160
	+	0.67	35	18	17	108	179
	+	1.34	32	15	13	87	129
	+	3.35	45	12	16	107	114
	+	6.7	25	8	23	80	135
	+	13.4	25	9	19	91	148
	+	33.5	31	18	18	80	133

Table A-55
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4-DINITROTOLUENE
EXPERIMENT 39

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		67	11	22	32	92
	+		102	13	16	29	114
Positive controls							
	-	50	157	1252			
	-	100			9		554
	-	50				218	2138
2-Nitrofluorene	-	0.1					
AF2	-		381	79	777	1632	
2-Anthramine	+						
Pre-chlorination 2,4-Dinitrotoluene	-	1.2	104	11	9	38	115
	-	3.1	80	13	16	29	89
	-	6.2	104	14	10	24	84
	-	12.3	90	4	8	23	75
	-	30.8	82	5	11	25	97
	-						
	+	1.2	65	11	17	35	110
	+	3.1	77	18	19	34	86
	+	6.2	78	12	16	27	70
	+	12.3	86	14	25	38	76
	+	30.8	65	17	17	40	83

Table A-55 (concluded)

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Post-chlorination 2,4-Dinitrotoluene	-	1.2	89	9	8	30	101
	-	3.1	83	7	2	22	99
	-	6.2	95	5	8	21	110
	-	12.3	85	13	6	29	111
	-	30.8	93	8	11	19	96
	+	1.2	100	9	13	29	103
	+	3.1	119	12	18	27	91
	+	6.2	103	11	17	35	83
	+	12.3	118	19	14	46	100
	+	30.8	106	10	17	39	102

Table A-56

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
3,5-DINITROTOLUENE
 EXPERIMENT 17

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		42	23	15	125 166
	+		29	28	41	174 163
Positive controls						
β-Propiolactone	-	50	~1700			
9-Aminoacridine	-	100		50		
2-Nitrofluorene	-	50			~3500	
AF2	-	0.1				591 ~1750
2-Anthramine	+	20	~ 720	348	~7500	~800 >10000
Pre-chlorination	-	32	42	15	26	113 127
3,5-Dinitrotoluene	+	32	21	19	26	125 99
Post-chlorination	-	0.61	61	17	18	120 163
3,5-Dinitrotoluene	-	1.22	47	12	11	182 145
	-	3.05	49	16	20	118 176
	-	6.1	50	13	21	129 166
	-	12.2	49	29	19	117 153
	-	30.5	48	12	30	121 184
	+	0.61	24	31	30	104 181
	+	1.22	16	28	23	141 169
	+	3.05	21	23	25	135 159
	+	6.1	20	29	22	119 156
	+	12.2	20	23	21	101 87
	+	30.5	23	30	27	134 138

Table A-57
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
3,5-DINITROTOLUENE
EXPERIMENT 18

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		72	28	34	78
	+		24	54	41	90
Positive controls						
β-Propiolactone	-	50	800			
9-Aminoacridine	-	100		C*		
2-Nitrofluorene	-	50				
AF2	-	0.1				
2-Anthramine	+	20				
Pre-chlorination	-	4.2				
3,5-Dinitrotoluene	+	4.2	86	46	31	97
			32	70	27	92
Post-chlorination	-	0.08	84	36	31	109
3,5-Dinitrotoluene	-	0.16	105	36	47	82
	-	0.4	93	39	27	85
	-	0.8	121	35	24	65
	-	1.6	110	36	31	C
	-	4	87	42	25	70
	+	0.08	32	96	49	90
	+	0.16	34	69	52	100
	+	0.4	29	80	C	97
	+	0.8	40	65	41	83
	+	1.6	28	71	C	73
	+	4	37	59	C	96

* C, Contaminated.

Table A-58
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROTOLUENE
EXPERIMENT 18

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		72	28	34	78
	+		24	54	41	90
Positive controls						
β-Propiolactone	-	50	~800			
9-Aminoacridine	-	100		C*		
2-Nitrofluorene	-	50			C	
AF2	-	0.1				
2-Anthramine	+	20	~600	~550	~1500	~600
						~1000
						~1500
						~2000
Pre-chlorination	-	35	56	26	25	102
2,4,6-Trinitrotoluene	+	35	29	54	23	73
Post-chlorination	-	0.67	68	23	27	79
2,4,6-Trinitrotoluene	-	1.34	82	22	37	90
	-	3.35	75	23	31	63
	-	6.7	57	23	28	89
	-	13.4	75	28	33	97
	-	33.5	47	24	34	108
	+	0.67	25	61	38	83
	+	1.34	27	39	33	79
	+	3.35	22	46	24	77
	+	6.7	31	54	32	94
	+	13.4	27	59	39	74
	+	33.5	22	57	32	71

* C, Contaminated.

Table A-59
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROTOLUENE
EXPERIMENT 28

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		20	15	9	85
	+		21	10	22	74
Positive controls						
β-Propiolactone	-	50	2400			
9-Aminoacridine	-	100		1000		
2-Nitrofluorene	-	50			1500	
AF2	-	0.1				500
2-Anthramine	+	20	336	81	270	1200
Pre-chlorination						
2,4,6-Trinitrotoluene	-	21	32	17	30	66
	+	21	29	15	18	75
Post-chlorination						
2,4,6-Trinitrotoluene	-	0.4	25	11	20	75
	-	0.8	17	6	22	99
	-	2	46	10	18	84
	-	4	23	22	25	74
	-	8	38	17	18	84
	-	20	34	16	22	82
	+	0.4	38	16	22	90
	+	0.8	25	15	25	93
	+	2	26	21	18	78
	+	4	32	21	23	82
	+	8	35	20	24	88
	+	20	33	27	13	71

Table A-60

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROTOLUENE
 EXPERIMENT 27

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		37	28	23	83
	+		12	40	21	108
Positive controls						
β-Propiolactone	-	50	550			
9-Aminoacridine	-	100		430		
2-Nitrofluorene	-	50			65	
AF2	-	0.1				324
2-Anthramine	+	20	332	850	1800	1900
Pre-ozonation	-	7.5	33	27	15	103
2,4,6-Trinitrotoluene	+	7.5	17	17	29	131
Post-ozonation	-	0.13	46	26	19	97
2,4,6-Trinitrotoluene	-	0.26	41	24	18	92
	-	0.65	34	21	13	C*
	-	1.3	44	26	21	46
	-	2.6	35	17	20	33
	-	6.5	43	33	19	28
	+	0.13	23	24	17	39
	+	0.26	9	33	24	29
	+	0.65	20	38	31	54
	+	1.3	15	29	30	43
	+	2.6	25	22	24	53
	+	6.5	18	27	35	44

* C, Contaminated.

Table A-61
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROTOLUENE
EXPERIMENT 36

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control	-		28	5	9	22	C*
	+		14	2	15	21	C
Positive controls							
	-	50	224				C
	-	100		900			C
	-	50			1300		C
	-	0.1				451	C
2-Anthramine	+	20	416	322	1955	2200	C
Pre-ozonation 2,4,6-Trinitrotoluene	-	9.3	11	1	17	21	C
	+	9.3	5	3	17	19	C
Post-ozonation 2,4,6-Trinitrotoluene	-	1.6	29	4	11	21	C
	-	3.2	23	6	9	28	C
	-	4.8	23	7	6	22	C
	-	6.4	18	8	12	17	C
	-	8	22	6	12	29	C
	+	1.6	12	4	10	14	C
	+	3.2	11	6	6	16	C
	+	4.8	10	5	7	16	C
	+	6.4	10	6	6	9	C
	+	8	6	6	7	26	C

* C, Contaminated.

Table A-62
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROTOLUENE
EXPERIMENT 40

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate	
			TAL00	
Negative control	-		115	
	+		119	
Positive controls				
AF2	-	0.1	446	
2-Anthramine	+	10.1	1755	
Pre-ozonation	-	8.9	150	
2,4,6-Trinitrotoluene	+	8.9	154	
Post-ozonation	-	1.5	163	
2,4,6-Trinitrotoluene	-	3.0	164	
	-	4.5	186	
	-	6.0	151	
	-	7.5	153	
	+	1.5	130	
	+	3.0	173	
	+	4.5	181	
	+	6.0	174	
	+	7.5	194	

Table A-63
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITRORESORCINOL
EXPERIMENT 17

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		42	23	15	125 166
	+		29	28	41	174 163
Positive controls						
8-Propiolactone	-	50	-1700			
9-Aminoacridine	-	100		50		
2-Nitrofluorene	-	50			~3500	
AF-2	-	0.1				591 ~1750
2-Anthramine	+	20	~720	348	~7500	~800 >10,000
Pre-chlorination	-	191	45	24	26	112 165
2,4,6-Trinitroresorcinol	-	191	34	15	20	121 161
Post-chlorination	-	3.6	54	15	14	141 153
2,4,6-Trinitroresorcinol	-	7.2	58	23	15	117 158
	-	18	66	17	13	114 166
	-	36	71	19	15	119 183
	-	72	67	15	16	115 188
	-	180	47	16	23	116 161
	+	3.6	25	15	31	122 141
	+	7.2	18	16	33	121 143
	+	18	24	22	19	167 147
	+	36	28	19	29	108 156
	+	72	28	20	30	119 144
	+	180	36	19	27	134 161

Table A-64
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITRORESORCINOL
EXPERIMENT 28

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		20	15	9	85	117
	+		21	10	22	74	121
Positive controls							
β-Propiolactone	-	50	2400				
9-Aminoacridine	-	100		1000			
2-Nitrofluorene	-	50			1500		500
AF-2	-	0.1				260	2000
2-Anthramine	+	20	336	81	270	1200	
Pre-chlorination	-	130	56	13	8	83	151
2,4,6-Trinitroresorcinol	+	130	22	16	24	50	155
Post-chlorination	-	2.5	32	26	17	91	144
2,4,6-Trinitroresorcinol	-	5	38	9	16	68	126
	-	12.5	19	14	14	100	150
	-	25	39	15	16	70	119
	-	50	35	9	18	60	176
	-	125	51	11	16	81	146
	+	2.5	21	21	18	68	144
	+	5	33	20	18	48	136
	+	12.5	25	11	19	53	163
	+	25	20	13	18	38	170
	+	50	17	9	16	45	170
	+	125	30	15	21	80	179

Table A-65
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITRORESORCINOL
EXPERIMENT 27

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		34	7	12	14
	+		14	6	24	25
Positive controls						
	-	50	547	400	700	200
	-	100				300
	-	50				1200
	-	0.1				
AF-2	-	20	108	11	54	38
2-Anthramine	+					C
Pre-ozonation 2,4,6-Trinitroresorcinol	-	218.8	34	6	18	84
	+	218.8	15	12	27	C
Post-ozonation 2,4,6-Trinitroresorcinol	-	4.3	39	9	16	40
	-	8.6	36	4	30	68
	-	21.5	35	5	18	24
	-	42.9	29	11	23	23
	-	85.8	34	13	23	22
	-	214.4	33	28	37	20
	-					91
	-					74
Post-ozonation 2,4,6-Trinitroresorcinol	+	4.3	22	9	37	38
	+	8.6	21	8	34	61
	+	21.5	21	12	19	37
	+	42.9	30	11	39	25
	+	85.8	22	9	39	41
	+	214.4	27	23	25	40
	+					C

* C, Contaminated.

