ACUTE ORAL TOXICITY OF 1-ACETYLOCTAHYDRO-357-TRINITRO-1357-TETRAZOCINE (S (U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA C W WHITE ET AL. MAY 84 F/G 6/20 NL
ACUTE ORAL TOXICITY OF 1-ACETYLOCTAHYDRO-3,5,7-TRINITRO-
1,3,5,7-TETRAZOCINE (SEX) IN MALE AND FEMALE RATS

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DIVISION OF RESEARCH SUPPORT

MAY 1984

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129
Acute oral toxicity of 1-acetyloctahydro-1,5,7-trinitro-1,3,5,7-tetrazocine
in male and female rats--White and Zimmerman

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(Signature and date) 2 May 1984

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**Acute Oral Toxicity of 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine in Male and Female Rats**

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Fort Detrick  
Frederick, MD 21701

**Abstract**  
The acute oral toxicity potential of the explosives by-product, 1-acetyl-octahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX), was determined in male and female albino Fisher 344 rats by using a single dose, free-choice feeding method. The study was conducted in compliance with the Good Laboratory Practice Regulations. No compound related mortality was observed at a limit dose of 5.0 g/kg.
ABSTRACT

The acute oral toxicity potential of the explosives by-product, 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX), was determined in male and female albino Fisher 334 rats by using a single dose, free-choice feeding method. The study was conducted in compliance with the Good Laboratory Practice Regulations. No compound related mortality was observed at a limit dose of 5.0 g/kg.
PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

TESTING FACILITY: US Army Medical Research and Development Command
Lettermann Army Institute of Research
Division of Research Support
Presidio of San Francisco, CA 94129

SPONSOR: US Army Medical Research and Development Command
US Army Medical Bioengineering Research
and Development Laboratory
Fort Detrick, MD 21701

PROJECT: 612720.835AA Acute Mammalian Toxicology Testing,
APC, TL06

GLP STUDY NUMBER: 82005

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC, Diplomate of American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: Craig W. White, DVM, CPT VC

CO-PRINCIPAL INVESTIGATOR: Evelyn M. Zimmerman, SP5

PATHOLOGIST: MAJ Glen E. Marrs Jr., DVM, MS, VC, Diplomate of American College of Veterinary Pathologists

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: 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine (SEX)

INCLUSIVE STUDY DATES: 14 April - 10 May 1983

OBJECTIVE: To determine the acute oral toxicity potential of 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX) in male and female, albino, Fisher 344 rats.
ACKNOWLEDGMENTS

The authors wish to thank SP5 Leonard J. Sauers, MS, and SP5 Thomas P. Kellner, BS, for their assistance in the conduct of this study. In addition, we wish to thank Jesse Barkley Jr., US Army Medical Bioengineering Research and Development Laboratory, for his assistance as Project Consultant.
SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY:

We, the undersigned, believe the study number 82005 described in this report to be scientifically sound and the results in this report and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations for Non-clinical Laboratory Studies, outlined by the Food and Drug Administration.

DON W. KORTE JR. / DATE
MAJ, MS
Study Director

EVELYN M. ZIMMERMAN / DATE
SP4, USA
Co-Principal Investigator

CRAIG W. WHITE / DATE
CPT, VC
Principal Investigator

CAROLYN M. LEWIS, MS / DATE
DAC
Data Manager

GLEN E. MARRS / DATE
MAJ, VC
Pathologist
MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 82005 the following inspections were made:

15 Apr 83
26 Apr 83 (1000 hours)
26 Apr 83 (1030 hours)
2 May 83

The report and raw data for this study were audited on 21 Mar 84.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the Jul 83 report to Management and the Study Director.

