UNITED STATES ARMY
ENVIRONMENTAL HYGIENE
AGENCY
ABERDEEN PROVING GROUND, MD 21010

TOPICAL HAZARD EVALUATION PROGRAM
OF
CANDIDATE INSECT REPELLENTS AI3-38222a, AI3-38223a,
AI3-38226a, AND AI3-38230a
US DEPARTMENT OF AGRICULTURE PROPRIETARY CHEMICALS
STUDY NOS. 75-51-0323-83 THRU 75-51-0326-83
JULY 1981 - DECEMBER 1982

Approved for public release; distribution unlimited.

John V. Wade, DVM, CPT, VC

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Preliminary hazard evaluations of A13-38222a, A13-38223a, A13-38226a, and A13-38230a were performed by means of laboratory animal studies using New Zealand white rabbits, albino Hartley guinea pigs, and Sprague Dawley rats. Chemicals A13-38222a, A13-223a, and A13-38226a produced mild primary skin irritation of the intact skin and the skin surrounding an abrasion. Chemical A13-38230a did not cause skin irritation. Chemical A13-38223a was noninjurious to the eyes of rabbits. Chemicals A13-38222a and A13-38226a produced mild injury to the cornea. Chemical A13-38230a produced mild injury to the cornea, and in addition
20. Some injury to the conjunctiva. All chemicals were relatively nontoxic by ingestion and did not cause photoirritation or prove to be skin sensitizers. Ethanol solutions of chemicals AI3-38223a and AI3-38226a demonstrated some skin irritation during photoirritation studies.
EXECUTIVE SUMMARY

The purpose, essential findings and recommendations of the inclosed report follow:

a. Purpose. The purpose of this program is to provide guidance for further Entomological Testing of the Candidate Insect Repellents A13-38222a, A13-38223a, A13-38226a and A13-38230a by means of laboratory animal studies using Sprague-Dawley rats, New Zealand White rabbits, and Albino-Hartley guinea pigs.

b. Essential Findings. Chemicals A13-38222a, A13-38223a, and A13-38226a produced mild primary skin irritation of the intact skin and the skin surrounding an abrasion. Chemical A13-38230a did not cause skin irritation. Chemical A13-38223a was noninjurious to the eyes of rabbits. Chemicals A13-38222a and A13-38225a produced mild injury to the cornea. Chemical A13-38230a produced mild injury to the cornea, and in addition, some injury to the conjunctiva. All chemicals were relatively nontoxic by ingestion and did not cause photoirritation or prove to be skin sensitizers. Ethanol solutions of chemicals A13-38223a and A13-38225a demonstrated some skin irritation during photoirritation studies.

c. Major Recommendations. Recommend the chemicals for further testing as candidate insect repellents. If chemicals A13-38222a, A13-38226a, or A13-38230a are accidently introduced into the eyes, they should be flushed immediately with copious amounts of water. Ethanol solutions of chemicals A13-38223a and A13-38225a may cause skin irritation in some sensitive individuals. Personnel experiencing this reaction should wash off the solution as soon as possible.
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1. AUTHORITY.

   a. Letter, US Department of Agriculture - Agriculture Research, Southern
      Region, Insects Affecting Man and Animals Research Laboratory, Gainesville,
      Florida, 18 June 1981.

   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
      US Department of Agriculture, Agricultural Research, Science and Education
      Administrations; titled Coordination of Biological and Toxicological Testing

2. REFERENCE. Toxicology Division Standing Operating Procedures, US Army
   Environmental Hygiene Agency (USAEHA), 1981.

3. PURPOSE. The purpose of this program is to provide guidance for further
   entomological testing of the candidate insect repellents AI3-38222a,
   AI3-38223a, AI3-38226a and AI3-38230a, US Department of Agriculture (USDA)
   Proprietary Chemicals.