Table A-66
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITRORESORCINOL
EXPERIMENT 35

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		30	8	13	22
	+		18	12	19	39
Positive controls						
8-Propiolactone	-	50	1050			
9-Aminoacridine	-	100		218		
2-Nitrofluorene	-	50			2000	24
AF2	-	0.1				73
2-Anthramine	+	20	500	1270	2300	2540
Pre-ozonation	-	77	30	13	12	19
2,4,6-Trinitroresorcinol	+	77	22	10	21	33
						53
						C*
Post-ozonation	-	14	49	10	24	28
2,4,6-Trinitroresorcinol	-	28	52	18	25	38
	-	42	43	26	28	44
	-	56	42	46	27	40
	-	70	57	43	38	46
						78
	+	14	60	40	60	33
	+	28	23	22	50	40
	+	42	45	C	52	46
	+	56	29	40	45	65
	+	70	40	40	55	49
						101
						97

* C, Contaminated.

Table A-67
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITRORESORCINOL
 EXPERIMENT 40

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate	
			TA100	
Negative control	-		115	
	+		119	
Positive controls				
AF2	-	0.1	446	
2-Anthramine	+	10	1755	
Pre-ozonation	-	219	120	
2,4,6-Trinitroresorcinol	+	219	106	
Post-ozonation	-	4	137	
2,4,6-Trinitroresorcinol	-	9	130	
	-	21	144	
	-	43	139	
	-	86	120	
	-	214	148	
	+	4	105	
	+	9	117	
	+	21	100	
	+	43	116	
	+	86	116	
	+	214	123	

Table A-68
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROBENZONITRILE*
EXPERIMENT 26

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	- +		50 19	20 35	41 43	28 54 144 137
Positive controls						
β-Propiolactone	-	50 µg	1500			
2-Nitrofluorene	-	50			324	
9-Aminoacridine	-	100		440		
AF2	-	0.1				1500
2-Anthramine	+	20	217	500	3000	600 1000 1500 6000
Pre-chlorination	-	0.25 ml	61	11	22	95
2,4,6-Trinitrobenzonitrile*	+	0.25	29	24	34	106 218 310
Post-chlorination	-	0.005 ml	48	17	27	33
2,4,6-Trinitrobenzonitrile*	-	0.01	51	20	25	41
	-	0.025	57	23	27	30
	-	0.05	46	15	24	46
	-	0.1	38	21	23	50
	-	0.25	51	18	21	51 180
	+	0.005	15	33	46	43
	+	0.01	15	42	22	45
	+	0.025	20	23	28	41
	+	0.05	19	19	33	38
	+	0.1	27	38	31	51
	+	0.25	15	41	31	58 174 194

* 100% Decomposition to picric acid and other products.

Table A-69
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROBENZONITRILE*
EXPERIMENT 31

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		17	11	14	14	164
	+		8	9	15	20	147
Positive controls							
8-Propiolactone	-	50 µg	650				
9-Aminoacridine	-	100		250			
2-Nitrofluorene	-	50			291		
AF2	-	0.1				21	560
2-Anthramine	+	20	10	430	1600	94	1500
Pre-chlorination	-	0.25 ml	41	12	18	22	780
2,4,6-Trinitrobenzonitrile	+	0.25	17	13	23	18	585
Post-chlorination	-	0.05 ml	33	5	18	23	249
2,4,6-Trinitrobenzonitrile	-	0.1	37	8	20	17	348
	-	0.15	25	6	14	23	430
	-	0.2	54	9	9	34	496
	-	0.25	34	6	20	20	557
	+	0.05	14	10	19	18	236
	+	0.1	13	12	19	20	365
	+	0.15	28	12	17	25	415
	+	0.2	17	9	20	15	515
	+	0.25	37	7	20	35	558

* 100% Decomposition to picric acid and other products.

Table A-70
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROBENZONITRILE*
EXPERIMENT 38

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TA1537	TA1538	TA98	TA100
Negative control	-		11	13	24	121
	+		12	19	29	128
Positive controls						
β-Propiolactone	-	50 μg				
9-Aminoacridine	-	100	1165			
2-Nitrofluorene	-	50		882		
AF2	-	0.1			300	840
2-Anthramine	+	20	99	1166	2685	2910
Pre-chlorination	-	0.05 ml	10	15	34	152
2,4,6-Trinitrobenzonitrile	-	0.1	16	14	30	137
	-	0.15	7	43	0	222
	-	0.2	12	41	0	218
	-	0.25	11	25	11	230
	+	0.05	8	34	43	98
	+	0.1	13	22	42	102
	+	0.15	13	28	18	75
	+	0.2	19	33	40	116
	+	0.25	14	40	67	176

* 100% Decomposition to picric acid and other compounds.

Table A-70 (concluded)

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TA1537	TA1538	TA98	TA100
Post-chlorination 2,4,6-Trinitrobenzonitrile	-	0.05 ml	20	14	50	161
	-	0.1	25	28	81	176
	-	0.15	40	49	130	207
	-	0.20	35	52	163	265
	-	0.25	13	21	130	313
	+	0.05	21	30	36	120
	+	0.1	12	26	48	98
	+	0.15	15	30	33	150
	+	0.2	14	36	50	136
	+	0.25	20	33	88	140

Table A-71

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 26

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		50	20	41	28
	+		19	35	43	54
Positive controls						
β-Propiolactone	-	50 μg	1500			
9-Aminoacridine	-	100		440		
2-Nitrofluorene	-	50			324	
AF2	-	0.1				1500
2-Anthramine	+	20	217	500	3000	1000
						600
						1500
						6000
Pre-ozonation	-	0.25 ml	45	13	22	116
2,4,6-Trinitrobenzonitrile	+	0.25	32	27	37	83
						261
						296
Post-ozonation	-	0.005 ml	50	19	40	43
2,4,6-Trinitrobenzonitrile	-	0.01	32	22	25	36
	-	0.025	54	16	22	57
	-	0.05	69	18	18	60
	-	0.1	45	20	32	80
	-	0.25	50	17	31	226
						540
	+	0.005	25	28	42	42
	+	0.01	22	27	40	58
	+	0.025	23	23	40	35
	+	0.05	25	28	35	48
	+	0.1	26	22	32	77
	+	0.25	27	31	41	119
						550

* 100% Decomposition to picric acid and other compounds.

Table A-72

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROBENZONITRILE*
 EXPERIMENT 36

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA1539	TA100
Negative control	-		28	5	9	22	76
	+		14	2	15	21	82
Positive controls							
8-Propiolactone	-	50 µg	224				
9-Aminoacridine	-	100		900			
2-Nitrofluorene	-	50			1300		
AF2	-	0.1				451	1200
2-Anthramine	+	20	416	322	1955	2200	900
Pre-ozonation	-	0.005 ml	23	6	11	20	C+
2,4,6-Trinitrobenzonitrile	-	0.01	21	1	12	27	C
	-	0.025	18	6	7	27	C
	-	0.05	20	2	19	30	C
	-	0.1	20	8	20	54	C
	-	0.25	22	23	28	72	C
	+	0.005	8	4	4	23	C
	+	0.01	16	7	9	23	C
	+	0.025	32	5	6	18	C
	+	0.05	11	5	15	30	C
	+	0.1	13	6	12	26	C
	+	0.25	4	9	15	70	C

* 100% Decomposition to picric acid and other compounds.

+ C, Contaminated.

Table A-72 (concluded)

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Post-ozonation* 2,4,6-Trinitrobenzonitrile	-	0.005 ml	18	4	6	18	C
	-	0.01	15	13	8	19	C
	-	0.025	11	2	7	15	C
	-	0.05	13	7	5	42	C
	-	0.1	15	8	15	46	C
	-	0.25	11	10	21	110	C
	+	0.005	8	0	6	8	C
	+	0.01	10	1	6	4	C
	+	0.025	13	2	8	14	C
	+	0.05	14	3	2	6	C
	+	0.1	8	2	6	5	C
	+	0.25	7	8	4	9	C

* 100% Hydrolysis.

Table A-73

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 40

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate	
			TAL00	
Negative control	-		115	
	+		119	
Positive controls				
AF2	-	0.1 µg	446	
2-Anthramine	+	10	1755	
Pre-ozonation	-	0.005 ml	118	
2,4,6-Trinitrobenzonitrile	-	0.01	117	
	-	0.025	137	
	-	0.05	141	
	-	0.1	152	
	-	0.25	160	
	+	0.005	124	
	+	0.01	139	
	+	0.025	106	
	+	0.05	132	
	+	0.1	159	
	+	0.25	153	

* 100% Decomposition to picric acid and other compounds.

Table A-73 (concluded)

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate	
			Tal00	
Post-ozonation 2,4,6-Trinitrobenzonitrile	-	0.005 ml	133	
	-	0.01	140	
	-	0.025	127	
	-	0.05	141	
	-	0.1	129	
	-	0.25	142	
	+	0.005	92	
	+	0.01	133	
	+	0.025	150	
	+	0.05	119	
	+	0.1	92	
	+	0.25	126	

Table A-74
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROBENZALDEHYDE
EXPERIMENT 27

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		37	28	23	29
	+		12	40	21	44
Positive controls						
β-Propiolactone	-	50	550			
9-Aminoacridine	-	100		430		
2-Nitrofluorene	-	50			65	
AF2	-	0.1				324
2-Anthramine	+	20	332	850	1800	1200
Pre-chlorination	-					
2,4,6-Trinitrobenzaldehyde	+	2.9	57	36	19	48
		2.9	13	40	31	40
Post-chlorination	-					
2,4,6-Trinitrobenzaldehyde	-	0.06	44	28	22	30
	-	0.12	25	30	19	28
	-	0.3	37	25	18	33
	-	0.6	40	25	21	28
	-	1.2	61	29	28	33
	-	2.9	53	32	27	45
						112
	+	0.06	14	C	23	40
	+	0.12	17	C	23	34
	+	0.3	18	46	29	33
	+	0.6	20	46	25	47
	+	1.2	26	C	33	38
	+	2.9	43	36	33	47

* C, Contaminated.

Table A-75
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROBENZALDEHYDE
EXPERIMENT 31

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		17	11	14	164
	+		8	9	15	147
Positive controls						
β-Propiolactone	-	50	650	250		
9-Aminoacridine	-	100			291	
2-Nitrofluorene	-	50				560
AF2	-	0.1				1500
2-Anthramine	+	20	10	430	1600	
Pre-chlorination	-	15	18	14	51	267
2,4,6-Trinitrobenzaldehyde	+	15	12	8	20	230
Post-chlorination	-	2.2	84	60	60	257
	-	4.4	105	110	128	312
	-	6.6	122	189	190	379
	-	8.8	119	216	237	460
	-	11	152	227	210	455
	+	2.2	91	48	61	202
2,4,6-Trinitrobenzaldehyde	+	4.4	153	72	120	320
	+	6.6	157	133	182	360
	+	8.8	209	135	245	380
	+	11	198	219	240	380

Table A-76
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROBENZALDEHYDE
EXPERIMENT 37

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA98	TA100
Negative control	-		7	10	14	92
	+		5	7	18	77
Positive controls						
β-Propiolactone	-	50	262			
9-Aminoacridine	-	100		1405		
2-Nitrofluorene	-	50				
AF2	-	0.1			400	1176
2-Anthramine	+	20	330	247	1836	1290
Pre-chlorination	-	57	16	3	3	244
2,4,6-Trinitrobenzaldehyde	+	57	11	9	86	209
Post-chlorination	-	10	11	12	150	274
2,4,6-Trinitrobenzaldehyde	-	20	22	10	C	368
	-	30	21	3	13	414
	-	40	20	4	4	247
	-	50	23	0	9	280
	+	10	11	6	44	127
	+	20	10	8	78	177
	+	30	9	5	92	194
	+	40	14	8	84	215
	+	50	13	12	126	221

Table A-77
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROBENZALDEHYDE
EXPERIMENT 45

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate	
			TAL535	
Negative control	-		30	
	+		37	
Positive controls				
Sodium azide	-	1.0	632	
2-Anthramine	-	2.5	48	
	+	2.5	247	
Pre-chlorination	-	14.8	75	
2,4,6-Trinitrobenzaldehyde	+	14.8	26	
Post-chlorination	-	2.5	35	
2,4,6-Trinitrobenzaldehyde	-	5.0	39	
	-	7.6	50	
	-	10.1	70	
	-	12.6	72	
	+	2.5	29	
	+	5.0	32	
	+	7.6	29	
	+	10.1	30	
	+	12.6	39	

Table A-78
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROBENZALDEHYDE
EXPERIMENT 27

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		37	28	23	29	83
	+		12	40	21	44	108
Positive controls							
β-Propiolactone	-	50	550				
9-Aminoacridine	-	100		430			
2-Nitrofluorene	-	50			65		
AF2	-	0.1				257	324
2-Anthramine	+	20	332	850	1800	1200	1900
Pre-ozonation	-	3.2	31	25	14	41	111
2,4,6-Trinitrobenzaldehyde	+	3.2	13	30	33	45	133
Post-ozonation	-	0.04	17	52	20	26	117
2,4,6-Trinitrobenzaldehyde	-	0.08	15	18	23	30	109
	-	0.2	40	21	22	33	93
	-	0.4	31	31	17	52	115
	-	0.8	30	35	26	42	112
	-	2	37	21	14	49	122
	+	0.04	15	44	26	37	99
	+	0.08	17	43	27	41	113
	+	0.2	15	39	29	44	120
	+	0.4	21	38	24	38	116
	+	0.8	18	32	36	37	132
	+	2	16	23	24	C*	146

* C, Contaminated.

