

AD-A198 496

Research Development on Potential of  
Chemical Warfare in Guinea Pig

Author: [illegible]  
Editor: [illegible]  
Subject: [illegible]

Research Development Branch  
Toxicology Division



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The methods described in this report, the investigation adhered to the standards of the Department of the Army, as promulgated by the Department of the Army, Office of the Chief of Laboratory Animal Facilities and Care, Department of the Army, National Research Council.

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*John S. Beatrice* 24 Jul 64  
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## SECURITY CLASSIFICATION OF THIS PAGE

## REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 0704-0188

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|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|-----------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|--------------------------------|
| 1a. REPORT SECURITY CLASSIFICATION                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |       |                                                     | 1b. RESTRICTIVE MARKINGS                                                                                                    |                                                    |                                |
| 2a. SECURITY CLASSIFICATION AUTHORITY                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |       |                                                     | 3. DISTRIBUTION/AVAILABILITY OF REPORT<br>Approved for public release; distribution is unlimited.                           |                                                    |                                |
| 2b. DECLASSIFICATION/DOWNGRADING SCHEDULE                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |       |                                                     |                                                                                                                             |                                                    |                                |
| 4. PERFORMING ORGANIZATION REPORT NUMBER(S)<br>Institute Report No. 282                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |       |                                                     | 5. MONITORING ORGANIZATION REPORT NUMBER(S)                                                                                 |                                                    |                                |
| 6a. NAME OF PERFORMING ORGANIZATION<br>Mammalian Toxicology Branch<br>Division of Toxicology                                                                                                                                                                                                                                                                                                                                                                                                                                             |       | 6b. OFFICE SYMBOL<br>(If applicable)<br>SGRD-ULE-TO | 7a. NAME OF MONITORING ORGANIZATION<br>US Army Biomedical Research and Development<br>Laboratory                            |                                                    |                                |
| 6c. ADDRESS (City, State, and ZIP Code)<br>Letterman Army Institute of Research<br>Presidio of San Francisco, CA 94129-6800                                                                                                                                                                                                                                                                                                                                                                                                              |       |                                                     | 7b. ADDRESS (City, State, and ZIP Code)<br>Ft. Detrick<br>Frederick, MD 21701-5010                                          |                                                    |                                |
| 8a. NAME OF FUNDING/SPONSORING<br>ORGANIZATION US Army Medical<br>Research & Development Command                                                                                                                                                                                                                                                                                                                                                                                                                                         |       | 8b. OFFICE SYMBOL<br>(If applicable)                | 9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER                                                                             |                                                    |                                |
| 8c. ADDRESS (City, State, and ZIP Code)<br>Ft. Detrick<br>Frederick, MD 21701-5012                                                                                                                                                                                                                                                                                                                                                                                                                                                       |       |                                                     | 10. SOURCE OF FUNDING NUMBERS                                                                                               |                                                    |                                |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |       |                                                     | PROGRAM<br>ELEMENT NO.<br>62720A                                                                                            | PROJECT<br>NO.<br>835                              | TASK<br>NO.<br>AB              |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |       |                                                     | WORK UNIT<br>ACCESSION NO.<br>DA 303913                                                                                     |                                                    |                                |
| 11. TITLE (Include Security Classification)<br>Dermal Sensitization Potential of Guanidine Nitrate in Guinea Pigs                                                                                                                                                                                                                                                                                                                                                                                                                        |       |                                                     |                                                                                                                             |                                                    |                                |
| 12. PERSONAL AUTHOR(S)<br>Gerald F.S. Hiatt, Earl W. Morgan, and Don W. Korte, Jr.                                                                                                                                                                                                                                                                                                                                                                                                                                                       |       |                                                     |                                                                                                                             |                                                    |                                |
| 13a. TYPE OF REPORT<br>Institute                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |       | 13b. TIME COVERED<br>FROM 3 Jul 84 to 27 Aug 84     |                                                                                                                             | 14. DATE OF REPORT (Year, Month, Day)<br>July 1988 |                                |
| 15. PAGE COUNT<br>25                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |       |                                                     |                                                                                                                             |                                                    |                                |
| 16. SUPPLEMENTARY NOTATION                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |       |                                                     |                                                                                                                             |                                                    |                                |
| 17. COSATI CODES                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |       |                                                     | 18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)                                           |                                                    |                                |
| FIELD                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | GROUP | SUB-GROUP                                           | Dermal sensitization; Mammalian toxicology; Guanidine nitrate;<br>Buehler test; Guinea pigs; Nitroguanidine; Munitions (KT) |                                                    |                                |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |       |                                                     |                                                                                                                             |                                                    |                                |
| 19. ABSTRACT (Continue on reverse if necessary and identify by block number)<br>Guanidine nitrate, an intermediate product in the synthesis of the triple-base propellant component, nitroguanidine, was evaluated for its potential to produce dermal sensitization in male guinea pigs. The Buehler test, which utilizes repeated closed patch inductions with the test compound, was used for this evaluation. No evidence of guanidine nitrate-induced sensitization was obtained in the study. <i>Keywords: exposure, pyrolysis</i> |       |                                                     |                                                                                                                             |                                                    |                                |
| 20. DISTRIBUTION/AVAILABILITY OF ABSTRACT<br><input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS                                                                                                                                                                                                                                                                                                                                                        |       |                                                     | 21. ABSTRACT SECURITY CLASSIFICATION                                                                                        |                                                    |                                |
| 22a. NAME OF RESPONSIBLE INDIVIDUAL<br>Edwin S. Beatrice COL, MC                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |       |                                                     | 22b. TELEPHONE (Include Area Code)<br>(415) 561-3600                                                                        |                                                    | 22c. OFFICE SYMBOL<br>SGRD-ULZ |