NELSON R. POWERS, Ph.D.
DAC
Chief, Quality Assurance Unit
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Acute Oral Toxicity of 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine--White and Zimmerman

The manufacture of the explosives, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), at the Holston Army Ammunition Plant (HSAAP) results in the formation of a by-product, 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX). It is formed by the nitrolysis and acetylation of hexamine. As a result, quantities of SEX are discharged from HSAAP. HSAAP is the only known producer of SEX. Its discharge, while partially mitigated by pollution abatement facilities at HSAAP, will continue and could increase during times of mobilization. Information on the chemical, physical, and toxicological properties of SEX is limited. Many of its properties can only be inferred by comparisons with RDX and HMX. Although no specific data are available, SEX, based on structural comparisons, appears to be more hydrophilic than either RDX or HMX and thus, potentially, a more serious toxicological threat than RDX or HMX to the aquatic life in the Holston River. This report summarizes the results from one of a series of studies being conducted at the Letterman Army Institute of Research (LAIR) to assess the toxicological hazards of SEX (1-3).

Objective of Study

The objective of the study is to determine the acute oral toxicity of 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX) in male and female, albino, Fisher 344 rats.

METHODS

Test Substance

The SEX was received from SRI-International (333 Ravenswood Ave, Menlo Park, CA 94025) on 7 January 1983. The bulk of the material (approximately 800 g) was stored at the Presidio Central Magazine Storage Facility. The magazine bunker is an underground, reinforced concrete structure used for the storage of explosive materials. Approximately 200 g was stored at the laboratory in a flame proof cabinet at room temperature.
White--2

Chemical name:

1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine

Chemical Abstract Service Registry Number: 13980-00-2

Structural formula:

\[
\begin{array}{c}
\text{O} \\
\text{C-CH}_3 \\
\text{N-CH}_2 \\
\text{H}_2C \quad \text{N-N=O}_2 \\
\text{O}_2N \quad \text{N-CH}_2 \\
\text{H}_2C \quad \text{N-N=O}_2 \\
\end{array}
\]

Empirical formula: \( C_6H_{11}N_7O_7 \)

Other test substance information appears in Appendix A.

Animal Data

A total of 44 (22 males, 22 females) Fisher 344 rats were received from Charles River Breeding Laboratory (Kingston, NY). Additional animal data are found in Appendix B.

Environmental Conditions

A commercially available certified rodent ration and tap water were provided ad libitum for the animals during the study. Appendix C is a listing of the environmental conditions of this study.

Dosing

Animals were randomly assigned to three study groups consisting of seven male and seven female rats per group. The study groups were designated as vehicle-control, cage-control and 5.0-g/kg limit-dose groups. The 5.0 g/kg dose is considered a no mortality limit dose (4). The limit dose was based upon the results of a pilot study performed between 14 March and 31 March 1983. Commercial grade peanut butter was used as the vehicle because of the insolubility and poor suspension properties of SEX.
The limit-dose group and the vehicle control group animals were conditioned to consume 5.0 g of peanut butter daily for 5 days before the day of dosing. All test animals were weighed on the day before dosing and the weight-based dosage was calculated. All limit-dose and vehicle control group animals were dosed on 26 April 1988. The calculated dose of the test compound was weighed on a balance, mixed with 5.0 g peanut butter, and placed in the individual rat cages in disposable petri dishes. The peanut butter/dose mixture was left in the cages until it was consumed by the rats.

The dosing procedure was conducted without animal sedation or anesthesia.

Observations

Animals were observed daily throughout the quarantine/acclimation period. During the course of the study, recorded observations were conducted once daily. Additional observations were made on the day of dosing. Animals were observed daily after dosing, undisturbed in cages, outside of cages, and after their return to cages. The first regularly scheduled observation period commenced at 1200 hours on the day of dosing.

Duration of Study

The study period was 15 days with a 13-day quarantine/acclimation period before the study was begun.

Historical study events are listed in Appendix D.

Deviation from Original Protocol

The room temperature elevated to 26.7°C (80°F) during the quarantine period; however, this was not considered to impact the study results.

One animal from the 5.0 g/kg dose group was removed from this study because the rat did not consume the entire dose amount. This particular animal also diluted the remaining dosing material with water from the automatic watering system. This made it impossible to determine the percent of the dose the animal received.

RESULTS

Mortality

All animals allocated to this study survived the entire test period. No compound-related deaths occurred at a limit dose of 5.0 g/kg.
Clinical Observations

The only clinical sign observed in this study was that of irritability which was observed in three of the fourteen animals dosed. No animal exhibited signs of acute toxicity and/or mortality at the limit dose of 5.0 g/kg.