Table A-79
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
2,4,6-TRINITROBENZALDEHYDE
EXPERIMENT 36

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		28	5	8	C*
	+		14	2	15	C
Positive controls						
8-Propiolactone	-	50	224			C
9-Aminoacridine	-	100		900		C
2-Nitrofluorene	-	50			1300	C
AF2	-	0.1			451	C
2-Anthramine	+	20	416	322	1955	C
Pre-ozonation	-	17.8	43	50	145	C
2,4,6-Trinitrobenzaldehyde	+	17.8	15	7	15	C
Post-ozonation	-	3.2	33	8	31	C
2,4,6-Trinitrobenzaldehyde	-	6.4	28	22	60	C
	-	9.6	28	25	77	C
	-	12.8	33	24	126	C
	-	16	35	44	146	C
	+	3.2	12	6	10	C
	+	6.4	13	6	12	C
	+	9.6	15	5	18	C
	+	12.8	11	6	12	C
	+	16	11	7	20	C

* C, Contaminated.

Table A-80

IN VITRO ASSAYS WITH *SALMONELLA* TYPHIMURIUM
2,4,6-TRINITROBENZALDEHYDE
 EXPERIMENT 40

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		20	15	9	19	115
	+		9	14	23	28	119
Positive controls							
8-Propiolactone	-	50	456				
9-Aminoacridine	-	100		546			
2-Nitrofluorene	-	50			1295		
AF2	-	0.1				98	446
2-Anthramine	+	10	604	444	2560	2320	1755
Pre-ozonation							
2,4,6-Trinitrobenzaldehyde	-	3.3	27	13	48	38	196
	-	6.5	16	15	56	84	249
	-	9.8	24	11	84	150	308
	-	13.1	17	33	137	243	297
	-	16.4	19	22	287	345	407
	+	3.3	12	7	29	17	100
	+	6.5	11	13	25	27	157
	+	9.8	8	15	27	44	178
	+	13.1	20	13	17	38	212
	+	16.4	19	15	24	34	189

Table A-80 (concluded)

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate	
			TA100	
Post-ozonation	-	2.7		260
2,4,6-Trinitrobenzaldehyde	-	5.4		350
	-	8.1		390
	-	10.8		463
	-	13.5		452
	+	2.7		167
	+	5.4		192
	+	8.1		244
	+	10.8		298
	+	13.5		293

APPENDIX B

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

APPENDIX B--IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE

B-1	1,3,5-Trinitrobenzene--Experiment 25.	138
B-2	1,3,5-Trinitrobenzene--Experiment 30.	139
B-3	1,3,5-Trinitrobenzene--Experiment 26.	140
B-4	1,3,5-Trinitrobenzene--Experiment 35.	141
B-5	1,3-Dinitrobenzene--Experiment 23	142
B-6	1,3-Dinitrobenzene--Experiment 30	143
B-7	1,3-Dinitrobenzene--Experiment 27	144
B-8	1,3-Dinitrobenzene--Experiment 36	145
B-9	Trinitroglycerine--Experiment 25	146
B-10	Trinitroglycerine--Experiment 34	147
B-11	PETN--Experiment 24	148
B-12	PETN--Experiment 32	149
B-13	PETN--Experiment 26	150
B-14	PETN--Experiment 35	151
B-15	Condensate Water--Experiment 19	152
B-16	Condensate Water--Experiment 29	153
B-17	Syringaldazine--Experiment 20	154
B-18	Syringaldazine--Experiment 33	155
B-19	HMX--Experiment 21.	156
B-20	HMX--Experiment 30.	157
B-21	RDX--Experiment 21.	158
B-22	RDX--Experiment 29.	159
B-23	DPO--Experiment 19.	160
B-24	DPO--Experiment 24.	161
B-25	DPO--Experiment 33.	162
B-26	DPS--Experiment 19.	163
B-27	DPS--Experiment 24.	164
B-28	DPS--Experiment 33.	165
B-29	7-50 LAP--Experiment 20	166
B-30	7-50 LAP--Experiment 29	167

B-31	7-50 LAP--Experiment 25.	168
B-32	7-50 LAP--Experiment 34.	169
B-33	7-50 LAP--Experiment 41.	170
B-34	7-100 LAP--Experiment 20	171
B-35	7-100 LAP--Experiment 44	172
B-36	7-100 LAP--Experiment 25	173
B-37	7-100 LAP--Experiment 34	174
B-38	9-100 LAP--Experiment 44	175
B-39	2,6-Dinitrotoluene--Experiment 21.	176
B-40	2,6-Dinitrotoluene--Experiment 23.	177
B-41	2,6-Dinitrotoluene--Experiment 27.	178
B-42	2,6-Dinitrotoluene--Experiment 34.	179
B-43	2,4-Dinitrotoluene--Experiment 18.	180
B-44	2,4-Dinitrotoluene--Experiment 28.	181
B-45	3,5-Dinitrotoluene--Experiment 17.	182
B-46	3,5-Dinitrotoluene--Experiment 18.	183
B-47	2,4,6-Trinitrotoluene--Experiment 18	184
B-48	2,4,6-Trinitrotoluene--Experiment 28	185
B-49	2,4,6-Trinitrotoluene--Experiment 27	186
B-50	2,4,6-Trinitrotoluene--Experiment 36	187
B-51	2,4,6-Trinitroresorcinol--Experiment 17.	188
B-52	2,4,6-Trinitroresorcinol--Experiment 28.	189
B-53	2,4,6-Trinitroresorcinol--Experiment 28.	190
B-54	2,4,6-Trinitroresorcinol--Experiment 35.	191
B-55	2,4,6-Trinitrobenzonitrile--Experiment 26.	192
B-56	2,4,6-Trinitrobenzonitrile--Experiment 31.	193
B-57	2,4,6-Trinitrobenzonitrile--Experiment 26.	194
B-58	2,4,6-Trinitrobenzonitrile--Experiment 36.	195
B-59	2,4,6-Trinitrobenzaldehyde--Experiment 27.	196
B-60	2,4,6-Trinitrobenzaldehyde--Experiment 31.	197
B-61	2,4,6-Trinitrobenzaldehyde--Experiment 27.	198
B-62	2,4,6-Trinitrobenzaldehyde--Experiment 36.	199
B-63	<u>Saccharomyces cerevisiae</u> Experimental Positive Controls	200

Table B-1

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE*
1,3,5-TRINITROBENZENE

EXPERIMENT 25

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		5.8	100	6.0	10
	+		5.0	100	3.5	7.0
Pre-chlorination	-	0.007	6.2	107	4.0	6.5
1,3,5-Trinitrobenzene	+	0.007	6.3	126	5.0	7.9
Post-chlorination	-	0.007	6.7	116	7.0	10
1,3,5-Trinitrobenzene	-	0.0035	6.4	110	5.0	7.8
	-	0.0017	6.0	103	4.0	6.7
	-	0.0007	5.9	102	9.0	15
	-	0.00014	6.1	105	3.0	4.9
	+	0.007	6.3	126	6.0	9.5
	+	0.0035	6.1	122	10.0	16
	+	0.0017	5.5	110	7.0	13.0
	+	0.0007	6.3	126	7.0	11.0
	+	0.00014	6.9	138	6.0	8.7

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The positive control values for the *Saccharomyces cerevisiae* D3 experiments are presented on Table 148.

Table B-2

IN VITRO ASSAYS WITH SACHAROMYCES CEREVISIAE
1,3,5-TRINITROBENZENE
 EXPERIMENT 30

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.1	100	6.3	10
	+		6.9	100	6.3	9.1
Pre-chlorination 1,3,5-Trinitrobenzene	-	0.012	7.2	118	5.0	7.0
	+	0.012	7.5	109	7.0	9.3
Post-chlorination 1,3,5-Trinitrobenzene	-	0.012	7.3	120	6.0	8.2
	-	0.006	8.3	136	3.0	3.6
	-	0.003	7.7	126	15	19
	-	0.0012	6.6	108	5.0	7.6
	-	0.00024	6.9	113	18	26
	+	0.012	7.4	107	8.0	11
	+	0.006	8.1	117	11	14
	+	0.003	7.6	110	6.0	7.9
	+	0.0012	9.3	135	19	20
	+	0.00024	8.4	122	7.0	8.3

Table B-3

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
1,3,5-TRINITROBENZENE

EXPERIMENT 26

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		12.0	100	7.5	6.3
	+		9.1	100	8.5	9.3
Pre-ozonation 1,3,5-Trinitrobenzene	-	0.015	4.6	38	5.0	11
	+	0.015	4.5	49	5.0	11
Post-ozonation 1,3,5-Trinitrobenzene	-	0.014	4.6	38	4.0	8.7
	-	0.007	6.0	50	5.0	8.3
	-	0.0035	5.0	42	5.0	10
	-	0.0014	5.1	43	6.0	12
	-	0.0003	4.0	33	1.0	2.5
	+	0.014	4.7	52	3.0	6.4
	+	0.007	4.5	49	6.0	13
	+	0.0035	4.2	46	5.0	12
	+	0.0014	4.8	53	5.0	10
	+	0.0003	4.0	44	4.0	10

Table B-4

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
1,3,5-TRINITROBENZENE

EXPERIMENT 35

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.6	100	3.8	5.8
	+		8.0	100	3.7	4.6
Pre-ozonation 1,3,5-Trinitrobenzene	-	0.014	7.1	108	8.0	11
	+	0.014	6.8	85	5.0	7.4
Post-ozonation 1,3,5-Trinitrobenzene	-	0.014	6.7	102	5.0	7.5
	-	0.0105	7.1	108	4.0	5.6
	-	0.007	7.1	108	8.0	11
	-	0.0035	6.3	95	5.0	7.9
	-	0.0014	8.1	123	4.0	4.9
	+	0.014	6.3	79	3.0	4.8
	+	0.0105	8.3	104	6.3	7.6
	+	0.007	8.8	110	9.0	10
	+	0.0035	6.6	83	9.0	14
	+	0.0014	C*	C	C	C

