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ACUTE TOXICITY EVALUATION OF NITROAROMATIC COMPOUNDS

PREPARED BY:

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March 1991

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The nitroaromatics 1,3-dinitrobenzene (DNB), 1,2,5-trinitrobenzene (TNB) and N-methyl-N,2,4,6-tetranitroaniline (tetryl) have been detected as environmental contaminants of water and soil near production waste sites and at military test grounds. Acute toxicity evaluations were carried out with these compounds to develop environmental and health effects criteria. Dermal and eye irritation tests and acute dermal toxicity tests in rabbits, acute oral toxicity tests in rats and dermal sensitization (Buehler) tests in guinea pigs were conducted according to EPA standard protocols. The sensitization tests showed that DNB and tetryl are not skin sensitizers while TNB caused a mild allergic reaction. None of these compounds produced skin irritation but positive (DNB) to severe (TNB, tetryl) eye irritation potentials were observed. TNB and tetryl were not toxic at 2 g/kg when applied to rabbit skin for 24 hours. However, the dermal LD50 of DNB was **

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**19. Abstract (Cont.)

1.99 g/kg for combined sexes. The oral LD50's of DNB, TNB and tetryl were 59 mg/kg, 284 mg/kg and greater than 5 g/kg respectively in rats for combined sexes. The LD50 of DNB for male and female rats were less than 62 mg/kg and 63.4 mg/kg respectively. The LD50 of TNB for male and female rats were 298 mg/kg and 275 mg/kg respectively. The oral LD50 of DNB, TNB and tetryl were 80 mg/kg, 804 mg/kg and greater than 5 g/kg respectively in mice for combined sexes. The LD50 of DNB for male and female mice were 74.7 mg/kg and 84.5 mg/kg respectively. The LD50 of TNB for male and female were greater than 900 mg/kg and 702 mg/kg respectively. These results show that DNB is more toxic than TNB and tetryl when exposed orally and dermally while TNB and tetryl were more severe eye irratants than DNB.

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FOREWORD

This project was sponsored by the U.S. Army Medical Research and Development Command and the Army Material Command. Citation of commercial organizations and trade names in this report does not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

Animal Care Program Certification

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", NIH Guide Supplement for Grants and Contracts, Vol.14, No.8, June 25, 1985 and the "Laboratory Animal Welfare Act" (Public Law 91-579)

George B. FitzGerald, Ph.D.
Director of Animal Toxicology

Good Laboratory Practice Certification

All tests were conducted according to the protocol. All changes in the protocol have been documented. All reported results were inspected and found to accurately reflect the original data. These studies were performed according to the Good Laboratory Practice Regulations of the FDA (21 CFR 58.1-58.219, 1989)

George B. FitzGerald, Ph.D.
Director of Animal Toxicology

Quality Assurance Inspection Statement

The study report was reviewed by the Quality Assurance Unit. Q.A. unit also performed audits at different phases of these studies and findings were reported to the Study Director and management.

Nancy DiGuilio, M.S.
Quality Assurance

EXECUTIVE SUMMARY

A series of Acute Toxicity studies were performed by Toxikon Corp. for the U.S. Army Medical Research and Development Command. These studies were designed to compare the toxicity of three test substances: 1,3-dinitrobenzene (DNB), 1,3,5-trinitrobenzene (TNB) and N-Methyl-N,2,4,6-tetranitroaniline. The test studies performed included: Primary Dermal Irritation, Primary Ocular Irritation, Acute Oral toxicity in rats and mice, Acute Dermal toxicity and Buehler Topical Closed Patch skin sensitization.

The following Table summarizes the findings of these studies.

SUMMARY OF ACUTE TOXICITY STUDIES

	RESPONSE		
TEST	DNB	TNB	TETRYL
Primary Dermal	non-irritant	non-irritant	non-irritant
Primary Eye	irritant	corrosive	corrosive
Acute Oral (rat)	LD50 (mg/kg)	LD50 (mg/kg)	non-toxic
Combined Sex	59.5	275	
Male	< 62.0	298	
Female	63.4	284	
Acute Oral (mice)	LD50 (mg/kg)	LD50 (mg/kg)	non-toxic
Combined Sex	80.4	804	
Male	74.7	>900	
Female	84.5	702	
Acute Dermal	LD50 (g/kg)	non-toxic	non-toxic
Combined Sex	1.99		
Male	2.08		
Female	1.90		
Buehler	non-sensitizer	mild sensitizer	non-sensitizer

The results indicate that DNB and TNB exhibit systemic toxicity when administered orally to rodents. Mice were less sensitive to these two test substances than rats. DNB was found to have 5 to 10 times more toxic than TNB when administered orally. DNB exhibited systemic toxicity when applied to the skin of rabbits. Both TNB and Tetryl were found to be corrosive to the ocular

tissue of rabbits and DNB was found to be an eye irritant. Only TNB exhibited an allergenic response to the skin of guinea pigs and was classified as a mild sensitizer. None of the test substances were found to be dermal irritants

INTRODUCTION

This study compared the toxicity of three test substance, 1,3-dinitrobenzene (DNB), 1,3,5-trinitrobenzene (TNB) and N-Methyl-N,2,4,6-tetranitroaniline (tetryl), utilizing a set of acute toxicity test systems. The test substances were examined using the Primary Dermal Irritation, Primary Ocular Irritation, Acute Oral toxicity in rats and Acute Oral toxicity in mice, Acute Dermal toxicity, and the Buehler Topical Closed Patch procedures. These studies were conducted by Toxikon Corp. for the U.S. Army Medical Research and Development Command.

MATERIALS AND METHODS

MATERIALS

Animals

Young adult New Zealand White rabbits (Oryctolagus cuniculus) were obtained from a registered commercial breeding laboratory (Pine Acres, Norton, MA). At the start of the study the animal were in the weight range of 2.0 and 3.0 kgs and were approximately 11 weeks of age. They were individually housed using suspended steel cages marked with the corresponding animal number. The animal were supplied with rabbit feed (Agway Prolab, Waverly, NY) and municipal tap water, ad libitum.

Rats (rattus norvegicus), Fisher 344, purchased from Charles River Breeding Laboratories (Wilmington, MA), were used in this study. The animals at the start of the study were in the weight range between 200 and 300 grams. All females were nulliparous and non-pregnant.

Outbred Swiss mice (Mus musculus), purchased from Charles River Breeding Laboratories (Wilmington, MA), were used in this study. The animals at the start of the study were in the weight range between 17 and 25 grams and approximately 8-12 weeks of age. All females were nulliparous and non-pregnant.

Female (nulliparous, non-pregnant) and Male Duncan-Hartley guinea pigs (Cavia Porcellus) in the weight range 250-400 grams were used. Animals were purchased from a registered commercial breeding laboratory (Elm Hill Breeding Labs, Chelmsford, MA). The guinea pigs were group housed in polycarbonate cages marked with the corresponding animal number.

Upon receipt, animals were placed in quarantine for a period of 3 to 5 days (rabbits), 5 or 13 days (mice), 7 or 8 days (rats) and 13 days (guinea pig).

Animals were housed in an AAALAC accredited facility maintained at 68 +/- 3 F with a relative humidity of 50 +/- 20% a minimum of 10 to 13 complete air exchanges per hour and a 12 hour light/dark cycle (full spectrum lights). The laboratory and animal rooms were maintained as limited access facilities.

Test Article

The test substance, 1,3-dinitrobenzene (99.89%), was obtained from the Sigma Chemical Co. St. Louis, MO.

The test substances, N-Methyl-N,2,4,6-tetranitroaniline (tetryl) (99.45%) and 1,3,5,-trinitrobenzene (99.67%), were supplied by the U.S. Army Biomedical Laboratories. The source and purity of the test substances are given in Appendix IV.

The test substances were stored at room temperature and appropriate safety precautions were observed for potentially toxic and explosive substances.

METHODS

The toxicity tests performed on each test article is summarized in the following table.

DNB ACUTE TOXICITY STUDIES	TNB ACUTE TOXICITY STUDIES
Primary dermal	Primary dermal
Primary ocular	Primary ocular
Acute oral (rats) (Range Finding, LD50)	Acute oral (rats) (Range Finding, LD50)
Acute oral (mice) (Range Finding, LD50)	Acute oral (mice) (Range Finding, LD50)
Acute dermal (Limit, LD50)	Acute dermal (Limit)
Buehler - Skin Sensitization	Buehler - Skin Sensitization

TETRYL ACUTE TOXICITY STUDIES
Primary dermal
Primary ocular
Acute dermal
Acute oral (rats) (Limit)
Acute oral (mice) (Limit)
Buehler - Skin Sensitization

Animals selected for testing were not subjected to any previous experimental procedures. Selected animals were chosen from a larger pool of animals after a complete examination.

Rabbits were identified by ear tattoo and were weighed to the nearest ten grams. Rats, guinea pigs and mice were identified by ear punch and weighed to the nearest tenth gram.

Clinical observations were conducted daily for all test and included all clinical, toxicologic, and pharmacologic signs.

At the completion of each study all animals were sacrificed by an approved method of euthanasia, i.e. for rabbits, IV injection of Somlethol, J.A. Webster, Sterling MA, or CO_2 inhalation for the smaller rodents.

Primary Dermal Irritation

A dose of 0.5 g of the test substance was applied to one intact and abraded skin site per rabbit The test substance was crushed to a fine powder prior to dosing.

In the present study, neither a vehicle control group nor an untreated control group was used concurrently with the test group. Each animal served as its own control with two untreated sites per animal prepared (intact and abraded).

The application sites were prepared by clipping the skin of the trunk free of hair approximately 24 hours before application of the test substance. One application site for each test or control substance on each animal was abraded by making minor incisions through the stratum corneum, but not sufficient to disturb the derma (that is not sufficiently deep to produce bleeding). The second application site was intact skin.

The test substance (0.5 g) was applied to small area (approximately 6 cm^2) of skin and covered with a gauze patch, which was held in place with Vetrap bandaging. The test substance was kept in contact with the skin for 24 hours. After the exposure period, the test substance was carefully rinsed from the area of application with water to prevent altering the existing response or the integrity of the epidermis.

Animals were weighed at the beginning and at the end of the observation period.

Animals were observed for signs of erythema and edema 30-60 minutes after the 24h exposure period, and then at 48 and 72 hours after the exposure period. Observations were scored according to the "Draize Scale for Scoring Skin Reactions" (see Appendix I).

Observation values were calculated by averaging the scores for each of 6 individual animals. This was performed by adding the

scores for each animal for erythema and edema at 30-60 minutes after the 24h exposure, 48 hours, and 72 hours (at a minimum). This total was divided by 3. The calculated value for each animal was added together for a total of six animals. The total was divided by 6 to obtain the Primary Irritation Index. This was done for both the abraded and intact skin site for each animal.

A test substance with a Primary Irritation Index of 0 is considered a non-irritant. A test substance with an index of 2 or less is considered a slight irritant. Test substances with indices greater than 2 and less than 5 are moderate irritants. Any test substances with an index of 5 or more are considered severe irritants. Those substances that destroy the structure of the intact skin or change it irreversibly are considered corrosive.

Primary Ocular Irritation

Both eyes of each rabbit were examined for macroscopic findings with fluorescein dye procedure within 24 hours preceding application of the test substance. Observations were scored according to the "Draize Scale for Ocular Lesions" described in Appendix II. Only rabbits with a total score of 0 were accepted for use in the study.

A dose of a 0.1 g of test substance (crushed to a fine dust) was applied to each test eye. Six animals were treated by instilling the test substance on the left eye of each animal, the upper and lower lids were gently held together for approximately one second to prevent loss of the test substance; then the animal was returned to its the cage. The right eye remained untreated, and thus served as a control.

Eyes were examined at 1, 24, 48, 72 and 96 hours. Fluorescein staining was also used during each examination, exclusive of the 1 hour observation period. At 24 h, the test and control eyes of each animal were gently flushed with 0.9% Sodium Chloride Injection USP. Readings were discontinued after 96 h. No local anesthetic was used in any phase of the study.

Acute Oral Toxicity in Rats and Mice

<u>1,3-Dinitrobenzene</u>

Due to available information from the Sponsor on the toxicity of the 1,3-dinitrobenzene, the Limit Test was not performed (DNB, LD50 value of approximately 83 mg/kg).

Dosage - Oral Range Finding

Two animals (rat or mice), 1 male and 1 female were used at each of the 5 dose levels, i.e. rat (30, 55, 80, 105, and 130 mg/kg), mice (20, 40, 80, 160, 320 mg/kg); so that in the final LD50

Study, no more than 3 dose groups of 10 animals each were required. A vehicle control group was not run concurrently.