DD Form 1473, JUN 86

Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

## ABSTRACT

Guanidine nitrate, an intermediate product in the synthesis of the triple-base propellant component, nitroguanidine, was evaluated for its potential to produce dermal sensitization in male guinea pigs. The Buehler test, which utilizes repeated closed patch inductions with the test compound, was used for this evaluation. No evidence of guanidine nitrate-induced sensitization was obtained in the study.

Key Words: Dermal Sensitization, Mammalian Toxicology, Guanidine Nitrate, Buehler Test, Guinea Pigs, Nitroguanidine, Munitions

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| Accession For      |                                            |
| NTIS GRA&I         | <input checked="checked" type="checkbox"/> |
| DTIC TAB           | <input type="checkbox"/>                   |
| Unannounced        | <input type="checkbox"/>                   |
| Justification      |                                            |
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| Distribution/      |                                            |
| Availability Codes |                                            |
| Dist               | Avail and/or<br>Special                    |
| A-1                |                                            |



## PREFACE

TYPE REPORT: Dermal Sensitization GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command  
Letterman Army Institute of Research  
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command  
US Army Biomedical Research and Development Laboratory  
Fort Detrick, MD 21701-5010  
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NO.: 84019

STUDY DIRECTOR: Don W. Korte Jr, PhD, MAJ, MSC

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Toxicology.

REPORT AND DATA MANAGEMENT: A copy of the final report,  
study protocols, raw data,  
retired SOPs, and an aliquot  
of the test compound will be  
retained in the LAIR  
Archives.

TEST SUBSTANCE: Guanidine Nitrate

INCLUSIVE STUDY DATES: 3 July - 27 August 1984

OBJECTIVE: The objective of the study was to evaluate the  
dermal sensitization potential of guanidine  
nitrate in guinea pigs.

### **ACKNOWLEDGMENTS**

SGT Steven K. Sano, Max Goldman, PhD, and Joy Bauserman assisted with the research. Richard D. Spieler, Richard Katona, and Charlotte Speckman provided animal care and managed the facilities. Callie B. Crosby, Lynda Araiza, and JoAnn Nishimoto provided office management during the performance of the study and preparation of the report.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE  
STUDY

We, the undersigned, declare that GLP Study 84019 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

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ATTENTION OF:

SGRD-ULZ-QA (70-1n)

13 July 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 84019

1. This is to certify that in relation to GLP Study 84019, the following inspections were made:

|               |                   |
|---------------|-------------------|
| 6 March 1984  | - Protocol Review |
| 1 August 1984 | - Dosing          |
| 1 August 1984 | - Patch/Removal   |
| 9 August 1984 | - Scoring         |

2. The institute report entitled "Dermal Sensitization Potential of Guanidine Nitrate in Guinea Pigs," Toxicology Series 100, was audited on 23 March 1987.

*Carolyn M. Lewis*

CAROLYN M. LEWIS  
Chief, Quality Assurance

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# Dermal Sensitization Potential of Guanidine Nitrate in Guinea Pigs--Hiatt et al

## INTRODUCTION

Guanidine nitrate is an intermediate product in the synthesis of nitroguanidine. Nitroguanidine is a primary component of US Army triple-base propellants and is now being produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique propellants generated by US Army munitions-manufacturing facilities, reviewed the nitroguanidine data base and identified significant gaps in the toxicity data (1). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products.

### Objective of Study

The objective of this study was to evaluate the dermal sensitization potential of guanidine nitrate in guinea pigs.

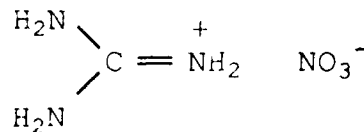
## MATERIALS

### Test Substance

Chemical name: Guanidine Nitrate

Chemical Abstracts Service Registry No.: 506-93-4

Chemical structure:



Molecular formula:  $\text{CH}_5\text{N}_3\text{HNO}_3$

Other test substance information is presented in Appendix A.

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#### Vehicle for Test Substance

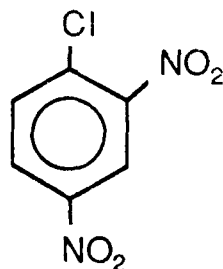
Sterile isotonic saline (Travenol Laboratories, Deerfield, IL) was used as the vehicle for guanidine nitrate. The expiration date for this lot (7C950X0) was October 1983.

#### Positive Control

Chemical name: Dinitrochlorobenzene (DNCB)

Chemical Abstracts Service Registry No.: 97-00-7

Chemical structure:



Molecular formula: C<sub>6</sub>H<sub>3</sub>N<sub>2</sub>O<sub>4</sub>Cl

Other positive control substance information is presented in Appendix A.

#### Vehicle for Positive Control

The vehicle for DNCB was a propylene glycol (3%) and isotonic saline (97%) mixture. Propylene glycol (lot number 36485) was obtained from Certified Laboratories, Inc., (Philadelphia, PA). Saline was the same as for the guanidine nitrate vehicle.

