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Document Title	INITIAL SUBMISSION: LETTER FROM DOW CHEM CO TO USEPA, SUBMISSION OF U.S. ARMY DOCUMENTS RE TOXICOLOGICAL EVALUATION OF 2,3-DIMETHYL 2,3-DINITROBUTANE, W/ATTCHMNTS AND DATED 4/4/2001		
Chemical Category	2,3-DIMETHYL 2,3-DINITROBUTANE		

8EHQ-0401-14893

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The Dow Chemical Company  
Midland, Michigan 48674

2001 APR -4 PM 3:42

1803 Building  
April 4, 2001

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8EHQ-01-14893

CONTAINS NO CONFIDENTIAL  
BUSINESS INFORMATION

Document Control Officer (7407)  
Office of Toxic Substances  
Office of Pollution Prevention and Toxics  
U.S. Environmental Protection Agency  
401 M Street, S.W.  
Washington, DC 20460  
Attn: TSCA 8(e) Coordinator

Re: 2,3-Dimethyl-2,3-dinitrobutane (DMDNB)  
CAS # 3964-18-9

2001 APR 10 AM 10:42  
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Dear Sir/Madam:

The following information is being submitted by The Dow Chemical Company (Dow) pursuant to current guidance issued by EPA indicating EPA's interpretation of Section 8(e) of the Toxic Substances Control Act. Dow has made no determination as to whether a significant risk of injury to health or the environment is actually presented by the findings.

DMDNB is apparently a taggant used to mark explosives for purposes of detection. Recently, Dow became aware of the attached U.S. Army documents summarizing toxicology and other studies on DMDNB:

- "Toxicological Evaluation of 2,3-Dimethyl 2,3-dinitrobutane, Study No. 75-51-YJ92-93"(undated)
- "Toxicological Study No. 75-51-YJ92-93, The Acute Toxicity of 2,3-Dimethyl-2,3-dinitrobutane" (October 1992)

The referenced studies were apparently commissioned by or conducted by the U.S. Army's Environmental Hygiene Agency (USAEHA).

EPA is already aware of some or all of the studies referenced in the summary documents. One of the studies referenced in the undated summary document was apparently conducted



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by personnel from EPA's Environmental Monitoring Systems Laboratory, published as Reddy, et al. "Carcinogenic Evaluation of 2,3-Dimethyl-2,3-dinitrobutane via the Mouse Skin Bioassay", *Journal of Applied Toxicology*, 14(3), 231-233 (1994). The Reddy et al. study refers to some of the other studies referenced in the summary documents, and in addition refers to a mouse study not referenced in the summary documents. The Reddy et al. study cites the following:

H. L. Snodgrass, J. P. Houde and J. G. Harvey, *The Acute Toxicity of 2,3-Dimethyl-2,3-dinitrobutane*, Toxicological Study No. 75-51-YJ92-93. U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD. (1992).

EPA personnel may have been aware of the remaining referenced studies as well.

Among the effects of DMDNB noted in the summary documents are the following:

- Hyperexcitability in acute oral and intraperitoneal rat and guinea pig studies. Among surviving (non-moribund) animals, hyperexcitability was seen in only one rat, and aggressiveness was seen in one other rat.
- Extreme irritability and tail biting in a 4-hour rat inhalation study. It is unclear how many of the surviving (non-moribund) rats exhibited these effects.
- Hyperexcitability at 700 ppm (or 600 ppm—the undated summary document refers to both as the high dose level) and 200 ppm (nominal concentrations in the diet) in a 90-day rat study. At the high dose, it is not clear whether hyperexcitability was seen in surviving rats, or only in moribund animals (75% mortality was reported). At 200 ppm, the hyperexcitability was described as mild and intermittent, and was noted in about 40% of males and females. USAEHA considered 200 ppm to be the no observable adverse effect level (NOAEL).
- Hyperexcitability and convulsions, among other effects, were observed in dams in an oral developmental study in rats. The study was conducted at 125 ppm, 250 ppm, and 500 ppm in the feed. It is unclear at what level(s) these particular maternal effects were noted, or in how many animals. The undated summary document indicates that exposure to DMDNB does not cause teratological effects.

Tremors and convulsions were seen in acute toxicity studies of DMDNB in mice and rats submitted by Eastman Kodak, 8EHQ-92-7143. It is unclear how many of the surviving (non-moribund) animals exhibited these effects.

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Dow has no additional information on these studies. The undated summary document indicates that all raw data and copies of all reports are available in the archives of USAEHA, Bldg E1570, Aberdeen Proving Ground, Maryland 21010-5422. For more information, please contact USAEHA directly.

Sincerely,



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EH&S Product Regulatory Management  
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Attachments

**Toxicological Evaluation of 2,3-Dimethyl 2,3-dinitrobutane  
Study No. 75-51-YJ92-93**

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## FORWARD

In conducting the studies described herein, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," U.S. Department of Health, Education and Welfare Publication No. (NIH) 85-23, 1985.

The studies reported herein were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

All Toxicological studies performed at USAEHA for 2,3-Dimethyl-2,3-dinitrobutane (DMDNB) were under purview of the USAEHA Quality Assurance Office from Jan 1992 thru Aug 1993. The Toxicological studies inspected by the USAEHA Quality Assurance Office for DMDNB include:

1. Acute oral toxicity in rats
2. Skin irritation studies in rabbits
3. Eye irritation studies in rabbits
4. Skin sensitization studies in guinea pigs
5. 90-day feeding studies in rats
6. Metabolism studies
7. Ames bacterial mutagenesis assays
8. Rabbit and rat teratology studies
9. Acute inhalation studies in rats.

All raw data and copies of all reports are available in the archives of the U.S. Army Environmental Hygiene Agency, Bldg E1570, Aberdeen Proving Ground, Maryland 21010-5422.

## EXECUTIVE SUMMARY

The purpose of the studies described in this report was to determine the toxic potential of 2,3-dimethyl-2,3-dinitrobutane (DMDNB) in various species. This included studies on (1) short-term toxic responses, (2) subchronic toxicity, (3) dose response data, (4) evaluation of reversibility of toxic responses, (5) evaluation of metabolic parameters and target organs, (6) determination of mutagenicity, (7) wildlife effects and (8) carcinogenicity. A number of difference studies are included in the work performed on this project and are described in the following sections.

### Short-term Toxicity

Acute oral and intraperitoneal (IP) toxicity studies (Approximate Lethal Dosages - ALD) were conducted using Sprague-Dawley rats and Hartley Guinea pigs. The ALD of the test procedure is considered the lowest dose that causes death during a 14-day observation period following exposure. ALDs of DMDNB orally in male and female rats, and in female guinea pigs was 87 mg/kg. The IP ALDs in male and female rats were 50 and 38 mg/kg, respectively. The appearance of toxic signs was rapid with deaths occurring between 30 minutes and 72 hours after treatment. Signs at fatal doses included lethargy, convulsions, prostration and death. Few signs were observed in surviving animals except hemolacrymation and hyperexcitability.

Acute dermal toxicity studies were conducted with New Zealand White rabbits. A single 24-hour occlusive exposure to the skin of male and female rabbits with a 14-day observation period was used. No effects occurred at a dose of 2000 mg/kg, the highest dose tested. No compound related lesions were observed in rabbits at necropsy performed at day 14.

The skin and eye irritation studies were conducted using New Zealand White rabbits. Technical DMDNB (dry; finely ground) did not produce primary irritation on the skin of rabbits during a 24-hour occlusive exposure. DMDNB did not induce irritation to the conjunctival sac or the cornea of eyes at 24, 48 and 72 hours post exposure.

Skin sensitization studies were conducted in female Hartley strain guinea pigs. Dermal applied DMDNB (10 percent in acetone) did not cause a skin sensitization response to a challenge dose following a 3-week induction.

Photochemical skin irritation studies were conducted with New Zealand White rabbits. Irradiation of a dermally applied DMDNB solution (10 percent in acetone) did not produce a photochemical irritation response in the skin of rabbit.

#### 90-Day Feeding Study

A 90-day feeding study with DMDNB was conducted in male and female rats. The substance was fed to rats, ground in their diet, at nominal concentrations of 0, 22, 66, 200 or 700 ppm. A recovery group (200 ppm level) was returned to a nontreated diet after the 90-day study and held for an additional four weeks to assess the reversal of DMDNB toxicity. In all animals clinical signs were observed daily and body weight changes and food consumption were measured each week. At the end of the 90-day treatment, hematological and clinical chemistry changes were measured. The effects of subchronic DMDNB treatment on rat organ and tissue systems was observed daily and body weight changes and food consumption were measured each week. At the end of the 90-day treatment, hematological and clinical chemistry changes were measured. The effects of subchronic DMDNB treatment on rat organ and tissue systems was observed grossly at necropsy and later evaluated microscopically.

