DERMAL SENSITIZATION OF 1-ACETYLOCTAHYDRO-3,5,7-TRINITRO-1,3,5,7-TETRAZOCINE

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DIVISION OF RESEARCH SUPPORT

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

AUGUST 1984

Toxicology Series 72
Dermal Sensitization of 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-
Tetrazocine (SEX) -- Johnson, Lewis and Korte

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In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

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The explosive by-product, 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX), was tested for dermal sensitization potential on guinea pigs. The study was conducted in compliance with the Good Laboratory Practice Regulations. The absence of erythema in the test animals during the study indicated SEX is a non-sensitizer when applied topically in saline according to the closed patch dermal sensitization technique of Buehler.
The explosive by-product, 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX), was tested for dermal sensitization potential on guinea pigs. The study was conducted in compliance with the Good Laboratory Practice Regulations. The absence of erythema in the test animals during the study indicated SEX is a non-sensitizer when applied topically in saline according to the closed patch dermal sensitization technique of Buehler.

Key Words: 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7- Tetrazocine (SEX), Dermal Sensitization
PREFACE

TYPE REPORT: Dermal Sensitization GLP Report

TESTING FACILITY: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Division of Research Support
Presidio of San Francisco, CA 94129

SPONSOR: U.S. Army Medical Research and Development Command
U.S. Army Medical Bioengineering Research and Development Laboratory
Fort Detrick, Frederick, MD 21701

PROJECT: 612720.835AA Acute Mammalian Toxicology Testing
APC TL06

GLP STUDY NO.: 82004

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

PRINCIPAL INVESTIGATOR: Carolyn M. Lewis, MS

CO-PRINCIPAL INVESTIGATOR: Yvonne C. Johnson, BS

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

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

INCLUSIVE STUDY DATES: 6 Jul - 18 Aug 1983

OBJECTIVE: The objective of the study was to evaluate the dermal sensitization potential of 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine in guinea pigs.
ACKNOWLEDGMENTS

The authors wish to thank SP5 Evelyn Zimmerman, Susan Hernandez, Michael Sands, and Richard Spieler for their assistance in the weighing, dosing, observing, and caring for the animals. We also wish to thank CPT Martha A. Hanes, DVM, and SP5 Leonard J. Sauers, MS, for their initial testing of patches with the test compound. Finally, we wish to thank Jesse Barkley Jr., US Army 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 82004 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

CAROLYN M. LEWIS, MS /DATE
DAC
Principal Investigator

YVONNE C. JOHNSON, BS /DATE
DAC
Co-Principal Investigator
MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 82004 the following inspections were made:

15 Apr 83
13 May 83
10 Jun 83
29 Jun 83
02 Aug 83
10 Aug 83

The report and raw data for this study were audited on 29 May 84.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the 5 July 83 and 29 Nov 83 report to Management and the Study Director.

NELSON R. POWERS, Ph.D.
DAC
Chief, Quality Assurance Unit
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>i</td>
</tr>
<tr>
<td>Preface</td>
<td>iii</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>iv</td>
</tr>
<tr>
<td>Signatures of Principal Scientists</td>
<td>v</td>
</tr>
<tr>
<td>Report of Quality Assurance Unit</td>
<td>vi</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>vii</td>
</tr>
</tbody>
</table>

## BODY OF REPORT

### INTRODUCTION

Objective of Study

### MATERIALS

- Test Substance
- Vehicle
- Animal Data
- Husbandry

### METHODS

- Acclimation and Group Assignment
- Dose Levels
- Compound Preparation
- Test Procedures

### RESULTS

5

### DISCUSSION

8

### CONCLUSION

8

### RECOMMENDATION

8

### REFERENCES

9
Table of Contents (continued)

APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendix A, Chemical Data</td>
<td>13</td>
</tr>
<tr>
<td>Appendix B, Deviations in Husbandry</td>
<td>19</td>
</tr>
<tr>
<td>Appendix C, Historical Listing of Events</td>
<td>21</td>
</tr>
<tr>
<td>Appendix D, Dermal Sensitization Tables</td>
<td>23</td>
</tr>
</tbody>
</table>

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Dermal Sensitization of 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine (SEX) Johnson et al

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 unavoidable formation of the by-product, 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX). It is formed during nitrolysis of hexamine. During this process, a portion of the hexamine is also acetylated by the acetic acid/acetic anhydride solvent. As a result, significant quantities of SEX are discharged from HSAAP. HSAAP is the only known producer of SEX. Its discharge, while partially mitigated by present and planned pollution abatement facilities at HSAAP, will continue and could increase at mobilization. Information on the chemical, physical, and toxicological properties of SEX is limited. Many of its properties can only be inferred by structural comparisons with RDX or HMX; however, no specific values are available. These comparisons suggest that SEX has a greater potential to affect aquatic environments than either RDX or HMX and this may produce adverse effects on aquatic life in the Holston River (1). The present study represents one study in a series of toxicological studies to be conducted at the Letterman Army Institute of Research (LAIR) to assess the toxicological hazards of SEX.