Dosage - Oral LD50

The test substance was ground to a fine powder and suspended in corn oil, at concentrations of 62 mg/kg, 68 mg/kg, and 74 mg/kg (rats) and 50 mg/kg, 70 mg/kg and 90 mg/kg (mice) which were determined based upon the results of the range finding studies. The dose levels were prepared so that each group was dosed at a constant volume (10 ml/kg). Three groups of rats and mice, consisting of 5 male and 5 female, were used in this study.

1,3,5-Trinitrobenzene

Due to available information from the Sponsor on the toxicity of the test substance, the Limit Test was not performed; LD50 value of approximately 450 mg/kg (rats) and 572 mg/kg (mice).

Dosage - Oral Range Finding

Two animals (rat or mice), 1 male and 1 female were used at each of the 5 dose levels, i.e. rat (200, 350, 500, 650, and 800 mg/kg), mice (125, 2500, 500, 1000, 2000 mg/kg); so that in the final LD50 Study, no more than 3 dose groups of 10 animals each were required. A vehicle control group was not run concurrently.

Dosage - Oral LD50

The test substance was ground to a fine powder and suspended in corn oil, at concentrations of 185 mg/kg, 260 mg/kg, and 335 mg/kg (rats) and 500 mg/kg, 700 mg/kg and 900 mg/kg (mice) which were determined based upon the results of the range finding studies. The dose levels were prepared so that each group was dosed at a constant volume (10 ml/kg). Three groups of rats and mice, consisting of 5 male and 5 female, were used in this study.

N-Methyl-N,2,4,6-tetranitroaniline (Tetryl)

The acute oral limit study was performed on 5 males and 5 females. Food was withheld from the animals the night prior to dosing. For the limit test, a single dose of the test substance at 5 g/kg body weight was administered.

Oral Toxicity Test

Food was withheld from the animals the night prior to dosing. On the day of the test, animals were weighed to the nearest 0.1 gram, and administered a single dose of the test substance by intragastric intubation, with a ball tip gavaging needle with syringe. The maximum volume dosed did not exceed 10 ml/kg body weight. Following dosing, the animals were returned to their cages and supplied with feed and water ad libitum.

Animals were observed for toxicity and occurrence of deaths at least once daily for 14 days following the administration of the test substance. Animals were observed for all clinical, toxicologic, and pharmacologic signs.

Individual body weights were determined on the fasted animals shortly before the test substance was administered on Day 0, again on Day 7, and on Day 14 prior to sacrifice, or at death. A gross necropsy was performed on all animals on the day of their death.

Acute Dermal Toxicity Limit Study

A limit test was performed on 10 rabbits, 5 male and 5 female, at a dose level of 2.0 g/kg. A dose of a 2.0 g/kg body weight of test substance was applied to each test animal. The test substance was administered as received. In the present study neither a vehicle control group nor an untreated control group was used concurrently with the test group.

The application sites were prepared by clipping the skin of the trunk free of hair approximately 24 hours before application of the test substance.

The test substance (ground to a fine powder) was introduced under gauze patches two single layers thick and applied directly to the skin of 10% of the body surface of a group of ten animals. The animals were immobilized while patches were secured in place by wrapping the entire trunk of the animal with a semi-occlusive bandage (Vetrap^{R} 3M, St. Louis, MN). The animals were returned to their cages for the exposure period. The test substance was kept in contact with the skin for 24 hours.

At the completion of the exposure period, the wrapping was removed and the skin was wiped and rinsed with physiologic saline to remove any test substance still remaining. The animals were observed for signs of erythema and edema.

<u>1,3-Dinitrobenzene</u>

Dermal LD50

Since mortality was observed during the dermal limit test (2g/kg) for DNB, a complete dermal LD_{50} study was required. A range finding study was not performed.

In the LD50 study, 3 dose levels were utilized, for a total of 30 rabbits (15 of each sex). Three dose levels, 2.25 g/kg, 2.00 g/kg, and 1.75 g/kg, were applied to the test animals, ten animals per dose level.

The LD50 study was performed as described for the limit test.

Buehler Topical Closed Patch

Animals were assigned by random number to the groups shown in the following Table.

BUEHLER SENSITIZATION TEST

Group	Treatment Induction	Challenge	No. Males	No. Females	Total No.
1. Primary Irritation*	(Four concentrations of the test substance applied to 4 sites on each animal (100%, 50%, 25%, 10%) -scored at 24, 48, and 72 h.		2	2	4
2. Experimental	3 weekly doses of test substance	one dose on week 05 - virgin site	10	10	20
3. Negative Control (0.9% Sodium Chloride)	nil	one dose on week 05 - virgin site	5	5	10
4. Positive Control+	DNCB - 3 weekly doses	DNCB - one dose on week 05 - virgin site	5	5	10
Total Number			22	22	44

*For determination of highest non-irritant dose for use as the challenging dose.
+ 0.4 ml of 0.1% DNCB in acetone for induction and 0.4 ml of 0.05% DNCB for challenge.

Before each application of test or control sample, an area of approximately 2 cm x 2 cm was shaved on the animal's left shoulder.

Induction Phase

Closed patches for the experimental group were prepared as follows: 0.4 g of test substance was applied directly to the skin and covered with a gauze pad and kept in place with Vetrap®.

Test and control substances were similarly applied. The patches were removed after 6 hours of exposure and any residual material was washed off with water.

The test substance was applied once per week for 3 consecutive weeks (days 0, 7 and 14) on one side of the animal. The positive control substance (DNCB) was applied in the same manner. Induction scoring was performed 24 hours after the test substance application.

Primary Irritation

Before the induction phase was completed, the primary irritation study was performed with four (4) previously unexposed animals. The test substance, diluted in 0.9% Sodium Chloride, was applied to 4 sites on each animal at 100%, 75%, 50%, and 10% concentration for 24 hours. The responses were read within 2 hours after the 24 hour exposure period, and at 48 and 72 hours.

Challenge

The day preceding the challenge dose on week 5 a virgin skin site of about 2 x 2 cm was shaved on the back of animals of the experimental and control groups.

The challenge test was performed in the same way as the induction phase except that the skin was exposed to the test material for 24 hours. One virgin site was prepared per animal. Reading of the skin area was repeated 48 and 72 hours after the challenge, and the skin reactions were graded. The Draize Scale used for scoring the primary irritation study is contained in Appendix I.

It was not considered necessary to rechallenge the sensitized guinea pigs at 1 and 2 weeks after the primary challenge (weeks 06 and 07).

RESULTS

1,3-DINTROBENZENE

Primary Dermal Irritation (Table 1)

All of the test animals exhibited a gain in body weight during the observation period. No overt signs of toxicity were evident during the course of the study. No animals exhibited signs of erythema or edema during the study, Table 1. The Primary Dermal Irritation Index (PDII) for the test substance and control for the intact and abraded sites was 0.00.

Primary Ocular Irritation (Table 2)

All of the animals exhibited a gain in body weight during the course of the study. No overt signs of toxicity were evident during the course of the study.

Ocular Irritation - Draize: (Table 2)

Two animals exhibited corneal opacity (Grade 2) at 24h. Two animal exhibited redness (Grade 2) at 1 h, 24 and and one animal showed redness in its treated eye at 48 h. All signs of irritation were absent by 72 h. No irritation was observed in the control eye of any animal during the observation period.

Fluorescein Staining: No fluorescein staining was evident in any of the test animals at any observation point.

Acute Oral Toxicity in Rats

Range Finding (Table 3)

Mortality (6/10 animals) was observed in three (80, 105 and 130 mg/kg) highest dose levels by Day 1. Both surviving males and one surviving female gained body weight. One surviving female lost body weight during the study period. No unusual lesions were noted during necropsy of any of animals. Clinical signs noted for the surviving animals included piloerection and lethargy.

Based upon the results of the range finding 3 doses were suggested for the LD50 study; 74 mg/kg, 68 mg/kg and 62 mg/kg.

LD50 (Table 4)

Dose group - 74 mg/kg:
All animals died by Day 2 of the study. Both the males and the females in this dose level had a death weight less than their Day 0 weight. Clinical signs of lethargic activity and dyspnea were exhibited prior to all animals' death on Day 0 (4h observation) or on Day 1.

Dose group - 68 mg/kg:
The one surviving animal of this dose level gained weight; the animals that died exhibited decreased weight from their initial Day 0 weight. Clinical Observations - The single surviving animal exhibited dyspnea, lethargy, prostration, dry scant feces and lacrimation. The nine animals that died exhibited lethargy and/or dyspnea prior to death. Nine out of the ten animals died by Day 2 of the study. One animal (male) survived the 14 day observation period.

Dose group - 62 mg/kg:
The three surviving animals gained body weight during the observation period. Animals found dead weighed less than their initial weight on Day 0 of the study. The surviving three animals were lethargic until Day 4. The animals that died showed signs of dyspnea and lethargic activity. Seven of the animals (5 males and 2 females) died by Day 2 of the observation period.

No unusual lesions were noted at necropsy in any of the animals at any of the dose levels.

The LD50 of DNB has been determined to be 59.5 mg/kg for combined sexes. The LD50 for male and female were less than 62 mg/kg and 63.4 mg/kg respectively utilizing the method of Litchfield and Wilcoxon.

Acute Oral Mice

Range Finding (Table 5)

Mortality (8/10 animals) was observed at all five dose (20, 40, 80, 60, and 320 mg/kg) levels through Day 8. The two females at the lowest dose levels survived for the duration of the observation period and both gained body weight. Clinical signs observed included tachypnea, piloerection, ptosis, lethargy and/or unusual locomotion in all of the animals that survived beyond the 4 hour observation point. No unusual lesions were noted during necropsy of any of animals.

Based upon the results of the range finding 3 doses were suggested of the LD50 study; 90 mg/kg, 70 mg/kg and 50 mg/kg.

LD50 (Table 6)

Dose group - 90 mg/kg:
The four surviving animals (2 males and 2 females) gained body weight during the observation period. Of the six animals found dead, five gained and one lost body weight. Clinical signs exhibited during the 14 day observation period include somnolence, tachypnea, tremors, lethargy, and squinting.

Dose group - 70 mg/kg:
All animals gained body weight during the observation period. Clinical signs exhibited during the 14 day observation period

include somnolence, tachypnea, tremors, lethargy, piloerection, and squinting. Four of the animals (3 males and 1 female) died by Day 6.

Dose group - 50 mg/kg:
All animals gained body weight during the observation period. No clinical signs were exhibited during the 14 day observation period. No mortality was observed during the observation period.

At necropsy, no unusual lesions were observed in any of the animals at any of the dose levels.

The LD50 of DNB has been determined to be 80.4 mg/kg for combined sexes. The LD50 for male and female mice were 74.7 mg/kg and 84.5 mg/kg respectively utilizing the method of Litchfield and Wilcoxon.

Acute Dermal Toxicity

Limit (Table 7)

All of the surviving animals exhibited a gain in body weight during the observation period. No overt signs of toxicity were evident in the surviving animals during the course of the study. Four of the test animals were found dead on study Day 3 and all of these animals had lost weight. The remaining test animals survived the 14 day test period. All tissues examined at gross necropsy were normal. No erythema or edema was exhibited 30 minutes following bandage removal.

Based upon the results of the limit study 3 doses were suggested of the LD50 study; 2.25 g/kg, 2.00 g/kg, and 1.75 g/kg.

LD50 (Table 8)

All of the 15 surviving animals exhibited a gain in body weight during the observation period. For the animals found dead during the study, 7 gained weight and 8 animals either lost weight or had no change in weight. Prior to death, all animals exhibited tonic - clonic or asphyxial convulsions. No overt signs of toxicity were evident in the surviving animals during the course of the study. All tissues examined at gross necropsy were normal. No erythema or edema was exhibited following bandage removal.

At 2.25 g/kg, 5 animals died by Day 2, and one animal died on day 3 and one on day 4 (7/10 animals died). Five animals died at 2.00 g/kg dose level, three on day 3 and one animal on day 4 and one on day 5. At the lowest dose level, 1.75 g/kg, 3 of 10 animals died, one on day 4 and two on day 5. The LD50 value was determined using the method of Wilcoxon and Litchfield to be 1.99 g/kg for combined sexes. The LD50 for male and female rabbits were 2.08 g/kg and 1.9 g/kg respectively.

Dermal (Buehler) Sensitization (Table 9)

All animals gained weight during the course of the study. No systemic signs of toxicity were evident during the course of the study. No signs of erythema or edema were present in any of the test or control animals throughout the induction scoring phase. No animals died during the induction or challenge phases.

The Preliminary Irritation Study showed no erythema nor edema at any of sites treated with the test substance. Therefore, the test material was utilized at 100% concentration for the sensitization study.

No signs of erythema/edema were evident in any of the animals in the control or experimental groups at either the induction or challenge phase of the study. All 10 animals in the positive control groups exhibited signs of erythema (2.0 - 3.0) at the challenge phase.