4. SUMMARY OF FINDINGS. Hazard evaluations of the candidate repellents
   AI3-38222a, AI3-38223a, AI3-38226a and AI3-38230a, USDA Proprietary Chemicals
   were conducted by this Agency using New Zealand White rabbits for skin and
   eye studies, Sprague-Dawley rats for determination of oral toxicity, and
   Albino-Hartley guinea pigs for skin sensitivity testing. A tabular
   presentation of animal toxicity data developed in this Agency follows.*†

* In conducting the studies described in this report, the investigators
   adhered to the "Guide for the Care and Use of Laboratory Animals," US
   Department of Health, Education and Welfare Publication No. (NIH) 80-23,
   revised 1978.
† The studies reported herein were performed in animal facilities fully
   accredited by the American Association for the Accreditation of Laboratory
   Animal Care.

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TABLE. PRESENTATION OF DATA

<table>
<thead>
<tr>
<th>Test</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SKIN IRRITATION STUDIES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Rabbits</strong></td>
<td></td>
<td></td>
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<tr>
<td>Single 24-hour application to intact and abraded skin of New Zealand White rabbits.</td>
<td>Chemicals A13-38222a, A13-38223a, and A13-38226a produced mild primary skin irritation of the intact skin and the skin surrounding an abrasion.</td>
<td>USAEHA Category II (ref Appendix A)</td>
</tr>
<tr>
<td>0.5 mL technical grade chemical applied to each of six rabbits.</td>
<td>Chemical A13-38230a did not cause skin irritation.</td>
<td>USAEHA Category I (ref Appendix A)</td>
</tr>
<tr>
<td><strong>EYE IRRITATION STUDIES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Rabbits</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single 24-hour application of 0.1 mL technical grade chemical to one eye of each of nine New Zealand White rabbits. Three of the nine rabbits had the eye flushed with warm water for 1 minute 25 seconds after application.</td>
<td>Chemical A13-38223a was noninjurious to the eyes of rabbits. Chemicals A13-38222a and A13-38226a produced injury to the cornea. Chemical A13-38230a produced mild injury to the cornea, and in addition some injury to the conjunctiva. The irritant effects of chemicals A13-38222a, A13-38226a, and A13-28230a were decreased by immediate flushing with water.</td>
<td>USAEHA Category A (ref Appendix A) USAEHA Category B (ref Appendix A) USAEHA Category C (ref Appendix A)</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Test</th>
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<tbody>
<tr>
<td>APPROXIMATE LETHAL DOSE (ALD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rats (male)-no diluent</td>
<td>AI3-38222a &gt;4306 mg/kg</td>
<td>These chemicals are relatively nontoxic by ingestion.</td>
</tr>
<tr>
<td></td>
<td>AI3-38223a &gt;6459 mg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AI3-38226a &gt;2871 mg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AI3-38230a 1914 mg/kg</td>
<td></td>
</tr>
</tbody>
</table>

PHOTOCHEMICAL SKIN IRRITATION STUDIES

Rabbits

A single 0.05 mL application of a 25 percent (w/v) solution of each tested chemical and 10 percent (w/v) oil of Bergamot solution (positive control) in 95 percent ethyl alcohol were applied to the intact skin of six rabbits. Five minutes after application, the rabbits were exposed to ultraviolet (UV) light (365 nm) for 30 minutes at a distance of 10-15 cm. Ethanol solutions of AI3-38223a caused moderate skin irritation, AI3-38226a caused slight skin irritation at both UV and non-UV skin sites.

Control

Following UV exposures of the rabbits, 0.05 mL of test chemical, positive control (oil of Bergamot), and diluent were applied to additional skin areas to serve as unirradiated control sites. Application areas were checked for skin irritations at 24, 48 and 72 hours. Positive control application and irradiation caused greater irritant effects than in unirradiated skin areas.
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**SENSITIZATION STUDIES**

**Guinea Pigs (Male)**

Intradermal (ID) injections of 0.1 mL of a 0.1 percent solution (w/v) of each chemical or of dinitrochlorobenzene (DNCB)* in a mixture containing 1 volume of propylene glycol and 29 volumes of saline.

Ten test guinea pigs for each chemical were given 10 sensitizing doses over a 3-week period. After 2-weeks rest, they were challenged with ID injections of each test compound.  

Ten positive control guinea pigs were sensitized over 3 weeks with DNCB. After 2-weeks rest, they were challenged with ID injections of DNCB.