Objective of the Study

The objective of the study was to evaluate the dermal sensitization potential of 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX) in guinea pigs

MATERIALS

Test Substance

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

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

\[
\begin{align*}
\text{NO}_2 \\
\text{H}_2\text{C}-\text{N}^-\text{CH}_2\text{O} \\
\text{O}_2\text{N}-\text{N}^-\text{C}-\text{CH}_3 \\
\text{H}_2\text{C}-\text{N}^-\text{CH}_2 \\
\text{NO}_2
\end{align*}
\]

Empirical formula: \(\text{C}_6\text{H}_{11}\text{N}_7\text{O}_7\)

Purity: 99.9%

Additional chemical data and results of chemical analyses appear in Appendix A.

Vehicle

Chemical name: USP Grade Physiological Saline, 0.9%

Source: Travenol Lab, Inc., Deerfield, IL 60015

Lot number: 8C865A4 used in formulation of the test compound. 2C655S1 used in formulation of the positive control.

Expiration date: December 1984

Date opened: 19 July 1983

Rationale: Saline is non-irritating and non-sensitizing

Animal Data

Forty male young adult Hartley guinea pigs (Charles River Breeding Laboratories, Inc., Wilmington, MA) were used for the dermal sensitization test. The animals weighed between 300 and 370 g upon receipt and between 400 and 500 g by the first dosing. Each guinea pig was ear tagged (LAIR SOP-OP-ARG-1).

Husbandry

The guinea pigs were housed individually in stainless steel, screen-bottom (no-bedding), battery type cages with automatic flushing. Water was provided ad libitum to the cage battery by automatic lick dispensers connected to a central line. During the
wrappings from getting wet. The animals were fed ad libitum Purina Certified Guinea Pig Chow No. 5026 (Lot Numbers APR21832A and MAY16832A). The temperature in the room was maintained between 21 to 26 C. The relative humidity varied between 40% and 60%. The photoperiod in the animal room was between 0630 and 1900 hours each day. A few minor deviations in these conditions are discussed in Appendix B. However, these should not have significantly affected the results of this study.

METHODS

Acclimation and Group Assignment

The guinea pigs were quarantined for twelve days before being assigned to groups. During the quarantine period, they were checked daily for signs of illness and weighed twice weekly. It was noticed upon their arrival that nine animals had calcification around one or both tarsal joints. Since this was such a prevalent condition in these animals, one was selected for quality control necropsy. No unusual findings were reported. As there was no evidence to indicate that this problem would affect the outcome of this study, its occurrence was not considered during the randomization of the guinea pigs into groups.

Ten animals were assigned to each of four groups using a stratified randomization technique based on their weights. The MINITAB statistical software (2) on the Data General Eclipse C/330 was used to rank animals according to their weight. Extra animals were eliminated from the extremes (i.e., those whose weights deviated furthest from the mean). The RANDOM program (LAIR SOP-OP-ISG-21) on the C/330 was used to generate ten random sequences of numbers one through four.

Dose Levels

The test substance was a solid, therefore, it was suspended in 0.9% sodium chloride before application (4). The positive control substance, dinitrochlorobenzene (DNCB), was used at 0.1% concentration. During the induction phase, the experimental group had a dose of 0.5 g in 0.5 ml saline applied topically under a one-inch square gauze patch once a week for three weeks (19 July 83, 26 July 83, 2 Aug 83). The positive control and vehicle control groups had a 0.5 ml dose applied in the same manner on the same schedule.

The animals were rested for two weeks following the third induction dose and then were given the challenge dose (16 Aug 83). The experimental group had a 0.5 g dose in 0.5 ml saline applied to the old site on the left side and to a new site on the right side. The positive control group had a 0.5 ml dose applied to both sides also. The vehicle control group had a 0.5 ml dose applied to the left side only. In addition, the negative control group for the test
Johnson--4

compound had a 0.5 g dose in 0.5 ml saline applied to the left side only.