#### Animal Data

Forty-six male guinea pigs, Hartley strain (Charles River Breeding Laboratories, Wilmington, MA) were studied. They were identified individually with ear tags numbered 84E047 to 84E092, inclusive. Two animals were selected for quality control necropsy evaluation on receipt. Four of the animals were selected for a pilot study to determine a nonirritating dose level. Animal weights on receipt (3 Jul 84) ranged from 178 to 225 g. Additional animal data appear in Appendix B.

L. sbandry

Guinea pigs assigned to this study were caged individually in stainless steel, wire mesh cages in racks equipped with automatically flushing dump tanks. The diet, fed *ad libitum*, consisted of Certified Purina Guinea Pig Chow Diet 5026 (Ralston Purina Company, Checkerboard Square, St. Louis, MO); water was provided by continuous drip from a central line. Temperature within the animal room was initially maintained in the range 18.9 to 22.2°C. In response to evidence of respiratory infection in a few of the test animals, room temperature was increased on 25 Jul 84 and maintained in the range of 20.0 to 23.8°C. Relative humidity was maintained in the range of 42 to 69%, with occasional spikes as high as 88% during steam line adjustments and room washing. The photoperiod was 12 hours of light per day.

**METHODS**

This study was conducted in accordance with LAIR SOP-OP-STX-82 "Buehler Dermal Sensitization Test" (2) and EPA guidelines (3).

Group Assignment/Acclimation

The guinea pigs were quarantined for 15 days before administration of the first induction dose. During the quarantine period, they were checked daily for signs of illness and weighed once a week. Ten animals were assigned to each of four groups by a stratified randomization technique based on their body weights.

Dosage Levels

Three animal groups comprise the basis for this report. Dermal sensitization potential was evaluated in a test group receiving three weekly induction doses of 10% guanidine nitrate and, after a two-week delay, a challenge dose at the same concentration. Dinitrochlorobenzene, a known potent sensitizing agent (4), was applied to another group, at a 0.1% concentration, as a positive control. A negative control group received 10% guanidine nitrate only on the day of challenge dosing.

Compound Preparation

Guanidine nitrate was moderately soluble in isotonic saline resulting in a milky solution with some fine suspension. The dinitrochlorobenzene dosing solution was

prepared by first adding 30 mg DNCB to 1.0 ml of propylene glycol and heating until it dissolved (approximately 40°C). To this, 29 ml of 0.9% sodium chloride solution were added, to give a final concentration of 0.1% (w/v). This solution was heated to 65°C and vortexed before application to keep the DNCB in solution. DNCB solutions were prepared fresh for each application day.

#### Test Procedures

The closed patch dermal sensitization test procedures utilized in this study were developed by Buehler and Griffith (5-7) to mimic the repeated-insult patch test for humans. Test compounds were applied for six hours under a closed patch once a week for three weeks during the induction phase. The same application site was used for each induction dose. To distinguish between reactions from repeated insult and sensitization, duplicate patches of the challenge dose were applied, one on the old site and one on a new site. To distinguish between reactions from primary irritation and sensitization, a negative control group was added which received only the challenge dose.

During the induction phase, the test and positive control groups were dosed with 0.5 ml of the appropriate solution/suspension applied topically under a 2.5-cm<sup>2</sup> gauze patch. This procedure was performed for three consecutive weeks (18, 25 Jul, and 1 Aug for the DNCB-positive control group; 25 Jul, 1 Aug, and 8 Aug for the 10% guanidine nitrate test group). Twenty-four hours before each dosing an 8-cm<sup>2</sup> area on the left flank of the animal was clipped with electric clippers (Oster® Model A5, size 40 blade, Sunbeam Corp., Milwaukee, WI) and then shaved with an electric razor (Norelco® Speed Razor Model HP1134/S, North American Phillips Corp., Stamford, CT). The patch was taped with Blenderm® hypoallergenic surgical tape (3M Corp., St. Paul, MN) to the same site each time, and the animal was wrapped several times with Vetrap® (3M Corp., St. Paul, MN). The patch was left in place for six hours. When the wrap and patch were removed, the area under the patch was marked off with a felt-tip surgical marking pen for ease of scoring.

Animals were challenged two weeks (15 Aug for the DNCB-positive control and negative control groups; 22 Aug for the 10% guanidine nitrate group) following the third induction dose. Test group and positive control group animals received two 0.5-ml doses each, one applied to the old site on the left flank and the other to a new site on the right flank. Negative control animals received only a single 0.5-ml dose,

applied to the left flank. Procedures for clipping, shaving, and wrapping and the exposure period remained the same.

In Buehler's procedure, skin reactions are scored 24 and 48 hours after the challenge dose only. In the present study, skin reactions were scored 24 and 48 hours after each induction dose as well. Skin reactions were assigned scores according to Buehler's grading system: 0 (no reaction), 1 (slight erythema), 2 (moderate erythema), and 3 (marked erythema). Results are expressed in terms of both incidence (the number of animals showing responses of 1 or greater at either 24 or 48 hours) and severity (the sum of the test scores divided by the number of animals tested). Results from the left flank are compared with right flank and with the negative control group.