The test substance caused alopecia, hyperexcitability and deaths (about 75%) in rats fed 600 ppm DMDNB for 90 days. At 200 ppm DMDNB, alopecia was observed in 10% of the animals and mild, intermittent hyperexcitability was noted in about 40% of male and female rats. The two lower dosage levels (66 and 22 ppm) produced little if any observable effects. Rat body weights for the 600 and 200 ppm dosage levels were initially depressed, and despite a return to normal food consumption rats, remained lower than controls throughout the study. Body weights, weight gains and food consumption for all rats treated at 66 and 22 ppm DMDNB were within normal limits. At the end of the 90-day the 90-day treatment, red blood cell counts and hematocrit levels in treated rats showed a mild but significant depression when compared to controls. There were, however, no dose-related effects on clinical chemistry values in the same animals. At necropsy, male and female rats dying before the scheduled necropsy (600 ppm level only) presented lesions compatible with terminal cardiac dysfunction. No specific cause of death could be identified.

Male rats surviving until the end of the study, and at all treatment levels, had swollen kidneys which may have been associated with the observed presence of hyaline droplet degeneration of the renal cortex. This lesion is commonly reported for male rats treated with hydrocarbon compounds and may not be an accurate indicator of toxicity in man. This effect on the kidney was absent in all of the female rats and males in the recovery group. Enlargement of the liver in both male and female rats treated with DMDNB appeared to be dose-related. The morphological change was associated with metabolism of the test substance, or an adaptive mechanism, and may not indicate hepatotoxicity in the classical sense.

Based upon the effects of continuous DMDNB treatment to male and female rats for 90-days, the no observed adverse effect level (NOAEL) is 200 ppm DMDNB. The toxic effects appear to be reversible when exposure is discontinued.

#### Developmental Studies

Developmental (teratology effects of DMDNB were studied in rats and rabbits exposed by the oral route on gestation days 6-15 (rats) or 6-18 (rabbits). Rats were exposed to DMDNB in their feed at levels of 125 ppm, 250 ppm and 550 ppm; equal to a dosage of 6.4, 8.6 and 14.4 mg/kg/day, respectively. Rabbits were dosed by intragastric intubation at levels of 0.7, 3.5 and 7.0 mg/kg/day.

Maternal effects in rats included decreased body weights, decreased body weight gain; hyperexcitability and convulsions. There were increased incidences of fetal resorptions in rats at 500 ppm (14.4 mg/kg/day). The lowest dose level at which the maternal effects were observed was at 125 ppm (6.4 mg/kg/day) in rats. The NOAEL for maternal effects was not established for rats. The NOAEL for developmental toxicity was 250 ppm (8.6 mg/kg/day) for rats.

Rabbits: Depressed body weights in maternal rabbits occurred at 7.0 mg/kg/day. There was a significant decrease in the number of litters at the 7.0 mg/kg/day dosage level. No other adverse developmental effects were observed at any dose level. The NOAEL for maternal effects was 3.5 mg/kg/day and the NOAEL for effects on the fetus was also 3.5 mg/kg/day.

Both the rat and rabbit developmental studies indicated that exposure to DMDNB does not cause teratological effects.

#### Inhalation

Groups of young adult male and female albino Sprague-Dawley rats were exposed head only for a single four-hour period to several airborne concentration of DMDNB dust. The mass median diameter (MMD) of DMDNB particles in the exposure chamber was 1.6 micra. Surviving animals were extremely irritable with signs of tail biting, blood around nose and mouth, and body weight loss. There were no gross findings in the survivors at necropsy. The four-hour LC<sub>50</sub> was determined to be  $290 \pm 10$  mg/m<sup>3</sup> with a slope of  $4.85 \pm 1.45$ .

### Metabolism

A metabolism study was conducted to obtain data on the absorption, distribution and excretion of [<sup>14</sup>C] DMDNB in the rat following intraperitoneal (IP) and oral administration. Two single dosage levels were given based on fractions of 1/5 and 1/25 of each of their respective approximate lethal doses (ALD).

The bioelimination of [<sup>14</sup>C] was very slow. About 50% of the oral doses was recovered in urine during the 3-day collection period, while about 40% was eliminated in urine following IP dosing.

Tissue and organ deposition data showed that DMDNB was highly concentrated in the kidney with secondary deposition in the fat. The data also indicated that the metabolism of DMDNB was more dependent upon DMDNB dosage than the route of exposure.

### Mutagenicity Studies

DMDNB was evaluated for mutagenic activity using various assays. In the Ames Salmonella/Microsome Reverse Mutation Plate assay, the test article, DMDNB exhibited no mutagenic response in all five bacteria test strains, both with the presence and absence of exogenous metabolic activation.

Mutagenic activity of DMDNB in a L5178Y TK+/- Mouse lymphoma assay could not be resolved to completion due to the inability to obtain high solution concentrations because of limited solubility. At this level, however, no mutagenic activity was observed.

Results from a Chromosome Aberration Assay in Chinese Hamster Ovary (CHO) Cells showed that DMDNB was clastogenic in the presence of but not in the absence of metabolic activation.

Multiple treatments with DMDNB in the Rodent Bone Marrow Micronucleus Assay in male B<sub>6</sub>C<sub>3</sub>F<sub>1</sub> mice did not result in a significantly increased frequency of micronucleated polychromatic erythrocytes (PCE). Neither did it depress the percentage of PCE in the bone marrow of mice. The test was negative.

### Rodent Dominant Lethal Assay

Genetic toxicity of DMDNB was evaluated employing the mouse dominant lethal assay. This test examines directly in the gametic tissue of an intact animals the mutagenic potential of a test article.

Treatment of CD-1 male mice with subacute doses of test article, 2,3-dimethyl-2,3-dinitrobutane induced a significant and dose dependent increase, only in the fourth week of mating, in (a) the proportion of females with early fetal deaths and (b) the mutagenic index. In the absence of a consistent trend in the response of specific maturation stages of male germ cell, it was

concluded that 2,3-dimethyl-2,3-dinitrobutane has no mutagenic effect in the postmeiotic/meiotic stages of spermatogenesis in mice. The positive response obtained from the CP treatment group was consistent with those reported for other studies.

### Avian Toxicity

Three avian studies were conducted to determine the effects of DMDNB on wildlife. A LC50 Bobwhite Quail Stud determined the effects of a 5-day dietary exposure to DMDNB, while a LD50 Bobwhite Quail study was run to determine the effects of single graded oral doses of DMDNB to these birds. Mallard ducks were used to determine the LC50 in ducklings of a 5-day dietary exposure to DMDNB.

A no-observed-effect level (NOEL) was not achieved in the LC50 Bobwhite Quail Study. The 9-day acute dietary LC50 of DMDNB was determined to be 1,664 ppm, with 95 percent confidence limits of 1,249 and 2,218 ppm. Food consumption rates in test birds were depressed by about 50 percent or more, compared to controls, during the 5-day treatment period. Consumption, however, returned to normal rates when birds were fed untreated food during the 4-day recovery period. Gross pathology examinations of birds dying during the investigation generally revealed hemorrhaging intestines were noted in 11 of the 20 birds evaluated. The prominent clinical sign in responding birds was lethargy which appeared by test day 4. Complete remission of all signs of toxicity was achieved in survivors by test day 7.

A NOEL was not achieved in the LC50 Mallard Duckling study. The 8-day acute dietary LC50 of DMDNB was determined to be between 312 and 625 ppm. No deaths occurred at the lowest DMDNB treatment level (312 ppm) but 9/10 birds died at the next higher level (625 ppm). At the remaining three higher dietary levels, all the birds died by test day 4. Signs included lethargy and inactivity which usually preceded death. Gross pathological examinations noted internal hemorrhaging in most of the birds dying on test and in 5 of the 25 survivors.

A NOEL was not achieved in the LD50 Bobwhite Quail study. The acute oral LD50 of DMDNB was determined to be 39 mg/kg of body weight, with 95 percent confidence limits of 31 and 47 mg/kg of body weight. Deaths occurred only at the two high doses: 31.6 mg/kg (2/10) and at 46.4 mg/kg (8/10). There was no difference in mortality based upon sex. Body weight depressions was noted at all treatment groups (except the low dose) at days 3 through 7. All had recovered normal food consumption and weight gain by day 21. Lethargy was the primary clinical sign observed in affected birds. This effect cleared by day 8 in surviving birds. Pathological examinations of the 10 birds that died during the investigation revealed abnormal findings in all 10 birds. Six exhibited internal hemorrhaging. The remaining in both survivors and nonsurvivors were of a miscellaneous nature.