DISCUSSION

1,3-DINITROBENZENE

The test substance, DNB, was tested for their dermal irritancy potential. According to the established criteria and guidelines, the test substance is considered non-irritating to the intact and abraded skin of laboratory rabbits.

DNB did cause slight changes, ocular opacity and redness, to the ocular tissue of rabbits. The irritation was absent by 72 hr. The test substance is therefore considered an eye irritant.

An acute oral toxicity study performed with rats indicated an LD50 for combined sexes to be 59.5 mg/kg. An acute oral toxicity study performed with mice indicated an LD50 for combined sexes to be 80.4 mg/kg.

The test substance, 1,3-dinitrobenzene, was found to have acute dermal toxicity at 2 g/kg. An acute dermal LD50 study determined that the LD50 for dermal toxicity for DNB is 1.99 g/kg.

1,3-Dinitrobenzene was assessed for its skin sensitization potential with the Buehler topical closed patch technique. A skin sensitizer is a material which elicits an allergic contact dermatitis. The results of this study indicated that the DNB is a non-sensitizer.

In summary (Table 10) the test substance, 1,3-dinitrobenzene was found to be non-toxic with respect to 3 acute toxicity criteria; skin sensitization, ocular irritation and dermal irritation. However, the LD50 for dermal toxicity was 1.99 g/kg, and the oral LD50 in rats was 59.9 mg/kg and for mice 80.4 mg/kg for combined sexes.

TABLE 1

PRIMARY DERMAL IRRITATION - DRAIZE SCORE

1,3-DINITROBENZENE

Animal #	Observation/Scores 24 hours* Erythema	24 hours* Edema	48 hours Erythema	48 hours Edema	72 hours Erythema	72 hours Edema	Divided By 3
INTACT SITE							
990	0	0	0	0	0	0	0
991	0	0	0	0	0	0	0
992	0	0	0	0	0	0	0
993	0	0	0	0	0	0	0
994	0	0	0	0	0	0	0
995	0	0	0	0	0	0	0
Primary Dermal Irritation Index (PDII) = 0.00							
ABRADED SITE							
990	0	0	0	0	0	0	0
991	0	0	0	0	0	0	0
992	0	0	0	0	0	0	0
993	0	0	0	0	0	0	0
994	0	0	0	0	0	0	0
995	0	0	0	0	0	0	0
Primary Dermal Irritation Index (PDII) = 0.00							

Note: PDII equals sum for all animals divided by 6.
* Readings taken 30-60 minutes after the 24 hour exposure treatment.

TABLE 2

LESION SUMMARY SHEET (TREATED EYE)
POSITIVE SCORES (TSCA)*
1,3-DINITROBENZENE

Lesion	1h	24h	48h	72h	96h
CORNEA					
Opacity	0/6	2/6	0/6	0/6	0/6
IRITIS	0/6	0/6	0/6	0/6	0/6
CONJUNCTIVA					
Redness	3/6	2/6	1/6	0/6	0/6
Chemosis	0/6	0/6	0/6	0/6	0/6

* Number of Positive Responses/Total Number of Animals (see Appendix II)

TABLE 3

ACUTE ORAL - RANGE FINDING - RATS
1,3-DINITROBENZENE

Animal #	Sex	Dose Level mg/kg	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.*	Necropsy
1	Male	130	2.23	223.1	223.0[a]	-	-0.1[b]	Death Day 1	Normal
2	Male	105	2.20	220.3	218.1[a]	-	-2.2[b]	Death Day 1	Normal
3	Male	80	2.12	211.7	210.0[a]	-	-1.7[b]	Death Day 1	Normal
4	Male	55	2.25	224.6	220.0	248.1	23.5	1	Normal
5	Male	30	2.10	210.4	216.01	253.0	42.6	1,2	Normal
			MEAN ±SD	218.0 6.6					
6	Female	130	2.05	204.6	203.0[a]	-	-1.6[b]	Death Day 1	Normal
7	Female	105	2.06	206.1	205.2[a]	-	-0.9[b]	Death Day 1	Normal
8	Female	80	2.06	206.2	206.0[a]	-	-0.2[b]	Death Day 1	Normal
9	Female	55	2.17	216.5	169.1	193.5	-23.0	1	Normal
10	Female	30	2.02	201.5	196.0	211.8	10.3	1	Normal
			MEAN SD	207.0 5.7					

* Summary of clinical observations day 0 through day 14

1: Lethargy
2: Piloerection

a: Death Weight
b: Difference based on death weight

TABLE 4

ACUTE ORAL - LD50 - RATS
1,3-DINITROBENZENE

Animal #	Sex	Dose Level mg/kg	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.*	Necropsy
1	Male	74	2.56	255.8	255.0[a]	--	-0.8[b]	1, Death Day 1	Normal
2	Male	74	2.70	270.4	268.0[a]	--	-2.4[b]	2, Death Day 2	Normal
3	Male	74	2.83	283.3	280.1[a]	--	-3.2[b]	2, Death Day 2	Normal
4	Male	74	2.64	250.7	260.4[a]	--	9.7[b]	2, Death Day 2	Normal
5	Male	74	2.51	250.7	249.3[a]	--	-1.4[b]	2, Death Day 1	Normal
			MEAN	264.7					
			±SD	12.8					
6	Female	74	2.32	232.2	230.0[a]	--	-2.2[b]	2, Death Day 2	Normal
7	Female	74	2.30	230.1	228.0[a]	--	-2.1[b]	1, Death Day 1	Normal
8	Female	74	2.35	234.6	233.1[a]	--	-1.5[b]	1, Death Day 1	Normal
9	Female	74	2.27	227.1	225.0[a]	--	-2.1[b]	1, Death Day 1	Normal
10	Female	74	2.34	234.0	230.0[a]	--	-4.0[b]	1, Death Day 2	Normal
			MEAN	231.6					
			SD	3.1					

* Summary of clinical observations day 0 through day 14

1: *Dyspnea*
2: Lethargy

a: Death Weight
b: Weight change calculated from death weight

TABLE 4 (cont.)

ACUTE ORAL - LD50 - RATS
1,3-DINITROBENZENE

Animal #	Sex	Dose Level mg/kg	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.*	Necropsy
11	Male	68	2.66	263.0	263.0[a]	--	-2.7[b]	1, Death Day 1	Normal
12	Male	68	2.74	273.8	270.1[a]	--	-3.7[b]	2, Death Day 2	Normal
13	Male	68	2.70	269.5	267.5[a]	--	-2.0[b]	2, Death Day 2	Normal
14	Male	68	2.71	271.0	269.1[a]	--	-1.9[b]	2, Death Day 2	Normal
15	Male	68	2.79	279.4	278.0[a]	--	-1.4[b]	1, Death Day 1	Normal
			MEAN ±SD	271.9 5.1					
16	Female	68	2.26	226.3	224.0[a]	--	-2.3[b]	2, Death Day 2	Normal
17	Female	68	2.25	229.2	243.1	261.1	31.9	1,2,3,4,5	Normal
18	Female	68	2.27	226.6	225.0[a]	--	-1.6[b]	1, Death Day 1	Normal
19	Female	68	2.30	230.3	228.1[a]	--	-2.2[b]	2, Death Day 2	Normal
20	Female	68	2.23	222.6	220.0[a]	--	-2.6[b]	1, Death Day 1	Normal
			MEAN SD	227.0 3.0					

* Summary of clinical observations day 0 through day 14

1: Dyspnea
2: Lethargy
3: Prostration
4: Lacrimation
5: Solid, dry feces

a: Death Weight
b: Weight change calculated from death weight

TABLE 4 (cont.)

ACUTE ORAL - LD50 - RATS
1,3-DINITROBENZENE

Animal #	Sex	Dose Level mg/kg	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.*	Necropsy
21	Male	62	2.70	270.3	268.1[a]	--	-2.2[b]	1, Death Day 1	Normal
22	Male	62	2.75	275.1	273.3[a]	--	-1.8[b]	1, Death Day 1	Normal
23	Male	62	2.63	263.2	260.0[a]	--	-3.2[b]	1, Death Day 1	Normal
24	Male	62	2.67	266.6	264.1[a]	--	-2.5[b]	1,2 Death Day 2	Normal
25	Male	62	2.49	248.8	245.9[a]	--	-2.9[b]	2, Death Day 2	Normal
			MEAN ±SD	264.8 10.0					
26	Female	62	2.30	230.1	227.1[a]	--	-3.0[b]	2, Death Day 2	Normal
27	Female	62	2.29	229.4	225.3[a]	--	-4.1[b]	2, Death Day 2	Normal
28	Female	62	2.19	219.0	254.9	288.6	69.6	2	Normal
29	Female	62	2.33	233.3	251.8	280.9	47.6	2	Normal
30	Female	62	2.24	224.1	241.3	269.1	85.0	2	Normal
			MEAN SD	227.2 5.6					

* Summary of clinical observations day 0 through day 14

1: Dyspnea
2: Lethargic Activity

a: Death weight
b: Weight change calculated from death weight

TABLE 5

ACUTE ORAL - RANGE FINDING - MICE
1,3-DINITROBENZENE

Animal #	Sex	Dose Level* mg/kg	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.*	Necropsy
1	Male	320	0.12	23.9[a]	-	-	-	6	Normal
2	Male	160	0.11	22.2[a]	-	-	-	6	Normal
3	Male	80	0.12	24.3[a]	-	-	-	6	Normal
4	Male	40	0.12	23.8	25.1[b]	-	+1.3[c]	1,2,3,6	Normal
5	Male	20	0.12	23.3	26.1	25.3[d]	+2.0[c]	1,2,3,4,5,6	Normal
			MEAN	23.5					
			±SD	0.8					
6	Female	320	0.10	19.4[a]	-	-	-	6	Normal
7	Female	160	0.11	22.0[a]	-	-	-	6	Normal
8	Female	80	0.09	18.1[a]	-	-	-	6	Normal
9	Female	40	0.10	19.2	24.3	26.9	+7.7	1,2	Normal
10	Female	20	0.09	17.9	23.9	26.1	8.2	1,2,3	Normal
			MEAN	19.3					
			SD	1.6					

* Summary of clinical observations day 0 through day 14

1: Tachypnea
2: Piloerection
3: Ptosis
4: Lethargy
5: Unusual locomotion
6: Death

a: Death by the 4 hour observation point, no death weight required
b: Death weight on Day 3, 04/27/90
c: Difference based on death weight
d: Death weight on Day 8, 05/02/90

TABLE 6

ACUTE ORAL - LD50 - MICE
1,3-DINITROBENZENE

Animal #	Sex	Dose Level mg/kg	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.**	Necropsy
1	Male	50	0.12	23.5	27.8	30.5	7.0	None	Normal
2	Male	50	0.12	23.9	26.1	29.7	5.8	None	Normal
3	Male	50	0.12	24.9	28.9	31.21	6.3	None	Normal
4	Male	50	0.11	21.8	24.7	28.6	6.8	None	Normal
5	Male	50	0.12	23.7	26.9	28.8	5.1	None	Normal
			MEAN	23.5	26.9	29.8			
			±SD	1.3	1.6	1.1			
6	Female	50	0.12	24.8	28.0	29.9	5.1	None	Normal
7	Female	50	0.12	24.3	28.2	31.2	6.9	None	Normal
8	Female	50	0.12	23.9	27.4	30.5	6.6	None	Normal
9	Female	50	0.12	24.8	28.9	31.0	6.2	None	Normal
10	Female	50	0.12	24.1	28.6	30.9	6.8	None	Normal
			MEAN	24.4	28.2	30.7			
			SD	0.4	0.6	0.5			

** Summary of clinical observations day 0 through day 14

TABLE 6 (cont.)

ACUTE ORAL - LD50 - MICE
1,3-DINITROBENZENE

Animal #	Sex	Dose Level mg/kg	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.**	Necropsy
11	Male	70	0.12	24.0	25.3[a]	-	1.3[b]	1,2,3,4,5,6,7	Normal
12	Male	70	0.12	23.1	23.6[a]	-	0.5[b]	1,2,3,5,7	Normal
13	Male	70	0.12	24.3	26.9	30.1	5.8	None	Normal
14	Male	70	0.11	22.8	23.0[a]	-	0.2[b]	1,2,3,5,7	Normal
15	Male	70	0.11	22.9	25.1	28.7	5.8	None	Normal
			MEAN	23.4					
			±SD	0.7					
16	Female	70	0.11	21.9	23.4[a]	-	1.5[b]	1,2,3,5,7	Normal
17	Female	70	0.11	22.9	25.0	28.2	5.3	1,2,3	Normal
18	Female	70	0.11	21.5	24.8	26.6	5.1	None	Normal
19	Female	70	0.11	22.6	25.9	29.1	6.5	None	Normal
20	Female	70	0.12	24.8	28.1	29.6	4.8	None	Normal
			MEAN	22.7	26.0[c]	28.4[c]			
			SD	1.3	1.5	1.3			

** Summary of clinical observations day 0 through day 14
1: Squinting
2: Somnolence
3: Tachypnea
4: Lethargy
5: Tremors
6: Piloerection
7: Death

a: Death weight
b: Difference based on death weight
c: Mean & Standard deviation did not include death weight

TABLE 6 (cont.)

