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TOPICAL HAZARD EVALUATION PROGRAM OF CANDIDATE INSECT  
REPELLENTS A13-3042. (U) ARMY ENVIRONMENTAL HYGIENE  
AGENCY ABERDEEN PROVING GROUND NO. . M H WEEKS JAN 88  
USAEHA-75-51-0457-88

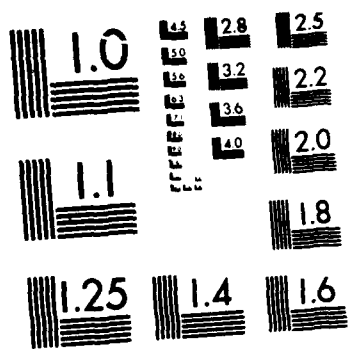
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# UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010-5422

TOPICAL HAZARD EVALUATION PROGRAM  
OF  
CANDIDATE INSECT REPELLENTS A13-38426,  
A13-39008, A13-39010, A13-30106, A13-38264,  
A13-38265, A13-38098, A13-38099 AND A13-5903,  
U.S. DEPARTMENT OF AGRICULTURAL PROPRIETARY CHEMICALS  
STUDY NOS. 75-51-0457-88, 75-51-0477-88, 75-51-0479-88,  
75-51-0516-88, 75-51-0539-88, 75-51-0540-88,  
75-51-0542-88, 75-51-0543-88, 75-51-0545-88,  
JANUARY 1988

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DEPARTMENT OF THE ARMY  
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY  
ABERDEEN PROVING GROUND, MARYLAND 21010-6422



REPLY TO  
ATTENTION OF

HSHB-MO-T

12 February 1988

MEMORANDUM FOR: Executive Director, Armed Forces Pest Management Board,  
Forest Glen Section, WRAMC, Washington, DC 20307-5001

SUBJECT: Topical Hazard Evaluation Program of Candidate Insect Repellents,  
AI3-38426, AI3-39008, AI3-39010, AI3-30106, AI3-38264, AI3-38265,  
AI3-38098, and AI3-38099, and AI3-5903, U.S. Department of Agriculture  
Proprietary Chemicals, Study Nos. 75-51-0457-88, 75-51-0477-88,  
75-51-0479-88, 75-51-0516-88, 75-51-0539-88, 75-51-0540-88, 75-51-0542-88,  
75-51-0543-88, and 75-51-0545-88, January 1988

EXECUTIVE SUMMARY

The purpose and recommendations of the enclosed report follow:

1. PURPOSE. To provide guidance for further entomological testing of the subject candidate insect repellents by means of laboratory animal studies. In addition, these data may be useful in developing preliminary safety guidelines for handling these compounds.

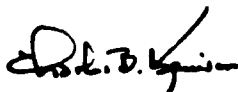
2. RECOMMENDATIONS. Based on professional scientific judgement, the following recommendations are offered.

a. Approve compounds AI3-38426, AI3-39008, AI3-39010, AI3-30106, AI3-38264, AI3-38265, AI3-38098, AI3-38099 and AI3-5903 for further entomological testing.

b. Further recommend compounds AI3-38426, AI3-39008, and AI3-39010 should be used with caution around the eyes and mucosa.

c. Recommend compounds AI3-38426, AI3-39010, AI3-38265 and AI3-38099 be resubmitted for mutagenic assay dependent upon recommendations from field studies.

Encl

  
CHARLES B. KENISON  
Colonel, MS  
Commanding

CF:  
HQDA(DASG-PSP-E) (w/encl)  
Dir, Advisory Cen on TOX, NRC (2 cy) (w/encl)  
Comdt, AHS (HSHA-IPM) (w/encl)  
USDA, ARS (Dr. Terrence McGovern)  
USDA, ARS-Southern Region (3 cy) (w/encl)  
USDA, ARS-Southern Region (Cdr Santana) (w/encl)  
Cdr, USAMMDA (SGRD-UMA) (COL Schiefer) (w/encl)  
Cdr, USAMMDA (SGRD-UMB) (LTC Roberts) (w/encl)