* C, Contaminated

Table B-5

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE*
1,3-DINITROBENZENE

EXPERIMENT 23

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.7	100	7.0	10
	+		6.1	100	10	16
Pre-chlorination 1,3-Dinitrobenzene	-	0.005	6.4	96	6.0	9.4
	+	0.005	8.0	131	5.0	6.0
Post-chlorination 1,3-Dinitrobenzene	-	0.005	6.2	93	6.0	9.7
	-	0.0025	6.2	93	9.0	15
	-	0.0012	5.8	87	6.0	10
	-	0.0005	6.1	91	11	18
	-	0.0001	6.9	103	4.0	5.8
	+	0.005	7.8	128	8.0	10
	+	0.0025	5.1	84	8.0	16
	+	0.0012	5.9	97	6.0	10
	+	0.0005	5.0	82	8.0	16
	+	0.0001	6.5	107	11	17

Table B-6
IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
1,3-DINITROBENZENE
EXPERIMENT 30

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.1	100	6.3	10
	+		6.9	100	6.3	9.1
Pre-chlorination 1,3-Dinitrobenzene	-		7.9	130	7.0	8.9
	+	0.0074 0.0074	7.8	113	18	23
Post-chlorination 1,3-Dinitrobenzene	-	0.0074	C*	C	C	C
	-	0.0037	8.2	134	5.0	6.1
	-	0.0018	9.1	149	5.0	5.5
	-	0.00074	6.0	98	7.0	12
	-	0.00015	6.9	113	14	20
	+	0.0074	8.2	119	11	13
	+	0.0037	9.1	132	6.3	6.9
	+	0.0018	8.7	126	8.0	9.2
	+	0.00074	6.4	93	4.0	6.3
	+	0.00015	5.9	86	6.0	10

* C, Contaminated

Table B-7

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
1,3-DINITROBENZENE
 EXPERIMENT 27

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		4.4	100	3.5	8.0
	+		4.6	100	3.5	7.6
Pre-ozonation 1,3-Dinitrobenzene	-	0.002	10.0	227	5.0	5.0
	+	0.002	4.6	100	3.0	6.5
Post-ozonation 1,3-Dinitrobenzene	-	0.0015	4.2	95	5.0	12
	-	0.0008	4.3	98	2.0	4.7
	-	0.0004	5.0	114	2.0	4.0
	-	0.00015	4.4	100	3.0	6.8
	-	0.00003	3.9	89	1.3	3.3
	+	0.0015	4.4	96	1.0	2.3
	+	0.0008	5.4	117	9.0	17
	+	0.0004	6.4	139	3.0	4.7
	+	0.00015	4.5	98	4.0	8.9
	+	0.00003	4.1	89	6.0	15

Table B-8

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE*
1,3-DINITROBENZENE

EXPERIMENT 36

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Survivors
Negative control	-		7.6	100	5.0	6.6
	+		8.8	100	7.0	8.0
Pre-ozonation 1,3-Dinitrobenzene	-	0.004	7.9	104	5.0	6.3
	+	0.004	6.8	77	8.8	13
Post-ozonation 1,3-Dinitrobenzene 145	-	0.0036	7.4	97	3.0	4.1
	-	0.0027	7.2	95	8.0	11
	-	0.0018	7.9	104	5.0	6.3
	-	0.0009	6.4	84	5.0	7.8
	-	0.00036	5.2	68	6.3	12
	+	0.0036	7.0	80	5.0	7.1
	+	0.0027	7.3	83	3.0	4.1
	+	0.0018	7.9	90	6.7	8.5
	+	0.0009	7.5	85	11	15
	+	0.00036	6.2	70	3.8	6.1

Table B-9

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
TRINITROGLYCERINE*

EXPERIMENT 25

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.8	100	6.0	10
	+		5.0	100	3.5	7.0
Pre-chlorination Trinitrolycerine	-	1	6.2	107	2.0	3.2
	+	1	6.3	126	4.0	6.3
Post-chlorination Trinitrolycerine	-	1	6.5	112	6.0	9.2
	-	0.5	6.6	114	7.0	11.0
	-	0.25	6.3	109	6.0	9.5
	-	0.1	6.7	116	14	21
	-	0.02	5.5	95	3.0	5.5
	+	1	6.2	124	3.0	4.3
	+	0.5	6.4	128	1.0	1.6
	+	0.25	6.4	128	2.0	3.1
	+	0.1	5.9	118	1.0	1.7
	+	0.02	5.8	116	7.0	12

* Saturated solution

Table B-10

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
TRINITROGLYCERINE*

EXPERIMENT 34

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.8	100	13	22
	+		5.2	100	5.8	11
Pre-chlorination Trinitroglycerine	-	1	5.7	98	11	19
	+	1	5.3	98	13	25
Post-chlorination Trinitroglycerine	-	1	5.3	91	12	23
	-	0.75	7.8	134	16	21
	-	0.50	+	+	+	+
	-	0.25	6.4	110	7.5	12
	-	0.1	6.0	103	13	22
	+	1	4.5	87	8.0	18
	+	0.75	4.7	90	7.0	15
	+	0.50	5.2	100	8.0	15
	+	0.25	6.3	121	3.0	4.8
	+	0.1	9.3	179	9.0	9.7

* Saturated solution
+Dilution error

Table B-11

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
PENTAERYTHRITOL TETRANITRATE*

EXPERIMENT 24

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.9	100	5.5	9.3
	+		5.4	100	6.5	12
Pre-chlorination PETN	-	1	6.1	103	6.0	9.8
	+	1	6.0	111	2.0	3.3
Post-chlorination PETN	-	1	4.9	83	4.0	8.2
	-	0.5	5.2	88	4.0	7.7
	-	0.25	4.6	78	7.0	15
	-	0.1	6.1	103	4.0	6.6
	-	0.02	6.1	103	7.0	11
	+	1	9.2	170	6.0	6.5
	+	0.5	5.2	96	7.0	13
	+	0.25	5.5	102	5.0	9.1
	+	0.1	6.4	119	7.0	11
	+	0.02	6.1	113	5.0	8.2

* Saturated solution

Table B-12

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
PENTAERYTHRITOL TETRANITRATE*

EXPERIMENT 32

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		5.0	100	5.5	11
	+		5.7	100	2.5	4.4
Pre-chlorination PETN	-	1	5.6	112	4.0	7.1
	+	1	4.8	84	4.0	8.3
Post-chlorination PETN	-	1	5.4	108	3.0	5.6
	-	0.75	5.7	114	11	19
	-	0.5	5.5	110	2.0	3.6
	-	0.25	6.4	128	2.0	3.1
	-	0.1	5.8	116	3.0	5.2
	+	1	5.7	100	5.0	8.8
	+	0.75	6.4	112	1.0	1.6
	+	0.5	5.5	96	4.0	7.3
	+	0.25	6.4	112	2.0	3.1
	+	0.1	5.2	91	2.0	3.8

* Saturated solution

Table B-13

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
PENTAERYTHRITOL TETRANITRATE*

EXPERIMENT 26

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		12.0	100	7.5	6.3
	+		9.1	100	8.5	9.3
Pre-ozonation PETN	-	1	5.2	43	2.0	3.8
	+	1	4.8	53	11	23
Post-ozonation PETN	-	1	8.0	67	4.0	5.0
	-	0.5	5.3	44	7.0	13
	-	0.25	5.6	47	11	20
	-	0.1	5.2	43	9.0	17
	-	0.02	4.9	41	5.0	10
	+	1	5.5	60	8.0	15
	+	0.5	5.1	56	3.0	5.9
	+	0.25	7.2	79	7.0	9.7
	+	0.1	4.8	53	8.0	17
	+	0.02	4.8	53	4.0	8.3

* Saturated solution

Table B-14

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
PENTAERYTHRITOL TETRANITRATE*

EXPERIMENT 35

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.6	100	3.8	5.8
	+		8.0	100	3.7	4.6
Pre-ozonation PETN	-	1	6.8	103	3.0	4.4
	+	1	7.5	94	3.0	4.0
Post-ozonation PETN	-	1	7.2	109	4.0	5.6
	-	0.75	6.2	94	6.0	9.7
	-	0.5	7.2	109	5.0	6.9
	-	0.25	6.8	103	5.0	7.4
	-	0.1	6.9	105	7.0	10
151	+	1	6.9	86	6.0	8.7
	+	0.75	5.0	63	7.0	14
	+	0.5	7.5	94	5.0	6.7
	+	0.25	5.5	69	5.0	9.1
	+	0.1	8.5	106	5.0	5.9

* Saturated solution

Table B-15
IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
CONDENSATE WATER
 EXPERIMENT 19

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		7.8	100	3.0	3.9
	+		6.6	100	3.0	4.6
Pre-chlorination Condensate water	-	0.0037	7.3	94	7.0	9.6
	+	0.0037	6.4	98	6.0	9.4
Post-chlorination Condensate water	-	0.0037	6.5	84	6.0	9.2
	-	0.0019	6.9	88	3.0	4.4
	-	0.0009	7.1	91	1.0	1.4
	-	0.00037	6.7	86	6.0	9.0
	-	0.00007	7.5	97	7.0	9.3
	+	0.0037	7.4	112	4.0	5.4
	+	0.0019	7.1	108	6.0	8.5
	+	0.0009	5.9	90	9.0	15
	+	0.00037	7.1	108	2.0	2.8
	+	0.00007	7.0	106	1.0	1.4

Table B-16

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
CONDENSATE WATER
EXPERIMENT 29

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.3	100	5.0	9.4
	+		5.9	100	3.5	6.0
Pre-chlorination Condensate water	-	0.0027	5.2	98	2.0	3.9
	+	0.0027	5.6	95	4.0	7.1
Post-chlorination Condensate water	-	0.0027	4.1	77	3.0	7.3
	-	0.002	5.6	106	3.0	5.4
	-	0.0014	4.6	87	8.0	17
	-	0.0007	6.0	113	3.3	5.5
	-	0.00027	5.3	100	5.0	9.4
	+	0.0027	5.2	88	1.0	1.9
	+	0.002	4.8	81	6.0	13
	+	0.0014	5.5	93	5.0	9.1
	+	0.0007	6.1	103	8.0	13
	+	0.00027	4.5	76	7.5	17

Table B-17

IN VITRO ASSAYS WITH SACHAROMYCES CEREVISIAE
SYRINGALDAZINE*

EXPERIMENT 20

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		6.8	100	6.0	8.9
	+		6.6	100	2.5	3.8
Pre-chlorination Syringaldazine	-	1	7.3	108	5.0	6.9
	+	1	6.2	93	1.0	1.6
Post-chlorination Syringaldazine	-	1	6.2	91	4.0	6.5
	-	0.5	6.2	91	4.0	6.5
	-	0.25	5.6	83	3.0	5.4
	-	0.1	6.1	91	8.0	13
	-	0.02	C†	C	C	C
	+	1	7.1	107	6.0	8.5
	+	0.5	6.6	99	3.0	4.6
	+	0.25	4.3	66	4.0	9.2
	+	0.1	6.3	96	8.3	13
	+	0.02	5.5	83	9.0	16

* Compound photolyzed.
†C, Contaminated.