Some modifications of Buehler's procedures were made. Instead of placing animals in restraint during the 6-hour exposure period, the animals were wrapped several times with an elasticized tape to hold the patch in place. Consequently, the animals were able to move about freely in their cage during the exposure period. Buehler and Griffith (7) also recommended depilating the day before the challenge dose. For consistency with induction procedures, this step was replaced by clipping the animals.

A historical listing of study events appears in Appendix C.

#### Deviations from Study Protocol

This study was conducted in accordance with the protocol and applicable amendments with the following exceptions:

A 0.5 level (very slight erythema) was added to the scoring system to allow for borderline responses.

The DNCB solution was maintained at approximately 65°C before dosing the guinea pigs. This was necessary to keep the DNCB in solution but did not result in thermal insult to the animals' skin as the aliquot for dosing cooled quickly during pipetting and application to the patch. Appreciable sensitization was produced by DNCB with this method.

A pilot study using 100%, 10%, 1% and 0.1% concentrations of guanidine nitrate, to evaluate the acute dermal irritation potential of guanidine nitrate in guinea pigs, was performed in four animals before the formal study. Irritation produced by the 100% suspension was equivocal in this pilot study; the initial formal induction dosing was

therefore performed at the 100% level. However, significant irritation occurred in the test animals in response to this first induction application, and this group of animals was removed from the study. In accordance with the SOP, a vehicle control group had received saline only at this first induction dosing. These animals from the vehicle control were substituted for the animals removed from the study, forming a new test group. This new test group was dosed with a 10% suspension which was nonirritating.

Two animals died during the study period, one (84F065) from the DNCB-positive control group and the other (84F084) from the negative control group. A third animal (84F075) from the discontinued 100% guanidine nitrate group also died. Postmortem findings revealed preexisting adrenal and hepatic lesions for animal 84F065, viral pneumonia for animal 84F075, and pulmonary edema and myocardial hemorrhage for animal 84F084.

It is believed that these deviations from the protocol did not adversely affect the study, as reflected by the results in the negative and positive control groups.

#### Raw Data and Final Report Storage

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

## **RESULTS**

### Guanidine Nitrate

Tables 1 and 2 summarize the incidence of reactions 24 and 48 hours after each dose. No reaction was observed in response to guanidine nitrate, either at 24 or 48 hours. This lack of response is reflected in Tables 3 and 4, which report the severity of skin reactions at 24 and 48 hours. Response severity for each group is calculated by summing the scores of responding animals and dividing by the total number of animals within that group. For guanidine nitrate no responses were obtained, and therefore severity scores were zero at all times.

TABLE 1

Incidences of Skin Reactions after 24 Hours

| Test Group           | Induction |        |       | Challenge |       |
|----------------------|-----------|--------|-------|-----------|-------|
|                      | First     | Second | Third | Left      | Right |
| Guanidine Nitrate    | 0/10      | 0/10   | 0/10  | 0/10      | 0/10  |
| Negative Control*    | ---       | ---    | ---   | 1/9       | ---   |
| DNCB <sup>&gt;</sup> | 1/10      | 7/9    | 9/9   | 9/9       | 9/9   |

\* The Negative Control Group received only a challenge dose of the test compound. Group size decreased due to non-compound-related death.

<sup>></sup> Group size decreased due to non-compound-related death.

TABLE 2

Incidences of Skin Reactions after 48 Hours

| Test Group           | Induction |        |       | Challenge |       |
|----------------------|-----------|--------|-------|-----------|-------|
|                      | First     | Second | Third | Left      | Right |
| Guanidine Nitrate    | 0/10      | 0/10   | 0/10  | 0/10      | 0/10  |
| Negative Control*    | ---       | ---    | ---   | 0/9       | ---   |
| DNCB <sup>&gt;</sup> | 0/10      | 5/9    | 7/9   | 6/9       | 7/9   |

\* The Negative Control Group received only a challenge dose of the test compound. Group size decreased due to non-compound-related death.

<sup>></sup> Group size decreased due to non-compound-related death.

TABLE 3

## Severity of Skin Reactions after 24 Hours

| Test Group        | Induction |        |       | Challenge |       |
|-------------------|-----------|--------|-------|-----------|-------|
|                   | First     | Second | Third | Left      | Right |
| Guanidine Nitrate | 0.0       | 0.0    | 0.0   | 0.0       | 0.0   |
| Negative Control* | ---       | ---    | ---   | 0.06      | ---   |
| DNCB              | 0.1       | 0.78   | 1.22  | 1.11      | 1.33  |

\* The Negative Control Group received only a challenge dose of the test compound.

TABLE 4

## Severity of Skin Reactions after 48 Hours

| Test Group        | Induction |        |       | Challenge |       |
|-------------------|-----------|--------|-------|-----------|-------|
|                   | First     | Second | Third | Left      | Right |
| Guanidine Nitrate | 0.0       | 0.0    | 0.0   | 0.0       | 0.0   |
| Negative Control* | ---       | ---    | ---   | 0.0       | ---   |
| DNCB              | 0.0       | 0.56   | 0.78  | 0.78      | 1.11  |

\* The Negative Control Group received only a challenge dose of the test compound.