## REPORT DOCUMENTATION PAGE

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| 1a. REPORT SECURITY CLASSIFICATION<br>Unclassified   |   | 1b. RESTRICTIVE MARKINGS   |                                 |
| 2a. SECURITY CLASSIFICATION AUTHORITY  |   | 3. DISTRIBUTION / AVAILABILITY OF REPORT<br>Approved for public release; distribution unlimited. |                                 |
| 2b. DECLASSIFICATION / DOWNGRADING SCHEDULE  |   |  |                                 |
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| 6c. ADDRESS (City, State, and ZIP Code)<br>Aberdeen Proving Ground, MD 21010-5422  |   | 7b. ADDRESS (City, State, and ZIP Code)  |                                 |
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| 11. TITLE (Include Security Classification) Unclassified - "Topical Hazard Evaluation Program of Candidate Insect Repellents   |   |  |                                 |
| 12. PERSONAL AUTHOR(S)<br>Maurice H. Weeks   |   |  |                                 |
| 13a. TYPE OF REPORT<br>Study   | 13b. TIME COVERED<br>FROM -- TO --                | 14. DATE OF REPORT (Year, Month, Day)<br>January 1988  | 15. PAGE COUNT<br>13            |
| 16. SUPPLEMENTARY NOTATION<br>The purpose of this report is  |   |  |                                 |
| 17. COSATI CODES   |   | 18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)                |                                 |
| FIELD  | GROUP   | SUB-GROUP  |                                 |
|  |   | Insect Repellents  |                                 |
| 19. ABSTRACT (Continue on reverse if necessary and identify by block number)   |   |  |                                 |
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| b. Further recommend compounds AI3-38426, AI3-39008, and AI3-39010 should be used with caution around the eyes and mucosa.   |   |  |                                 |
| c. <del>Recommend</del> compounds AI3-38426, AI3-39010, AI3-38265, and AI3-38099 <sup>should</sup> be resubmitted for mutagenic assay dependent upon recommendations from field studies.   |   |  |                                 |
| 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<br>Unclassified   |                                 |
| 22a. NAME OF RESPONSIBLE INDIVIDUAL<br>Maurice H. Weeks  |   | 22b. TELEPHONE (Include Area Code)<br>(301) 671-3980   | 22c. OFFICE SYMBOL<br>HSHB-MO-T |



DEPARTMENT OF THE ARMY  
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY  
ABERDEEN PROVING GROUND, MARYLAND 21010-5422



REPLY TO  
ATTENTION OF

HSHB-MO-T

TOPICAL HAZARD EVALUATION PROGRAM  
OF  
CANDIDATE INSECT REPELLENTS AI3-38426,  
AI3-39008, AI3-39010, AI3-30106, AI3-38264,  
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75-51-0542-88, 75-51-0543-88, 75-51-0545-88,  
JANUARY 1988

1. AUTHORITY.

- a. Memorandum of Understanding between the U.S. Army Environmental Hygiene Agency; the U.S. Army Health Services Command; the Department of The Army, Office of The Surgeon General; the Armed Forces Pest Control Board; and the U.S. Department of Agriculture, Agricultural Research, Science and Education Administration; titled, Coordination of Biological and Toxicological Testing of Pesticides, effective 23 January 1979.
- b. Letter, U.S. Department of Agriculture, Agricultural Research Service, Northeastern Region, Beltsville Agricultural Research Service, Beltsville, Maryland, 10 June 1983, subject: Chemical Transmittal.
- c. Letter, U.S. Department of Agriculture, Agricultural Research Service, Northeastern Region, Beltsville Agricultural Research Service, Beltsville, Maryland, 3 September 1986, subject: Chemical Request.
- d. Letter, U.S. Department of Agriculture, Agricultural Research Service, Northeastern Region, Beltsville Agricultural Research Service, Beltsville, Maryland, 6 December 1983, subject: Chemicals Synthesized for PHE.
- e. Letter, U.S. Department of Agriculture, Agricultural Research Service, Southern Region, Insects Affecting Man and Animals Research Laboratory, Gainesville, Florida, 1 August 1984, subject: Repellents for Preliminary Hazard Evaluation.
- f. Letter, U.S. Department of Agriculture, Agricultural Research Service, Northeastern Region, Beltsville Agricultural Research Service, Beltsville, Maryland, 26 November 1984, subject: Repeat THEP Tests.
- g. Letter, U.S. Department of Agriculture, Agricultural Research Service, Southern Region, Insects Affecting Man and Animals Research Laboratory, Gainesville, Florida, 6 December 1984, subject: Repellents for Preliminary Hazard Evaluation.