<table>
<thead>
<tr>
<th>Test</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Challenge doses of the tested chemicals did not produce a sensitization reaction.</td>
<td>The tested chemicals did not produce sensitization reactions under test conditions and are not expected to produce sensitization reactions in man.</td>
</tr>
<tr>
<td></td>
<td>Challenge dose of DNCB in positive control guinea pigs produced a marked sensitization reaction in 10 out of 10 guinea pigs.</td>
<td>DNCB produced a marked reaction, indicating these guinea pigs respond to sensitizing agents.</td>
</tr>
</tbody>
</table>

* A known skin sensitizer.

5. **CONCLUSION.** Chemicals A13-38222a, A13-38223a, and A13-38226a produced mild primary skin irritation of the intact skin and the skin surrounding an abrasion. Chemical A13-38230a did not cause skin irritation. Chemical A13-38223a was noninjurious to the eyes of rabbits. Chemicals A13-38222a and A13-38226a produced mild injury to the cornea. Chemical A13-38230a produced mild injury to the cornea, and in addition some injury to the conjunctiva.

All chemicals were relatively nontoxic by ingestion and did not cause photoirritation or prove to be skin sensitizers. Ethanol solutions of
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chemicals A13-38223a and A13-38226a demonstrated some skin irritation during photoirritation studies. These studies were monitored by the Analytical Quality Assurance Office (see Appendix B).

6. RECOMMENDATION. Recommend that the following USDA proprietary chemicals be approved for further testing as candidate insect repellents: A13-38222a, A13-38223a, A13-38226a, and A13-38230a (paragraph 1b, this study). Chemicals A13-38222a, A13-38226a, or A13-38230a should be flushed immediately with copious amounts of water if accidently introduced into the eyes. Ethanol solutions of chemicals A13-38223a and A13-38226a may cause skin irritation in some sensitive individuals. Personnel experiencing this reaction should wash off the solution as soon as possible.

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APPENDIX A

TOPICAL HAZARD EVALUATION PROGRAM
DEFINITIONS OF CATEGORIES OF COMPOUNDS BEING
CONSIDERED FOR ACUTE SKIN APPLICATION

CATEGORY I - Compounds producing no primary irritation of the intact skin or
no greater than mild primary irritation of the skin surrounding an abrasion.
(INTERPRETATION: No restriction for acute application to the human skin.)

CATEGORY II - Compounds producing mild primary irritation of the intact skin
and the skin surrounding an abrasion. (INTERPRETATION: Should be used only
on human skin found by examination to have no abrasions or may be used as a
clothing impregnant.)

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

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

CATEGORY V - Compounds impossible to classify because of staining of the skin
or other masking effects owing to physical properties of the compound.
(INTERPRETATION: Not suitable for use on humans.)

EYE CATEGORIES:

A. Compounds noninjurious to the eye. INTERPRETATION: Irritation of
human eyes is not expected if the compound should accidentally get into the
eyes, provided it is washed out as soon as possible.

B. Compounds producing mild injury to the cornea. INTERPRETATION:
Should be used with caution around the eyes.

C. Compounds producing mild injury to the cornea, and in addition some
injury to the conjunctiva. INTERPRETATION: Should be used with caution
around the eyes and mucosa.

D. Compounds producing moderate injury to the cornea. INTERPRETATION:
Should be used with extreme caution around the eyes.

E. Compounds producing moderate injury to the cornea, and in addition
producing some injury to the conjunctiva. INTERPRETATION: Should be used
with extreme caution around the eyes and mucosa.

F. Compounds producing severe injury to the cornea and to the
conjunctiva. INTERPRETATION: Should be used with extreme caution. It is
recommended that use be restricted to areas other than the face.
APPENDIX B

ANALYTICAL QUALITY ASSURANCE

The Analytical Quality Assurance Office certifies the following with regard to this study:

a. This study was conducted in accordance with:

   (1) Standing Operating Procedures developed by the Toxicology Division, USAEHA.


b. Facilities were inspected during its operational phase to ensure compliance with paragraph a above.

c. The information presented in this report accurately reflects the raw data generated during the course of conducting the study.

PAUL V. SNEERINGER, Ph.D.
Chief, Analytical Quality Assurance Office