### Positive Control

Dinitrochlorobenzene produced a marked response at all time points after the first induction dose. Between 70% and 100% of the DNCB-treated animals exhibited a response 24 hours following the second or third induction and challenge doses. Between 50% and 80% of these animals still exhibited a response 48 hours following the same doses; the reactions were therefore persistent. Severity scores for these responses to DNCB ranged from 0.7 to 1.33 at the 24-hour scoring period (Table 3). The highest score, 1.33, was observed on the right (non-induction) flank in response to the challenge dose. By 48 hours the reactions had subsided somewhat; consequently, the severity range decreased to between 0.56 and 1.11 (Table 4).

### Negative Control

Only one response was observed in the negative control (challenge dose of guanidine nitrate) group, a 0.5 (borderline) score on one animal at 24 hours.

Individual 24-hour and 48-hour scores for all animals appear, by group, in Appendix D.

## **DISCUSSION**

### Dermal Irritation and Sensitization

Most skin reactions occurring from contact with chemicals can be classified as either irritation or sensitization. Both reactions present as inflammation of the skin; the difference between irritation and sensitization is the mechanism responsible for this inflammation. Primary irritation is direct inflammation in response to injury to the skin produced by the eliciting chemical. Irritation is a locally mediated response ranging from mild reversible inflammation to severe ulceration progressing to necrosis. Sensitization is manifested as indirect inflammation mediated by components of the immune system in response to activation by the eliciting chemical (8). Dermal sensitization is usually a delayed hypersensitivity or cellular immunologic reaction. Although both types of reactions can appear grossly similar in experimental animals and may even be produced by the same agent, it is possible to distinguish between them. Irritation is an immediate response and can be produced upon first contact with the chemical, whereas sensitization requires at least one innocuous "conditioning" exposure before a reaction can be elicited.

Irritative responses usually require a relatively high concentration or dose of the offending chemical, whereas sensitization reactions may occur in response to minute quantities. Essentially all individuals in a population will express an irritative response to a reactive chemical, provided the dose is high enough, whereas only a fraction of the population normally becomes sensitized to the same chemical. A fully developed response can be produced by first contact with an irritant, but initial contact with a sensitizer produces no reaction (a conditioning exposure is necessary). Unless there is accumulation of damage, subsequent exposures to an irritant produce inflammation of essentially similar intensity/severity, whereas the reaction to a sensitizer often increases over 2 to 4 exposures after the initial contact. An irritant produces inflammation of rapid onset with short duration, whereas a sensitization reaction is somewhat delayed and prolonged. The inflammatory response to an irritant may spread beyond the area of contact, whereas sensitization reactions are usually circumscribed.

The features of irritation and sensitization were applied by Buehler and Griffith (5-7) to establish guidelines for differentiation between the two. In evaluating a dermal sensitization study they recommend comparing the results from a challenge dose in the experimental group with those for the negative control group:

Irritative Responses:

- occur in a large proportion of test animals.
- develop in response to the first or second exposure.
- usually fade within 24 to 48 hours, unless damage is severe.
- may be stronger at challenge to a previously unexposed area of skin (contralateral flank).

Sensitization Reactions:

- occur in only a few animals, unless the compound is a potent sensitizer.
- are absent after the initial (conditioning) exposure, but appear in response to subsequent exposures.
- develop slowly, the intensity/severity of inflammation often is greater at 72 to 96 than at 24 to 48 hours.
- increase in intensity/severity from one exposure to the next (at sites previously exposed or unexposed).

Dermal irritancy potential is evaluated by the method of Draize et al (9) in which the chemical is applied once, at high concentration, and the resulting acute inflammatory reaction is graded. Evaluation of sensitizing potential is

accomplished by repeated application, at lower non-irritating concentrations, over a few weeks. There is then a latent period, usually two weeks, to allow the immune system to elaborate and increase its specific response to the chemical. A challenge dose is then given, and the resulting inflammatory response is graded. Analysis of the incidence, severity, and timing of the response to the challenge dose estimates the sensitizing potential of the study compound.

#### Guanidine Nitrate

Guanidine nitrate was evaluated for its ability to elicit a delayed-hypersensitivity or cellular immunologic reaction via contact with the skin. Guanidine nitrate produced no response indicative of the potential to elicit dermal sensitization when evaluated according to the method (5-7) of Buehler and Griffith. This finding closely parallels the result of an earlier study on the hydrochloride salt of guanidine (10). In that study, guanidine hydrochloride exhibited no sensitizing potential in the Buehler Dermal Sensitization Test.

Sensitization produced by guanidine nitrate would have been detected by this study. A hypersensitivity-type response was reliably elicited by DNCB in the present group of animals. This response to DNCB was characteristic of that observed previously within the Institute (10). Although DNCB is capable of producing primary irritation, the characteristics of the responses observed in this study are indicative of a reaction due to sensitization. The concentration of DNCB used for induction and challenge is too low to produce primary irritation. Also the response to DNCB was observed primarily after two or more exposures, and the severity generally increased with the number of previous exposures.

#### **CONCLUSION**

Guanidine nitrate, based on a zero percent sensitization rate in this study, exhibited no potential for inducing dermal sensitization.