Table B-18

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
SYRINGALDAZINE
EXPERIMENT 33

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		4.6	100	9.9	22
	+		4.8	100	12	25
Pre-chlorination Syringaldazine	-	0.0049	4.1	89	2.0	4.9
	+	0.0049	4.4	92	<1.0	2.3
Post-chlorination Syringaldazine	-	0.0032	9.1	198	6.0	6.6
	-	0.0024	5.1	111	7.0	14
	-	0.0016	5.2	113	10	19
	-	0.0008	C*	C	C	C
	-	0.00032	5.9	128	5.0	8.5
	+	0.0032	5.7	119	8.8	15
	+	0.0024	4.8	100	8.0	17
	+	0.0016	4.4	92	6.0	14
	+	0.0008	C	C	C	C
	+	0.00032	4.4	92	7.5	17

* C, Contaminated

Table B-19

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
HMX*
 EXPERIMENT 21

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants Per ml ($\times 10^{-3}$)	Survivors Per 10^5
			Cells per ml ($\times 10^{-7}$)	Percent		
Negative control	-		6.5	100	6.5	10
	+		6.0	100	5.0	8.3
Pre-chlorination HMX	-	1	7.2	111	7.0	9.7
	+	1	7.3	120	9.0	12
Post-chlorination HMX	-	1	6.8	105	8.0	12
	-	0.5	6.5	100	10	15
	-	0.25	7.7	118	8.0	10
	-	0.1	8.4	129	13	15
	-	0.02	9.2	142	5.0	5.4
	+	1	6.6	110	4.0	6.0
	+	0.5	6.5	107	5.0	7.7
	+	0.25	7.4	123	5.0	6.7
	+	0.1	7.3	121	6.0	8.2
	+	0.02	7.2	119	6.0	8.3

* Saturated solution

Table B-20

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
HMX*

EXPERIMENT 30

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.1	100	6.3	10
	+		6.9	100	6.3	9.1
Pre-chlorination HMX	-	1	6.2	102	6.0	9.7
	+	1	6.1	88	6.0	9.9
Post-chlorination HMX	-	1	7.2	118	4.0	5.6
	-	0.5	7.1	116	3.8	5.3
	-	0.25	9.8	161	4.0	4.1
	-	0.1	8.2	134	6.0	7.3
	-	0.02	6.1	100	5.0	8.2
	+	1	6.3	91	1.0	1.6
	+	0.5	6.1	88	6.0	9.9
	+	0.25	7.9	114	10	13
	+	0.1	9.3	135	2.5	2.7
	+	0.02	7.0	101	2.0	2.9

* Saturated solution

Table R-21

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE*
RDX

EXPERIMENT 21

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.5	100	6.5	10
	+		6.0	100	5.0	8.3
Pre-chlorination RDX	-	0.0019	7.2	111	7.0	9.7
	+	0.0019	5.7	94	3.0	5.3
Post-chlorination RDX	-	0.0019	6.0	92	6.0	10
	-	0.0010	6.2	95	4.0	6.5
	-	0.0005	6.5	100	4.0	6.1
	-	0.00019	6.2	96	11	18
	-	0.00004	C*	C	C	C
	+	0.0019	5.8	96	6.0	10
	+	0.0010	6.0	99	8.0	13
	+	0.0005	6.1	101	7.0	11
	+	0.0019	C	C	C	C
	+	0.0004	C	C	C	C

* C, Contaminated

Table B-22

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE*
RDX

EXPERIMENT 29

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-3}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		5.3	100	5.0	9.4
	+		5.9	100	3.5	6.0
Pre-chlorination RDX	-	0.0023	6.0	113	3.0	5.0
	+	0.0023	6.6	112	4.0	6.1
Post-chlorination RDX	-	0.0023	7.1	134	4.0	5.7
	-	0.0017	5.0	94	2.0	4.0
	-	0.0011	6.2	117	5.0	8.1
	-	0.0006	5.3	100	1.0	1.9
	-	0.00023	6.5	123	1.0	1.5
	+	0.0023	5.8	98	4.0	6.9
	+	0.0017	5.0	85	5.0	9.9
	+	0.0011	4.9	83	5.0	10
	+	0.0006	6.0	102	1.0	1.7
	+	0.00023	6.4	121	4.0	6.3

Table 3-23

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE*
N,N-DIETHYL-P-PHENYLENEDIAMINE OXALATE*

EXPERIMENT 19

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		7.8	100	3.0	3.9
	+		6.6	100	3.0	4.6
Pre-chlorination DPO	-	1	1.6	20	2.0	13
	+	1	3.7	56	6.0	16
Post-chlorination DPO	-	1	1.1	14	1.0	9.1
	-	0.5	6.2	80	4.0	6.4
	-	0.25	5.4	69	3.0	5.6
	-	0.1	6.0	77	4.0	6.7
	-	0.02	5.8	74	3.0	5.2
	+	1	2.4	37	2.0	8.3
	+	0.5	5.4	82	4.0	7.4
	+	0.25	6.0	92	3.0	5.0
	+	0.1	5.8	89	4.0	6.9
	+	0.02	6.0	91	4.0	6.7

* Compound photolyzed

Table B-24

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE*
N,N-DIETHYL-*p*-PHENYLENEDIAMINE OXALATE*

EXPERIMENT 24

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.9	100	5.5	9.3
	+		5.3	100	6.5	12
Pre-chlorination DPO	-	1	T†	T	T	T
	+	1	2.3	43	2.0	8.7
Post-chlorination DPO	-	1	T	T	T	T
	-	0.5	T	T	T	T
	-	0.25	0.7	12	1.0	14
	-	0.1	3.8	64	2.0	5.3
	-	0.02	5.2	88	6.0	12
	+	1	1.9	36	4.0	21
	+	0.5	1.6	30	2.0	13
	+	0.25	3.7	70	6.0	16
	+	0.1	7.0	132	5.0	7.1
	+	0.02	4.4	83	4.0	9.1

* Compound photolyzed

†T, Toxic

Table B-25

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
N,N-DIETHYL-p-PHENYLENEDIAMINE OXALATE

EXPERIMENT 33

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		4.6	100	9.9	22
	+		4.8	100	12	25
Pre-chlorination DPO	-	0.009	5.1	111	8.0	16
	+	0.009	4.8	100	6.3	13
Post-chlorination DPO	-	0.0072	4.3	93	6.0	14
	-	0.0054	4.9	107	7.0	14
	-	0.0036	4.8	104	4.0	8.3
	-	0.0018	5.1	111	11	22
	-	0.00072	5.0	109	12	24
	+	0.0072	4.4	92	5.0	11
	+	0.0054	5.0	104	7.0	14
	+	0.0036	4.7	98	9.0	19
	+	0.0018	5.1	106	4.0	7.8
	+	0.00072	5.1	106	4.0	7.8

Table P-26

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
N,N-DIMETHYL-p-PHENYLENEDIAMINE SULFATE*

EXPERIMENT 19

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		7.8	100	3.0	3.9
	+		6.6	100	3.0	4.6
Pre-chlorination DPS	-	1	7.5	96	8.0	11
	+	1	5.4	82	4.0	7.5
Post-chlorination DPS	-	1	6.6	85	3.0	4.6
	-	0.5	6.9	88	1.0	1.4
	-	0.25	6.1	78	4.0	6.6
	-	0.1	8.3	106	1.0	1.2
	-	0.02	7.9	101	1.0	1.3
163	+	1	5.5	83	6.0	11
	+	0.5	7.6	115	6.0	7.9
	+	0.25	6.1	92	7.0	11
	+	0.1	8.0	121	2.0	2.5
	+	0.02	7.9	120	2.0	2.5

* Compound photolyzed

Table B-27

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
N,N-DIMETHYL-p-PHENYLENEDIAMINE SULFATE*

EXPERIMENT 24

Compound	Metabolic Activation	Milliliters solution Added	Survivors		Mitotic Recombinants Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
			Cells per ml ($\times 10^{-7}$)	Percent		
Negative control	-		5.9	100	5.5	9.3
	+		5.3	100	6.5	12
Pre-chlorination DPS	-	1	T†	T	T	T
	+	1	2.8	47	6.0	21
Post-chlorination DPS	-	1	T	T	T	T
	-	0.5	T	T	T	T
	-	0.25	1.2	20	1.0	8.3
	-	0.1	3.7	63	4.0	11
	-	0.02	13	22	4.0	3.1
	+	1	2.4	45	1.0	4.2
	+	0.5	1.3	25	1.0	7.7
	+	0.25	2.5	47	3.0	12
	+	0.1	4.9	92	4.0	8.2
	+	0.02	4.7	89	2.0	4.3

* Compound photolyzed

†T, Toxic

Table B-28

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
N,N-DIMETHYL-p-PHENYLENEDIAMINE SULFATE

EXPERIMENT 33

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		4.6	100	9.9	22
	+		4.8	100	12	25
Pre-chlorination DPS	-	0.0056	4.7	102	15	32
	+	0.0056	5.2	108	11	21
Post-chlorination DPS	-	0.004	3.9	85	13	33
	-	0.003	4.3	93	11	26
	-	0.002	5.0	109	10	20
	-	0.001	4.3	93	9.0	21
	-	0.0004	5.0	109	5.0	10
	+	0.004	4.4	92	8.0	18
	+	0.003	4.6	96	11	24
	+	0.002	7.5	156	7.0	9.3
	+	0.001	4.6	96	6.0	13
	+	0.0004	4.8	100	7.0	15

Table B-29

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-50 LAP

EXPERIMENT 20

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		6.8	100	6.0	8.9
	+		6.6	100	2.5	3.8
Pre-chlorination 7-50 LAP	-	0.0068	5.6	83	8.0	14
	+	0.0068	4.9	75	12	24
Post-chlorination 7-50 LAP	-	0.0068	5.9	88	2.0	3.4
	-	0.0034	6.8	100	3.0	4.4
	-	0.0017	5.5	81	7.0	13
	-	0.00068	4.7	69	7.0	15
	-	0.00014	5.8	86	4.0	6.9
	+	0.0068	5.6	85	5.0	9.0
	+	0.0034	4.7	71	6.0	13
	+	0.0017	4.9	74	6.0	12
	+	0.00068	5.5	83	4.0	7.3
	+	0.00014	4.8	73	8.0	17

Table B-30

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-50 LAP

EXPERIMENT 29

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.3	100	5.0	9.4
	+		5.9	100	3.5	6.0
Pre-chlorination 7-50 LAP	-	0.0014	9.9	187	3.8	3.8
	+	0.0014	5.2	88	4.0	7.7
Post-chlorination 7-50 LAP	-	0.0014	5.1	96	6.3	12
	-	0.0011	5.8	109	3.0	5.2
	-	0.00070	5.4	102	3.0	5.6
	-	0.00035	6.0	113	6.0	10
	-	0.00014	5.5	104	1.0	1.8
	+	0.0014	5.1	86	3.0	5.9
	+	0.0011	5.0	85	2.0	4.0
	+	0.00070	5.6	95	4.0	7.2
	+	0.00035	5.6	95	5.0	9.0
	+	0.00014	6.0	102	2.0	3.4

Table B-31

IN VITRO ASSAYS WITH SACHAROMYCES CEREVISIAE
7-50 LAP

EXPERIMENT 25

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		5.8	100	6.0	10
	+		5.0	100	3.5	7.0
Pre-ozonation 7-50 LAP	-	0.00024	4.3	74	8.0	19
	+	0.00024	4.7	94	5.0	11
Post-ozonation 7-50 LAP	-	0.000023	5.8	100	6.0	10
	-	0.000011	5.2	90	6.0	12
	-	0.000006	6.5	112	2.0	3.1
	-	0.000002	5.5	95	6.0	11
	-	0.0000005	4.4	76	5.0	11
	+	0.000023	5.1	102	6.0	12
	+	0.000011	4.9	98	1.0	2.0
	+	0.000006	4.8	96	1.0	2.1
	+	0.000002	4.8	96	4.0	8.3
	+	0.0000005	4.9	98	8.0	16

Table B-32

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-50 LAP

EXPERIMENT 34

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.8	100	13	22
	+		5.2	100	5.8	11
Pre-ozonation 7-50 LAP	-	0.0016	6.2	107	13	21
	+	0.0016	5.5	106	18	33
Post-ozonation 7-50 LAP	-	0.0011	5.4	93	16	30
	-	0.0008	5.9	102	8.0	14
	-	0.0006	5.4	93	11	20
	-	0.0003	5.7	98	7.0	12
	-	0.0001	2.9	50	5.0	17
	+	0.0011	6.5	125	7.0	11
	+	0.0008	7.1	137	11	15
	+	0.0006	4.7	90	3.0	6.4
	+	0.0003	6.0	115	4.0	6.7
	+	0.0001	4.6	88	6.0	13

