PRELIMINARY ASSESSMENT OF THE RELATIVE TOXICITY OF A13-20837 IN ANIMALS(U) ARMY ENVIRONMENTAL HYGIENE AGENCY ABERDEEN PROVING GROUND MD G J LEACH SEP 86
Preliminary Assessment of the Relative Toxicity of A13-20837 in Animals
Studyno. 75-51-0528-86
March 1985 - July 1986
**Title:** Preliminary Assessment of the Relative Toxicity of A13-20837 in Animals, Study No. 75-51-0528-86

**Personal Author(s):** Glenn J. Leach

**Type of Report:** Study

**Time Covered:** From Mar 85 to Jul 86 (86 Sep)

**Abstract:**

To provide preliminary toxicity data for the candidate cockroach repellent A13-30827. These data are intended to provide guidance in selecting compounds for further entomological and toxicological evaluation. In addition, the data may be useful in developing preliminary safety guidelines for handling this compound.

EXECUTIVE SUMMARY

The purpose and a summary of the recommendations of the enclosed report follow:

a. Purpose. To provide preliminary toxicity data for the candidate cockroach repellent A13-20837. These data are intended to provide guidance in selecting compounds for further entomological and toxicological evaluation. In addition, the data may be useful in developing preliminary safety guidelines for handling this compound.

b. Recommendations. Based on professional scientific judgment, the following recommendations are offered.

(1) A13-20837 should be considered for more extensive entomological and toxicological testing.

(2) Personnel handling this compound should avoid contact with the skin and eyes. In case of contact, the area should be flushed with plenty of water.

FOR THE COMMANDER:

N. JOE THOMPSON
Colonel, MC
Director, Occupational and Environmental Health

Encl

CF:
HQDA(DASG-PSP) (wo/encl)
Comdt, AHS (HSHA-IPM) (w/encl)
Dir, Advisory Cen on Tox, NRC (2 cy) (w/encl)
USDA, ARS (Dr. Terrence McGovern) (w/encl)
USDA, ARS - Southern Region (w/encl)
Cdr, USMRDC (SGRD-DPM/COL Reinert) (w/encl)
Preliminary Assessment of the Relative Toxicity of A13-20837 in Animals

STUDY NO. 75-51-0528-86
MARCH 1985 - JULY 1986

1. AUTHORITY.


b. Memorandum of Understanding between the US Army Environmental Hygiene Agency; the US Army Health Services Command; the Department of the Army, Office of The Surgeon General; the Armed Forces Pest Control Board and the Department of Agriculture, Agricultural Research, Science and Education Administration; titled, Coordination of Biological and Toxicological Testing of Pesticides, effective 23 January 1979.

2. REFERENCES.


c. Standing Operating Procedures, HSHB-OT, Toxicology Division, USAEHA.


3. PURPOSE. To provide preliminary toxicity data for the candidate cockroach repellent A13-20837. This report summarizes the toxicological data for USDA candidate cockroach repellent A13-20837. These data are intended to be used in selecting compounds for more extensive entomological and toxicological testing. The data may also be used in establishing preliminary safety guidelines for handling the material.

4. BACKGROUND.

a. General. The preliminary toxicological evaluation of candidate cockroach repellents consists of a series of acute screening tests designed to assess potential hazards from single exposures by various routes of administration. The test battery included:

Use of company names does not imply endorsement by the US Army, but is intended only to assist in identification of a specific product.
Study No. 75-51-0528-86, March 1985 - July 1986

- Rat oral approximate lethal dose (ALD);
- Primary irritation (skin and eye);
- Dermal sensitization;
- Saturated vapor (inhalation hazard);
- Physiological screen;
- Mutagenicity (Ames test).

b. Project Information.

(1) All raw data from this study may be found in project file number 75-51-0528-86 or USAEHA Laboratory Notebooks Numbered 101 and 106.

(2) In conducting the studies described in this report, the investigators adhered to reference 2a. In addition, these studies were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

5. PROCEDURES.

a. Test Compound. Two lots (c & d) of AI3-20837 were synthesized and supplied for use in the toxicological evaluations by Dr. Terrance McGovern, USDA, Beltsville, Maryland. AI3-20837 is a clear oily liquid with a sweet odor. It exhibits low solubility in water but is soluble in acetone and other organic solvents. It has a molecular weight of 197 and boils at 83 °C at 0.1 mm Hg.

b. Methods.

(1) Acute Toxicity Tests. Detailed descriptions of the methodology for tests (a) through (d) listed below are published in reference 2b. Methodology for test (e) is published in reference 2c.

(a) Rat ALD.
(b) Skin irritancy.
(c) Eye irritancy.
(d) Dermal sensitization (Buehler technique).
(e) Saturated vapor.