ACUTE ORAL - LD50 - MICE
1,3-DINITROBENZENE

Animal #	Sex	Dose Level* mg/kg	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.**	Necropsy
21	Male	90	0.10	19.8	20.4[a]	-	0.6[b]	1,2,3,6	Normal
22	Male	90	0.11	22.8	24.5	27.6	4.8	None	Normal
23	Male	90	0.12	23.4	22.8[a]	-	-0.6[b]	1,2,3,6	Normal
24	Male	90	0.12	23.5	22.9[a]	-	-0.6[b]	1,2,3,6	Normal
25	Male	90	0.12	24.1	25.9	27.3	3.2	None	Normal
			MEAN	22.7					
			±SD	1.7					
26	Female	90	0.12	23.1	24.3[a]	-	1.2[b]	1,2,3,4,6	Normal
27	Female	90	0.11	22.3	25.1	28.1	3.0	None	Normal
28	Female	90	0.11	22.6	23.4[a]	-	0.8[b]	1,2,3,4,5,6	Normal
29	Female	90	0.11	23.9	26.7	28.6	4.7	None	Normal
30	Female	90	0.12	24.8	25.0[a]	-	0.2[b]	1,2,3,4,5,6	Normal
			MEAN	23.3					
			SD	1.0					

** Summary of clinical observations day 0 through day 14

1: Squinting
2: Somnolence
3: Tachypnea
4: Lethargy
5: Tremors
6: Death

a: Death weight
b: Difference based on death weight

TABLE 7

ACUTE DERMAL - LIMIT TEST
1,3-DINITROBENZENE
DOSE 2.0 g/kg

Animal #	Sex	Body Weight (Kg) Day 0	Body Weight (Kg) Day 14	Change in Weight (Kg)	Day of Death	Signs* Toxicity	Abnormality at Necropsy
1075	Male	2.23	2.21	-0.02	DAY 3	0	None
1076	Male	2.40	2.35	-0.05	DAY 3	0	None
1077	Male	2.36	2.50	0.14	-	0	None
1078	Male	2.21	2.37	0.16	-	0	None
1079	Male	2.02	2.29	0.27	-	0	None
	Mean	2.24					
	±SD	0.15					
1080	Female	2.01	2.31	0.30	-	0	None
1081	Female	2.17	2.39	0.22	-	0	None
1082	Female	2.08	2.05	-0.03	DAY 3	0	None
1083	Female	2.13	2.08	-0.05	DAY 3	0	None
1084	Female	2.03	2.30	0.27	-	0	None
	Mean	2.08					
	±SD	0.07					

*Summary of 14 days of observation

TABLE 8

ACUTE DERMAL - LD50
1,3-DINITROBENZENE
Dose Level: 2.25 g/kg

Animal #	Sex	Body Weight (Kg) Day 0	Day 7	Day 14	Change in Weight (Kg)	Day of Death	Signs* Toxicity	Abnormalities at Necropsy
2597	Male	2.91	2.99	3.10	0.19	--	None	None
2598	Male	2.20	2.21[a]	----	0.01[b]	Day 2	1,2,3	None
2599	Male	2.40	2.42[b]	----	0.02[b]	Day 2	1,2,3	None
2600	Male	2.80	2.85	2.99	0.14	--	None	None
2601	Male	2.54	2.54[a]	----	0.00[b]	Day 2	2,3	None
	Mean	2.57						
	±SD	0.29						
2602	Female	2.66	2.64[a]	----	-0.02[b]	Day 2	1,2,3	None
2603	Female	2.59	2.58[a]	----	-0.01[b]	Day 2	3	None
2604	Female	2.78	2.79[a]	----	0.01[b]	Day 3	2,3	None
2605	Female	2.35	2.42	2.53	0.07	--	None	None
2606	Female	2.50	2.49[a]	----	-0.01[b]	Day 4	2,3	None
	Mean	2.58						
	±SD	0.16						

a Death Weight
b Based on Death Weight

1: Tonic - clonic convulsions
2: Asphyxial convulsions
3: Death

TABLE 8 (Cont.)

ACUTE DERMAL - LD50
1,3-DINITROBENZENE
Dose Level: 2.00 g/kg

Animal #	Sex	Body Weight (Kg) Day 0	Day 7	Day 14	Change in Weight (Kg)	Day of Death	Signs* Toxicity	Abnormalities at Necropsy
2607	Male	2.98	3.01[a]	----	0.03[b]	Day 5	1,2,3	None
2608	Male	2.68	2.72	2.77	0.09	--	None	None
2609	Male	2.47	2.47[a]	----	0.00[b]	Day 3	2,3	None
2610	Male	2.67	2.74	2.83	0.17	--	None	None
2611	Male	2.45	2.51	2.60	0.15	--	None	None
	Mean	2.65						
	±SD	0.21						
2612	Female	2.44	2.49	2.53	0.09	--	None	None
2613	Female	2.88	2.90[a]	----	0.02[b]	Day 3	1,2,3	None
2614	Female	2.37	2.45	2.49	0.08	--	None	None
2615	Female	2.55	2.55[a]	----	0.00[b]	Day 3	1,2,3	None
2616	Female	2.30	2.28[a]	----	-0.02[b]	Day 4	1,2,3	None
	Mean	2.51						
	±SD	0.23						

a Death Weight
b Based on Death Weight

*Summary of 14 days of observation

1: Tonic - clonic convulsions
2: Asphyxial convulsion
3: Death

TABLE 8 (Cont.)

ACUTE DERMAL - LD50
1,3-DINITROBENZENE
Dose Level: 1.75 g/kg

Animal #	Sex	Body Weight (Kg) Day 0	Day 7	Day 14	Change in Weight (Kg)	Day of Death	Signs* Toxicity	Abnormalities at Necropsy
2617	Male	2.61	2.71	2.80	0.19	--	None	None
2618	Male	2.74	2.80	2.88	0.14	--	None	None
2619	Male	2.25	2.27[a]	----	0.02[b]	Day 4	2,3	None
2620	Male	2.62	2.66[a]	----	0.04[b]	Day 5	2,3	None
2621	Male	2.97	2.99	3.11	0.14	--	None	None
	Mean	2.64						
	±SD	0.26						
2622	Female	2.99	3.08	3.15	0.16	--	None	None
2623	Female	2.64	2.72	2.80	0.16	--	None	None
2624	Female	2.73	2.80	2.90	0.17	--	None	None
2625	Female	2.75	2.82	2.91	0.16	--	None	None
2626	Female	2.88	2.85[a]	----	-0.03[b]	Day 5	1,2,3	None
	Mean	2.80						
	±SD	0.14						

* Death Weight

*Summary of 14 days of observation

1: Tonic - clonic convulsions
2: Asphyxial convulsions
3: Death

** Change in weight determined for death weight or final body weight for surviving animals

TABLE 9

SUMMARY FOR BUEHLER SENSITIZATION TEST
CHALLENGE SCORES

1,3-DINITROBENZENE

Group		Mean Wt Change +/- Std	Number of Responses* 24h	48h	72h
Treated	Females	125.9 +/- 33.0	0/10	0/10	0/10
Treated	Males	148.2 +/- 20.1	0/10	0/10	0/10
Negative	Females	137.7 +/- 21.2	0/5	0/5	0/5
Control	Males	165.4 +/- 11.4	0/5	0/5	0/5
Positive	Females	126.6 +/- 17.6	5/5	5/5	5/5
Control	Males	113.5 +/- 15.0	5/5	5/5	5/5

*Number of Responses/Total Number of Animals

TABLE 10

SUMMARY OF ACUTE TOXICITY STUDIES

1,3-DINITROBENZENE

Test	Response
Primary Dermal	non-irritant
Primary Eye	irritant
Acute Oral (rat)	
Combined Sex	LD50 = 59.5 mg/kg
Male	LD50 = <62 mg/kg
Female	LD50 = 63.4 mg/kg
Acute Oral (mice)	
Combined Sex	LD50 = 80.4 mg/kg
Male	LD50 = 74.7 mg/kg
Female	LD50 = 84.5 mg/kg
Acute Dermal (rabbits)	
Combined Sex	LD50 = 1.99 g/kg
Male	LD50 = 2.08 g/kg
Female	LD50 = 1.90 g/kg
Buehler	non-sensitizer

RESULTS (cont.)

1,3,5-TRINITROBENZENE

Primary Dermal Irritation (Table 11)

All of the test animals exhibited a gain in body weight during the observation period. No overt signs of toxicity were evident during the course of the study. No animals exhibited signs of erythema or edema during the study, Table 11. The Primary Dermal Irritation Index (PDII) for the test substance and control for the intact and abraded sites was 0.00.

Primary Ocular Irritation (Table 12)

All of the animals exhibited a gain in body weight during the course of the study. No overt signs of toxicity were evident during the course of the study.

Ocular Irritation - Draize: (Table 12)

The treated eyes of all six rabbits received scores of severe (grade 3 or 4) redness and chemosis at the 1h, 24h, 48h, 72h and 96h observation point. All rabbits received scores (grade 4, area 4) of severe cornea opacity and extreme fluorescein staining at the 24h and continuing through the 96h observation point. No irritation was observed in the control eye of any animal during the observation period.

Extreme fluorescein staining was evident in all of the test animals. Pannus (corneal vasculation) was noted at the 96h observation in 6/6 treated eyes. Vessel growth extended 1-2 mm into the cornea.

Acute Oral Toxicity in Rats

Range Finding (Table 13)

Mortality (8/10 animals) was observed at the four highest (350, 500, 650 and 800 mg/kg) dose levels by Day 1. The surviving male gained body weight while the surviving female lost body weight during the study period. One male and one female that died during the study gained weight, the other dying animals all lost weight. No unusual lesions were noted during necropsy of any of animals. Clinical signs noted for the animals that died during the study included piloerection somnolence and hyperactivity. The surviving female was hyperactive through Day 6.

Based upon the results of the range finding 3 doses were suggested of the LD50 study; 185 mg/kg, 260 mg and 335 mg/kg.

LD50 (Table 14)

Dose group - 335 mg/kg:
All animals died by Day 2 of the study. Both the males and the females in this dose level had a death weight less than their Day 0 weight. All males exhibited signs of dyspnea and hyperactivity on Day 0 (4h observation). On Day 1, prostration, dyspnea and ataxia were observed prior to death. On Day 0 all females exhibited signs of catalepsy.

Necropsy - The findings of the gross necropsy of the animals that died at this dose level include:

Stomach - black depressed lesions ca. 1-2 mm in diameter, (8/10)
Small Intestines - contents stained orange-yellow, (7/10)
Brain - small quantity of blood around cerebellum, (6/10)
Lungs - lobes are dark red/brown and blotchy, (4/10)
Kidneys - medullae are dark red, (2/10)

Dose group - 260 mg/kg:
Two out of the ten animals (both females) died by Day 7 of the study. The eight surviving animals at this dose level gained weight; the animals that died exhibited a slight increase in weight from their initial Day 0 weight.

None of the males exhibited any clinical signs at any of the observation points. None of the females exhibited any clinical signs on Day 0 (4h observation point). All females were lethargic Day 1 through Day 4. Additional clinical signs, yellow-orange staining around the urogenital orifice and alopecia around eyes, were observed from Day 4 through Day 7 in the surviving females. No clinical signs were observed from Day 10 to Day 14.

Necropsy - Gross necropsy revealed depressed black lesions, ca. 1-2 mm in diameter in the stomach of the two animals that died on day 7. No unusual lesions were noted in any of the surviving (8/10) animals.

Dose group - 185 mg/kg:
All animals survived at this dose level. One of the ten surviving animals had a decrease in body weight, all other animals at this dose level gained weight. The animals at this dose level exhibited no clinical signs during the 14 day observation period.

Necropsy - No unusual lesions were noted in any of the animals.

The LD50 of the TNB has been determined to be 284 mg/kg for combined sexes utilizing the method of Linear Regression. The LD50 for male and female rats were 298 mg/kg and 275 mg/kg respectively in rats.

Acute Oral Toxicity in Mice

Range Finding (Table 15)

Mortality (4/10 animals) was observed at the two highest dose (1, 2 g/kg) levels through Day 1. All surviving animals gained weight. The one animals that died at Day 1 lost weight. Clinical signs observed included tachypnea, piloerection, ptosis, and lethargy in the animals that survived beyond the 4 hour observation point. No unusual lesions were noted during necropsy of any of animals.