**Compound Preparation**

The test compound, SEX, was moistened with 0.5 ml of 0.9% sodium chloride immediately before application. The dinitrochlorobenzene dosing solution was prepared by first adding 30 mg DNCB to 1 ml of propylene glycol and heating it until it dissolved. 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 40 °C and vortexed before application to keep the DNCB in solution. The same solution was used for all four applications.

**Test Procedures**

The closed patch dermal sensitization test developed by Buehler and Griffith (3–6) was used for this study. The Buehler test was used instead of the standard Landsteiner Draize (7–8) test because the test compound was highly insoluble in any non-irritating solution, therefore, intradermal injections were impractical.

Following Buehler's technique, the test compounds were applied under a closed patch once a week for three weeks during the induction phase. The same application site was used for each induction dose. The day before each dosing a three-inch square area on the left side of the animal was clipped with electric clippers (Oster® Model A5, size 40 blade, Sunbeam Corp., Milwaukee, WI 53217) and then shaved with an electric razor (Norelco® Speed Razor Model HP1134/S, North American Phillips Corp., Stamford, CT 06904). The patch was taped (Durapore® hypoallergenic surgical tape, 3M Corp., St. Paul, MN 55144) to the same site each time. The animal was wrapped several times with Conform® elastic tape (The Kendall Company, Boston, MA 02101) to occlude the patch. The patch was left in place for six hours. When the patch was removed, the area under the patch was marked off for scoring.

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. The procedures for clipping, shaving, wrapping, and exposure period for the challenge dose remained the same.

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

Some modifications of Buehler's procedures were made. Instead of placing animals in restrainers during the 6-hour exposure period for each application, the animals were wrapped several times with elasticized adhesive tape to hold the patch in place and occlude it. Consequently, the animals were able to move about freely in their cage during the exposure period. Buehler and Griffith (5) also recommended depilatating the hair the day before the challenge dose was applied, but we felt this might cause some skin irritation by itself and any residue left from the depilatory cream could possibly react with the test compound.

A historical listing of study events appears in Appendix C.

RESULTS

The incidence of reactions 24 and 48 hours after each dose are summarized in Tables 1 and 2. There was no reaction in the test group, negative control group or vehicle control group after any of the three induction doses, or the challenge dose. After the second induction dose, all ten animals in the positive control group demonstrated a reaction, and continued to after all subsequent dosings. There was no difference in incidence of reaction between the 24 and 48 hour observations.

The severity of skin reactions 24 and 48 hours after each dose is summarized in Tables 3 and 4. Again, there were no reactions observed in the test group, negative control group or the vehicle control group following either the induction doses or the challenge dose.

The positive control group showed the greatest increase in 24 and 48 hour scores after the second induction dose, with a smaller increase following the third induction dose. There was a decrease in both scores on the left side from the third induction dose to the challenge dose. The reactions on the right side after the challenge dose were only slightly lower than the left side.

The individual 24-hour and 48-hour scores for all the doses appear in Appendix D by group.
TABLE 1
Incidences of Skin Reactions
after Twenty-Four Hours

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Induction First</th>
<th>Induction Second</th>
<th>Induction Third</th>
<th>Challenge Left</th>
<th>Challenge Right</th>
<th>Negative Control*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEX</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Saline</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>DNCB</td>
<td>0/10</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td>--</td>
</tr>
</tbody>
</table>

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

TABLE 2
Incidences of Skin Reactions
after Forty-Eight Hours

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Induction First</th>
<th>Induction Second</th>
<th>Induction Third</th>
<th>Challenge Left</th>
<th>Challenge Right</th>
<th>Negative Control*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEX</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Saline</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>DNCB</td>
<td>0/10</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td>--</td>
</tr>
</tbody>
</table>

* The Negative Control Group received a challenge dose of the test compound.
### TABLE 3
**Severity of Skin Reactions**
**after Twenty-Four Hours**

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Induction</th>
<th>Challenge</th>
<th>Negative Control+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First</td>
<td>Second</td>
<td>Third</td>
</tr>
<tr>
<td>SEX</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Saline</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DNCB</td>
<td>0</td>
<td>1.3</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* Mean values based on a scale of: 1=Slight Erythema; 2=Moderate Erythema; 3=Severe Erythema

+ The Negative Control Group received a challenge dose of the test compound.