Table B-33

IN VITRO ASSAYS WITH SACHAROMYCES CEREVISIAE
7-50 LAP

EXPERIMENT 41

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.7	100	2.0	3.5
	+		5.5	100	7.0	13
Pre-ozonation 7-50 LAP	-	.0013	7.0	123	3.0	4.3
	+	.0013	5.1	93	4.0	7.8
Post-ozonation 7-50 LAP	-	.0012	6.9	121	7.0	10
	-	.0006	6.1	107	10	16
	-	.0003	7.1	125	7.0	9.8
	-	.0001	5.6	98	6.0	11
	+	.0012	4.7	85	5.0	11
	+	.0006	5.1	93	2.0	3.9
	+	.0003	5.4	98	6.0	11
	+	.0001	6.5	118	6.0	9.2

Table B-34

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-100 LAP*

EXPERIMENT 20

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml (x 10 ⁻³)	Percent	Per ml (x 10 ⁻³)	Per 10 ³ Survivors
Negative control	-		6.8	100	6.0	8.9
	+		6.6	100	2.5	3.8
Pre-chlorination 7-100 LAP	-	1	6.4	95	12	19
	+	1	5.8	88	6.0	10
Post-chlorination 7-100 LAP	-	1	5.0	74	6.0	12
	-	0.5	6.2	92	1.0	1.6
	-	0.25	6.8	101	2.0	2.9
	-	0.1	7.3	109	2.0	2.7
	-	0.02	6.7	99	6.0	3.0
	+	1	C†	C	C	C
	+	0.5	5.6	85	4.0	7.1
	+	0.25	6.8	103	3.0	4.4
	+	0.1	6.5	99	9.0	14
	+	0.02	7.2	109	4.0	5.6

* Solution saturated

†C, Contaminated

Table B-35
IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-100 LAP*
EXPERIMENT 44

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml (x 10 ⁻⁷)	Percent	Per ml (x 10 ⁻³)	Per 10 ⁵ Survivors
Negative control	-		4.9	100	4.5	9.2
	+		4.7	100	2.0	4.3
Pre-chlorination 7-100 LAP	-	1.0	4.9	100	6.0	12
	+	1.0	5.3	113	<1.0	1.9
Post-chlorination 7-100 LAP	-	1.0	4.0	82	6.0	15
	-	0.5	5.1	104	4.0	7.9
	-	0.25	5.4	110	9.0	17
	-	0.1	6.6	135	4.0	6.0
	-	0.02	3.2	65	4.0	13
	+	1.0	4.8	102	7.0	15
	+	0.5	3.5	74	4.0	11
	+	0.25	+	+	+	+
	+	0.1	4.9	104	2.0	4.1
	+	0.02	5.2	111	4.0	7.7

* 100% Photolyzed
† Dilution error

Table B-36
IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-100 LAP*

EXPERIMENT 25

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.8	100	6.0	10
	+		5.0	100	3.5	7.0
Pre-ozonation 7-100 LAP	-	1	4.8	83	3.0	6.3
	+	1	4.6	92	10	22
Post-ozonation 7-100 LAP	-	1	5.2	90	5.0	9.6
	-	0.5	5.2	90	6.0	12
	-	0.25	5.4	93	2.0	3.7
	-	0.1	5.0	86	3.0	6.0
	-	0.02	5.1	88	8.0	16
	+	1	4.7	94	4.0	8.5
	+	0.5	4.2	84	4.0	9.5
	+	0.25	4.8	96	5.0	10
	+	0.1	5.8	116	6.0	10
	+	0.02	5.2	104	6.0	12

* Saturated solution

Table B-37

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-100 LAP*

EXPERIMENT 34

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		5.8	100	13	22
	+		5.2	100	5.8	11
Pre-ozonation 7-100 LAP	-	1	6.6	114	10	15
	+	1	5.8	112	8.0	14
Post-ozonation 7-100 LAP	-	1	5.3	91	8.0	15
	-	0.75	4.5	78	5.0	11
	-	0.5	4.1	71	11	27
	-	0.25	5.7	98	14	25
	-	0.1	5.2	90	15	29
	+	1	4.3	83	9.0	21
	+	0.75	4.9	94	14	29
	+	0.5	7.0	135	10	14
	+	0.25	C†	C	C	C
	+	0.1	4.7	90	6.7	14

* Saturated solution
†C, Contaminated

Table B-38

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
9-100 LAP*

EXPERIMENT 44

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		4.9	100	4.5	9.2
	+		4.7	100	2.0	4.3
Pre-chlorination 9-100 LAP	-	1.0	4.5	92	1.0	2.2
	+	1.0	6.4	136	6.0	9.4
Post-chlorination 9-100 LAP	-	1.0	6.3	129	2.0	3.2
	-	0.5	5.9	120	7.0	12
	-	0.25	6.3	129	<1.0	1.6
	-	0.1	6.2	127	2.0	3.2
	-	0.02	6.2	127	5.0	8.1
	+	1.0	4.1	87	6.0	14.5
	+	0.5	4.1	87	5.0	12
	+	0.25	3.1	66	1.0	3.3
	+	0.1	4.6	98	6.0	13
	+	0.02	4.7	100	4.0	8.6

*100% Photolyzed

Table B-39

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,6-DINITROTOLUENE

EXPERIMENT 21

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.5	100	6.5	10
	+		6.0	100	5.0	8.3
Pre-chlorination 2,6-Dinitrotoluene	-	0.000096	6.5	100	1.0	1.5
	+	0.000096	6.9	114	5.0	4.4
Post-chlorination 2,6-Dinitrotoluene	-	0.000096	10	157	5.0	4.9
	-	0.000048	7.1	109	6.0	8.5
	-	0.000024	7.9	121	6.0	7.6
	-	0.000096	15	237	2.0	1.3
	-	0.000019	7.2	111	1.0	1.4
	+	0.000096	8.9	147	5.0	5.6
	+	0.000048	8.2	135	4.0	4.9
	+	0.000024	6.3	105	4.0	6.3
	+	0.000096	13	212	2.0	1.6
	+	0.000019	6.0	99	2.0	3.4

Table B-40

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,6-DINITROTOLUENE

EXPERIMENT 23

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.7	100	7.0	10
	+		6.1	100	10	16
Pre-chlorination 2,6-Dinitrotoluene	-	0.007	6.6	99	2.0	3.0
	+	0.007	5.3	87	3.0	5.7
Post-chlorination 2,6-Dinitrotoluene	-	0.007	6.7	100	6.0	9.0
	-	0.0035	5.3	79	5.0	9.4
	-	0.0018	5.2	78	1.0	1.9
	-	0.0007	5.6	84	5.0	8.9
	-	0.00014	5.8	87	3.0	5.2
	+	0.007	6.5	107	2.0	3.1
	+	0.0035	5.5	90	8.0	15
	+	0.0018	5.9	97	6.0	10
	+	0.0007	6.5	107	4.0	6.2
	+	0.00014	5.4	89	8.0	15

Table B-4]

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,6-DINITROTOLUENE
 EXPERIMENT 27

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.4	100	3.0	5.6
	+		4.8	100	4.5	9.4
Pre-ozonation 2,6-Dinitrotoluene	-	0.0008	8.6	159	7.0	8.1
	+	0.0008	4.3	90	4.0	9.3
Post-ozonation 2,6-Dinitrotoluene	-	0.00035	4.2	78	1.0	2.4
	-	0.00018	4.3	80	5.0	12
	-	0.00009	4.0	74	5.0	13
	-	0.000035	4.5	83	2.0	4.4
	-	0.000007	4.0	74	11	28
	+	0.00035	4.1	85	6.0	15
	+	0.00018	4.4	92	3.0	6.8
	+	0.00009	4.7	98	5.0	11
	+	0.000035	4.1	85	2.0	4.9
	+	0.000007	4.0	83	8.0	20

Table B-42

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE*
2,6-DINITROTOLUENE

EXPERIMENT 34

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.8	100	13	22
	+		5.2	100	5.8	11
Pre-ozonation 2,6-Dinitrotoluene	-	0.006	6.0	103	13	22
	+	0.006	5.9	113	9.0	15
Post-ozonation 2,6-Dinitrotoluene	-	0.0048	5.9	102	13	22
	-	0.0036	5.2	90	6.0	12
	-	0.0024	5.9	102	11	19
	-	0.0012	5.3	91	13	25
	-	0.00048	5.3	91	10	19
	+	0.0048	6.1	117	12	20
	+	0.0036	5.4	104	15	28
	+	0.0024	5.5	106	17	31
	+	0.0012	5.7	110	12	21
	+	0.00048	6.0	115	14	23

Table B-43
IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4-DINITROTOLUENE

EXPERIMENT 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.8	100	5.0	8.6
	+		5.5	100	3.5	6.3
Pre-chlorination 2,4-Dinitrotoluene	-	0.008	4.0	69	11	28
	+	0.008	5.2	93	7.0	13
Post-chlorination 2,4-Dinitrotoluene	-	0.008	5.1	87	8.0	16
	-	0.004	4.0	69	9.0	23
	-	0.002	4.8	83	5.0	10.
	-	0.0008	4.2	72	2.0	4.8
	-	0.00016	3.4	58	1.0	3.0
	+		5.5	99	5.0	9.1
	+	0.004	5.4	97	3.0	5.6
	+	0.002	4.4	80	8.0	18
	+	0.0008	5.3	96	4.0	7.5
	+	0.00016	4.2	76	7.0	17

Table B-44

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4-DINITROTOLUENE

EXPERIMENT 28

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		4.8	100	5.5	11
	+		5.9	100	5.5	9.3
Pre-chlorination 2,4-Dinitrotoluene	-	0.006	6.1	127	1.0	1.7
	+	0.006	6.8	115	2.0	2.9
Post-chlorination 2,4-Dinitrotoluene	-	0.006	3.6	75	3.0	8.3
	-	0.003	5.7	119	5.0	8.7
	-	0.0015	5.7	119	1.0	1.8
	-	0.0006	6.1	127	3.0	4.9
	-	0.00012	5.4	113	6.0	11
	+	0.006	4.7	80	<1.0	2.1
	+	0.003	6.0	102	6.0	10
	+	0.0015	6.6	112	3.0	4.5
	+	0.0006	6.0	102	2.0	3.3
	+	0.00012	4.8	81	<1.0	2.1

Table B-45
IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
3,5-DINITROTOLUENE
EXPERIMENT 17

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10 ⁻⁷)	Percent	Per ml (x 10 ⁻³)	Survivors
Negative control	-		5.4	100	8.0	15
	+		6.3	100	6.5	10
Pre-chlorination 3,5-Dinitrotoluene	-	0.005	4.7	88	8.0	17
	+	0.005	5.0	80	4.0	8.0
Post-chlorination 3,5-Dinitrotoluene	-	0.005	5.4	99	5.0	9.3
	-	0.0025	5.3	98	5.0	9.5
	-	0.0015	4.2	78	6.0	14
	-	0.0005	5.2	96	4.0	7.7
	-	0.0001	9.9	184	1.0	1.0
	+	0.005	5.6	89	1.0	1.8
	+	0.0025	5.4	86	7.0	13
	+	0.0015	4.7	75	5.0	11
	+	0.0005	5.7	91	3.0	5.3
	+	0.0001	5.1	81	4.0	7.9