Gross Pathological Observations

No gross lesions attributable to SEX were observed at necropsy. A report of gross pathological observations appears in Appendix E.

DISCUSSION

The test compound, 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX) should be classified as relatively non-toxic by the oral route of administration (5). This, in all probability, can be attributed to the insolubility of the test compound. It is highly probable that the compound has little bioavailability as it is insoluble in aqueous and lipid solvent systems (3).

CONCLUSION

The test compound 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX) was classified as non-toxic, when administered orally, based upon this acute-limit test.

RECOMMENDATION

Any further safety testing planned for SEX should take into account the low oral toxicity of SEX which is probably attributable to its low solubility.
REFERENCES


CHEMICAL DATA

1. Chemical name: 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine (SEX)
   1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine;
   1,3,5,7-Tetrazocine, 1-Acetyloctahydro-3,5,7-Trinitro (CA Name);
   Octahydro-1-Acetyl-3,5,7-Trinitro-S-Tetramine;
   1-Aceto-3,5,7-Trinitro-1,3,5,7-Tetrazacyclooctane;
   1-(N)-Acetyl-3,5,7-Trinitrocyclotetramethylenetetramine

Chemical Abstract Service Registry No.: 13980-00-2

Structural formula:

Empirical formula: C6H11N7O7
Molecular weight: 293.2 g/mole (calculated).
Physical state: Solid at 20°C
Melting Point: 224.2 - 224.7°C
Density: 1.785 g/cc at 21°C
pH: N/A nonaqueous
Compound Refractory Index: Unknown
Stability: After 48 hours at 75°C, there was no change (NMR, IR, color, or weight) in SEX samples tested.
Purity: 99.9%
Manufacturer: SRI International
Menlo Park, CA 94205

APPENDIX A
2. Name: Peanut Butter

Ingredients: Peanuts, Dextrose, Salt, Hydrogenated Palm Oil, Mono and Diglycerides.

Physical state: Light brown, creamy style.

Name of contaminants percentages: None known.

Manufacturer: Golden State Brand
Laura Scudder
Snack Foods Division
IC Industries Company
Anaheim, CA

Lot Number: 3061
ANIMAL DATA

Species: Laboratory rat

Strain: Albino, Fisher 344

Source: Charles River Breeding Laboratories Inc., Kingston, NY

Sex: Male and Female

Date of Birth: Males - 7 Feb 83, Females - 14 Jan 83

Method of Randomization: Weight bias, stratified animal allocation (SOP OP-ISG-21).

Animals in each group: 14 animals, 7 males and 7 females

Condition of animals at start of study: Normal

Body weight range: Males 196-215 g, Females 159-174 g

Identification procedures: Ear tagged (SOP-OP-ARG-1)

Pretest conditioning:

1. Quarantine from 13 Apr 83 - 25 Apr 83.

2. Animals were trained to consume 5-6 g of peanut butter from 21 Apr to 25 Apr 83.

Justification: Rats are a proven sensitive animal model for this test.

APPENDIX B
ENVIRONMENTAL CONDITIONS

Caging: Number/cage = 1; Type of cage = stainless steel, wire mesh bottom, battery type, no bedding, automatic flush.