### TABLE 4
**Severity of Skin Reactions**
**after Forty-Eight Hours**

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Induction</th>
<th>Challenge</th>
<th>Negative Control+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First</td>
<td>Second</td>
<td>Third</td>
</tr>
<tr>
<td>SEX</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Saline</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DNCB</td>
<td>0</td>
<td>1.0</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* Mean values based on a scale of: 1=Slight Erythema; 2=Moderate Erythema; 3=Severe Erythema

+ The Negative Control Group received a challenge dose of the test compound.
DISCUSSION

Based on the results of this study, in which there was a negative response in all animals of the experimental group, it can be concluded that the test compound, SEX, is neither a sensitizer or a primary irritant. The absence of erythema in the vehicle control group confirms that saline is also a non-sensitizer. It is possible that the insolubility of SEX in saline may have influenced the results of this study. The compound, moistened with saline, remained on the surface of the skin as a paste with little, if any, absorption by the skin. The lack of absorption by the skin may have been a factor in the outcome, and could warrant further investigation.

According to the guidelines provided by Griffith (7), the incidence and severity of the skin reactions in the positive control group confirm that DNCB was a sensitizer. There was a sudden increase in the incidence and severity from the first to the second and third applications. There was a 100% response to the challenge dose on the right side after 24 and 48 hours. Reactions to the DNCB were slight to moderate with only one animal (83E00322) exhibiting a severe reaction which may have indicated a pre-existing sensitization.

CONCLUSION

The test compound 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX) possesses no sensitizing potential under the conditions of the study.

RECOMMENDATION

The insolubility of SEX in saline could possibly have prevented the occurrence of any positive skin reactions. It may be worthwhile to re-examine the sensitizing potential of this compound when administered in such vehicles as acetone or dimethyl sulfoxide (DMSO) in which it is more soluble.
REFERENCES


CHARACTERIZATION OF SEX

SEX appears sufficiently stable in normal nitrolysis media to exist as a contaminant in RDX/HMX manufacturing process. The characteristics of SEX are as follows:

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

\[
\begin{align*}
\text{Structural Formula:} & \quad \text{NO}_2 \\
& \quad \text{H}_2\text{C}-\text{N}-\text{CH}_2, \quad \text{O} \\
& \quad \text{O}_2\text{N}-\text{N} - \text{C-CH}_3, \\
& \quad \text{H}_3\text{C}-\text{N}-\text{CH}_2, \\
& \quad \text{NO}_2
\end{align*}
\]

Empirical Formula: \( \text{C}_7\text{H}_5\text{N}_2\text{O}_3 \)

Elemental Analysis: Calculated: \( \text{C}, 24.57; \text{H}, 3.75; \text{N}, 33.45 \)
(C, 24.21; H, 3.76; N, 33.45)

Melting Point: 237°-237.5°C (DEC)

Density: 1.785 g/cm³ at 21°C

Molecular Weight: 293 (Calculated)


Impact Sensitivity (drop weight test): Greater than 300 kg-cm compared with 148 kg-cm for pure HMX. SEX is sensitive to direct strong hammer blows. During our investigations SEX has exhibited no instability, but because of the hammer results should be handled as a potential explosive, like HMX.

Infrared Spectrum: See Figure 1.

Proton NMR Spectrum: See Figure 2.

Chemical Properties: SEX gives a positive Franchimont nitramine reaction, but a negative Liebermann nitroso test. Decomposition in hydroxide fails to produce free \( \text{CH}_3\text{COO}^- \) for a lanthanum nitrate test.
However, if SEX is decomposed in 96% sulfuric acid, the distillate gives a lanthanum nitrate test.

SEX appears inert to boiling acetic anhydride and unaffected by treatment with ammonium nitrate-nitric acid mixtures. Absolute nitric acid at 50°-60°C converts SEX to HMX. Warm 70% nitric acid destroys the compound rapidly, as does 10% aqueous sodium hydroxide and 28% ammonia.

Purity: The purity of SEX was determined by analytical HPLC with a Spectra-Physics 3500B Liquid Chromatograph. A Waters RC-100, C18 cartridge with a mobile phase of 80/20 water/methanol was used for DADN/SEX/HMX mixtures. An internal standard of RDX was used with 1/Rf values of 1.5 for HMX, 1.5 for SEX, and 1.7 for DADN. Hot-column chromatographed SEX contained no detectable amounts of DADN (starting material) and only 1% to 2% HMX (sole contaminant). High pressure liquid chromatographed material contained no DADN or HMX. Also, no other contaminants were detected by analytical HPLC, ensuring a 99.9+% purity of SEX.