## Topical Hazard Evaluation Program, January 1988

### 2. REFERENCES.

a. Toxicology Division, Topical Hazard Evaluation Program Procedural Guide, October 1985.

b. Toxicology Division Standing Operating Procedures, U.S. Army Environmental Hygiene Agency (USAEHA), 1982.

3. PURPOSE. The purpose of this program is to provide guidance for further entomological testing of Candidate Insect Repellents, U.S. Department of Agriculture (USDA) Proprietary Chemicals.

### 4. MATERIALS AND METHODS. \*†

a. Testing for primary skin irritation, photochemical skin irritation, and primary eye irritation was conducted using New Zealand white rabbits from Hazleton-Dutchland Laboratories, Denver, Pennsylvania. Albino-Hartley guinea pigs also from Hazleton-Dutchland Laboratories were used for sensitization studies, and Sprague-Dawley rats from Charles River Laboratories, Wilmington, Massachusetts were used for determination of Approximate Lethal Doses (ALD's) (reference paragraph 2a,b).

b. All samples except AI3-30106 were synthesized by Dr. Terrence P. McGovern, Organic Chemical Synthesis Laboratory, USDA, Beltsville, Maryland (reference paragraph 1b, c, d, e, g). AI3-30106 aGb was obtained from Agriculture Canada (reference 1e).

c. Mutagenicity evaluation of selected compounds was performed in a Ames Salmonella/Microsome Reverse Mutation Assay.

### 5. FINDINGS.

a. Skin irritation studies were performed by single 24-hour application of 0.5 mL doses technical grade chemical to occlude intact and abraded skin of six rabbits. A tabular presentation of the skin irritation data developed on the subject candidate insect repellents follows.

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\* In conducting the studies described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," U.S. Department of Health, Education and Welfare Publication No. (NIH) 85-23, 1985.

† 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 1. SKIN IRRITATION DATA

| Compound      | Results   | Category<br>(Reference Appendix B) |
|---------------|---|------------------------------------|
| AI3-38426-d   | Compound noninjurious to the skin   | I                                  |
| AI3-39008-a   | Compound produced mild irritation of the intact skin and the skin surrounding an abrasion. No irritation at 7 days. | II                                 |
| AI3-39010-b   | Compound produced mild erythema and slight edema of the intact and abraded skin. No irritation at 7 days.           | II                                 |
| AI3-30106-aGb | Compound produced slight erythema and edema of the intact and abraded skin. No irritation at 7 days.                | II                                 |
| AI3-38264-b   | Compound noninjurious to the intact skin with slight redness of the abraded skin.                                   | I                                  |
| AI3-38265-b   | Compound noninjurious to the skin.  | I                                  |
| AI3-38098-b   | Compound produced very slight erythema and edema of the intact and abraded skin. No irritation at 7 days.           | II                                 |
| AI3-38099-b   | Compound noninjurious to the skin.  | I                                  |
| AI3-5903-Gb   | Compound produced very slight irritation with no irritation at 7 days.  | I                                  |

b. Eye irritation studies were performed by administering single 0.1 mL doses of technical grade chemical to one eye of each of six rabbits. A tabular presentation of the eye irritation data developed on the subject candidate insect repellents follows.