### Aquatic Toxicity

The objective of these studies was to evaluate the acute toxicity of DMDNB to the Bluegill, the Rainbow trout and to Daphnia. The toxicity to Bluegill

(*Lepomis macrochirus*) and to Rainbow Trout (*concorhynchus mybiss*) was determined during a 96-hour exposure period under static test conditions. The acute toxicity of DMDNB to cladoceran (*Daphnia magna*) was determined during a 48-hour exposure period under static test conditions.

The 96-hour LC50 value for bluegill exposure to DMDNB was 8.8 mg/L (C.L. 7.2-12 mg). The 96-hour no mortality concentration was 4.3 mg DMDNB/L. For rainbow trout the 96-hour LC50 was 4.6 mg DMDNB/L (C.L. 3.6-6.0 mg). The 96-hour no mortality concentration was 3.6 mg DMDNB/L. The 48-hour EC50 value for daphnids exposed to DMDNB was >120 mg/DMDNB/L, the highest concentration tested. The 48-hour no mortality/immobility concentration was 120 mg DMDNB/L.

Carcinogenic Evaluation

Studies were conducted to determine the ability of DMDNB to initiate skin tumor formation in a sensitive two-stage skin tumor animal model system employing female SENCAR mice. Animals receiving repeated intragastric administrations of the test article were subsequently dosed with repeated topical applications of a known promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA) and evaluated for skin tumor formation.

Female SENCAR mice initiated with DMDNB and promoted with TPA via the SENCAR mouse skin bioassay did not exhibit a significant increase in skin tumors. The mice were dosed at 20 mg/kg divided into six intragastric doses over two weeks and promoted three times per week for 20 weeks.

Permissible Exposure Limit (PEL)

Occupational PELs are considered necessary to protect the health and welfare of employees who are potentially exposed to substances in the workplace. Promulgation of a well documented PEL represents one of the most significant steps to ensure the adequacy of health protection for workers.

No current regulations or advisory exists of a PEL for DMDNB. The following suggested procedure for development of a PEL considers the results from rat and rabbit toxicity data.

A suggested PEL of 0.15 mg/m<sup>3</sup> for DMDNB was derived from the 90-day rat feeding study. A dietary no-observable-adverse-effect-level (NOAEL) of 200 ppm was used. The derivation of the PEL was as follows:

Ingestion

- NOAEL = 200 ppm (0.2 mg DMDNB/g feed)
- Intake = 20 g/day (rat)
- Daily dose = 4 mg/da/~~0.1 kg rat~~ 0.2 kg rat
- = 20 mg/kg/da.
- = 1400 mg/da/70 kg man

Inhalation

- Minute volume = 20 L/min (man)
- = 28,800 L/da

Dose =  $\frac{1400 \text{ mg/da}}{28,800 \text{ L/da}}$   
 = 0.049 mg/L/da or 49 mg/m<sup>3</sup>

PEL =  $\frac{49 \text{ mg/m}^3}{1000 \text{ (safety factor)}}$

PEL (24 hr) = 0.05 mg/m<sup>3</sup>

PEL (8 hr) = 0.15 mg/m<sup>3</sup>

A less documented method for derivation of a PEL is the use of results from the rabbit teratology study. In this study the oral administration of DMDNB, 7.0 mg/kg/day, to mated female rabbits produced a depressed fertility index when compared to controls and other DMDNB dosage groups. The low number of litters in that group reflects either an inhibition of implantation or, more probably, early embryonic death. This finding suggests that oral administration of DMDNB, 7.0 mg/kg/day, during the period of major organogenesis represents the lowest observed adverse effect level (LOAEL) of DMDNB for developmental toxicity in rabbits. The NOAEL for DMDNB developmental toxicity would then be 3.5 mg/kg/day. Based upon the results of this study, the derivation of the PEL of 0.26 mg/m<sup>3</sup> for humans is as follows:

Ingestion

Daily Dose (NOAEL) = 3.5 mg/kg/day  
 = 245 mg/day/70 kg man

Inhalation:

Minute volume = 20 L/min (man)  
 = 28,800 L/day

Dose =  $\frac{245 \text{ mg/da}}{28,800 \text{ L/da}}$   
 = 0.0085 mg/L/day or 8.5 mg/m<sup>3</sup>

PEL =  $\frac{8.5 \text{ mg/m}^3}{100 \text{ (safety factor)}}$

PEL (24 hr) = 0.085 mg/m<sup>3</sup>

PEL (8 hr) = 0.26 mg/m<sup>3</sup>

Either method for derivation of a PEL is acceptable. The dependent factors are the use of safety factors and the length of a exposure study. In addition, monitoring of occupational areas should give some idea of the extent of the potential exposure condition.

### Conclusions

Based upon the collective information, the greatest hazard to occupationally exposed individuals would result from DMDNB ingestion and from inhalation of the dry DMDNB aerosol.

There was not evidence of tumor irritation from DMDNB exposure in the SENCAR mouse skin bioassay.

Developmental studies in rabbits and rats demonstrated that DMDNB does not cause teratogenic effects in animals and should not be considered a developmental hazard to humans.

The NOAEL in the rat 90-day feeding was 200 ppm (0.2 mg DMDNB/g feed). The occupational PEL (8-hour) suggested from this study is 0.15 mg/m<sup>3</sup>.

BACKGROUND

DMDNB	PETN	RDX
<p>CAS No. 3964-18-4</p> <p>DMDNB is intended to be used in Army items.</p>	<p>CAS No. 78-11-5</p> <p>Highly explosive organic compound used in the manufacture of detonators and as a "long-acting" vasodilator. Cross-sensitivity with nitroglycerin has been described.</p>	<p>CAS No. 121-82-4</p> <p>An explosive polynitramine commonly known as cyclonite or (British code name for Research Department or Royal Demolition Explosive. It has been extensively used as a high-impact explosive in military munition formulations during and since World War II. It is also used as a rat poison.</p>

HEALTH HAZARD DATA

DMDNB	PETN	ROX
<p><u>Short-term Exposure</u></p> <p>Oral ALD* - 87 mg/kg (rat)</p> <p>I.P.** ALD - 38-50 mg/kg (rat)</p> <p>Dermal ALD - 780-800 mg/kg (guinea pig)</p> <p>Dermal ALD - &gt;2 gm/kg (rabbit)</p> <p>Skin irritation - no irritation (rabbit)</p> <p>Eye irritation - no irritation (rabbit)</p> <p>Skin sensitization - non sensitizer (guinea pigs)</p> <p>Convulsions, tremors and salivation at lethal dosages.</p>	<p>Oral LDLO*** - 7 gm/kg (mouse)</p> <p>I.P. LD50° - &gt; 5 gm/kg (mouse)</p> <p>Skin irritation - dermatitis</p> <p>Similar to nitrite toxicity, acute exposures may cause methemoglobin, vasodilation and vascular collapse. Cyanosis, coma, convulsions and death may occur at high doses.</p>	<p>Oral LD50 - 59 mg/kg (mouse)</p> <p>Oral LD50 - 100 mg/kg (rat)</p> <p>I.P. LDLO - 10 mg/kg (rat)</p> <p>Dermal LD50 &gt; 2 gm/kg (rabbit)</p> <p>Skin irritation - no irritation</p> <p>Skin sensitization - no evidence of sensitization</p> <p>Acute doses produce convulsions, labored breathing and other CNS effects. Dermatitis and erythema may result from contact.</p>

Signs & Symptoms

- \* ALD - Approximate Lethal Dose
- ++ I.P. - Intraperitoneal
- +++ LDLO - Lowest Reported Lethal Dose
- LD50 Lethal Dose 50%

DMDNB	PETN	RDX
<p><u>LONGER-TERM EXPOSURE</u></p> <p>90-day feeding study - Rats</p> <p>NOAEL - 200 ppm NOEL - 0.3 To 0.6 mg/kg/da</p> <p>Toxic signs alopecia, weight loss, death at high levels</p> <p>Developmental Effects - Rats - oral 20-days - 500 ppm. No teratologic effects.</p> <p>500 ppm slight weight loss in dams.</p>	<p>One year feeding study - Rats</p> <p>2 mg/kg/da caused no effects</p> <p>Developmental effects - no published data</p>	<p>90-day feeding study - Rats</p> <p>LOAEL - 160 mg/kg/da NOAEL - 80 mg/kg/da</p> <p>Developmental effects - rat - oral 10 gm/kg - 22 days of pregnancy. Effects on newborn - stillbirth reduced live birth index.</p> <p>Developmental effects: 20g/kg 6-15 day pregnancy fetotoxic in rats, but no teratogenic effects.</p>

LOAEL - Lowest observed adverse effect level  
NOAEL - No observable adverse effect level