Stability: Decomposes at 232°C (printout from differential scanning calorimeter attached). After 72 hours at 75°C there was no change in composition (IR, NMR and color) or weight loss.

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*Rf = response factor.
FIGURE 1  INFRARED SPECTRUM OF 99.9+ % SEX

APPENDIX A (cont.)
FIGURE 2  PROTON NMR SPECTRUM OF 99.9+ % SEX

APPENDIX A (cont.)
DEVIATIONS IN HUSBANDRY

1. The water to the cages was accidently left off one night (8 July 1983) during the quarantine period.

2. The relative humidity was 70% ± 10% for one week (6-11 August 1983) during the rest period between the third induction dose and the challenge dose. However, the hygrothermograph may have been calibrated incorrectly during this week since the next week the hygrothermograph was recalibrated and the humidity was 10% lower.
## HISTORICAL LISTING OF STUDY EVENTS

<table>
<thead>
<tr>
<th>Date</th>
<th>Day</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Jul 83</td>
<td>AO</td>
<td>Animals arrive. Animals examined, placed in cages and fed.</td>
</tr>
<tr>
<td>7 Jul 83</td>
<td>Al</td>
<td>Animals ear tagged and weighed. Quality control animals submitted for necropsy.</td>
</tr>
<tr>
<td>7 Jul - 18 Aug 83</td>
<td>Al-30</td>
<td>Animals checked daily.</td>
</tr>
<tr>
<td>11,14,18,21 Jul 83</td>
<td>A5,A8,</td>
<td>Animals weighed.</td>
</tr>
<tr>
<td>25,28 Jul - 1,4,8,11,15, 18 Aug 83</td>
<td>A12,2,6, 9,13,16, 20,23,27, 30</td>
<td>All animals except negative control group, given induction dose.</td>
</tr>
<tr>
<td>14 Jul 83</td>
<td>A8</td>
<td>Animals randomized into groups.</td>
</tr>
<tr>
<td>18,25 Jul 1 Aug 83</td>
<td>A12,6, 13</td>
<td>All animals, except negative control group, clipped and shaved.</td>
</tr>
<tr>
<td>19,26 Jul 2 Aug 83</td>
<td>0,7,14</td>
<td>All animals except negative control group, scored for 24-hour skin reaction.</td>
</tr>
<tr>
<td>20,27 Jul 3 Aug 83</td>
<td>1,8,15</td>
<td>All animals, except negative control group, scored for 24-hour skin reaction.</td>
</tr>
<tr>
<td>21,28 Jul 4 Aug 83</td>
<td>2,9,16</td>
<td>All animals, except negative control group, scored for 48-hour skin reaction.</td>
</tr>
<tr>
<td>15 Aug 83</td>
<td>27</td>
<td>All animals clipped and shaved.</td>
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<tr>
<td>16 Aug 83</td>
<td>28</td>
<td>All animals given challenge dose.</td>
</tr>
<tr>
<td>17 Aug 83</td>
<td>29</td>
<td>All animals scored for 24-hour skin reaction.</td>
</tr>
<tr>
<td>18 Aug 83</td>
<td>30</td>
<td>All animals scored for 48-hour skin reaction.</td>
</tr>
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LIST OF TABLES

DERMAL SENSITIZATION

Table 1, Dinitrochlorobenzene............................ 25
Table 2, Sodium Chloride............................................ 26
Table 3, SEX, Negative Control............................ 27
Table 4, SEX....................................................... 28

APPENDIX D
### TABLE 1

**GLP Study # 82004**

**BUHLER SENSITIZATION TEST**

<table>
<thead>
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<th>Group Number</th>
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**APPENDIX D (cont.)**
**TABLE 2**

GLP Study # 82004

BUEHLER SENSITIZATION TEST

Group Number 2  
Chemical Name 0.9% Sodium Chloride

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APPENDIX D (cont.)
# TABLE 3

**SUMMARY OF SKIN REACTION AFTER THE CHALLENGE DOSE FOR THE NEGATIVE CONTROL GROUP**

GLP Study Number 82004

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<thead>
<tr>
<th>Group 3</th>
<th>Chemical Name: SEX</th>
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**APPENDIX D (cont.)**
### TABLE 4

**GLP Study #** 82004  
**BUEHLER SENSITIZATION TEST**

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<th>Group Number</th>
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<th>Challenge Dose</th>
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<tr>
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