Based upon the results of the range finding 3 doses were suggested of the LD50 study; 900 mg/kg, 700 mg and 500 mg/kg.

LD50 (Table 16)

Dose group - 900 mg/kg:
The four surviving animals (all males) gained body weight during the observation period. All six animals (5 females, 1 male) found dead, gained body weight. Clinical signs exhibited by all but one animal included dyspnea, and squinting. One surviving male showed no clinical signs.

The necropsy found no unusual lesions were noted in any of the animals.

Dose group - 700 mg/kg:
All surviving animals gained body weight during the observation period, while all animals that died at this dose level lost body weight. Clinical signs exhibited during the 14 day observation period include dyspnea, and prostration. Four of the animals (all females) died by Day 1.

Dose group - 500 mg/kg:
All animals gained body weight during the observation period. No clinical signs were exhibited during the 14 day observation period. No mortality was observed during the observation period.

The LD50 of the TNB has been determined to be 804 mg/kg for combined sexes of mice. The LD50 for male and female were greater than 900 mg/kg and 702 mg/kg respectively utilizing the method of Litchfield and Wilcoxon.

Acute Dermal Toxicity

Limit (Table 17)

All of the animals exhibited a gain in body weight during the observation period. No overt signs of toxicity were evident during the course of the study. All test animals survived the study. Gross necropsy revealed no unusual lesions and no

erythema or edema was exhibited 30 minutes following bandage removal for the animals treated with the test article.

Dermal (Buehler) Sensitization (Table 18)

All animals gained weight during the course of the study. No systemic signs of toxicity were evident during the course of the study. No signs of erythema or edema were present in any of the test or control animals throughout the induction scoring phase. No animals died during the induction or challenge phases.

The Preliminary Irritation Study showed no erythema nor edema at any of sites treated with the test substance. Therefore, the test material was utilized at 100% concentration for the sensitization study.

Signs of erythema (0.5 - 1.0) were observed in 17 of 20 test animals. No signs of erythema were evident in the negative control group. All 10 animals in the positive control groups exhibited signs of erythema (2.0 - 3.0).

DISCUSSION

1,3,5-TRINITROBENZENE

The test article, TNB, was tested for its dermal irritancy potential. According to the established criteria and guidelines, the TNB is considered non-irritating to the intact and abraded skin of laboratory rabbits.

The test substance, 1,3,5-trinitrobenzene, caused severe changes to the ocular tissue of the laboratory animals. Macroscopic lesions were evident in the treated eyes of all six of the animals at 96 hours. Due to the severity of the scores present at the 96h observation point, the lesions were considered irreversible and the study was discontinued to minimize animal discomfort. Therefore, the test substance is considered corrosive according to the criteria of the study protocol.

An acute oral toxicity study was performed with 1, 3,5-trinitrobenzene at 185, 260, and 335 mg/kg in rats. The LD50 value for combined sexes was 275 mg/kg.

A oral LD50 study was performed with 1,3,5-trinitrobenzene at 500, 700, and 900 mg/kg in mice. The LD50 was determined to be 804 mg/kg for the combined sexes.

The test articles, TNB, was found to be non-toxic following a single dose dermal application.

The 1,3,5-trinitrobenzene was assessed as a skin sensitizer with the Buehler topical closed patch technique. A skin sensitizer is a material which elicits an allergic contact

dermatitis. In humans, sensitization is characterized by pruritis, erythema, edema, papules, vesicles, bullae, or a combination of these changes. In rabbits and other species the reaction may be limited to only erythema and edema.
Challenge with the test article following 3 weeks of induction caused erythema in 16/20 treated animals with a combined severity of 11/20. No reactions were noted in the negative control group, and 10/10 animals reacted in the positive control group. Consequently, under the conditions of this study the test substance, 1,3,5-trinitrobenzene is considered a mild sensitizer.

In summary (Table 19) the test substance, 1,3,5-trinitrobenzene was found to be non-toxic with respect to acute dermal toxicity and dermal irritation. TNB was found to be a mild skin sensitizer. It was corrosive to the ocular tissue of rabbits. The oral LD50 in rats was 275 mg/kg and for mice 804 mg/kg for combined sexes.

TABLE 11

PRIMARY DERMAL IRRITATION - DRAIZE SCORE

1,3,5-TRINITROBENZENE

Animal #	Observation/Scores 24 hours* Erythema	Edema	48 hours Erythema	Edema	72 hours Erythema	Edema	Divided By 3
INTACT SITE							
990	0	0	0	0	0	0	0
991	0	0	0	0	0	0	0
992	0	0	0	0	0	0	0
993	0	0	0	0	0	0	0
994	0	0	0	0	0	0	0
995	0	0	0	0	0	0	0
Primary Dermal Irritation Index (PDII) = 0.00							
ABRADED SITE							
990	0	0	0	0	0	0	0
991	0	0	0	0	0	0	0
992	0	0	0	0	0	0	0
993	0	0	0	0	0	0	0
994	0	0	0	0	0	0	0
995	0	0	0	0	0	0	0
Primary Dermal Irritation Index (PDII) = 0.00							

Note: PDII equals sum for all animals divided by 6.
* Readings taken 30-60 minutes after the 24 hour exposure treatment.

TABLE 12

LESION SUMMARY SHEET (TREATED EYE)
POSITIVE SCORES (TSCA)[a]

1,3,5-TRINITROBENZENE

Lesion	1h	24h	48h	72h	96h
CORNEA					
Opacity	6/6	6/6	6/6	6/6	6/6[b]
IRITIS	6/6	6/6	6/6	6/6	6/6
CONJUNCTIVA					
Redness	6/6	6/6	6/6	6/6	6/6
Chemosis	6/6	6/6	6/6	6/6	6/6

[a] Number of Positive Responses/Total Number of Animals (Appendix II).

[b] All treated eyes at this time point showed signs of pannus.

TABLE 13

ACUTE ORAL - RANGE FINDING - RATS

1,3,5-TRINITROBENZENE

Animal #	Sex	Dose Level mg/kg	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.*	Necropsy
1	Male	800	2.11	210.7	209.4[a]	-----	-1.3[b]	Death Day 1	Normal
2	Male	650	2.09	208.5	208.1[a]	-----	-0.4[b]	Death Day 1	Normal
3	Male	500	2.10	210.3	210.2[a]	-----	-0.1[b]	Death Day 1	Normal
4	Male	350	2.11	210.6	211.7[a]	-----	1.1[b]	Death Day 1	Normal
5	Male	200	2.09	209.3	216.0	256.6	47.3	1,2,3	Normal
			MEAN ±SD	209.9 0.9					
6	Female	800	2.07	207.0	206.4[a]	------	-0.6[b]	Death Day 1	Normal
7	Female	650	2.14	213.8	210.8[a]	------	-3.0[b]	Death Day 1	Normal
8	Female	500	2.05	204.6	209.1[a]	------	4.5[b]	Death Day 1	Normal
9	Female	350	2.02	202.3	200.4[a]	------	-1.9[b]	Death Day 1	Normal
10	Female	200	2.14	213.5	179.9	186.8	-26.7	2	Normal
			MEAN ±SD	208.2 5.2					

* Summary of clinical observations day 0 through day 14

1: Somnolence
2: Hyperactivity
3: Piloerection

a: Death Weight
b: Difference based on death weight

TABLE 14

ACUTE ORAL - LD50 - RATS

1,3,5-TRINITROBENZENE

Animal #	Sex	Dose Level mg/kg	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.*	Necropsy
1	Male	335	2.1	209.3	199.7[a]	--	-9.6[b]	1,2 Death Day 1	Abnormal
2	Male	335	2.1	210.9	199.4[a]	--	-11.5[b]	1,2,3 Death Day 1	Abnormal
3	Male	335	2.1	212.3	197.1[a]	--	-15.2[b]	1,2 Death Day 1	Abnormal
4	Male	335	2.1	213.7	199.6[a]	--	-14.1[b]	1,2,3 Death Day 1	Abnormal
5	Male	335	2.1	212.7	198.3[a]	--	-14.4[b]	1,2,4 Death Day 1	Abnormal
			MEAN ±SD	211.8 1.7					
6	Female	335	2.1	215.4	204.4[a]	--	-11.0[b]	1,5 Death Day 2	Abnormal
7	Female	335	2.0	200.7	190.5[a]	--	-10.2[b]	5 Death Day 1	Abnormal
8	Female	335	2.0	204.2	195.8[a]	--	-8.4[b]	5 Death Day 1	Abnormal
9	Female	335	2.0	204.1	194.1[a]	--	-10.0[b]	5 Death Day 1	Abnormal
10	Female	335	2.2	215.7	205.7[a]	--	-10.0[b]	5 Death Day 1	Abnormal
			MEAN ±SD	208.0 7.0					

* Summary of clinical observations day 0 through day 14

1: Dyspnea
2: Hyperactivty
3: Prostration
4: Ataxia
5: Catalepsy

a: Death Weight
b: Weight change calculated from death weight

TABLE 14 (cont.)

ACUTE ORAL - LD50 - RATS

1,3,5-TRINITROBENZENE

Animal #	Sex	Dose Level mg/kg	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.*	Necropsy
11	Male	260	2.0	203.5	228.8	241.6	+38.1	None	Normal
12	Male	260	2.1	211.3	244.6	267.2	+55.9	None	Normal
13	Male	260	2.1	209.9	233.3	246.3	+36.4	None	Normal
14	Male	260	2.2	218.1	251.2	274.0	+55.9	None	Normal
15	Male	260	2.2	218.7	240.1	252.9	+34.2	None	Normal
			MEAN	212.3	239.6	256.4			
			±SD	6.3	8.9	13.8			
16	Female	260	2.1	210.8	211.2[a]	--	+0.4[b]	1,3 Death Day 7	Abnormal
17	Female	260	2.1	208.3	210.7	216.7	+8.4	1,3	Normal
18	Female	260	2.1	209.5	212.2	217.6	+8.1	1,3	Normal
19	Female	260	2.0	200.3	202.0[a]	--	+1.7[b]	1,2 Death Day 7	Abnormal
20	Female	260	2.1	207.8	210.6	220.6	+12.8	1 Death Day 1	Normal
			MEAN	207.3					
			SD	4.1					

* Summary of clinical observations day 0 through day 14

1: Hyperactivity
2: Alopecia around eye
3: Orange-yellow staining of urogenital orifice

a: Death Weight
b: Weight change calculated from death weight
c: Mean and ±SD does not include death weight

TABLE 14 (cont.)

ACUTE ORAL - LD50 - RATS

1,3,5-TRINITROBENZENE

Animal #	Sex	Dose Level mg/kg	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.*	Necropsy
21	Male	185	2.1	211.8	245.6	261.4	+49.6	None	Normal
22	Male	185	2.1	208.8	240.7	255.1	+46.3	None	Normal
23	Male	185	2.2	217.4	250.8	266.6	+49.2	None	Normal
24	Male	185	2.1	211.1	238.2	257.3	+46.2	None	Normal
25	Male	185	2.1	211.6	247.2	259.5	+47.9	None	Normal
			MEAN	212.1	244.5	260.0			
			±SD	3.3	5.2	4.5			
26	Female	185	2.2	218.7	230.5	231.7	13.0	None	Normal
27	Female	185	2.0	204.1	209.1	215.0	10.9	None	Normal
28	Female	185	2.1	212.6	218.8	223.0	10.4	None	Normal
29	Female	185	2.1	209.8	222.3	225.7	15.9	None	Normal
30	Female	185	2.1	207.7	209.5	204.9	-2.8	None	Normal
			MEAN	210.6	218.0	220.1			
			±SD	5.7	9.3	10.7			

* Summary of clinical observations day 0 through day 14

TABLE 15

ACUTE ORAL - RANGE FINDING - MICE

1,3,5-TRINITROBENZENE

Animal #	Sex	Dose Level* (g/kg)	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.*	Necropsy
1	Male	2.0	0.113	22.6[a]	--	--	--	Death 4h	Normal
2	Male	1.0	0.1145	22.9	22.7[b]	--	-0.2[c]	1,2,3 Death Day 2	Normal
3	Male	0.5	0.1240	24.8	26.1	29.5	+4.7	1,2,3	Normal
4	Male	0.25	0.1195	23.9	27.0	31.6	+7.7	1,2,3	Normal
5	Male	0.125	0.1025	20.5	23.9	25.2	+4.7	1,2	Normal
			MEAN	22.9					
			±SD	1.6					
6	Female	2.0	0.096	19.2[a]	--	--	--	Death 4h	Normal
7	Female	1.0	0.098	19.6[a]	--	--	--	Death 4h	Normal
8	Female	0.5	0.955	19.1	24.2	27.9	+8.8	1,2,3	Normal
9	Female	0.25	0.0985	19.7	24 9	28.5	+8.8	1,2	Normal
10	Female	0.125	0.0965	19.3	24.1	28.6	+9.3	1,2,	Normal
			MEAN	19.4					
			±SD	0.3					