Diet: Purina Certified Rodent Chow No. 5002 ad lib,

Water: Central line to cage battery with automatic lick dispensers

Temperature: 22 ± 1°C

Relative Humidity: 50% ± 10%

Photoperiod: 0530 - 2000 hours per day
**HISTORICAL LISTING OF STUDY EVENTS**

<table>
<thead>
<tr>
<th>Date</th>
<th>Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-31 Mar 83</td>
<td>Pilot study accomplished.</td>
</tr>
<tr>
<td>13 Apr 83</td>
<td>Animals arrive at LAIR. They were sexed, observed for illness, ear tagged, weighed and caged in the GLP Suite.</td>
</tr>
<tr>
<td>14-25 Apr 83</td>
<td>Animals checked once daily.</td>
</tr>
<tr>
<td>22 Apr 83</td>
<td>Animals weighed and randomized into dose groups.</td>
</tr>
<tr>
<td>21-25 Apr 83</td>
<td>Animals weighed daily and conditioned to dosing procedure by feeding and recording peanut butter intake.</td>
</tr>
<tr>
<td>25 Apr 83</td>
<td>Animals weighed and dose calculated. Feed removed at 1630 hours.</td>
</tr>
<tr>
<td>26 Apr 83</td>
<td>Animals dosed by mixing test compound with 5-6 g peanut butter. Observations conducted one to two hours after dosing and at 1600 hours. Animals observed for clinical signs which were recorded. Rat chow made available as soon as the peanut butter was consumed.</td>
</tr>
<tr>
<td>27 Apr - 9 May 83</td>
<td>Animals observed for clinical signs at 0930 hours.</td>
</tr>
<tr>
<td>29 Apr - 2,6 May 83</td>
<td>All animals weighed.</td>
</tr>
<tr>
<td>9 May 83</td>
<td>Food removed at 1600 hours.</td>
</tr>
<tr>
<td>10 May 83</td>
<td>Animals observed for clinical signs at 0700 hours and weighed. Animals delivered to the PSG Necropsy Suite for sacrifice and gross necropsy by 0830 hours.</td>
</tr>
</tbody>
</table>

APPENDIX D
PATHOLOGY REPORT

GLP Study 82005

Acute Oral Toxicity Limit Study in Male Rats of Octahydro-1-((N))-
acetyl-3,5,7-trinitro-1,3,5,7-tetrazine (SEX), (CAS #13980-00-2)

History: The male Fisher 344 rats in this study were divided into
3 groups. All groups but the cage controls were fed 5-6 g of
peanut butter vehicle or 5-6 g of peanut butter vehicle mixed with
test compound at a dose rate of 5.0 g/kg body weight. The material
dosed with and the number of rats in each group were as follow:

- Group 1 (Cage controls) - 7 rats
- Group 2 (Vehicle controls) - 7 rats
- Group 3 (5.0 g/kg SEX) - 7 rats

One of 7 rats in group 3 was misdosed and was removed from the
study. All other rats survived until completion of the study, 14
days after dosing. The rats were killed by exsanguination from
severed axillary vessels while under anesthesia produced by
intraperitoneal injection of pentobarbital.

Gross necropsy findings: Necropsies revealed no test compound
related gross lesions in male rats that were killed at completion of
the study. Seven of 7 rats in group 1, 7/7* rats in group 2, and
6/6 rats in group 3 had bilaterally enlarged inguinal (prepuceal)
glands that were turgid and filled with yellow-green granular
material that was most likely inspissated secretory material.
External examination revealed a subcutaneous nodule** in the
inguinal area of 1/7 rats in group 1 and 1/6 rats in group 3. The
skin over the nodule in the rat in group 3 was umbilicated and
ulcerated. The eyes of 1/7 rats in group 2 had red-black optic
disks and red retinal foci and the right eye of 1/6 rats in group 3
had a red retinal focus. The red-black optic disks and the red
retinal foci may have represented hemorrhage. A cause for the
lesions in the prepucial glands and the eyes was not determined.

*Number of rats affected/Number of rats in the group.

**Microscopic examination of the subcutaneous nodules in the
inguinal area of the rat in group 1 and the rat in group 3 revealed
ruptured prepucial glands that had spilled secretion material into
adjacent subcutis. Both the subcutis and the lumen of the gland
contained abundant pyohistiocytic inflammatory cell infiltrate and
cell debris.
Summary: No test related gross or microscopic lesions were observed in male Fisher 344 rats that were cage controls, vehicle controls, or dosed with 5.0 mg of SEX.