DMDNB	PETN	RDX
<p><b>Mutagenicity</b></p> <p>Ames test           negative  Mouse Lymphoma    negative  Micronucleus       negative  Chinese Hamster    +activated  Ovary (CHO)        -non-activated</p> <p>Dominant Lethal    negative  - mouse</p> <p>Sencar Mouse       negative  tumor study</p>	<p>Ames                negative  Sister chromatid  exchange  Hamster ovary  positive @ 160 mg/L</p> <p>Dominant Lethal - no  report</p> <p>Tumorigenic effects  - Rat  Route oral - dose  12875 mg/kg/2yr  thyroid tumors  Equivocal  tumorigenic agent</p> <p>Mouse</p> <p>a 103 wk study -  negative for tumor  formation</p>	<p>Not found to be  mutagenic</p> <p>Lifetime feeding  studies in rats and  mice showed  testicular  degeneration and  prostate  inflammation.  Decrease fertility  in a two-generation  reproductive study  in rats.</p> <p>Induces  hepatocellular  carcinomas and a  denomas in mice.</p>
<p>Metabolism         in-life  (<sup>14</sup>C labeled)       complete  evaluation  in progress</p>	<p>Metabolism - animal  studies not reported</p>	<p>Metabolism -  completely absorbed  when ingested in  rats. Highest  levels are found in  kidneys, liver,  brain and heart.  RDX metabolized by  the liver and its  metabolites excreted  in urine.</p>





DEPARTMENT OF THE ARMY  
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY  
ABERDEEN PROVING GROUND, MARYLAND 21010-6422



REPLY TO  
ATTENTION OF

EXECUTIVE SUMMARY  
TOXICOLOGICAL STUDY NO. 75-51-YJ92-93  
THE ACUTE TOXICITY OF 2,3-DIMETHYL-2,3-DINITROBUTANE  
OCTOBER 1992

1. **PURPOSE.** The purpose of this study was to determine the acute toxicity of 2,3-dimethyl-2,3-dinitrobutane (DMDNB).
2. **FINDINGS.** The DMDNB is not a primary skin or eye irritant to animals following a single exposure nor does it cause skin sensitization or photochemical reaction. It is a toxic hazard by ingestion having an oral approximate lethal dose between 50 and 87 mg/kg in rats and guinea pigs. The material is not readily absorbed by the skin nor do its vapors have any adverse effects in animals when inhaled for 8 hours. The material was mutagenic in one of four assays but required activation to elicit the positive response. To the avian species, ducks and quail, DMDNB is moderately toxic.
3. **RECOMMENDATIONS.** Pending the results of planned and in-progress toxicity studies in animals, it is recommended that personnel who may be exposed to DMDNB be protected from inhaling the dry material and/or ingesting the substance in any form.



DEPARTMENT OF THE ARMY  
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY  
ABERDEEN PROVING GROUND, MARYLAND 21010-2422



REPLY TO  
ATTENTION OF

HSHE-MO-T

TOXICOLOGICAL STUDY NO. 75-51-YJ92-93  
THE ACUTE TOXICITY OF 2,3-DIMETHYL-2,3-DINITROBUTANE  
OCTOBER 1992

1. REFERENCES. See Appendix A for a listing of references.
2. AUTHORITY. Letter, U.S. Army, Office of the Surgeon General, 23 September 1991, subject: Request for AEHA Assistance.
3. PURPOSE. The studies were conducted to determine the acute toxicity of 2,3-dimethyl-2,3-dinitrobutane (DMDNB).
4. GENERAL. The DMDNB is intended to be used in Army items. During production and storage of these items, exposure to DMDNB may occur. Before the Government Contractor will initiate production of these items, the toxicity of the DMDNB must be established. This report provides the results of the initial acute or short-term tests. Subchronic tests, involving repeated exposures, are in progress and will be reported separately.
5. MATERIALS.

a. Test Substance. The test substance, DMDNB, was provided by the sponsor, the U.S. Army Armament Research, Development and Engineering Center, Picatinny Arsenal, New Jersey. It was a white crystalline material with little, if any, odor. It is listed in the TSCA inventory as CAS No. 3964-18-4. Three "production" lots (Z0312, Z1957, and Z1958) and a "pure product" lot (Z0311) were received. The four samples were analyzed by the Organic Environmental Chemistry Division, U.S. Army Environmental Hygiene Agency (USAEHA), using IR spectroscopy and gas chromatography with electron capture detector. When compared to the pure product sample, the analyses of the three production lots resulted in a purity of 96 percent or greater.

Use of trademarked names does not imply endorsement by the U.S. Army but is intended only to assist in identification of a specific product.

Toxicological Study No. 75-51-YJ92-93, Oct 92

b. Animals.\*†

(1) Testing for primary eye and skin irritation, photochemical skin irritation, and dermal toxicity was conducted using New Zealand White rabbits (2.8-4.5 kg) obtained from Hazelton-Dutchland Laboratories, Denver, Pennsylvania. For the dermal toxicity test, male and female groups were identified. In the remaining studies, the sexes were mixed. Female Albino-Hartley guinea pigs (375-425 g) also from Hazelton-Dutchland Laboratories, were used for skin sensitization studies and for determination of oral toxicity. Male and female Sprague-Dawley rats (175-225 g) obtained from the Charles River Laboratories, Wilmington, Massachusetts, were used for oral toxicity testing and for inhalation studies. The animals were in acceptable health after quality control determinations were made during a 2-week quarantine period.

(2) Rabbits, guinea pigs, and rats were housed individually in wire-bottom stainless steel cages. Drinking quality water and feed (Purina® Certified Rabbit Chow 5322; Purina Certified Guinea Pig Chow 5025, and Purina Certified Rodent Chow 5002) were available ad libitum. Ambient temperatures in the animal rooms were maintained at 21 to 25 °C with relative humidity between 40 and 60 percent. The light/dark cycle was 12-hour intervals.

c. Contract Studies.

(1) Mutagenicity Studies were performed under commercial contract DAAD05-91-C-0018 by Integrated Laboratory Systems, Research Triangle Park, North Carolina.

(2) Avian Toxicity Studies were performed under commercial contract DAAD05-90-P-9510 by Bio-Life Associates, Nellisville, Wisconsin.

(3) Histopathological evaluation of animal tissues was performed by George A. Parker, D.V.M., Ltd., Sterling, Virginia, under commercial contract DAAD05-90-C-0040.

\* In conducting the studies described herein, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," U.S. Department of Health Education and Welfare Publication No. (NIH) 85-23, 1985.

† The studies reported herein were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

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## 6. METHODS.

a. Primary Skin Irritation. An acute dermal irritation test, based upon the method of Draize, was conducted in rabbits. The procedure (reference 1) involved the single application of 0.5 gm of DMDNB to the clipped backs of each of six rabbits. The material was finely ground and moistened slightly with saline and placed on a 2X2 inch gauze pad. The pad was applied to the skin surface then overwrapped with Coban<sup>®</sup>, an occlusive covering. Exposure was for 24 hours, after which the coverings were removed and irritation scored one hour later. Evaluations were also made at 48 and 72 hours and at 7 days. Scoring of irritation was based on the Draize method in which erythema and edema were evaluated on a scale of 0 to 4 for severity. Categorizing the responses was based on the mean of the sum of the 24- and 48-hour scores.

b. Primary Eye Irritation. Eye irritation studies were performed in rabbits following a standard method (reference 2). A single 0.1 gm dose of the dry, finely ground DMDNB was administered to the conjunctival sac of one eye of each of six rabbits. Eyes were scored for irritation at 24, 48, and 72 hours after treatment and again at 7 days. Scoring of irritation effects followed the method of Draize in which the total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctiva. Categorizing the responses was based on the 24-hour evaluation.

c. Photochemical Skin Irritation. A photochemical skin irritation study was performed to assess the potential of DMDNB to become chemically reactive when exposed to sunlight. Studies were performed (reference 3) by applying 0.05 mL of the test solution (10 percent in acetone) to the right side of the clipped backs of six rabbits. After 5 minutes, the backs were exposed to ultraviolet (UV) light (365 nm) for about 15 minutes at a distance of 10-15 cm. Following UV exposure, the left side of the same animal's back was treated identically to the right side, except that it was not irradiated. Oil of Bergamot, a known photoirritant, was included in the exposure regimen to assure the responsiveness of the test system. Photochemical irritation, as determined by the net difference between irradiated and nonirradiated scores was evaluated at 24, 48, and 72 hours post-exposure. Scoring of erythema and edema was based upon the method of Draize.

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<sup>®</sup> Coban is a registered trademark of Minnesota Mining and Manufacturing Co., St. Paul, Minnesota.