Table B-46

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
3,5-DINITROTOLUENE

EXPERIMENT 18

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		5.8	100	5.0	8.6
	+		5.5	100	3.5	6.3
Pre-chlorination 3,5-Dinitrotoluene	-	0.00067	4.7	82	6.0	13
	+	0.00067	4.8	87	3.0	6.3
Post-chlorination 3,5-Dinitrotoluene	-	0.00067	4.7	81	5.0	11
	-	0.00034	4.2	73	3.0	7.1
	-	0.00017	4.5	77	2.0	4.5
	-	0.000067	3.9	69	2.0	5.2
	-	0.000015	4.3	74	5.0	12
	+	0.00067	4.6	83	5.0	11
	+	0.00034	5.0	90	4.0	8.1
	+	0.00017	4.4	80	2.0	4.6
	+	0.000067	5.2	94	4.0	7.7
	+	0.000015	4.7	86	5.0	11

Table B-48

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROTOLUENE

EXPERIMENT 28

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		4.8	100	5.5	11
	+		5.9	100	5.5	9.3
Pre-chlorination 2,4,6-Trinitrotoluene	-	0.003	6.3	131	8.0	13
	+	0.003	5.1	86	8.3	16
Post-chlorination 2,4,6-Trinitrotoluene	-	0.003	6.5	135	10	15
	-	0.0015	6.1	127	5.0	8.2
	-	0.008	4.9	102	4.0	8.2
	-	0.0003	4.9	102	4.0	8.1
	-	0.00006	5.2	108	6.0	11
	+	0.003	5.4	92	1.0	1.9
	+	0.0015	5.1	86	3.0	5.9
	+	0.008	4.3	73	3.0	5.7
	+	0.0003	5.3	90	3.0	5.7
	+	0.00006	6.2	105	2.0	3.2

Table B-47

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE*
2,4,6-TRINITROTOLUENE

EXPERIMENT 18

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-2}$)	Per 10^5 Survivors
Negative control	-		5.8	100	5.0	8.6
	+		5.5	100	3.5	6.3
Pre-chlorination 2,4,6-Trinitrotoluene	-	0.006	5.4	93	3.0	5.6
	+	0.006	4.9	88	1.0	2.1
Post-chlorination 2,4,6-Trinitrotoluene	-	0.006	5.5	94	5.0	9.1
	-	0.003	4.8	83	6.0	13
	-	0.0015	4.9	84	1.0	2.1
	-	0.0006	5.0	87	5.0	10.0
	-	0.00012	4.8	83	6.0	13
	+	0.006	6.4	116	3.0	4.7
	+	0.003	4.7	85	4.0	8.5
	+	0.0015	4.0	72	5.0	13
	+	0.0006	4.0	73	3.0	7.4
	+	0.00012	5.0	91	3.0	6.0

Table B-49

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROTOLUENE

EXPERIMENT 27

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.4	100	3.0	5.6
	+		4.8	100	4.5	9.4
Pre-ozonation 2,4,6-Trinitrotoluene	-	0.0012	5.3	98	1.0	1.9
	+	0.0012	4.5	94	4.0	8.9
Post-ozonation 2,4,6-Trinitrotoluene	-	0.001	5.5	102	4.0	7.3
	-	0.0005	5.7	106	3.0	5.3
	-	0.00025	4.2	78	7.0	17
	-	0.0001	4.3	80	1.0	2.3
	-	0.00002	4.8	89	2.0	4.2
	+	0.001	5.2	108	3.0	5.8
	+	0.0005	5.2	108	5.0	9.6
	+	0.00025	5.0	104	9.0	18
	+	0.0001	4.8	100	8.0	17
	+	0.00002	4.4	92	6.0	14

Table B-50

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROTOLUENE

EXPERIMENT 36

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		7.6	100	5.0	6.6
	+		8.8	100	7.0	8.0
Pre-ozonation 2,4,6-Trinitrotoluene	-	0.0015	11	145	4.0	3.6
	+	0.0015	9.9	113	6.3	6.4
Post-ozonation 2,4,6-Trinitrotoluene	-	0.0013	9.4	124	2.0	2.1
	-	0.0009	9.0	118	3.0	3.3
	-	0.0006	9.7	128	8.0	8.2
	-	0.0003	9.5	125	3.0	3.2
	-	0.00013	9.7	128	9.0	9.3
	+	0.0013	8.2	93	7.5	9.1
	+	0.0009	9.3	106	7.0	7.5
	+	0.0006	8.3	94	4.0	4.8
	+	0.0003	9.9	113	3.8	3.8
	+	0.00013	10	114	4.0	4.0

Table B-51

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITRORESORCINOL

EXPERIMENT 17

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.4	100	8.0	15
	+		6.3	100	6.5	10
Pre-chlorination 2,4,6-Trinitroresorcinol	-	0.03	6.0	111	8.0	13
	+	0.03	5.2	82	5.0	9.7
Post-chlorination 2,4,6-Trinitroresorcinol	-	0.028	5.8	107	3.0	5.2
	-	0.014	5.3	98	5.0	9.5
	-	0.007	4.9	91	4.0	8.1
	-	0.0028	5.2	96	5.0	9.7
	-	0.00056	5.6	103	5.0	9.0
	+	0.028	5.8	92	5.0	8.7
	+	0.014	5.9	93	5.0	8.5
	+	0.007	5.1	82	5.0	9.8
	+	0.0028	6.0	96	1.0	1.7
	+	0.00056	5.0	79	2.0	4.0

Table B-52

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE*
2,4,6-TRINITRORESORCINOL

EXPERIMENT 28

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		4.8	100	5.5	11
	+		5.9	100	5.5	9.3
Pre-chlorination 2,4,6-Trinitroresorcinol	-	0.02	5.8	121	6.0	10
	+	0.02	6.4	108	4.0	6.3
Post-chlorination 2,4,6-Trinitroresorcinol	-	0.19	6.3	131	2.0	3.2
	-	0.10	5.7	119	4.0	7.0
	-	0.005	7.3	152	2.0	2.7
	-	0.0019	6.8	142	7.0	10
	-	0.0004	7.8	163	4.0	5.1
	+	0.19	6.2	105	9.0	15
	+	0.10	6.3	107	4.0	6.3
	+	0.005	8.2	139	4.0	4.9
	+	0.0019	6.7	114	7.0	10
	+	0.0004	7.9	134	3.0	3.8

Table B-53

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITRORESORCINOL

EXPERIMENT 28

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-3}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		4.4	100	3.5	8.0
	+		4.6	100	3.5	7.6
Pre-ozonation 2,4,6-Trinitroresorcinol	-	0.033	5.3	120	8.0	15
	+	0.033	4.1	89	4.0	9.8
Post-ozonation 2,4,6-Trinitroresorcinol	-	0.029	5.4	123	4.0	7.4
	-	0.015	5.4	123	8.0	15
	-	0.007	7.6	173	4.0	5.3
	-	0.0029	4.6	105	3.0	6.5
	-	0.0006	5.0	114	5.0	10
	+	0.029	5.4	117	7.0	13
	+	0.015	8.7	189	5.0	5.7
	+	0.007	7.5	163	3.0	4.0
	+	0.0029	5.6	122	5.0	8.9
	+	0.0006	4.7	102	6.0	13

Table B-54

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITRORESORCINOL

EXPERIMENT 35

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.6	100	3.8	5.8
	+		8.0	100	3.7	4.6
Pre-ozonation 2,4,6-Trinitroresorcinol	-	0.012	7.5	114	5.0	6.7
	+	0.012	7.6	95	9.0	12
Post-ozonation 2,4,6-Trinitroresorcinol	-	0.010	7.0	106	9.0	13
	-	0.0075	7.4	112	6.0	8.1
	-	0.0050	7.1	108	6.0	8.5
	-	0.0025	6.2	94	6.3	10
	-	0.0010	6.1	92	9.0	15
	+	0.010	5.4	68	3.0	5.6
	+	0.0075	6.4	80	3.0	4.7
	+	0.0050	5.7	71	6.0	11
	+	0.0025	6.1	76	6.0	9.8
	+	0.0010	6.5	81	5.0	7.7

Table B-55

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE*
2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 26

Compound	Metabolic Activation	Survivors		Mitotic Recombinants	
		Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-	12	100	7.5	6.3
	+	9.1	100	8.5	9.3
Pre-chlorination 2,4,6-Trinitrobenzo- nitrile	-	6.3	53	5.0	7.9
	+	5.8	64	4.0	6.9
Post-chlorination 2,4,6-Trinitrobenzo- nitrile	-	5.7	48	3.0	5.3
	0.5	5.5	46	5.0	9.1
	0.25	7.3	61	3.0	4.1
	0.1	20	167	8.0	4.0
	0.02	32	267	3.0	0.9
	+	5.3	58	7.0	13
192	+	6.2	68	9.0	15
	+	6.3	69	8.0	13
	+	12	132	2.0	1.5
	+	16	176	8.0	5.0

* Compound 100% decomposition to picric acid and other compounds.

Table B-56

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 31

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		7.0	100	5.0	7.1
	+		7.0	100	5.5	7.9
Pre-chlorination 2,4,6-Trinitrobenzo- nitrile	-	1	7.7	110	3.0	3.9
	+	1	7.5	107	9.0	12
Post-chlorination 2,4,6-Trinitrobenzo- nitrile	-	1	6.6	94	9.0	14
	-	0.75	6.4	91	4.0	6.3
	-	0.5	7.8	111	5.0	6.4
	-	0.25	7.2	103	4.0	5.6
	-	0.1	5.7	81	3.0	5.3
	+	1	6.5	93	4.0	6.2
	+	0.75	6.8	97	2.0	2.9
	+	0.5	6.1	87	6.0	9.8
	+	0.25	6.5	93	9.0	14
	+	0.1	5.3	76	5.0	9.4

* Compound 100% decomposition into picric acid and other compounds.

Table B-57

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE*
2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 26

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml (x 10 ⁻⁷)	Percent	Per ml (x 10 ⁻³)	Per 10 ⁵ Survivors
Negative control	-		12	100	7.5	6.3
	+		9.1	100	8.5	9.3
Pre-ozonation 2,4,6-Trinitrobenzo- nitrile	-	1	6.3	53	7.0	11
	+	1	5.3	58	2.0	3.8
Post-ozonation 2,4,6-Trinitrobenzo- 194 nitrile	-	1	6.7	56	9.0	13
	-	0.5	6.9	58	3.8	5.5
	-	0.25	6.2	52	4.0	6.5
	-	0.1	7.8	65	2.0	2.6
	-	0.02	6.5	54	5.0	7.7
	+	1	5.2	57	7.0	13
	+	0.5	7.0	77	1.3	1.9
	+	0.25	6.3	69	6.0	9.5
	+	0.1	7.7	85	4.0	5.2
	+	0.02	6.7	74	7.0	10

* Compound 100% decomposition to picric acid and other compounds.

Table B-58

IN VITRO ASSAYS WITH SACCCHAROMYCES CEREVISIAE
2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 36

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		7.6	100	5.0	6.6
	+		8.8	100	7.0	8.0
Pre-ozonation 2,4,6-Trinitrobenzo- nitrile	-	1	7.4	97	4.0	5.4
	+	1	7.9	90	3.0	3.8
Post-ozonation 2,4,6-Trinitrobenzo- nitrile	-	1	9.8	129	7.0	7.1
	-	0.75	9.9	130	1.0	1.0
	-	0.5	9.6	126	3.0	3.1
	-	0.25	8.7	114	3.0	3.4
	-	0.1	9.5	125	3.0	3.2
	+	1	7.8	89	7.0	9.0
	+	0.75	9.8	111	6.0	6.1
	+	0.5	9.4	107	5.0	5.3
	+	0.25	8.1	92	4.0	4.9
	+	0.1	7.2	82	1.7	2.4

* 100% Decomposition to picric acid and other compounds.