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|                                               |    |
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| Appendix C. Historical Listing of Events..... | 19 |
| Appendix D. Individual Dermal Scores.....     | 22 |

## Appendix A: CHEMICAL DATA

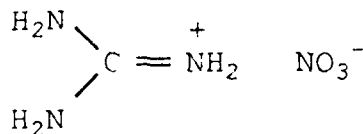
Chemical Name: Guanidine Nitrate

Lot Number: 123820

Chemical Abstracts Service Registry No.: 506-93-4

LAIR Code: TP030

Chemical Structure:



Molecular Formula:  $\text{CH}_6\text{N}_3\cdot\text{NO}_3$

Molecular Weight: 122.1

Physical State: White crystalline powder

Melting Point:  $214^\circ\text{C}^1$

Analytical Data:

Infrared spectrophotometry was performed and the spectrum obtained<sup>2</sup> was identical to the Sadtler spectrum<sup>3</sup> for Guanidine Nitrate. Major absorption peaks were observed at 3400 (broad), 3200, 1665, 1575, 1400, 1385, and  $825\text{ cm}^{-1}$ . The grade of material obtained for this study is referred to as the Ultralog Grade by the manufacturer. The label on the bulk container states that the purity is at least 99.99%.

Source: Chemical Dynamics Corporation  
Hadley Road, PO Box 395  
South Plainfield, NJ

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<sup>1</sup> Windholz M, ed., The Merck Index. 9th ed., Rahway, NJ: Merck and Co., Inc., 1976: Monograph Number 4414.

<sup>2</sup> Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p. 62. Letterman Army Institute of Research, Presidio of San Francisco, CA.

<sup>3</sup> Sadtler Research Laboratory, Inc., Sadtler standard spectra, Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared Spectrogram #14498.

**Appendix A (cont.): CHEMICAL DATA****Stability of Dosing Formulations:**

The stability of guanidine nitrate in aqueous solution is demonstrated by the absorbance values obtained for a standard solution containing 20 µg/ml of guanidine nitrate. This solution was prepared on 25 May and kept at room temperature over the period of analysis. From 25 May to 6 June, four assays of this solution were performed yielding statistically identical absorbance values.<sup>4</sup> Since the Voges-Proskauer assay is specific for unsubstituted and mono-substituted guanidines, the data demonstrate that aqueous solutions of guanidine nitrate are stable for a period of at least 12 days (Table 1).

TABLE 1: Stability Assay of a 20 µg/ml Standard Solution of Guanidine Nitrate

| Date of Analysis | Absorbance Values* |
|------------------|--------------------|
| 25 May 84        | 1.74 ± 0.02        |
| 29 May 84        | 1.76 ± 0.05        |
| 30 May 84        | 1.76 ± 0.02        |
| 6 Jun 84         | 1.76 ± 0.02        |

\* Values are mean ± S.D. for three replicates.

<sup>4</sup> Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, pp 55-57, 59. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

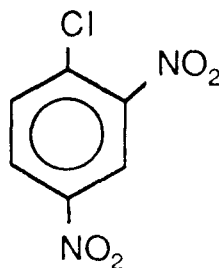
POSITIVE CONTROL

Chemical Name: 1-Chloro-2,4-dinitrobenzene

Alternate Chemical Name: 2,4-Dinitrochlorobenzene

Chemical Abstracts Service Registry Number: 97-00-7

Chemical Structure:



Molecular Formula: C<sub>6</sub>H<sub>3</sub>N<sub>2</sub>O<sub>4</sub>Cl

Molecular Weight: 202.6

Physical State: Yellow crystals

Melting Point: 52-54° C<sup>1</sup>

Purity:

The compound was designated as 95% pure by source.

Analytical Data:

Chemical analysis was performed as follows: Infrared spectra were obtained with a Perkin-Elmer 983 spectrometer.<sup>2</sup> Proton magnetic resonance (NMR) spectra were recorded on a Varian XL300 instrument with tetramethylsilane as the internal standard and chemical shifts expressed as parts per million (d).<sup>3</sup> Low resolution GC-MS analysis was performed with a Kratos MS-25RFA (30 m DB-1 capillary column).<sup>4</sup>

The following data were obtained: IR (KBr): 3445, 3104, 2877, 1963, 1829, 1801, 1756, 1705, 1604, 1591, 1542, 1349, 1246, 1156, 1046, 917, 902, 850, 835, 749, 732 cm<sup>-1</sup>. The IR spectrum was very close to the Sadler reference spectrum.<sup>5</sup> Differences were due to the much finer spectral resolution obtained on the P-E 983 instrument. NMR (CDCl<sub>3</sub>): d 7.78 (1 H, d, J = 8.7 Hz), 8.38 (1 H, q, J<sub>ortho</sub> = 8.7 Hz, J<sub>meta</sub> = 3.6 Hz), 8.74 (1 H, d, J<sub>meta</sub> = 2.4 Hz). The spectrum of DNCB was identical to the Aldrich reference spectrum.<sup>6</sup> GC-MS Analysis: A plot of the total ion current versus scan

number showed one major peak for DNCB with only traces of other compounds (not identified). Molecular ion masses ( $m/z$ ) of 202 and 204 confirmed the identity of the major peak as DNCB.<sup>7</sup>

Lot Number: 11F-0543

Source: Sigma Chemical Co.  
St. Louis, MO

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<sup>1</sup>Windholz M, ed. The Merck Index. 10th ed. Rahway, NJ: Merck and Co., Inc., 1983:300.

<sup>2</sup>Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, pp. 9-10. Letterman Army Institute of Research, Presidio of San Francisco, CA.