## Toxicological Study No. 75-51-YJ92-93, Oct 92

d. Skin Sensitization. Sensitization studies were performed to determine the potential of DMDNB for causing skin sensitization reactions in humans. The test procedure (reference 4) was based on the method of Buehler. The test material (DMDNB, 10 percent in acetone) was applied under Webril® patches to the shaved backs of 20 guinea pigs. The occlusive exposure was for 6 hours, once per week, for 3 weeks. This was considered the "induction" phase. Following a 2-week rest period the animals were "challenged", meaning that a single application of the test material was applied to a naive site. Ten additional naive animals also received the challenge dose. Twenty-four and 48 hours after the challenge, the skin was depilated, then irritation scored 3 hours later. The appearance of erythema and/or edema at the challenge site which is significantly greater than that observed on the naive animals (no induction) is considered an allergic response.

e. Oral Toxicity. An approximate lethal dose (ALD) study, which uses a small number of animals, was performed to determine the minimum lethal dose of DMDNB. Single oral graded doses were given by gavage to male and female rats and to female guinea pigs. For oral administration, DMDNB was suspended in either distilled water or corn oil at concentrations of 50 mg/mL. Gum tragacanth was added to each vehicle (5 percent) to enhance suspension. After treatment, the animals were examined daily for the onset and reversal of toxic signs. Animals surviving the 14-day observation period were sacrificed for gross pathological examination. The ALD of the test substance is considered the lowest dose that causes death during the 14-day observation period.

f. Dermal Toxicity. A "limit" study was performed in animals to determine the toxicity of DMDNB resulting from dermal absorption. A single dose of 2,000 mg/kg of DMDNB was applied to the clipped backs of six male and six female rabbits. The material was finely ground and moistened slightly with saline and placed on a 4X4 inch gauze pad. The pad was taped to the skin surface then overwrapped with Coban, an occlusive covering. Exposure was for 24 hours, after which the coverings were removed and remaining DMDNB removed from the skin. Animals were examined daily for toxic signs through 14 days. Survivors were euthanized at day 14 and examined grossly for pathology.

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g. Saturated Vapor. The acute effects of inhaling DMDNB vapors were separately measured in male and female rats. Six male and six female rats were exposed to DMDNB vapors continuously for 8 hours. For testing, dried air at 10 psi passed through a rotameter, then into a 2 L flask with 3 angle necks which contained about 150 g of dry DMDNB. The flask was mounted on a heating mantle which maintained a constant temperature of 100 °C at the bottom of the flask. From the flask, the air passed through polyethylene tubing containing glass wool and into the inhalation exposure chamber. The exposure chamber was a 9 L glass desiccator with six individually screened compartments which held the rats. The animal chamber temperature ranged from 22 to 30 °C and the air flow was 1.0 L/min. Control groups of male and female rats were exposed in the same fashion but to room air only. Animals were observed daily for the onset of toxic signs and were weighed on days 0, 1, 3, 7, and 14. At the end of the 14-day observation period, the rats were euthanized and observed for gross pathology. Selected tissues were harvested for histopathological evaluation.

h. In Vitro Mutagenicity Assays.

(1) Ames Test. The DMDNB was evaluated for mutagenic activity using the Ames Salmonella/Microsome Reverse Mutation Plate Assay. The Ames test used Salmonella typhimurium indicator strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100. The sample was tested directly and in the presence of liver homogenates (S9 fraction) from rats treated with Aroclor® 1254. Concurrent positive and solvent controls were run along with five dose points of the test sample. Sterile dimethylsulfoxide (DMSO) was used as the diluent in preparing stock solutions. All tests were run in triplicate plates at doses of 10, 1, 0.5, 0.1, and 0.05 mg/plate.

(2) Mouse Lymphoma Test. The DMDNB was assessed for mutagenic activity in the L5178Y TK+/- Mouse Lymphoma Mutagenicity Assay in the presence and absence of Aroclor induced rat liver S9. The nonactivated cultures selected for cloning were treated with DMDNB at a maximum dose of 2,400 µg/mL. The S9 activated cultures selected for cloning were treated with doses up to 2,500 µg/mL. The diluent was sterile DMSO. The cultures were evaluated for elevated mutation frequency and clastogenic response.

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(3) CHO Cell Assay. The DMDNB was tested for its mutagenic potential in the Chromosome Aberration Assay in Chinese Hamster Ovary (CHO) Cells. In the nonactivated study, duplicate cultures of CHO cells were exposed to DMDNB (in DMSO) at 25, 50, 100, 200 and 400  $\mu\text{g}/\text{mL}$  for 18 hours. In the S9 activated study, cells were exposed to 100, 250, 500, 750, and 1000  $\mu\text{g}/\text{mL}$  for 4 hours, washed and incubated for another 8 hours. Metaphase cells were then harvested, stained, and examined. The percent of structurally damaged cells in the total population examined and the frequency of structural aberrations per cell was calculated. The positive control was Mitomycin C in the nonactivated portion and cyclophosphamide in the S9 activated portion.

(4) Micronucleus Assay (In Vivo). The potential for DMDNB for causing mutagenicity was assessed in the Rodent Bone Marrow Micronucleus Assay in male B6C3F<sub>1</sub> mice. Mice were treated by intraperitoneal injection of DMDNB in corn oil on days 1 through 3. Treatment doses were 0.0, 17.5, 35.0, and 70 mg/kg. The positive control was dimethylbenz[*a*]anthracene. Selected animals were euthanized 24 hours after each treatment day and the bone marrow removed. The frequency of micronuclei in polychromatic erythrocytes (immature red cells) was scored in addition to an evaluation of clastogenic and/or aneugenic damage.

i. Avian Toxicity.

(1) LC50 Bobwhite Quail. To determine the effects of DMDNB toxicity on wildlife, a 5-day dietary exposure in bobwhite quail was conducted. The test material was fed in the diet at 312, 625, 1200, 2500, and 5000 ppm DMDNB to five groups of ten 12-day-old quail. Treatment was for 5 days followed by a 4-day recovery period. Control groups received the stock diet only. Birds dying during the investigation were subjected to gross pathological examinations as were selected survivors from each treatment level.

(2) LC50 Mallard Ducklings. To determine the effects of DMDNB toxicity on wildlife, a 5-day dietary exposure in mallard ducklings was conducted. The test material was fed in the diet at 312, 625, 1200, 2500, and 5000 ppm DMDNB to five groups of ten 5-day-old ducklings. Treatment was for 5 days followed by a 3-day recovery period. Control groups received the stock diet only. Birds dying during the investigation were subjected to gross pathological examinations as were selected survivors from each treatment level.

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(3) LD50 Bobwhite Quail. To determine the effects of graded oral doses of DMDNB in bobwhite quail, six groups of 10 birds (5 males and 5 females per group) were treated via gelatin capsules containing the test material. Dose levels were 0, 10.0, 14.7, 21.5, 31.6, and 46.4 mg/kg DMDNB. Controls received only an empty gelatin capsule. All birds were observed on the day of dosing and for 20 consecutive days thereafter. Birds dying during the investigation were subjected to gross pathological examinations as were selected survivors from each treatment level.

## 7. RESULTS.

a. Primary Skin Irritation. Technical DMDNB (dry; finely ground) did not produce primary irritation in the skin of rabbits during a 24-hour occlusive exposure. The results indicated assignment to USAEHA Category I (see Appendix B) and to U.S. Environmental Protection Agency (EPA) Toxicity Category IV (see Appendix C). The individual animal data appear at Appendix D.

b. Primary Eye Irritation. Technical DMDNB (dry; finely ground) did not produce primary eye irritation in rabbits. The material was assigned USAEHA Category A (see Appendix E) and EPA Category IV. The individual animal data appear at Appendix F.

c. Photochemical Skin Irritation. Irradiation of a dermally applied DMDNB solution (10 percent in acetone) did not produce photochemical irritation to the skin of rabbits. See Appendix G for individual animal data. The positive control substance, Bergamot Oil, caused photochemical irritation in the same animals. (See Appendix H).

d. Skin Sensitization. Dermally applied DMDNB (10 percent in acetone) did not cause a skin sensitization response in guinea pigs. The individual animal data appear at Appendix I.

e. Oral Toxicity. The ALD of DMDNB orally in male and female rats, and female guinea pigs was 87 mg/kg. The two vehicles used to suspend the material, either corn oil or distilled water, had no apparent effect on toxicity. The appearance of toxic effects was rapid in the guinea pigs, occurring between 4 and 36 minutes after treatment. In rats, the earliest effects appeared at 14 minutes with deaths occurring between 30 minutes and 72 hours after treatment. Signs at fatal doses included lethargy, convulsions, prostration, and death. Few signs were observed in surviving animals except hemolacrymation and hyperexcitability in one rat and aggressiveness in another. Gross pathology observations were

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unremarkable. Several treated and control rats had mild congestion and diffuse hemorrhage of the lungs. Passive congestion of the liver was also observed in a few rats, but none of the observations could be conclusively associated with DMDNB treatment. Appendices J and K provide the individual animal data for male and female rats where corn oil was the vehicle. Appendices L and M provide the data for male rats and female guinea pigs where distilled water was the vehicle.

f. Dermal Toxicity. The 24-hour occlusive exposure of DMDNB to the skin of male and female rabbits did not produce observable effects at a dose of 2,000 mg/kg (see Appendices N and O). The ALD was >2,000 mg/kg which equates to EPA Category III. No compound-related lesions were observed in rabbits at necropsy performed at day 14. Due to the absence of effects at the "limit" dose, no further dermal toxicity tests were conducted.

g. Saturated Vapor. Male and female rats exposed to vapors of DMDNB heated to 100 °C were not effected by the single 8-hour exposure. No clinical signs were observed during the exposure or through the 14-day observation period. Body weights remained comparable to control animals as did organ-to-body weight ratios determined following necropsy. Histopathological evaluations performed on tissues harvested at necropsy were unremarkable. According to the pathologist, "There were no histopathologic findings that were considered to be a result of exposure to the test material [DMDNB]. All lesions were considered to be incidental findings or part of spontaneous disease complexes of laboratory rats."

h. In Vitro Mutagenicity Assays.