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Topical Hazard Evaluation Program, January 1988

TABLE 2. EYE IRRITATION DATA

| Compound      | Results   | Category<br>(Reference Appendix B) |
|---------------|---|------------------------------------|
| AI3-38426-d   | Compound produced moderate injury to the cornea and in addition some injury to the conjunctiva. Injury resolved within 7 days.        | (E)                                |
| AI3-39008-a   | Compound produced moderate injury to the cornea and in addition some injury to the conjunctiva. Injury resolved within 7 days.        | (E)                                |
| AI3-39010-b   | Compound produced mild injury to the cornea, iris and conjunctiva. Irritation resolved within 7 days.                                 | (E)                                |
| AI3-30106-aGb | Compound produced slight injury to the cornea, and in addition moderate injury to the conjunctiva. Irritation resolved within 7 days. | C                                  |
| AI3-38264-b   | Compound produced slight injury to the cornea, and in addition some injury to the conjunctiva. Irritation resolved within 7 days.     | C                                  |
| AI3-38265-b   | Compound produced very mild injury to the cornea.   | B                                  |
| AI3-38098-b   | Compound produced mild injury to the cornea and in addition some injury to the conjunctiva. Injury resolved within 7 days.            | C                                  |
| AI3-38099-b   | Compound produced mild injury to the cornea and in addition some injury to the conjunctiva. Injury resolved within 7 days.            | C                                  |
| AI3-5903-Gb   | Compound produced mild injury to the cornea and in addition some injury to the conjunctiva. All irritation resolved within 3-7 days.  | C                                  |

c. Photochemical skin irritation studies were performed by administering a single 0.05 mL dose of a 25 percent (w/v) solution of a chemical and a 10 percent (w/v) Oil of Bergamot solution (positive control) in 95 percent ethyl alcohol 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. Following UV exposures of the rabbits, 0.05 mL

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of test chemical, positive control and diluent were applied to additional skin areas to serve as unirradiated control sites. Application areas were checked for skin irritation at 24, 48 and 72 hours. A summary of the responses in rabbits from these chemicals follow.

TABLE 3. PHOTO CHEMICAL SKIN IRRITATION STUDIES.

| Compound      | Results             | Comment  |
|---------------|---------------------|--|
| AI3-38426-d   | No photo irritation | This chemical is not expected to cause photo irritation in man.          |
| AI3-39008-a   | No photo irritation | Alcohol solutions may cause slight irritation in sensitive individuals.  |
| AI3-39010-b   | No photo irritation |  |
| AI3-30106-aGb | No photo irritation | This compound is not expected to cause a photo chemical reaction in man. |
| AI3-38264-b   | No photo irritation | This chemical is not expected to cause photo irritation in man.          |
| AI3-38265-b   | No photo irritation | No photo irritation is expected in man.                                  |
| AI3-38098-b   | No photo irritation | No photo irritation is expected in man.                                  |
| AI3-38099-b   | No photo irritation | No photo irritation is expected in man.                                  |
| AI3-5903-Gb   | No photo irritation | No photo irritation is expected in man.                                  |

d. Approximate lethal dose (ALD) studies were performed by administering single oral graded dosages of technical grade material to young male rats. The purpose of the ALD is to determine the minimum lethal dose of a compound using a small number of animals. Animals are observed daily for signs up to 14 days after dosing. A tabular presentation of the ALD's developed on the subject candidate insect repellents follows.

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TABLE 4. APPROXIMATE LETHAL DOSE (ALD's)

| Compound      | Results     | Comment   |
|---------------|-------------|---|
| AI3-38426-d   | >3333 mg/kg | No signs.   |
| AI3-39008-a   | 2222 mg/kg  | Salivation, labored breathing, and death at high dosages. |
| AI3-39010-b   | >3333 mg/kg | No signs.   |
| AI3-30106-aGb | 3333 mg/kg  | Salivation and death at high dosages.                     |
| AI3-38264-b   | 2222 mg/kg  | Ataxia and death at high dosages.                         |
| AI3-38265-b   | 3333 mg/kg  | Prostration at high dosage.                               |
| AI3-38098-b   | >3333 mg/kg | Ataxia at high dosages.                                   |
| AI3-38099-b   | >3333 mg/kg | No signs.   |
| AI3-5903-Gb   | >3333 mg/kg | Lethary at high dosages.                                  |