<sup>3</sup>Ibid. pp. 11-12.

<sup>4</sup>Ibid. pp. 13-16.

<sup>5</sup>Sadtler Research Laboratory, Inc., Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared spectrogram #964.

<sup>6</sup>Pouchert CJ. The Aldrich Library of NMR Spectra. Vol. 1, 2nd ed. Milwaukee: Aldrich Chemical Co., 1981:1173, spectrum D.

<sup>7</sup>Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, pp. 13-15. Letterman Army Institute of Research, Presidio of San Francisco, CA.

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# **Appendix B: ANIMAL DATA**

Species: *Cavia porcellus*

Strain: Hartley

Source: Charles River Breeding Laboratories  
Wilmington, MA

Sex: Male

Date of Birth: 15 June 1984

Method of randomization: Weight bias, stratified animal  
allocation

Animals in each group: 10 male animals

Condition of animals at start of study: Normal

Identification procedures: Ear tag, tag numbers 84E047 to  
84E092 inclusive.

Pretest conditioning: Quarantine/acclimation 3-18 July 1984

Justification: The laboratory guinea pig has proven to be a  
sensitive and reliable model for detection of  
delayed hypersensitivity from dermal contact.

**Appendix C: HISTORICAL LISTING OF EVENTS**

| <u>Date</u>                          | <u>Event</u>                                                                                                                                                                                                     |
|--------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 3 Jul 84                             | Forty-six animals arrived at LAIR. Animals were examined, placed in cages, and fed. Animals were ear tagged and weighed. Two animals were submitted for necropsy as quality controls.                            |
| 3 Jul -<br>20 Aug 84                 | Animals were checked daily.                                                                                                                                                                                      |
| 10,17,24,<br>31 Jul,<br>14,21 Aug 84 | Animals were weighed.                                                                                                                                                                                            |
| 10 Jul 84                            | Four pilot animals were shaved. Pilot dosing was solution prepared.                                                                                                                                              |
| 11 Jul 84                            | Pilot animals were patch tested.                                                                                                                                                                                 |
| 12 Jul 84                            | Pilot animals were scored for 24-hour skin reaction.                                                                                                                                                             |
| 13 Jul 84                            | Pilot animals were scored for 48-hour skin reaction.                                                                                                                                                             |
| 16 Jul 84                            | Pilot results were evaluated, test concentration was determined, animals were randomized into groups.                                                                                                            |
| 18 Jul 84                            | Test animals, except negative control group, were given first induction dose.                                                                                                                                    |
| 19 Jul 84                            | Test animals, except negative control group, were scored for 24-hour skin reaction.                                                                                                                              |
| 20 Jul 84                            | Test animals, except negative control group, were scored for 48-hour reaction.                                                                                                                                   |
| 20 Jul 84                            | 100% guanidine nitrate suspension was determined to be too irritating for induction dosing. Test animals were replaced with those from saline control group. One test animal was found dead (stress, pneumonia). |

**Appendix C (cont.): HISTORICAL LISTING OF EVENTS**

| <u>Date</u> | <u>Event</u>                                                                                                                                                                                                                                            |
|-------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 24 Jul 84   | All animals, except negative control group, were clipped and shaved.                                                                                                                                                                                    |
| 25 Jul 84   | Positive control animals were given second induction dose. Test (10% guanidine nitrate) animals were given first induction dose. One DNCEB-treated animal was found dead (adrenal and hepatic lesions, stress). Room temperature setting was increased. |
| 26 Jul 84   | Test and positive control groups were scored for 24-hour skin reaction.                                                                                                                                                                                 |
| 27 Jul 84   | Test and positive control groups were scored for 48-hour skin reaction.                                                                                                                                                                                 |
| 31 Jul 84   | All animals, except negative control group, were clipped and shaved.                                                                                                                                                                                    |
| 1 Aug 84    | Positive control animals were given third induction dose. Test (10% guanidine nitrate) animals were given second induction dose.                                                                                                                        |
| 2 Aug 84    | Test and positive control groups were scored for 24-hour skin reaction.                                                                                                                                                                                 |
| 3 Aug 84    | Test and positive control groups were scored for 48-hour skin reaction.                                                                                                                                                                                 |
| 7 Aug 84    | Test group was clipped and shaved. Four pilot animals were sacrificed.                                                                                                                                                                                  |
| 8 Aug 84    | Test group (10% guanidine nitrate) was given third induction dose.                                                                                                                                                                                      |
| 9 Aug 84    | Test group was scored for 24-hour skin reaction.                                                                                                                                                                                                        |
| 10 Aug 84   | Test group was scored for 48-hour skin reaction.                                                                                                                                                                                                        |
| 14 Aug 84   | Positive and negative control groups were clipped and shaved.                                                                                                                                                                                           |
| 15 Aug 84   | Positive and negative control groups were given challenge dose. One negative control animal was found dead (stress).                                                                                                                                    |