(1) Ames Test. The test article, DMDNB, exhibited a negative mutagenic response in all five Salmonella tester strains, both in the presence and absence of exogenous metabolic activation.

(2) Mouse Lymphoma. The test material could not be tested to sufficiently high concentration to obtain a conclusive result in the Mouse Lymphoma Assay because of limited solubility. Test criteria calls for the chemical to yield a relative total growth (RTG) in the culture of about 10 to 20 percent. The lowest RTG values for DMDNB was about 40 percent. Although some, albeit insufficient, toxicity was observed under the conditions of testing there was no evidence of a clastogenic or mutagenic response; concentration-related increases in induced mutation frequencies were not observed in the absence or presence of metabolic activation.

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(3) CHO Assay. The test article, DMDNB, was clastogenic (positive response) in the presence of but not the absence of metabolic activation. Under these conditions, the lowest effective dose was 1,000  $\mu\text{g}/\text{mL}$ .

(4) Micronucleus Assay. Multiple treatments with DMDNB did not result in a significantly increased frequency of micronucleated polychromatic erythrocytes (PCE) and did not significantly depress the percentage of PCE in the bone marrow of male B6C3F1 mice. The test was negative.

i. Avian Toxicity.

(1) LC50 Bobwhite Quail. A no-observed-effect level (NOEL) was not achieved in this study. The 9-day acute dietary LC50 of DMDNB was determined to be 1,664 ppm, with 95 percent confidence limits of 1,249 and 2,218 ppm. Food consumption rates in test birds were depressed by about 50 percent or more, compared to controls, during the 5-day treatment period. Consumption, however, returned to normal rates when birds were fed untreated food during the 4-day recovery period. Gross pathology examinations of birds dying during the investigation generally revealed hemorrhaging in or around the crop area. In the surviving birds, hemorrhagic intestines were noted in 11 of the 20 birds evaluated. The prominent clinical sign in responding birds was lethargy which appeared by test day 4. Complete remission of all signs of toxicity was achieved in survivors by test day 7.

(2) LC50 Mallard Ducklings. A NOEL was not achieved in this study. The 8-day acute dietary LC50 of DMDNB was determined to be between 312 and 625 ppm. No deaths occurred at the lowest DMDNB treatment level (312 ppm) but 9/10 birds died at the higher level (625 ppm). At the remaining three higher dietary levels, all the birds died by test day 4. Signs included lethargy and inactivity which usually preceded death. Gross pathological examinations noted internal hemorrhaging in most of the birds dying on test and in 5 of the 25 survivors.

(3) LD50 Bobwhite Quail. A no-observed-effect level (NOEL) was not achieved in this study. The acute oral LD50 of DMDNB was determined to be 39 mg/kg of body weight, with 95 percent confidence limits of 31 and 47 mg/kg of body weight. Deaths occurred only at the two high doses: 31.6 mg/kg (2/10) and at 46.4 mg/kg (8/10). There was no difference in mortality based upon sex. Body weight depressions were noted at all treatment groups (except the low dose) at days 3 through 7. All had recovered normal food consumption and weight gain by day 21.

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Lethargy was the primary clinical sign observed in affected birds. This effect cleared by day 8 in surviving birds. Pathological examinations of the 10 birds that died during the investigation revealed abnormal findings in all 10 birds. Six exhibited internal hemorrhaging. The remaining findings in both survivors and nonsurvivors were of a miscellaneous nature.

8. DISCUSSION.

a. Acute toxicity testing is a means of determining the immediate health hazards associated with the use of a chemical substance. It further provides the direction and rationale for more in-depth investigations. Ideally, the tests address the three potential routes of human exposure: dermal, oral, and inhalation. In the case of DMDNB, skin contact is not an acute hazard based upon the absence of local or systemic effects observed in animals. No primary irritation to the skin or eyes, or skin sensitization, or photochemical reactivity has been observed following DMDNB treatment. The substance is minimally absorbed through the skin, probably due to its limited solubility in organic solvents and its near insolubility in water. Heating of DMDNB to 100 °C does not produce vapors of sufficient volume or toxicity to cause adverse effects in rats when inhaled for 8 hours. Orally, DMDNB is "very toxic" using the criterion described by Gleason (reference 7) in that the LD50 ranges from 50-500 mg/kg. The EPA criteria (Appendix C) for the same range equals Toxicity Category II.

b. Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is the major component in a formulation that DMDNB will be combined with. The chemical and toxic properties of DMDNB and RDX are remarkably similar (reference 8). Both are white crystalline materials which are nearly insoluble in water and have limited solubility only in polar organics such as acetone (<10 percent). Neither material is an acute hazard to the skin or eyes nor is there appreciable dermal absorption. When ingested, they produce nearly identical toxicities of between 50 to 125 mg/kg for most species tested. Absorption from the G.I. tract following ingestion is prolonged for either DMDNB or RDX, with deaths in rats occurring nearly 24 hours after treatment.

c. The mutagenicity studies performed with DMDNB were all negative except for the CHO assay with activation. This result indicates some clastogenic activity but only in the presence of the S9 promoter. This response was not observed in the mouse lymphoma test which also measures clastogenic activity. Two in vivo studies, Dominant Lethal and SENCAR mouse bioassays, already in progress, will help to resolve the significance of the single positive finding.

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d. Based upon the collective toxicity information to date, the greatest hazard to occupationally exposed personnel would result from DMDNB ingestion. The effects from inhaling the dry substance must also remain of concern until the toxic levels from this exposure route can be established.

9. RECOMMENDATIONS. Pending the results of planned and in-progress toxicity studies in animals, it is recommended that personnel who may be exposed to DMDNB be protected from inhaling the dry material and/or ingesting the substance in any form.

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APPENDIX A

REFERENCES

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2. Toxicology Division, SOP No. 7.91, USAEHA, HSHB-MO-T, Primary Eye Irritation Study.
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8. Etnier, E.L. (June 1986) Final Report, Water Quality Criteria for Hexahydro-2,3,5-trinitro-1,3,5-triazine (RDX), Report No. ORNL-6178, Oak Ridge National Laboratory, Oak Ridge, TN.

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APPENDIX B

USAEHA CATEGORIES FOR SKIN IRRITATION EFFECTS

CATEGORY I - Compounds producing no primary irritation of the intact skin or no greater than mild primary irritation of the skin surrounding an abrasion. Score Limits: Intact 0-0.5; Abraded 0.51-2.0; Total 0-2.0 (INTERPRETATION: No restriction for acute application to the human skin.)

CATEGORY II - Compounds producing mild primary irritation of the intact skin and the skin surrounding an abrasion. Score Limits: Intact >0.5; Total 0.51-2.0 (INTERPRETATION: Should be used only on human skin found by examination to have no abrasions or may be used as clothing impregnant.)

CATEGORY III - Compounds producing moderate primary irritation of the intact skin and the skin surrounding an abrasion. Score Limits: Total 2.1-5.0 (INTERPRETATION: Should not be used directly without a prophetic patch test having been conducted on humans to determine irritation potential to human skin. May be used without patch testing, with extreme caution, as clothing impregnants. Compound should be resubmitted in the form and at the intended use concentration so that its irritation potential can be reexamined using other test techniques on animals.)

CATEGORY IV - Compounds producing moderate to severe primary irritation of the intact skin and of the skin surrounding an abrasion. Score Limits: Total 2.1-7.9 (INTERPRETATION: Should be resubmitted for testing in the form and at the intended use concentration. Upon resubmission, its irritation potential will be reexamined using other test techniques on animals, prior to possible prophetic patch testing in humans, at concentrations which have been shown not to produce primary irritation in animals.)