GLEN E. MARRS, JR., DVM, VS
Diplomate, A.C.V.P.
MAJ, VC
Assistant Chief, Pathology Services Group
Division of Research Support

9 August 1983

TABLE I
Acute Oral Toxicity Limit Study in Male Rats of SEX,
(CAS #13980-00-2) - GLP Study 82005

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID# 83DOOxxx</td>
<td>2 3 3 3 3 3 3 2 2 3 3 3 3 2 2 2 2 3 3</td>
<td>3 2 3 3 3 3 3 2 2 2 2 3 3 3</td>
</tr>
<tr>
<td>9 0 1 1 2 2 2 9 9 0 1 2 2 2 8 8 9 9 0 1 1</td>
<td>3 2 3 3 3 3 3 2 2 2 2 3 3 3 3 2 2 2 2 3 3 3</td>
<td></td>
</tr>
<tr>
<td>5 2 0 3 1 2 9 6 7 8 5 3 4 6 7 9 2 3 4 2 6</td>
<td>3 2 3 3 3 3 3 2 2 2 2 3 3 3 3 2 2 2 2 3 3 3</td>
<td></td>
</tr>
</tbody>
</table>

Survived to Completion

| + + + + + + + + + + + + + + + + + + + + |

Removed - misdose

| + |

Inguinal (Prepucial) Glands, enlarged

| + + + + + + + + + + + + + + + + + + + + |

Skin:

(1) Subcutaneous inguinal nodule

| + |

(2) Umbilicated & ulcerated over inguinal nodule

| + |

Eye:

(1) Optic disk, red-black

| + |

(2) Retina, red fous/foli

| + |
PATHOLOGY REPORT

GLP Study 82005

Acute Oral Toxicity Limit Study in Female Rats of Octahydro-1-(N)-acetyl-3,5,7-trinitro-1,3,5,7-tetrazine (SEX), (CAS #13980-00-2)

History: The female Fisher 344 rats in this study were divided into 3 groups. All groups but the cage controls were fed 0-0% of peanut butter vehicle or 5-6 g of peanut butter vehicle mixed with test compound at a dose rate of 5.0 g/kg body weight. The material dosed with and the number of rats in each group were as follow:

- Group 1 (Cage controls) - 7 rats
- Group 2 (Vehicle controls) - 7 rats
- Group 3 (5.0 g/kg SEX) - 7 rats

All rats survived until completion of the study, 11 days after dosing. The rats were killed by exsanguination from severed axillary vessels while under anesthesia produced by intraperitoneal injection of pentobarbital.

Gross necropsy findings: Necropsies revealed no test compound related gross lesions in female rats that were killed at completion of the study. One of 7 rats in group 3 had a fluid filled cyst in one ovary and 1/7* rats in group 1, 1/7 rats in group 2, and 1/7 rats in group 3 had bilaterally enlarged inguinal (clitoral) glands that were turgid and were filled with yellow-green granular material. The ovarian cyst was considered to be an incidental finding. A cause for the enlargement of the clitoral glands was not determined.

Summary: No test related gross lesions were observed in female Fisher 344 rats that were cage controls, vehicle controls, or dosed with 5.0 mg/kg of SEX.

*Number of rats affected/Number of rats in the group

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Diplomate, A.C.V.P.
MAJ, VC
Assistant Chief, Pathology Services Group
Division of Research Support

9 August 1983
## TABLE I

Acute Oral Toxicity Limit Study in Female Rats of CRX

(Cat. #1999-90-3) - HSD Study #2005

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDS 9999xxx</td>
<td>* * * * * * *</td>
<td>* * * * * * *</td>
<td>* * * * * * *</td>
</tr>
<tr>
<td></td>
<td>* * * * * * *</td>
<td>* * * * * * *</td>
<td>* * * * * * *</td>
</tr>
<tr>
<td>Survived to Completion</td>
<td>+ + + + + + + + + + + +</td>
<td>+ + + + + + + + + + + +</td>
<td>+ + + + + + + + + + + +</td>
</tr>
<tr>
<td>No lesions recognized</td>
<td>+ + + + + + + + + + + +</td>
<td>+ + + + + + + + + + + +</td>
<td>+ + + + + + + + + + + +</td>
</tr>
<tr>
<td>Inguinal (Clitoral) glands, enlarged</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ovary: Tux, fluid-filled, unilateral</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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Commander
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