**Appendix C (cont.): HISTORICAL LISTING OF EVENTS**

| <u>Date</u> | <u>Event</u>                                                                |
|-------------|-----------------------------------------------------------------------------|
| 16 Aug 84   | Positive and negative control groups were scored for 24-hour skin reaction. |
| 17 Aug 84   | Positive and negative control groups were scored for 48-hour skin reaction. |
| 21 Aug 84   | Test group was clipped and shaved.                                          |
| 22 Aug 84   | Test (10% guanidine nitrate) group was given challenge dose.                |
| 23 Aug 84   | Test group was scored for 24-hour skin reaction.                            |
| 24 Aug 84   | Test group was scored for 48-hour skin reaction.                            |
| 27 Aug 84   | Thirty-seven study animals were sacrificed.                                 |

## Appendix D-1: INDIVIDUAL ANIMAL SCORES

GROUP : ONE

COMPOUND: GUANIDINE NITRATE

[illegible]

# Appendix D-2: INDIVIDUAL ANIMAL SCORES

| GROUP: TWQ    | COMPOUND: DINITROCHLOROBENZENE |      |                  |      |                 |      |                |      |      |             |
|---------------|--------------------------------|------|------------------|------|-----------------|------|----------------|------|------|-------------|
|               | FIRST INDUCTION                |      | SECOND INDUCTION |      | THIRD INDUCTION |      | CHALLENGE DOSE |      |      |             |
| ANIMAL NUMBER | 24 H                           | 48 H | 24 H             | 48 H | 24 H            | 48 H | LEFT FLANK     | 24 H | 48 H | RIGHT FLANK |
| 84E0060       | 0.0                            | 0.0  | 1                | 1    | 1               | 0.5  | 2              | 2    | 2    | 2           |
| 84E0063       | 0.0                            | 0.0  | 1                | 1    | 1               | 1    | 1              | 0.0  | 1    | 1           |
| 84E0065       | 0.0                            | 0.0  | N/A              | N/A  | N/A             | N/A  | N/A            | N/A  | N/A  | N/A         |
| 84E0072       | 0.0                            | 0.0  | 1                | 1    | 2               | 2    | 1              | 1    | 1    | 1           |
| 84E0080       | 0.0                            | 0.0  | 1                | 0.0  | 1               | 1    | 1              | 1    | 2    | 2           |
| 84E0082       | 0.0                            | 0.0  | 0.0              | 0.0  | 1               | 0.5  | 1              | 1    | 1    | 1           |
| 84E0083       | 1                              | 0.0  | 1                | 0.0  | 2               | 1    | 1              | 1    | 2    | 2           |
| 84E0085       | 0.0                            | 0.0  | 0.0              | 0.0  | 1               | 0.0  | 1              | 0.0  | 1    | 0.0         |
| 84E0086       | 0.0                            | 0.0  | 1                | 1    | 1               | 0.0  | 1              | 1    | 1    | 0.0         |
| 84E0089       | 0.0                            | 0.0  | 1                | 1    | 1               | 1    | 1              | 0.0  | 1    | 1           |
| AVERAGES      | 0.10                           | 0.0  | 0.78             | 0.56 | 1.22            | 0.78 | 1.11           | 0.78 | 1.33 | 1.11        |

# Appendix D-3: INDIVIDUAL ANIMAL SCORES

| GROUP: THREE  | COMPOUND: <u>NEGATIVE CONTROL</u> |      |                  |      |                 |      |                |             |      |      |
|---------------|-----------------------------------|------|------------------|------|-----------------|------|----------------|-------------|------|------|
|               | FIRST INDUCTION                   |      | SECOND INDUCTION |      | THIRD INDUCTION |      | CHALLENGE DOSE |             |      |      |
|               | 24 H                              | 48 H | 24 H             | 48 H | 24 H            | 48 H | LEFT FLANK     | RIGHT FLANK | 24 H | 48 H |
| ANIMAL NUMBER |                                   |      |                  |      |                 |      |                |             |      |      |
| 84E0051       | N/A                               | N/A  | N/A              | N/A  | N/A             | N/A  | 0.5            | 0.0         | N/A  | N/A  |
| 84E0052       | N/A                               | N/A  | N/A              | N/A  | N/A             | N/A  | 0.0            | 0.0         | N/A  | N/A  |
| 84E0057       | N/A                               | N/A  | N/A              | N/A  | N/A             | N/A  | 0.0            | 0.0         | N/A  | N/A  |
| 84E0066       | N/A                               | N/A  | N/A              | N/A  | N/A             | N/A  | 0.0            | 0.0         | N/A  | N/A  |
| 84E0071       | N/A                               | N/A  | N/A              | N/A  | N/A             | N/A  | 0.0            | 0.0         | N/A  | N/A  |
| 84E0072       | N/A                               | N/A  | N/A              | N/A  | N/A             | N/A  | 0.0            | 0.0         | N/A  | N/A  |
| 84E0076       | N/A                               | N/A  | N/A              | N/A  | N/A             | N/A  | 0.0            | 0.0         | N/A  | N/A  |
| 84E0079       | N/A                               | N/A  | N/A              | N/A  | N/A             | N/A  | 0.0            | 0.0         | N/A  | N/A  |
| 84E0084       | N/A                               | N/A  | N/A              | N/A  | N/A             | N/A  | N/A            | N/A         | N/A  | N/A  |
| 84E0089       | N/A                               | N/A  | N/A              | N/A  | N/A             | N/A  | 0.0            | 0.0         | N/A  | N/A  |
| AVERAGES      | N/A                               | N/A  | N/A              | N/A  | N/A             | N/A  | 0.06           | 0.00        | N/A  | N/A  |

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