* Summary of clinical observations day 0 through day 14

1: Tachypnea
2: Piloerection
3: Ptosis

a: Death by the 4 hour observation point, no death weight required
b: Death weight Day 2
c: Difference based on death weight
d: Mean and ±SD does not include death weight day 2

TABLE 16

ACUTE ORAL - LD50 - MICE

1,3,5-TRINITROBENZENE

Animal #	Sex	Dose Level g/kg	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.**	Necropsy
1	Male	0.9	0.23	23.0	22.8[a]	-	-0.2[b]	1,2 Death Day 1	Normal
2	Male	0.9	0.24	23.7	25.1	27.8	4.1	1,2	Normal
3	Male	0.9	0.25	24.9	26.3	29.2	4.3	1,2	Normal
4	Male	0.9	0.25	24.8	25.9	28.3	3.5	None	Normal
5	Male	0.9	0.24	24.4	26.0	29.4	5.0	1,2	Normal
			MEAN ±SD	24.2 0.8	25.8[c] 0.5	28.7[c] 0.7			
6	Female	0.9	0.23	23.4	22.9[a]	-	-0.5[b]	1,2 Death Day 1	Normal
7	Female	0.9	0.24	22.8	23.2[a]	-	+0.3[b]	1,2 Death Day 1	Normal
8	Female	0.9	0.22	21.5	20.7[a]	-	-0.8[b]	Death 4h	Normal
9	Female	0.9	0.22	23.5	21.9[a]	-	-1.6[b]	1,2 Death Day 1	Normal
10	Female	0.9	0.22	22.6	22.1[a]	-	-0.5[b]	1,2 Death Day 1	Normal
			MEAN ± SD	22.7 0.8					

** Summary of clinical observations day 0 through day 14
1: Dyspnea
2: Prostration

a: Death weight
b: Difference based on death weight
c: Mean & Standard deviation did not include death weight

TABLE 16 (cont.)

ACUTE ORAL - LD50 - MICE

1,3,5-TRINITROBENZENE

Animal #	Sex	Dose Level g/kg	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.**	Necropsy
11	Male	0.7	0.25	24.6	26.1	28.9	4.3	1,2	Normal
12	Male	0.7	0.25	24.8	26.3	27.2	2.4	1,2	Normal
13	Male	0.7	0.25	24.5	26.9	28.1	3.6	1,2	Normal
14	Male	0.7	0.25	24.9	25.8	28.6	3.7	None	Normal
15	Male	0.7	0.25	24.7	26.0	29.3	4.6	1,2	Normal
			MEAN	24.7	26.2	28.4			
			±SD	0.2	0.4	0.8			
16	Female	0.7	0.23	23.1	25.7	27.0	3.9	1,2	Normal
17	Female	0.7	0.23	23.0	22.8[a]	-	-0.2[b]	1,2 Death Day 1	Normal
18	Female	0.7	0.23	22.6	22.1[a]	-	-0.5[b]	1,2 Death Day 1	Normal
19	Female	0.7	0.24	23.9	23.4[a]	-	-0.5[b]	1,2 Death Day 1	Normal
20	Female	0.7	0.23	22.9	22.5[a]	-	-0.4[b]	1,2 Death Day 1	Normal
			MEAN	23.1					
			±SD	0.5					

** Summary of clinical observations day 0 through day 14
1: Dyspnea
2: Prostration

a: Death weight
b: Difference based on death weight

TABLE 16 (cont.)

ACUTE ORAL - LD50 - MICE

1,3,5-TRINITROBENZENE

Animal #	Sex	Dose Level g/kg	Dose (ml)	Wt (g) Day 0	Wt (g) Day 7	Wt (g) Day 14	Weight Change (g)	Clinical Obs.**	Necropsy
21	Male	0.5	0.25	24.9	29.1	31.6	6.7	None	Normal
22	Male	0.5	0.25	24.2	28.9	32.8	8.6	None	Normal
23	Male	0.5	0.25	24.9	28.7	30.2	5.3	None	Normal
24	Male	0.5	0.24	24.1	29.3	32.8	8.7	None	Normal
25	Male	0.5	0.24	24.1	29.0	31.6	7.5	None	Normal
			MEAN	24.4	29.0	31.8			
			±SD	0.4	0.2	1.1			
26	Female	0.5	0.22	21.8	23.7	25.9	4.1	None	Normal
27	Female	0.5	0.23	23.1	25.4	27.4	4.3	None	Normal
28	Female	0.5	0.22	21.7	23.6	24.7	3.0	None	Normal
29	Female	0.5	0.21	21.2	23.5	24.6	3.4	None	Normal
30	Female	0.5	0.23	22.6	24.7	26.3	3.7	None	Normal
			MEAN	22.1	24.2	25.8			
			±SD	0.8	0.8	1.2			

** Summary of clinical observations day 0 through day 14

TABLE 17

ACUTE DERMAL - LIMIT

1,3,5-TRINITROBENZENE

DOSE LEVEL 2.0 g/kg

Animal #	Sex	Signs* Toxicity	Abnormalities at Necropsy	Skin Site	Erythema/Edema Day 1
1085	Male	0	None	Intact	0/0
1086	Male	0	None	Intact	0/0
1087	Male	0	None	Intact	0/0
1088	Male	0	None	Intact	0/0
1089	Male	0	None	Intact	0/0
1090	Female	0	None	Intact	0/0
1091	Female	0	None	Intact	0/0
1092	Female	0	None	Intact	0/0
1093	Female	0	None	Intact	0/0
1094	Female	0	None	Intact	0/0

*Summary of 14 days of observation

TABLE 18

SUMMARY FOR BUEHLER SENSITIZATION TEST
CHALLENGE SCORES

1,3,5-TRINITROBENZENE

Group		Mean Wt Change +/- Std	24h I[a]	24h S[b]	48h I	48h S	72h I	72h S
Treated	Females	123.4 ± 17.8	8/10	5/10	8/10	4/10	9/10	4.5/10
Treated	Males	141.4 ± 17.7	8/10	6/10	8/10	5/10	8/10	4/10
Negative	Females	137.7 ± 23.7	0/5	--	0/5	--	0/5	--
Control	Males	165.4 ± 12.7	0/5	--	0/5	--	0/5	--
Positive	Females	113.5 ± 16.7	5/5	12/5	5/5	13/5	5/5	13/5
Control	Males	126.6 ± 19.7	5/5	11/5	5/5	11/5	5/5	11/5

[a] Incidence - number responding divided by number of animals.
[b] Severity - sum of test grades divided by number of animals.

TABLE 19

SUMMARY OF ACUTE TOXICITY STUDIES

1,3,5-TRINITROBENZENE

Test	Response
Primary Dermal	non-irritant
Primary Eye	corrosive
Acute Oral (rat)	
Combined Sex	LD50 = 284 mg/kg
Male	LD50 = 298 mg/kg
Female	LD50 = 275 mg/kg
Acute Oral (mice)	
Combined Sexes	LD50 = 804 mg/kg
Male	LD50 = >900 mg/kg
Female	LD50 = 702 mg/kg
Acute Dermal	non-toxic
Buehler	mild sensitizer

RESULTS (cont.)

N-METHYL-N,2,4,6-TETRANITROANILINE (TETRYL)

Primary Dermal Irritation (Table 20)

All of the test animals exhibited a gain in body weight during the observation period. No overt signs of toxicity were evident during the course of the study. No animals exhibited signs of erythema or edema during the study. The Primary Dermal Irritation Index (PDII) for the Tetryl and control for the intact and abraded sites was 0.00.

Primary Ocular Irritation (Table 21)

All of the animals exhibited a gain in body weight during the course of the study. No overt signs of toxicity were evident during the course of the study.

Ocular Irritation: (Draize) The treated (tetryl) eye of 6/6 rabbits received scores of severe (grade 3 or 4) redness, chemosis and/or discharge at the 1h, 24h, 48h, 72h and 96h observation points (Table 21).

Ocular Irritation (Fluorescein Staining)
The treated (tetryl) eye of 6/6 rabbits received scores of extreme (grade 4) fluorescein staining of greater than half of the cornea at the 24h, 48h, 72h and 96h observation points.

Additional Observations: Pannus (corneal vascularization) was noted at the 96h observation in 6/6 treated (tetryl) eyes. Vessel growth extended 1-2 mm into the cornea. Due to the severity of damage at the 96h observation point, the study was discontinued to minimize animal discomfort.

Acute Oral Toxicity in Rats (Table 22)

Nine animals lost body weight by Day 7 of the observation period. The female found dead on Day 5 had also lost weight. All the male animals gained weight while all the surviving females exhibited a loss in body weight.

Clinical signs were noted in all animals through the first 7 days of the observation period. No clinical signs were observed in the male rats after study Day 8. Two female rats showed clinical signs through Day 14, one through Day 11, and the fourth through Day 10. The female found dead on Day 5 had shown clinical signs up until death. Clinical signs included lethargy, piloerection, watery stool, wet tail, and nostril discharge, Table 22.

A single female animal died on Day 5 of the study. No other mortality was observed throughout the 14 day observation period. No unusual lesions were noted in any of the animals.

Acute Oral Toxicity in Mice (Table 23)

All animals gained body weight during the observation period. Clinical signs observed included tachypnea, piloerection, ptosis, catalepsy, and/or unusual locomotion in 10/10 test animals, on Days 1, 2, 3, or 4.

One death was observed on Day 4. No other mortality was observed throughout the 14 day observation period. No unusual lesions were noted in any of the animals.

Acute Dermal Toxicity (Table 24)

All of the animals exhibited a gain in body weight during the observation period. No overt signs of toxicity were evident during the course of the study. All test animals survived the study. Gross necropsy revealed no unusual lesions and no erythema or edema was exhibited 30 minutes following bandage removal for the animals treated with the test article, Table 24.

Dermal (Buehler) Sensitization (Table 25)

All animals gained weight during the course of the study. No systemic signs of toxicity were evident during the course of the study. No signs of erythema or edema were present in any of the test or control animals throughout the induction scoring phase. No animals died during the induction or challenge phases.

The Preliminary Irritation Study showed no erythema nor edema at any of sites treated with the test article. Therefore, the test material was utilized at 100% concentration for the sensitization study.

No signs of erythema/edema were evident in any of the animals in the control or experimental groups at either the induction or challenge phase of the study. All 10 animals in the positive control groups exhibited signs of erythema (2.0 - 3.0) at the challenge phase.

DISCUSSION

N-METHYL-N,2,4,6-TETRANITROANILINE

The test substance was evaluated for its potential to produce an allergic skin reaction with the Buehler topical closed patch technique. A skin sensitizer is a material which elicits an

allergic contact dermatitis. The results of this study indicated that the test substance has no sensitization potential.

N-Methyl-N,2,4,6-tetranitroaniline was tested for its dermal irritancy potential and was found to be non-irritating. The test substance was found to have no acute dermal (rabbit) toxicity at 2 g/kg. Tetryl was also found to be non-toxic when administered orally to rats and mice at 5 g/kg.

The test substance caused severe changes to the ocular tissue of the laboratory animals. Macroscopic lesions were evident in the treated eyes of all six of the animals at 96 hours. Due to the severity of the scores present at the 96h observation point, the lesions were considered irreversible and the study was discontinued to minimize animal discomfort. Therefore, the test substance is considered corrosive according to the criteria of the study protocol.

In summary (Table 26) the test substance, N-Methyl-N,2,4,6-tetranitroaniline was found to be non-toxic with respect to 4 acute toxicity criteria, skin sensitization, dermal irritation, and acute dermal and acute oral toxicity. It was, however, found to be corrosive to ocular tissue of rabbits.

TABLE 20

PRIMARY DERMAL IRRITATION - DRAIZE SCORE

METHYL-N,2,4,6-TETRANITROANILINE

Animal #	Observation/Scores 24 hours* Erythema	24 hours* Edema	48 hours Erythema	48 hours Edema	72 hours Erythema	72 hours Edema	Divided By 3
INTACT SITE							
1003	0	0	0	0	0	0	0
1004	0	0	0	0	0	0	0
1005	0	0	0	0	0	0	0
1006	0	0	0	0	0	0	0
1007	0	0	0	0	0	0	0
1008	0	0	0	0	0	0	0
Primary Dermal Irritation Index (PDII) = 0.00							
ABRADED SITE							
1003	0	0	0	0	0	0	0
1004	0	0	0	0	0	0	0
1005	0	0	0	0	0	0	0
1006	0	0	0	0	0	0	0
1007	0	0	0	0	0	0	0
1008	0	0	0	0	0	0	0
Primary Dermal Irritation Index (PDII) = 0.00							

Note: PDII equals sum for all animals divided by 6.
* Readings taken 30-60 minutes after the 24 hour exposure treatment.