(2) Mutagenicity. Mutagenicity testing was performed by Hazleton Biotechnologies Company under contract DAAD05-86-M-L723 with USAEHA. A complete description of the methodology and results may be found in the final report (reference 2d).
Physiological Screening. The physiological screening tests were designed to obtain basic information on the underlying mechanisms of action for this compound. Male Sprague Dawley rats weighing between 270 - 380 gms were anesthetized with sodium pentobarbital (30 mg/kg). A heparinized cannula (15 cm length of PE50 tubing) was inserted into the left carotid artery for blood pressure monitoring. A similar catheter was inserted into the right external jugular vein for drug injection. A Statham P23-AC fluid filled pressure transducer (Gould Instruments) was used for blood pressure monitoring. The signals were processed by a Buxco Model 6 Pulmonary Function Analyzer (Buxco Electronics) and printed on a Texas Instruments Silent 700 terminal. EKG's were monitored from LEAD II and fed through a pre-amplifier and Buxco EKG analyzer. A digital recording of wave heights and intervals was printed on a second Texas Instruments TI terminal. Following a short period of time, usually 10-15 minutes, stable physiological recordings were obtained and the animals were treated with challenge doses of standard pharmacological drugs including epinephrine, nor-epinephrine, acetylcholine and histamine. Saline injections served as a volume control. Preliminary experiments were performed in order to find optimum dosage levels. In most cases, the dosage chosen produced a marked change in blood pressure (10-50 mm Hg) lasting less than 5 minutes. Following the initial drug challenges, the test compound A13-20837 was injected intraperitoneally, and the drug challenges were repeated 15 minutes post injection. For each drug, the maximum change from baseline condition was recorded and the pre- and post-dosing values compared. In this way, each animal served as its own control. The data were analyzed using a two-way analysis of variance with repeated measures program on an IBM PC microcomputer. A least significant range test was used to compare pre and post-dosing values. A probability of less than 0.05 was used as the level of significance.

6. RESULTS.

a. ALD. The rat oral ALD was found to be 1480 mg/kg (Appendix A, Table A-1). This was the lowest dose that produced lethals. All animals treated with A13-20837 (as little as 293 mg/kg) exhibited marked salivation. Animals receiving the ALD or higher dose died between 10 minutes and 18 hours post administration.

b. Skin Irritation. Compound A13-20837 produced a total irritancy score of 4.75 in the Draize rabbit skin irritancy test. A description of the scoring system employed in these tests is provided at Appendix B. Based on this scoring system, A13-20837 would be considered a moderate to severe skin irritant. Scurf and/or eschar formation was evident at 1 week post application.

c. Eye Irritation. Based on our Draize eye test in rabbits, this compound is a mild eye irritant with a total irritancy score of 29. It produced injury to both cornea and conjunctiva; however, all but one rabbit was healed by 7 days post application. Washing the eyes with water immediately post application reduced the eye injury.

d. Skin Sensitization. Challenge doses of A13-20837 did not produce a reaction in pretreated guinea pigs and, based on these data, it is not considered to be a sensitizer.
Study No. 75-51-0528-86, March 1985 - July 1986

e. Saturated Vapor. As indicated in Table A-1, exposure to atmospheres of A13-20837 for 8 hours did not produce any mortalities during the exposure or for up to 14 days post exposure. Nominal chamber concentrations, based on amount of material volatilized, were 4.23 and 12.52 mg/L for the 22 °C and 100 °C bubblers, respectively. Rats exposed to the higher concentration exhibited excessive salivation and rapid breathing, suggesting that the compound is a respiratory irritant. Twenty-four hours post exposure, these animals appeared normal. Table A-2 (Appendix A) presents the body weight gain and organ-to-body weight ratios from this experiment. There were no significant differences in any of these parameters between the exposure groups.

f. Physiological Studies. Table A-3 (Appendix A) illustrates the cardiovascular effects of exposure to sublethal intraperitoneal injections of A13-20837. The values presented represent the maximum change from resting or baseline levels in response to injections of the challenge drug. The only statistically significant change was an apparent increase in epinephrine responsiveness in repellent-treated rats. This probably reflects a reduced heart rate in the baseline tests since in preliminary control experiments using saline as the test compound, rats typically exhibited a much higher heart rate in response to epinephrines in both the baseline and post exposure drug challenge.

g. Mutagenicity. A13-20837 did not exhibit mutagenic activity under the test conditions employed. It was negative in all test strains used (Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100) and at dosages ranging from .1 μL to 25 μL per plate both activated and nonactivated test systems (reference 2d).