Table B-59

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROBENZALDEHYDE

EXPERIMENT 27

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		5.4	100	3.0	5.6
	+		4.8	100	4.5	3.4
Pre-chlorination 2,4,6-Trinitrobenzaldehyde	-	0.0004	3.8	70	3.0	7.9
	+	0.0004	3.6	75	3.0	8.3
Post-chlorination 2,4,6-Trinitrobenzaldehyde	-	0.0004	2.6	48	2.0	7.7
	-	0.0002	3.2	59	2.0	6.3
	-	0.0001	3.3	61	3.0	9.1
	-	0.00004	3.5	65	3.0	8.6
	-	0.000008	3.7	69	6.0	16
	+	0.0004	5.1	106	3.0	5.9
	+	0.0002	3.3	69	3.0	9.1
	+	0.0001	3.4	71	3.0	8.8
	+	0.00004	3.2	67	2.0	6.3
	+	0.000008	3.6	75	2.0	5.6

Table B-60

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROBENZALDEHYDE

EXPERIMENT 31

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		7.0	100	5.0	7.1
	+		7.0	100	5.5	7.9
Pre-chlorination 2,4,6-Trinitrobenzaldehyde	-	0.002	C*	C	C	C
	+	0.002	7.3	104	5.0	6.8
Post-chlorination 2,4,6-Trinitrobenzaldehyde	-	0.0015	12	171	6.0	5.0
	-	0.0011	--†	--	--	--
	-	0.0007	5.9	84	3.0	5.1
	-	0.00035	5.9	84	3.0	5.1
	-	0.00015	C*	C*	C*	C*
	+	0.0015	9.5	136	4.0	4.2
	+	0.0011	--	--	--	--
	+	0.0007	6.8	97	7.0	10
	+	0.00035	5.9	84	4.0	6.8
	+	0.00015	10	143	3.0	3.0

* C, Contaminated

† Dilution error.

Table B-61

IN VITRO ASSAYS WITH SACHAROMYCES CEREVISIAE
2,4,6-TRINITROBENZALDEHYDE

EXPERIMENT 27

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.4	100	3.0	5.6
	+		4.8	100	4.5	9.4
Pre-ozonation 2,4,6-Trinitrobenzaldehyde	-	0.0005	2.9	54	5.0	17
	+	0.0005	3.3	69	3.0	9.1
Post-ozonation 2,4,6-Trinitrobenzaldehyde	-	0.00034	3.0	56	4.0	13
	-	0.00017	3.9	72	5.0	13
	-	0.00009	3.9	72	4.0	10
	-	0.000034	3.5	65	2.0	5.7
	-	0.000007	4.7	87	9.0	19
	+	0.00034	5.2	108	4.0	7.7
	+	0.00017	3.6	75	9.0	25
	+	0.00009	2.7	56	6.0	22
	+	0.000034	3.3	69	2.0	6.1
	+	0.000007	3.5	73	6.0	17

Table B-62

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROBENZALDEHYDE

EXPERIMENT 36

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Survivors Per 10^5
Negative control	-		7.6	100	5.0	6.6
	+		8.8	100	7.0	8.0
Pre-ozonation 2,4,6-Trinitrobenzaldehyde	-	0.0028	9.5	125	6.0	6.3
	+	0.0028	8.8	116	4.0	4.5
Post-ozonation 2,4,6-Trinitrobenzaldehyde	-	0.0025	9.3	122	1.0	1.1
	-	0.0018	7.4	97	6.0	8.1
	-	0.0012	7.7	101	13	17
	-	0.0006	9.3	122	4.0	4.3
	-	0.00025	8.5	112	5.0	5.9
	+	0.0025	8.2	93	9.0	11
	+	0.0018	8.0	91	5.0	6.3
	+	0.0012	7.9	90	6.0	7.6
	+	0.0006	8.7	99	6.0	6.9
	+	0.00025	8.8	100	4.0	4.5

Table B-63

SACCHAROMYCES CEREVISIAE EXPERIMENTAL POSITIVE CONTROLS

Exp. No.	Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
				Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
1	1,2,3,4-Diepoxylbutane	-	0.5	5.0	75	851	1702
		+	0.5	5.6	80	934	1668
2	1,2,3,4-Diepoxylbutane	-	0.5	8.2	178	590	720
		+	0.5	2.6	27	630	2471
3	1,2,3,4-Diepoxylbutane	-	0.5	1.1	19	580	5272
		+	0.5	0.4	8	176	4190
4	1,2,3,4-Diepoxylbutane	-	0.5	3.1	103	865	2808
		+	0.5	T*	T	T	T
5	1,2,3,4-Diepoxylbutane	-	0.5	4.2	55	213	504
		+	0.5	T	T	T	T
6	1,2,3,4-Diepoxylbutane	-	0.5	4.2	84	1013	2401
		+	0.5	6.4	112	988	1542
7	1,2,3,4-Diepoxylbutane	-	0.5	4.3	83	1088	2547
		+	0.5	4.5	92	1087	2443
8	1,2,3,4-Diepoxylbutane	-	0.5	7.2	91	1282	1788
		+	0.5	7.3	54	1680	2301
9	1,2,3,4-Diepoxylbutane	-	0.5	6.4	85	865	1352
		+	0.5	14.7	201	938	638
10	1,2,3,4-Diepoxylbutane	-	0.5	5.6	100	1253	2250
		+	0.5	5.2	96	1033	1979

*T, Toxic

Table B-63 (continued)

Exp. No.	Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
				Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
11	1,2,3,4-Diepoxycyclobutane	-	0.5	3.1	53	785	2532
		+	0.5	4.8	87	833	1753
12	1,2,3,4-Diepoxycyclobutane	-	0.5	4.5	71	1425	3181
		+	0.5	4.9	83	1228	2495
13	1,2,3,4-Diepoxycyclobutane	-	0.5	4.7	89	1108	2346
		+	0.5	T	T	T	T
14	1,2,3,4-Diepoxycyclobutane	-	0.5	4.4	85	1420	3215
		+	0.5	6.2	105	1368	2218
15	1,2,3,4-Diepoxycyclobutane	-	0.5	0.8	5	390	5200
		+	0.5	6.0	34	730	1217
16	1,2,3,4-Diepoxycyclobutane	-	0.5	2.8	44	978	3516
		+	0.5	2.9	49	738	2569
17	1,2,3,4-Diepoxycyclobutane	-	0.5	3.4	63	715	2115
		+	0.5	6.5	103	1590	2458
18	1,2,3,4-Diepoxycyclobutane	-	0.5	2.7	47	1128	4176
		+	0.5	1.8	33	660	3667
19	1,2,3,4-Diepoxycyclobutane	-	0.5	T	T	T	T
		+	0.5	5.6	85	1153	2058
20	1,2,3,4-Diepoxycyclobutane	-	0.5	2.9	43	1023	3526
		+	0.5	5.0	76	1318	2635
21	1,2,3,4-Diepoxycyclobutane	-	0.5	5.9	91	1545	2619
		+	0.5	6.9	115	1413	2047

Table B-63 (continued)

Exp. No.	Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
				Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10 ⁵ Survivors
22	1,2,3,4-Diepoxycyclohexane	-	0.5	4.4	67	1278	2923
		+	0.5	5.9	97	1663	2834
23	1,2,3,4-Diepoxycyclohexane	-	0.5	5.9	88	1148	1945
		+	0.5	5.6	92	1295	2313
24	1,2,3,4-Diepoxycyclohexane	-	0.5	4.5	76	1468	3261
		+	0.5	4.5	85	1515	3368
25	1,2,3,4-Diepoxycyclohexane	-	0.5	4.8	83	1498	3120
		+	0.5	4.2	84	1325	3155
26	1,2,3,4-Diepoxycyclohexane	-	0.5	5.5	44	995	1809
		+	0.5	3.0	33	1020	3400
27	1,2,3,4-Diepoxycyclohexane	-	0.5	4.4	81	1183	2688
		+	0.5	3.7	77	1048	2831
28	1,2,3,4-Diepoxycyclohexane	-	0.5	T	T	T	T
		+	0.5	4.7	80	1263	2686
29	1,2,3,4-Diepoxycyclohexane	-	0.5	5.3	100	1443	2722
		+	0.5	5.5	93	1253	2277
30	1,2,3,4-Diepoxycyclohexane	-	0.5	5.3	87	1705	3217
		+	0.5	5.7	83	1366	2398
31	1,2,3,4-Diepoxycyclohexane	-	0.5	6.2	89	1363	2198
		+	0.5	6.4	91	1350	2109
32	1,2,3,4-Diepoxycyclohexane	-	0.5	6.4	128	1085	1695
		+	0.5	5.7	100	1393	2443

Table B-63 (continued)

Exp. No.	Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
				Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10 ⁵ Survivors
33	1,2,3,4-Diepoxycyclohexane	-	0.5	3.3	72	835	2511
		+	0.5	3.2	67	580	1803
34	1,2,3,4-Diepoxycyclohexane	-	0.5	3.9	67	988	2543
		+	0.5	3.5	67	818	2319
35	1,2,3,4-Diepoxycyclohexane	-	0.5	6.4	97	785	1227
		+	0.5	4.9	61	734	1498
36	1,2,3,4-Diepoxycyclohexane	-	0.5	7.3	96	1088	1493
		+	0.5	5.2	59	1222	2342
37	Not tested						
38	Not tested						
39	Not tested						
40	Not tested						
41	1,2,3,4-Diepoxycyclohexane	-	0.5	0.5	14	150	3000
		+	0.5	2.3	18	910	3957
42	1,2,3,4-Diepoxycyclohexane	-	0.5	5.0	74	928	1855
		+	0.5	5.6	75	925	1652
43	1,2,3,4-Diepoxycyclohexane	-	0.5	5.4	95	1213	2246
		+	0.5	4.9	92	1065	2173
44	1,2,3,4-Diepoxycyclohexane	-	0.5	2.8	57	995	3596
		+	0.5	3.8	81	63	165
45	Not tested						

APPENDIX C

Abstract of Poster Presentation at
Sixteenth Annual Meeting, Society of
Toxicology, Toronto, Canada, March
27-30, 1977

MUNITIONS WASTEWATER TREATMENTS: DOES CHLORINATION OR OZONATION
OF INDIVIDUAL COMPONENTS PRODUCE MICROBIAL MUTAGENS? V. F. Simmon,
S. L. Eckford, A. F. Griffin, R. Spanggord, and G. W. Newell, SRI
International, Menlo Park, California*

*Abstract No. 157 in: Toxicology and Applied Pharmacology, 41,
197 (1977).

Abstract

A number of compounds present in wastewater from munitions plants were examined before and after ozonation or chlorination to determine whether any were mutagenic before treatment and whether such activity was affected by the treatment. Several photolytic as well as metabolic products of trinitrotoluene (TNT) also were examined for mutagenic activity. Test materials included TNT, TNT production wastewater and individual components of TNT wastewater (1,3-dinitrobenzene; 2,4-dinitrotoluene; 3,5-dinitrotoluene; 1,3,5-trinitrohexahydro-1,3,5-triazine (RDX); 1,3,5,7-tetranitrooctahydro-1,3,5,7-tetrazocine (HMX); components of photolysed TNT; pentaerythritol tetranitrate (PETN); and trinitro-resorcinol. The in vitro mutagenic assays used were the Ames Salmonella/microsome assay (strains TA1535, TA1537, TA1538, TA98, and TA100) and mitotic recombination in the yeast, Saccharomyces cerevisiae D3. A metabolic activation system using the post-mitochondrial supernatant fraction of liver from rats pretreated with Aroclor 1254 was included in each assay procedure. Materials found to be mutagenic prior to and after treatment were trinitrobenzene, trinitrobenzaldehyde, trinitrobenzonitrile, and 50% and 100% photolysed TNT wastewater. Neither ozonation nor chlorination significantly altered the mutagenic activity of the materials tested. (Supported by the U.S. Army Medical Research and Development Command, under Contract No. DAMD 17-76-C-6013.)

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