e. Sensitization studies were performed to determine the potential of the proposed insect repellents for causing sensitization reactions in humans. Albino guinea pigs were given dermal doses of test chemical over a 3 week period, rested for 2 weeks then challenged with the same compound and concentration. A positive control, dimtrochlorobenzene (DNCB) was run concurrently with the test substances. The test procedure for AI3-38264-b was based on the studies of Landsteiner, while the test procedure for the remaining chemicals was based on the studies of Buehler. The challenge doses of the tested chemicals did not produce a sensitization reaction. The tested chemicals are not expected to produce sensitization reactions in humans.

f. Mutagenicity Plate Assays. The objective of these studies was to evaluate a selected few of the chemical repellents for mutagenic activity in the Ames Salmonella/Microsome Assays using *Salmonella typhimurium* strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100. The assays were conducted using two plates per dose level in the presence and absence of a metabolic activation system. The following test materials, AI3-39008a, AI3-30106-aGc, AI3-38264-b, AI3-38098-b and AI3-5903-Gb, did not exhibit genetic activity in these assays and are not considered mutagenic under test conditions. Compounds AI3-38426-d, AI3-39010-b, AI3-38265-b and AI3-38099-b were not analyzed for mutagenic assay but will be nominated dependent upon results from future entomological field studies.

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6. A summary of the results from all of the above described studies are presented in Table 5.

TABLE 5. RESULTS OF PRESENT STUDIES

| Compound      | Skin Irritation | Eye Irritation | Sensitization | Photo     | ALD mg/kg | Ames    | Recommendation |
|---------------|-----------------|----------------|---------------|-----------|-----------|---------|----------------|
| AI3-38426-d   | I               | (E)            | non-sens      | non-photo | >3333     | -       | approval       |
| AI3-39008-a   | II              | (E)            | non-sens      | non-photo | 2222      | non-mut | approval       |
| AI3-39010-b   | II              | (E)            | non-sens      | non-photo | >3333     | -       | approval       |
| AI3-30106-aGb | II              | C              | non-sens      | non-photo | 3333      | non-mut | approval       |
| AI3-38264-b   | I               | C              | non-sens      | non-photo | 2222      | non-mut | approval       |
| AI3-38265-b   | I               | B              | non-sens      | non-photo | 3333      | -       | approval       |
| AI3-38098-b   | II              | C              | non-sens      | non-photo | >3333     | non-mut | approval       |
| AI3-38099-b   | I               | C              | non-sens      | non-photo | >3333     | -       | approval       |
| AI3-5903-Gb   | I               | C              | non-sens      | non-photo | >3333     | non-mut | approval       |

7. RECOMMENDATIONS.

a. Approve compounds AI3-38426, AI3-39008, AI3-39010, AI3-30106, AI3-38264, AI3-38265, AI3-38098, AI3-38099 and AI3-5903 for further entomological testing.

b. Recommend further that compounds AI3-38426, AI3-39010, AI3-38265 and AI3-38099 be resubmitted for mutagenic assay dependent upon recommendations from field studies.

*Maurice H. Weeks*  
MAURICE H. WEEKS  
Chief, Toxicology Division

APPENDIX A

ANALYTICAL QUALITY ASSURANCE

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

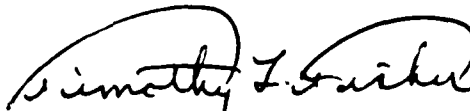
a. This study was conducted in accordance with:

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

(2) Title 21, Code of Federal Regulations (CFR), 1985 rev, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

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.



TIMOTHY L. FISHER  
Chief, Analytical Quality  
Assurance Office

APPENDIX B

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 prophetic 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. (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 prophetic 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 or compounds producing necrosis, vesiculation, or eschars. (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 (e.g., nose and mouth).

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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.

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