7. CONCLUSIONS. Compound A13-20837 is moderately toxic by the oral route of exposure. It is a mild eye irritant and a moderate to severe skin irritant. This compound presents no acute inhalation hazard at room temperatures though at higher temperatures or if the repellent is atomized as an aerosol, it may cause skin, eye and respiratory irritation. We found no indication of a sensitization reaction and it was not mutagenic in the Ames test. When administered at approximately 0.5 x the ALD to anesthetized, catherized rats, it did not produce any marked cardiovascular effects.

8. RECOMMENDATIONS. The following recommendations are based on professional scientific judgment.

a. A13-20837 should be considered for more extensive entomological and toxicological testing. Toxicological tests should include a more detailed evaluation of acute toxicity by multiple routes of administration, an assessment of the effects of repeated dosing and a complete evaluation of mutagenic potential.

b. Personnel handling this compound should avoid contact with the skin and eyes. In case of accidental contact, the area should be flushed with plenty of water.
9. ACKNOWLEDGEMENT. The project personnel shown in Appendix C assisted in the experiments.

GLENN J. LEACH
Biologist
Toxicology Division

APPROVED:

MAURICE H. WEEKS
Chief, Toxicology Division
APPENDIX A

RESULTS

TABLE A-1. SUMMARY OF TOXICITY DATA CANDIDATE COCKROACH REPELLENT AI3-20837

<table>
<thead>
<tr>
<th>ALD (Mg/Kg)</th>
<th>Skin Category</th>
<th>Eye Category</th>
<th>Sensitization</th>
<th>Sat</th>
<th>Physio</th>
<th>Ames</th>
</tr>
</thead>
<tbody>
<tr>
<td>1480</td>
<td>IV</td>
<td>C</td>
<td>Negative</td>
<td>No</td>
<td>No CV*</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*Cardiovascular - Parameters monitored included blood pressure, heart rate and electrocardiogram in anesthetized rats.
### TABLE A-2. SUMMARY OF SATURATED VAPOR RESULTS COMPOUND AI3-20837

<table>
<thead>
<tr>
<th>Parameter/Test Group</th>
<th>BW (grams)</th>
<th>LW x100 BW</th>
<th>KW x100 BW</th>
<th>HW x100 BW</th>
<th>LGW x100 BW</th>
<th>TW x100 BW</th>
<th>BRW x100 BW</th>
<th>SW x100 BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>249.33 ± 7.34</td>
<td>6.38 ± 0.27</td>
<td>1.15 ± 0.03</td>
<td>0.467 ± 0.022</td>
<td>0.766 ± 0.062</td>
<td>0.972 ± 0.039</td>
<td>0.749 ± 0.028</td>
<td>0.331 ± 0.018</td>
</tr>
<tr>
<td>Low Temp</td>
<td>244.50 ± 11.19</td>
<td>6.23 ± 0.26</td>
<td>1.16 ± 0.02</td>
<td>0.470 ± 0.006</td>
<td>0.831 ± 0.060</td>
<td>1.001 ± 0.055</td>
<td>0.776 ± 0.032</td>
<td>0.333 ± 0.016</td>
</tr>
<tr>
<td>High Temp</td>
<td>260.00 ± 7.32</td>
<td>5.96 ± 0.22</td>
<td>1.10 ± 0.03</td>
<td>0.467 ± 0.017</td>
<td>0.927 ± 0.066</td>
<td>0.934 ± 0.031</td>
<td>0.707 ± 0.010</td>
<td>0.359 ± 0.018</td>
</tr>
</tbody>
</table>

Body weight (BW) and organ to body weight ratios, saturated vapor test, compound AI3-20837. Organ weights abbreviated as follows: Liver weight (LW), kidney weight (KW), heart weight (HW), lung weight (LGW), testes weight (TW), brain weight (BRW), spleen weight (SW). Numbers presented represent the mean ± standard error of the mean for six animals. There were no statistically significant differences among the three groups in any of the measured parameters.
**TABLE 2.3: SUMMARY OF PHYSIOLOGICAL DATA, COMPOUND AE3-20937**