TABLE 21

PRIMARY OCULAR IRRITATION

LESION SUMMARY SHEET (TREATED EYE)

METHYL-N,2,4,6-TETRANITROANILINE

Lesion		1h	24h	48h	72h	96h
CORNEA						
	Opacity*	6/6	6/6	6/6	6/6	6/6
IRITIS		6/6	6/6	6/6	6/6	6/6
CONJUNCTIVA						
	Redness	6/6	6/6	6/6	6/6	6/6
	Chemosis	6/6	6/6	6/6	6/6	6/6
	Discharge	6/6	6/6	6/6	6/6	6/6

Number of Responses/Total Number of Animals

TABLE 22

ACUTE ORAL TOXICITY IN RATS

METHYL-N,2,4,6-TETRANITROANILINE

Animal #	Sex	Dose (g)*	Dose (ml)	Wt (g) Day 0 05/04/90	Wt (g) Day 7 05/10/90	Wt (g) Day 14 05/18/90	Weight Change (g)	Clinical Obs.**	Necropsy
1	Male	1.04	2.09	208.9	195.0	245.7	36.8	1,2,3,4	Normal
2	Male	1.07	2.14	214.0	184.1	240.3	26.3	1,2,3,4	Normal
3	Male	1.09	2.18	217.7	190.8	245.1	27.4	1,2,3	Normal
4	Male	1.05	2.10	209.9	196.0	249.9	40.0	1,2,3	Normal
5	Male	1.03	2.06	205.2	186.6	255.0	49.8	1,2,3	Normal
			MEAN	211.1	190.5	247.2			
			±SD	4.8	5.2	5.5			
6	Female	1.09	2.18	218.7	179.1[a]	-	-39.6[c]	1,2,6	Normal
7	Female	1.03	2.06	206.9	147.5	161.8	-45.1	1,2,3	Normal
8	Female	1.05	2.10	210.2	149.3	166.6	-43.6	1,2,3	Normal
9	Female	1.03	2.06	205.4	143.0	169.4	-36.0	1,3,5	Normal
10	Female	1.04	2.08	207.3	139.1	135.0	-72.3	1,2,3	Normal
			MEAN	209.7	144.7[b]	158.2			
			±SD	5.3	4.6	15.8			

* 5 g/kg (dose volume adjusted based on suspension concentration of 0.5 g/ml)

** Summary of clinical observations day 0 through day 14

1: Lethargy
2: Piloerection
3: Watery stool
4: Wet Tail
5: Nostril discharge
6: Death

a: Death weight
b: Death weight for animals #6 not included in Mean and SD calculation
c: Difference based on death weight

TABLE 23

ACUTE ORAL TOXICITY IN MICE

METHYL-N,2,4,6-TETRANITROANILINE

Animal #	Sex	Dose (g)*	Dose (ml)	Wt (g) Day 0 04/24/90	Wt (g) Day 7 05/01/90	Wt (g) Day 14 05/08/90	Weight Change (g)	Clinical Obs.**	Necropsy
1	Male	0.124	0.494	24.7	26.9	29.6	+4.9	1,2	Normal
2	Male	0.096	0.382	19.1	23.2	28.2	+9.1	1,2,3	Normal
3	Male	0.122	0.488	24.4	27.1	30.2	+5.8	1,2	Normal
4	Male	0.121	0.484	24.2	26.5	29.4	+5.2	1,2,3	Normal
5	Male	0.103	0.410	20.5	24.1	28.1	+7.6	1,2,3	Normal
			MEAN	22.6	25.6	29.1			
			±SD	2.6	1.8	0.9			
6	Female	0.099	0.394	19.7	21.7[a]	-	+2.0[c]	1,2,3,4,5,6	Normal
7	Female	0.095	0.380	19.0	23.7	26.9	+7.9	1,2,3	Normal
8	Female	0.098	0.392	19.6	24.0	27.3	+7.7	1,2,3	Normal
9	Female	0.091	0.364	18.2	23.6	26.9	+8.7	1,2	Normal
10	Female	0.910	0.362	18.1	22.9	24.7	+6.6	1,2	Normal
			MEAN	18.9	23.6[b]	26.4			
			±SD	0.8	0.5	1.2			

* 5 g/kg (dose volume adjusted based on suspension concentration of 0.25 g/ml)

** Summary of clinical observations day 0 through day 14

1: Tachypnea
2: Piloerection
3: Ptosis
4: Cataplesy
5: Unusual locomotion
6: Death

a: Death weight
b: Death weight for animal #6 not included in Mean and SD calculation
c: Difference based on death weight

TABLE 24

ACUTE DERMAL TOXICITY STUDY

METHYL-N,2,4,6-TETRANITROANILINE

DOSE LEVEL 2.0 g/kg

Animal #	Sex	Signs* Toxicity	Abnormalities at Necropsy	Skin Site	Erythema/Edema Day 1* 04/26/90
1095	Male	0	None	Intact	0/0
1096	Male	0	None	Intact	0/0
1097	Male	0	None	Intact	0/0
1098	Male	0	None	Intact	0/0
1099	Male	0	None	Intact	0/0
2000	Female	0	None	Intact	0/0
2001	Female	0	None	Intact	0/0
2002	Female	0	None	Intact	0/0
2003	Female	0	None	Intact	0/0
2004	Female	0	None	Intact	0/0

*Summary of 14 days of observation

TABLE 25

SUMMARY FOR BUEHLER SENSITIZATION TEST

METHYL-N,2,4,6-TETRANITROANILINE

Group		Mean Wt Change +/- Std	Number Responsing* 24h	48h	72h	96h
Treated	Females	125.9 +/- 33.0	0/10	0/10	0/10	0/10
Treated	Males	148.2 +/- 20.1	0/10	0/10	0/10	0/10
Negative	Females	137.7 +/- 21.2	0/5	0/5	0/5	0/5
Control	Males	165.4 +/- 11.4	0/5	0/5	0/5	0/5
Positive	Females	126.6 +/- 17.6	5/5	5/5	5/5	5/5
Control	Males	113.5 +/- 15.0	5/5	5/5	5/5	5/5

*Number of Responses/Total Number of Animals

TABLE 26

SUMMARY OF ACUTE TOXICITY STUDIES

METHYL-N,2,4,6-TETRANITROANILINE

Test	Response
Buehler	non-sensitizer
Primary Eye	corrosive
Primary Dermal	non-irritant
Acute Dermal	non-toxic
Acute Oral (rat)	non-toxic
Acute Oral (mice)	non-toxic

APPENDIX I

Draize Scale for Induction Scoring of Skin Reactions

Erythema and Eschar Formation	Value
No erythema	0
Very slight erythema (barely perceptible	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Total possible erythema score = 4

Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges are well defined by definite raising	2
Moderate edema (raised approximately 1mm	3
Severe edema (raised more than 1mm and extending beyond area of exposure	4

Total possible edema score = 4

APPENDIX II

DRAIZE SCALE FOR SCORING OCULAR LESIONS

CORNEA
A. OPACITY: degree of density (areas most dense taken for reading)

No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster, details or iris clearly visible	1*
Easily discernible translucent areas, details of iris slightly obscured	2*
Opalescent/nacreous areas, no details of iris visible size of pupil barely discernible	3*
Opaque cornea, iris not discernible through opacity	4*

B. AREAS OF CORNEA INVOLVED

One quarter or less, but not zero	1
Greater than one quarter, but less than one half	2
Greater than one half, but less than three quarters	3
Greater than three quarters, up to whole area	4

Cornea subtotal score equals AxBx5 Total Maximum = 80

IRIS
A. VALUES

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2*

Iris subtotal score equals A x 5; Total Maximum = 10

CONJUNCTIVA
A. REDNESS: refers to palpebral and bulbar conjunctivae excluding cornea and iris

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2*
Diffuse beefy red	3*

B. CHEMOSIS

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3*
Swelling with lids about half closed to completely closed	4*

* Positive Reactions for TSCA Guidelines.

C. DISCHARGE

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals	1
Discharge with moistening of lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

Subtotal score equals (A+B+C) x 2 Total Maximum = 20

The TOTAL MAXIMUM SCORE is the sum of the scores obtained for the cornea, iris, and conjunctiva. The total maximum score possible is 110.

APPENDIX III
FLUORESCEIN STAINING

A. INTENSITY OF STAINING	
Absence of fluorescein staining	0
Slight fluorescein staining confined to a small focus. With diffuse illumination the underlying structures are easily visible. The outline of the pupillary margin is as if there were no fluorescein staining	1
Moderate fluorescein staining confined to a small focus. With diffuse illumination the underlying structures are clearly visible, although there is some loss of detail	2
Marked fluorescein staining. Staining may involve a larger portion of the cornea. With diffuse illumination underlying structures are barely visible but not completely obliterated.	3
Extreme fluorescein staining. With diffuse illumination the underlying structures cannot be observed.	4
B. AREA INVOLVED	
No area of fluorescein	0
One quarter of less but not 0	1
Greater than one quarter, but less than one half	2
Greater than one half, but less than three quarters	3
Greater than three quarters, up to whole area	4

Appendix IV

Chemical Purity:

The test substance 1,3-dinitrobenzene (DNB), Sigma CAT. NO. D-6379, Lot NO. 107F-5012 was obtained from the Sigma Chemical Co. St. Louis, MO. The test substances 1,3,5-trinitrobenzene (TNB) and N-Methyl-N,2,4,6-tetranitoaniline (tetryl) were synthesized by Dr. W. Koppes and Dr. C.D. Bedford of Naval Surface Warefare Center, Silver Spring, MD. The purity of these compounds were checked by Mr. E. Bruggman and Mr. A. Rosencrance of U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, Frederick, MD. The following High Pressure Liquid Chromatography (HPLC) conditions were used.

HPLC CONDITIONS

Column: Microsorb C18 (Rainin Instruments Co., Woburn, MA)

Column Temperature: 50 degrees centigrade

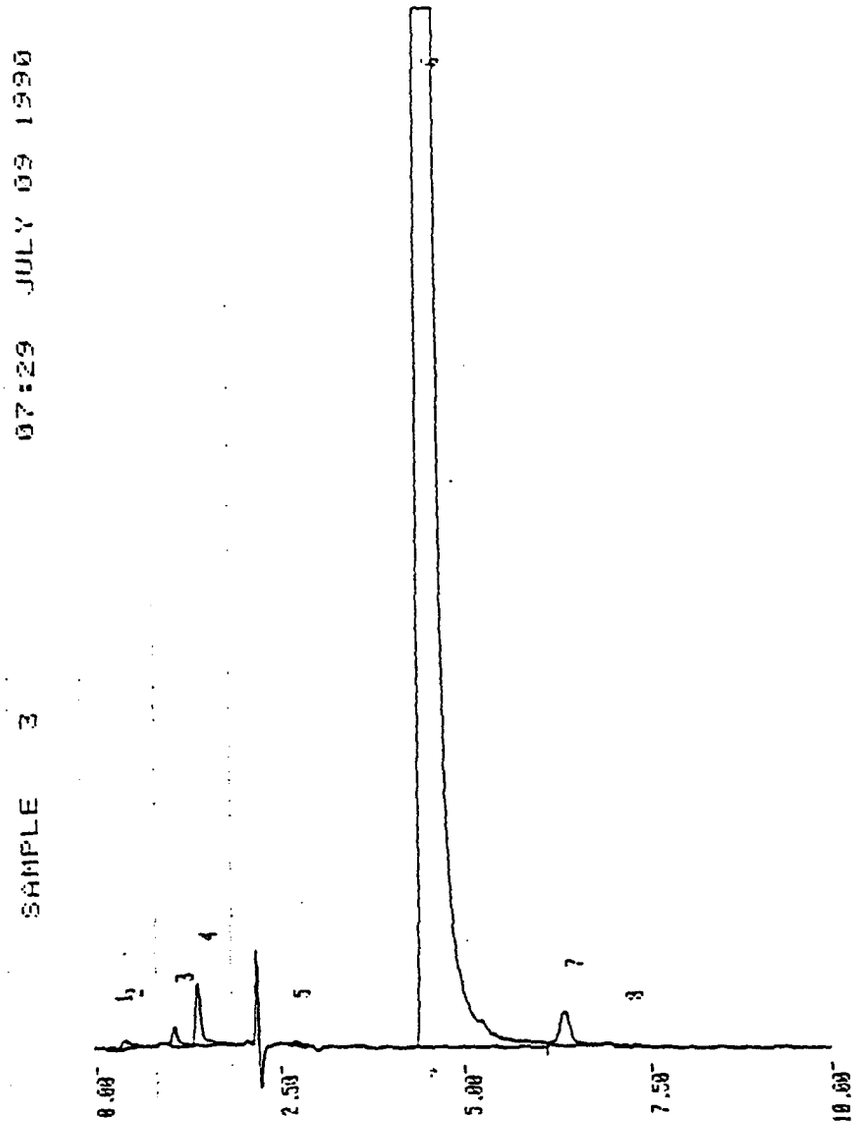
Mobile Phase: 57% Methanol/Water

Flow Rate: 1.2 ml/min.