CATEGORY V - Compounds impossible to classify because of staining of the skin or other masking effects owing to physical properties of the compound or compounds producing necrosis, vesiculation, or eschars. Score Limits: Total 8.0 or not scorable. (INTERPRETATION: Not suitable for use on humans.)

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## APPENDIX C

## EPA TOXICITY CATEGORY CRITERIA\*

Hazard Indicators	Category I	Category II	Category III	Category IV
Oral LD50	Up to and incl 50 mg/kg	>50 thru 500 mg/kg	>500 thru 5000 mg/kg	>5000 mg/kg
Dermal LD50	Up to and incl 200 mg/kg	>200 thru 2000 mg/kg	>2000 thru 20,000 mg/kg	>20,000 mg/kg
Inhal LC50 (Chmbr conc)	Up to and incl 0.2 mg/L	>0.2 thru 2.0 mg/L	>2.0 thru 20 mg/L	>20 mg/L
Eye Effects†	Corrosive (Irrevers destruct of ocular tiss) or corneal involv or irrit persist for more than 21 days	Corneal involv or irrit clng in 8-21 days	Corneal involv or irrit clng in 7 days or less	Min effects clng in less than 24 hrs
Skin Effects	Corrosive (Tiss destruct into dermis and/or scarring)	Sev irrit at 72 hrs (sev erythema or edema)	Mod irrit at 72 hrs (mod erythema)	Mild or slt irrit (no irrit or slt erythema)

\* 40 CFR 156.10. Labeling Requirements. Revised 1 July 1990.

† [EPA] PR Notice 81-3; Label Improvement Program: Change in Test Methods for and Categorization of Eye Irritation. September 29, 1981.

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## APPENDIX D

## PRIMARY SKIN IRRITATION STUDY

Study No. 75-51-YJ92-91 Protocol No. 91-9-3 SOP No. 91.8  
 Test Substance 2,3-Dimethyl-2,3-dinitrobutane (DMDNB)  
 Study Director M. Weeks Investigator H. Snodgrass

## INDIVIDUAL ANIMAL SCORES

TIME (Hours)	OBSERVATION	RABBIT NO. / SCORE						MEAN
		811	812	814	816	817	818	
24	Erythema	0	0	0	0	1	0	0.1
72		0	0	0	0	0	0	
7 DA		0	0	0	0	0	0	
Subtotal (24 and 72 hr)								0.1
24	Edema	0	0	0	0	0	0	0.0
72		0	0	0	0	0	0	
7 DA		0	0	0	0	0	0	
Subtotal (24 and 72 hr)								0.0
Total (24 and 72 hour)								0.1

0.5 g of the finely ground DMDNB was moistened with saline and placed on the animal's back under a gauze pad. The pad was covered with an occlusive wrap. After 24 hrs the covering was removed. Skin irritation was scored at 24 and 72 hours according to the method of Draize.

The test substance did not produce significant irritation to the skin of rabbits.

USAHA Category - I (Slight to no irritation)  
 EPA Category IV

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## APPENDIX E

### USA-EHA CATEGORIES FOR EYE IRRITATION EFFECTS

CATEGORY A - Compounds noninjurious to the eye. Score Limits: 0-10 (individual conjunctival score for chemosis, redness or discharge not to exceed 1). (INTERPRETATION: Irritation of human eyes is not expected if the compound should accidentally get into the eyes, provided it is washed out as soon as possible.)

CATEGORY B - Compounds producing mild injury to the cornea. Score Limits: 10-20 (individual conjunctival score for chemosis, redness or discharge not to exceed 1). (INTERPRETATION: To be used with caution around the eyes.)

CATEGORY C - Compounds producing mild injury to the cornea, and in addition some injury to the conjunctiva. Score Limits: 5-30 (individual conjunctival score for chemosis, redness or discharge exceed 1). (INTERPRETATION: To be used with caution around the eyes and mucosa [e.g., nose and mouth].)

CATEGORY D - Compounds producing moderate injury to the cornea. Score Limits: <20-50 (individual conjunctival score for chemosis, redness or discharge not to exceed 1). (INTERPRETATION: To be used with extreme caution around the eyes.)

CATEGORY E - Compounds producing moderate injury to the cornea, and in addition producing some injury to the conjunctiva. Score Limits: 20-50 (individual conjunctival score for chemosis, redness or discharge exceed 1). (INTERPRETATION: To be used with extreme caution around the eyes and mucosa.)

CATEGORY F - Compounds producing severe injury to the cornea and to the conjunctiva. Score Limits: 50 or greater. (INTERPRETATION: To be used only with extreme caution; it is recommended that use be restricted to areas other than the face.)

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APPENDIX F

PRIMARY EYE IRRITATION STUDY

Study No. 75-51-YJ92-91 Protocol No. 91-9-3 SOP No. 91.7
Test Substance 2,3-Dimethyl-2,3-dinitrobutane (DMDNB)
Study Director M. Weeks Investigator H. Snodgrass

INDIVIDUAL ANIMAL SCORES

Table with columns: TIME, STRUCTURE, RABBIT NO. / SCORE (813, 815, 819, 820, 821, 822), MEAN. Rows include 24 HR, 48 HR, 72 HR, and 7 DA for Cornea, Iris, and Conjunctiva.

0.1 g of the finely ground DMDMB was placed in the conjunctival sac of each eye. Scoring by the method of Draize began 24 hrs after application.
The test substance did not produce adverse eye effects in rabbits.
USAEHA Category - A (Noninjurious to the eye)
EPA Category IV

Toxicological Study No. 75-51-YJ92-93, Oct 92

APPENDIX G

PHOTOCHEMICAL SKIN IRRITATION

Study No. 75-51-YJ92-91 Protocol No. 91-9-3 SOP No. 91.12  
 Test Substance 2,3-Dimethyl-2,3-dinitrobutane (DMDNB)  
 Study Director M. Weeks Investigator H. Snodgrass

INDIVIDUAL ANIMAL SCORES

UV IRRADIATED SITES

No.	Erythema			Edema		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
	781	0	0	0	0	0
782	0	0	0	0	0	0
783	0	1	0	0	0	0
784	0	1	1	0	0	0
785	0	0	0	0	0	0
786	0	0	0	0	0	0
SUM	A 3			B 0		

NONIRRADIATED SITES

No.	Erythema			Edema		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
	781	0	0	0	0	0
782	0	0	0	0	0	0
783	0	0	0	0	0	0
784	0	0	1	0	0	0
785	0	0	0	0	0	0
786	0	0	0	0	0	0
SUM	A' 1			B' 0		

NET ERYTHEMA SCORE = (A - A') + No. of Observ. (18) 0.11  
 NET EDEMA SCORE = (B - B') + No. of Observ. (18) 0.00

0.05 mL of a DMDNB solution (ca 10% in acetone) was placed on opposite sides of the rabbit's back. One side was irradiated with UV-A light. At 24, 48, and 72 hours after irradiation, skin irritation was scored by the method of Draize and comparisons made between the UV and non UV exposed sites. Bergamot Oil (10%) was the positive control substance.

DMDNB did not produce photochemical irritation in the skin of rabbits.

Toxicological Study No. 75-51-YJ92-93, Oct 92

APPENDIX H

PHOTOCHEMICAL SKIN IRRITATION

Study No. 75-51-YJ92-91 Protocol No. 91-9-3 SOP No. 91.12  
 Test Substance Bergamot Oil (Positive Control substance)  
 Study Director M. Weeks Investigator H. Snodgrass

INDIVIDUAL ANIMAL SCORES

UV IRRADIATED SITES

No.	Erythema			Edema		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
	781	2	2	2	1	1
782	2	2	2	1	1	1
783	2	3	3	2	2	2
784	2	3	3	2	2	1
785	2	3	3	1	1	2
786	3	3	3	2	3	1
SUM A	45			B 27		

NONIRRADIATED SITES

No.	Erythema			Edema		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
	781	2	2	2	0	0
782	2	2	2	0	0	0
783	2	2	1	1	0	0
784	2	2	1	0	0	0
785	2	2	2	0	0	0
786	2	2	2	0	0	0
A'	34			B' 1		

NET ERYTHEMA SCORE = (A - A') + No. of Observ. (18) 0.6

NET OEDEMA SCORE = (B - B') + No. of Observ. (18) 1.44

0.05 mL of Bergamot Oil solution (10% in acetone) was placed on opposite sides of the rabbit's back. One side was irradiated with UV-A light. At 24, 48, and 72 hours after irradiation, skin irritation was scored by the method of Draize. The net erythema and edema scores were determined.

Bergamot Oil, the positive control substance, produced photochemical irritation in rabbits (i.e. net erythema score >1.0 and/or net edema ≥0.5).