<table>
<thead>
<tr>
<th></th>
<th>BP PRE</th>
<th>BP POST</th>
<th>HR PRE</th>
<th>HR POST</th>
<th>ORS PRE</th>
<th>ORS POST</th>
<th>OT PRE</th>
<th>OT POST</th>
<th>PU PRE</th>
<th>PU POST</th>
<th>RV PRE</th>
<th>RV POST</th>
<th>PH PRE</th>
<th>PH POST</th>
<th>PR PRE</th>
<th>PR POST</th>
<th>RH PRE</th>
<th>RH POST</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPI</td>
<td>166 ± 6</td>
<td>161 ± 5</td>
<td>150 ± 50</td>
<td>323 ± 15</td>
<td>32 ± 1</td>
<td>34 ± 1</td>
<td>68 ± 4</td>
<td>70 ± 3</td>
<td>19 ± 3</td>
<td>19 ± 3</td>
<td>18 ± 2</td>
<td>0.14 ± 0.03</td>
<td>0.10 ± 0.01</td>
<td>1.07 ± 0.15</td>
<td>1.02 ± 0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACT</td>
<td>67 ± 9</td>
<td>67 ± 4</td>
<td>312 ± 29</td>
<td>320 ± 17</td>
<td>32 ± 1</td>
<td>32 ± 1</td>
<td>68 ± 3</td>
<td>68 ± 4</td>
<td>18 ± 3</td>
<td>22 ± 1</td>
<td>20 ± 2</td>
<td>19 ± 1</td>
<td>0.15 ± 0.05</td>
<td>0.08 ± 0.01</td>
<td>0.84 ± 0.20</td>
<td>0.82 ± 0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>170 ± 6</td>
<td>132 ± 11</td>
<td>319 ± 10</td>
<td>371 ± 24</td>
<td>33 ± 1</td>
<td>35 ± 2</td>
<td>70 ± 4</td>
<td>69 ± 4</td>
<td>19 ± 3</td>
<td>22 ± 3</td>
<td>19 ± 1</td>
<td>0.08 ± 0.02</td>
<td>0.11 ± 0.01</td>
<td>0.99 ± 0.13</td>
<td>0.92 ± 0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIST</td>
<td>97 ± 5</td>
<td>90 ± 6</td>
<td>331 ± 31</td>
<td>167 ± 17</td>
<td>30 ± 1</td>
<td>34 ± 2</td>
<td>69 ± 3</td>
<td>68 ± 5</td>
<td>21 ± 2</td>
<td>27 ± 2</td>
<td>17 ± 2</td>
<td>18 ± 2</td>
<td>0.11 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>0.85 ± 0.19</td>
<td>0.85 ± 0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAL</td>
<td>127 ± 8</td>
<td>123 ± 11</td>
<td>358 ± 22</td>
<td>318 ± 16</td>
<td>31 ± 1</td>
<td>33 ± 2</td>
<td>74 ± 3</td>
<td>67 ± 5</td>
<td>22 ± 2</td>
<td>21 ± 1</td>
<td>18 ± 2</td>
<td>19 ± 1</td>
<td>0.12 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>0.82 ± 0.21</td>
<td>0.84 ± 0.25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes:** Values presented are the mean ± the standard error of the mean for five animals. Maximum changes in response to drug challenges were recorded. Data were analyzed with a two-way analysis of variance for repeated values. Table abbreviations are as follows: BP - blood pressure (mm Hg), HR - heart rate (beats/min), ORS - width of ORS complex (msec), PU - P wave (msec), RV - R wave (msec), PH - P height (millivolt), RV - R height (millivolt), PR - pre-treatment response, POST - response to drug challenge 15 min post-injection, Eph - epinephrine, ACT - acetylcholine, NE - nor epinephrine, HIST - histamine, SAL - saline. *Indicates significant difference when compared to pre-exposure results. (P < 0.05).
APPENDIX B
DEFINITIONS OF CATEGORIES OF SKIN AND EYE IRRITANTS

1. Skin irritants.
   a. **Category I** – Compounds producing no irritation of intact skin or no greater than mild primary irritation of the skin surrounding an abrasion.
   b. **Category II** – Compounds producing mild primary irritation of the intact skin and the skin surrounding an abrasion.
   c. **Category III** – Compounds producing moderate primary irritation of the intact skin and the skin surrounding an abrasion.
   d. **Category IV** – Compounds producing moderate to severe primary irritation of the intact skin and of the skin surrounding an abrasion and in addition, producing necrosis, vesiculation, and/or eschars.
   e. **Category V** – Compounds impossible to classify because of staining of the skin or other masking effects owing to physical properties of the compound.

2. Eye irritants.
   a. **Category A** – Compounds noninjurious to the eye.
   b. **Category B** – Compounds producing mild injury to the cornea.
   c. **Category C** – Compounds producing mild injury to the cornea and in addition some injury to the conjunctiva.
   d. **Category D** – Compounds producing moderate injury to the cornea.
   e. **Category E** – Compounds producing moderate injury to the cornea and in addition, some injury to the conjunctiva.
   f. **Category F** – Compounds producing severe injury to the cornea and to the conjunctiva.
APPENDIX C

PROJECT PERSONNEL

The experiments described in this report were performed by a multidisciplinary group under the direction of Glenn Leach. The group included the following:

1. Lynn M. Balczewski, SGT.
2. John G. Harvey, Bio Lab Tech.
4. R. David Russell, CPT, VC.
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