Detector: UV, 254nm

Injection Volume: 5 microliters

HPLC Chromatograms pages 66-69

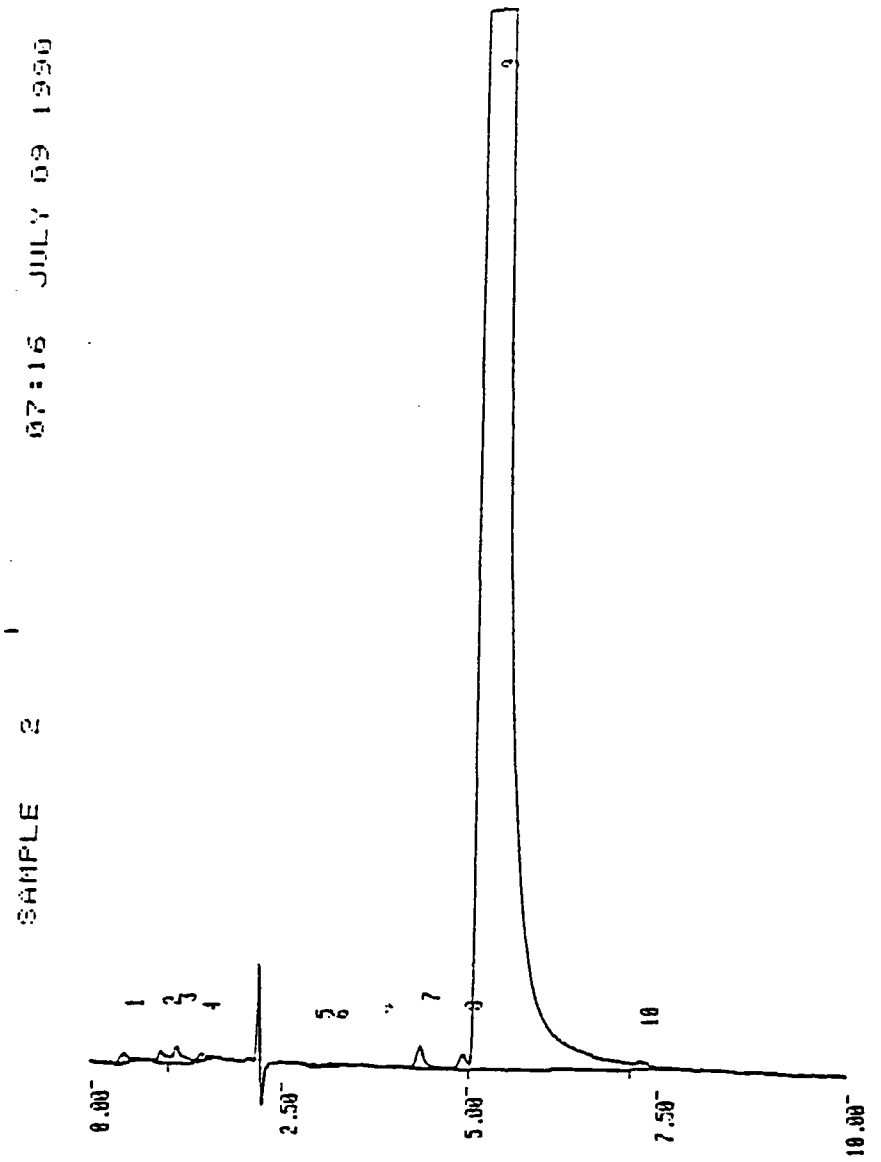


CAL. METHOD 00

	SF	PA	PB
	.100000E+03	.100000E+01	.100000E+01

NO.	NAME	RT	A OR H	MK	CONC
1		0.373	53		0.0024
2		0.422	418		0.0192
3		1.087	719		0.0331
4		1.397	2727		0.1257
5		2.702	136		0.0063
6		4.568	2161107	M	99.6758
7		6.391	2843		0.1311
8		7.178	129		0.0059
	TOTAL		2168135		100.0000

HPLC chromatogram of 350 mg/L TNB.

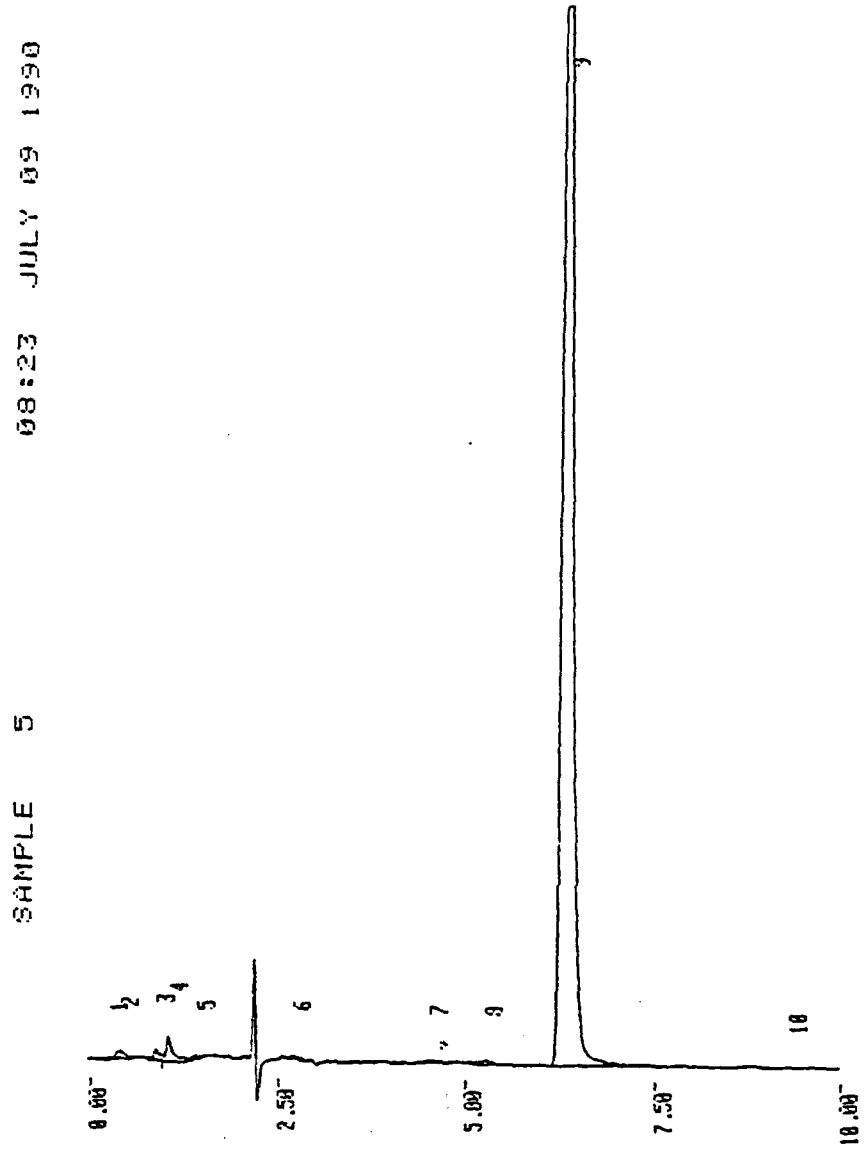


CAL. METHOD 00

	SF	PA	PB
	.100000E+03	.100000E+01	.100000E+01

NO.	NAME	RT	A OR H	MK	CONC
1		0.426	445		0.0087
2		0.902	447	M	0.0087
3		1.095	822		0.0161
4		1.410	355		0.0070
5		2.976	79		0.0015
6		3.181	161		0.0031
7		4.396	1157		0.0227
8		4.977	801	M	0.0157
9		5.268	5079379	M	99.8934
10		7.285	894		0.0175
	TOTAL		5084543		100.0000

HPLC chromatogram of 588 mg/L DNB (test tube #1).

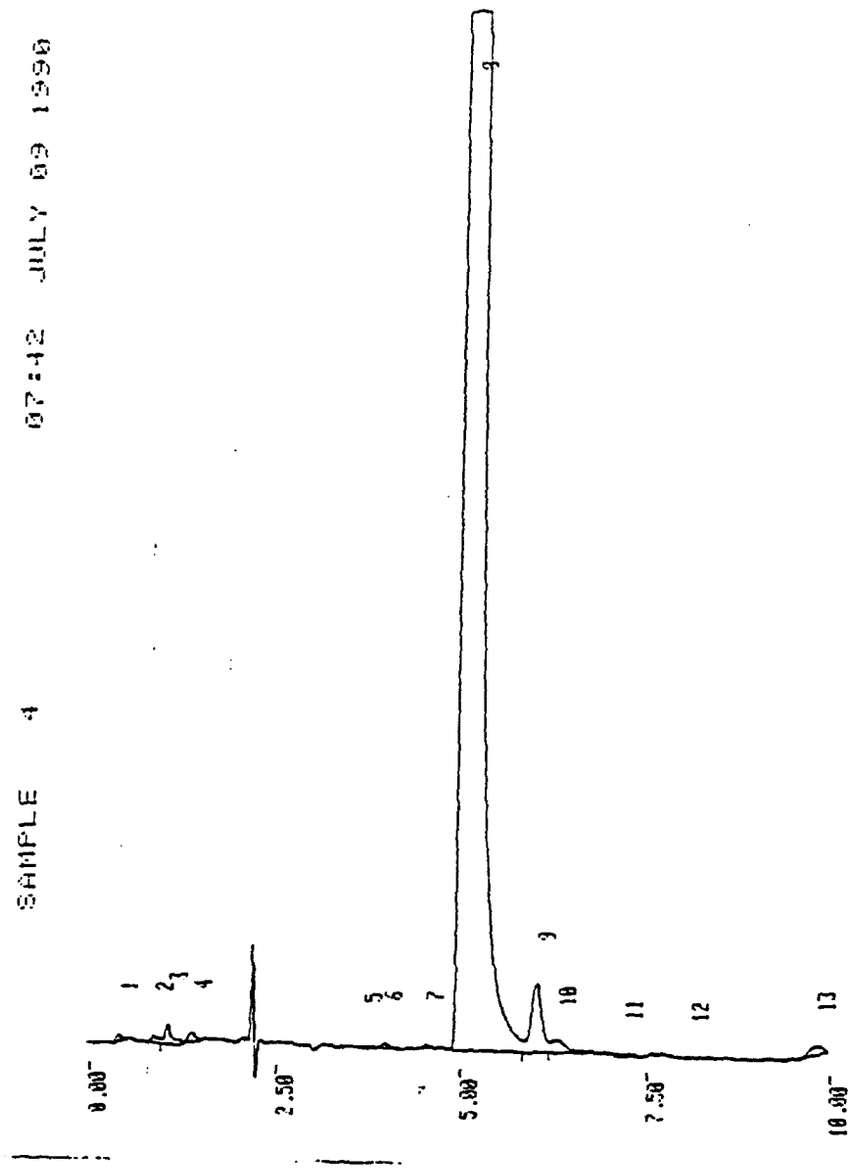


CAL. METHOD 00

	SF	PA	PB
	.100000E+03	.100000E+01	.100000E+01

NO.	NAME	RT	A OR H	MK	CONC
1		0.282	39		0.0419
2		0.427	474		0.5030
3		0.893	359	M	0.3803
4		1.063	882		0.9345
5		1.422	136		0.1444
6		2.722	92		0.0977
7		4.549	225		0.2389
8		5.283	347		0.3679
9		6.383	91810		97.2554
10		9.330	33		0.0355
	TOTAL		94401		100.0000

HPLC chromatogram of a TNT standard solution in methanol.



CAL. METHOD 00

SF	PA	PB
.100000$_{10}$+03	.100000$_{10}$+01	.100000$_{10}$+01

NO.	NAME	RT	A OR H	MK	CONC
1		0.427	367		0.0201
2		0.903	258	M	0.0142
3		1.092	772		0.0424
4		1.404	418		0.0230
5		3.727	75		0.0041
6		3.993	271		0.0148
7		4.553	342		0.0188
8		5.112	1809724	M	99.4580
9		6.047	5043	M	0.2771
10		6.317	1193		0.0655
11		7.220	151		0.0083
12		8.133	120		0.0066
13		9.843	845		0.0464
	TOTAL		1819585		100.0000

HPLC chromatogram of 365 mg/L tetryl.

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