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APPENDIX I

GUINEA PIG SENSITIZATION

Study No. 75-51-YJ92-91 Protocol No. 91-9-3 SOP No. 91.35  
 Test Substance 2,3-Dimethyl-2,3-dinitrobutane (DMDNB)  
 Induction Sol. 10% in acetone Challenge Sol. 10% in acetone  
 Study Director M. Weeks Investigator J. Harvey

Adml No.	Induction Group				Challenge				Naive Challenge				
	24 Hr Er*	24 Hr Ed*	48 Hr Er	48 Hr Ed	24 Hr Er	24 Hr Ed	48 Hr Er	48 Hr Ed	Adml No.	24 Hr Er	24 Hr Ed	48 Hr Er	48 Hr Ed
311	0	0	0	0	1	0	0	0	331	0	0	0	0
312	0	0	0	0	0	0	0	0	332	0	0	0	0
313	0	0	0	0	0	0	0	0	333	0	0	0	0
314	0	0	0	0	0	0	0	0	334	0	0	0	0
315	0	0	0	0	0	0	0	0	335	0	0	0	0
316	0	0	0	0	0	0	0	0	336	0	0	0	0
317	0	0	0	0	0	0	0	0	337	0	0	0	0
318	0	0	0	0	0	0	0	0	338	0	0	0	0
319	0	0	0	0	0	0	0	0	339	0	0	0	0
320	0	0	0	0	0	0	0	0	340	0	0	0	0
321	0	0	0	0	0	0	0	0					
322	0	0	0	0	0	0	0	0					
323	0	0	0	0	0	0	0	0					
324	0	0	0	0	0	0	0	0					
325	0	0	0	0	0	0	0	0					
326	0	0	0	0	0	0	0	0					
327	0	0	0	0	0	0	0	0					
328	0	0	0	0	0	0	0	0					
329	0	0	0	0	0	0	0	0					
330	0	0	0	0	0	0	0	0					
Incidence of Grade 2 erythema					0/20	0/20							
Incidence of Grade 1 erythema										0/10	0/10		
* Er - erythema (skin irritation) Ed - edema (skin irritation)													
The test substance <u>did not</u> produce skin sensitization in guinea pigs.													

Toxicological Study No. 75-51-YJ92-93, Oct 92

APPENDIX J

APPROXIMATE LETHAL DOSE (ALD)

Study No. 75-51-YJ92-91 Protocol No. 91-9-3 SOP No. 91.17  
 Test Substance 2,3-Dimethyl-2,3-dinitrobutane (DMDNB)  
 Route Oral - gavage Species Rat Sex Male  
 Conc. Test Substance (w/v) 50 mg/mL Diluent Corn oil (suspension) + Gum tragacanth (5%)  
 Study Director M. Weeks Investigator H. Snodgrass

INDIVIDUAL ANIMAL EFFECTS

Animl No.	Wght kg	Dose mg/kg	Vol ml	Effect S*/min to onset	Recov min	Death min
870	0.246	295	1.44	S1/60		75
880	0.248	195	0.97	S1/14		30
885	0.235	130	0.61	S1/103		143
899	0.243	87	0.41			21Hr O.N.
905	0.233	50	0.23	S2/24Hr S3/24Hr	72Hr	
907	0.233	38	0.18			
917	0.231	26	0.12			
860	0.225	5.85 mL/kg	1.32	Corn oil control		

Signs: S1- Convulsions S3- Hyperexcitable S5-  
 S2- Hemolacrimation S4- O.N.- Overnight

Mortality: 24 Hr 4/7 48 Hr 4/7 72 Hr 4/7 14 Da 4/7

ALD (mg/kg) 87 EPA Toxicity Category II



Toxicological Study No. 75-51-YJ92-93, Oct 92

APPENDIX L

APPROXIMATE LETHAL DOSE (ALD)

Study No. 75-51-YJ92-91 Protocol No. 91-9-3 SOP No. 91.17  
 Test Substance 2,3-Dimethyl-2,3-dinitrobutane (DMDNB)  
 Route Oral - gavage Species Rat Sex Male  
 Conc. Test Substance (w/v) 50 mg/mL Diluent Dist. water (suspens.) + Gum tragacanth (5%)  
 Study Director M. Weeks Investigator H. Snodgrass

INDIVIDUAL ANIMAL EFFECTS

Animl No.	Wght kg	Dose mg/kg	Vol mL	Effect S*/min to onset	Recov min	Death min
862	0.265	439	2.3	S1/163		19Hr O.N.
863	0.273	293	1.6			72Hr O.N.
864	0.254	195	1.0	S1/166		19Hr O.N.
865	0.247	130	0.6			19Hr O.N.
869	0.275	87	0.5			19Hr O.N.
874	0.220	50	0.2			
875	0.250	38	0.2			
902	0.280	26	0.15			

Signs: S1- Convulsions S3- S5-  
 S2- S4- O.N. - Overnight

Mortality: 24 Hr 4/8 48 Hr 4/8 72 Hr 5/8 14 Da 5/8

ALD (mg/kg) 87 EPA Toxicity Category II

Toxicological Study No. 75-51-YJ92-93, Oct 92

APPENDIX M

APPROXIMATE LETHAL DOSE (ALD)

Study No. 75-51-YJ92-91 Protocol No. 91-9-3 SOP No. 91.17  
 Test Substance 2,3-Dimethyl-2,3-dinitrobutane (DMDNB)  
 Route Oral - gavage Species Guinea pig Sex Female  
 Conc. Test Substance (w/v) 50 mg/mL Diluent Dist. water (suspens.) + Gum Tragacanth (5%)  
 Study Director M. Weeks Investigator J. Harvey

INDIVIDUAL ANIMAL EFFECTS

Anml No.	Wght kg	Dose mg/kg	Vol mL	Effect S*/min to onset	Recov min	Death min
091	0.360	38	0.27			
092	0.360	50	0.36			
093	0.324	87	0.56	S1/10 S2/15		36
094	0.354	130	0.97	S1/2 S2/5		13
095	0.366	195	1.42	S1/2 S1/5		17
096	0.354	293	2.07	S1/1 S2/1		27
097	0.374	440	3.29	S1/1 S2/2		04
098	0.368	Vehicle Control	3.29			

Signs: S1- Convulsions S3- S5-  
 S2- Prostrate S4- O.N.- Overnight

Mortality: 24 Hr 4/7 48 Hr 4/7 72 Hr 4/7 14 Da 4/7

ALD (mg/kg) 87 EPA Toxicity Category II

Toxicological Study No. 75-51-YJ92-93, Oct 92

APPENDIX N

APPROXIMATE LETHAL DOSE (ALD)

Study No. 75-51-YJ92-91 Protocol No. 91-9-3 SOP No. 91.17  
 Test Substance 2,3-Dimethyl-2,3-dinitrobutane (DMDNB)  
 Route Dermal Species Rabbit Sex Male  
 Conc. Test Substance (w/v) Neat Diluent None  
 Study Director M. Weeks Investigator H. Snodgrass

INDIVIDUAL ANIMAL EFFECTS

Anml No.	Wght kg	Dose mg/kg	Vol gm	Effect S*/min to onset	Recov min	Death min
8	2.66	2000	5.3			
9	3.01	2000	6.0			
10	3.44	2000	6.9			
11	2.93	2000	5.8			
13	2.75	2000	5.9			
14	2.96	2000	5.9			

Signs: S1- S2- S3- S4- S5- O.N.- Overnight

Mortality: 24 Hr 0/6 48 Hr 0/6 72 Hr 0/6 14 Da 0/6

ALD (mg/kg) >2000 EPA Toxicity Category III

Toxicological Study No. 75-51-YJ92-93, Oct 92

APPENDIX O

APPROXIMATE LETHAL DOSE (ALD)

Study No. 75-51-YJ92-91 Protocol No. 91-9-3 SOP No. 91.17  
 Test Substance 2,3-Dimethyl-2,3-dinitrobutane (DMDNB)  
 Route Dermal Species Rabbit Sex Female  
 Conc. Test Substance (w/v) Neat Diluent None  
 Study Director M. Weeks Investigator H. Snodgrass

INDIVIDUAL ANIMAL EFFECTS

Animl No.	Wght kg	Dose mg/kg	Vol gm	Effect S*/min to onset	Recov min	Death min
819	3.50	2000	7.00			
820	3.530	2000	7.06			
822	2.97	2000	5.94			
823	3.37	2000	6.74			
824	3.63	2000	7.26			
825	3.56	2000	7.12			

Signs: S1- S2- S3- S4- S5- O.N. - Overnight

Mortality: 24 Hr 0/6 48 Hr 0/6 72 Hr 0/6 14 Da 0/6

ALD (mg/kg) >2000 EPA Toxicity Category III



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