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TOPICAL HAZARD EVALUATION PROGRAM OF CANDIDATE INSECT
REPELLENT AI3-35770. (U) ARMY ENVIRONMENTAL HYGIENE
AGENCY ABERDEEN PROVING GROUND MD R D RUSSELL JUN 85
USAEHA-75-51-0435-85

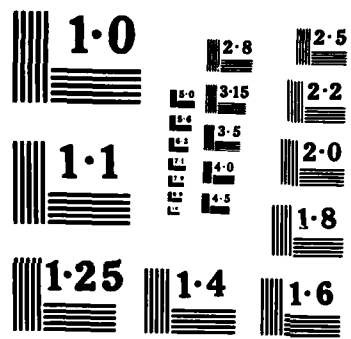
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**UNITED STATES ARMY
ENVIRONMENTAL HYGIENE
AGENCY**

ABERDEEN PROVING GROUND, MD 21010-5422

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PHASE 1
TOPICAL HAZARD EVALUATION PROGRAM
OF
CANDIDATE INSECT REPELLENT AI3-35770
HEXAHYDRO-1-[(2-METHYLCYCLOHEXYL)CARBONYL]-1-H-AZEPINE
STUDY NO. 75-51-0435-85
AUGUST 1984 - JUNE 1985

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Topical Hazard Evaluations of USDA Candidate Insect Repellent AI3-35770 were performed using New Zealand White rabbits, albino Hartley guinea pigs, and Sprague Dawley rats. Chemical AI3-35770 produced moderate to severe primary dermal irritation and necrosis in rabbits and guinea pigs. These effects were quantitatively worse in rabbits than in guinea pigs. This compound was moderately toxic to rats when given by the oral route.			

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 U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY 584-3980
 ABERDEEN PROVING GROUND, MARYLAND 21010-5422

REPLY TO
 ATTENTION OF

7 AUG 1985

HSMB-OT

SUBJECT: Phase 1, Topical Hazard Evaluation Program of Candidate Insect Repellent,
 AI3-35770, Hexahydro-1-[(2-Methylcyclohexyl)Carbonyl]-1-H-Azepine,
 Study No. 75-51-0435-85, August 1984 - June 1985

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

EXECUTIVE SUMMARY

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


a. Purpose. The purpose of this program is to provide guidance for further entomological testing of the candidate insect repellent AI3-35770 by means of laboratory animal studies using New Zealand White rabbits, Albino-Hartley guinea pigs and Sprague-Dawley rats.

b. Essential Findings. The US Department of Agriculture Candidate Insect Repellent AI3-35770 produced moderate to severe primary irritation of the intact skin and the skin surrounding an abrasion of New Zealand White rabbits and produced moderate primary irritation of intact skin and the skin surrounding an abrasion in guinea pigs. Chemical AI3-35770 caused epidermal necrosis and inflammation of the dermis which was quantitatively worse in rabbits than in guinea pigs. This chemical was moderately toxic in rats when given orally.

c. Major Recommendations. Complete human patch testing and evaluate the results prior to any further decision regarding future testing of this compound. This compound should not be used near mucus membranes (i.e. around face).

FOR THE COMMANDER:

Encl


 JOEL C. GAYDOS
 Colonel, MC
 Director, Occupational and
 Environmental Health

- CF:
 HQDA(DASG-PSP) (w/enc1)
 Cdr, HSC (HSCL-P) (w/enc1)
 Dir, Advisory Ctr on TOX, NRC (2 cy) (w/enc1)
 Comdt, AHS (HSHA-IPM) (w/enc1)
 USDA, ARS (Dr. Terrence McGovern) (w/enc1)
 USDA, ARS-Southern Region (w/enc1)
 USDA, ARS-Southern Region (SGRD-DPM/COL Reinert) (w/enc1)



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

REPLY TO
ATTENTION OF

HSHB-OT

PHASE 1
TOPICAL HAZARD EVALUATION PROGRAM
OF
CANDIDATE INSECT REPELLENT AI3-35770
HEXAHYDRO-1-[(2-METHYLCYCLOHEXYL)CARBONYL]-1-H-AZEPINE
STUDY NO. 75-51-0435-85*
AUGUST 1984 - JUNE 1985

1. AUTHORITY.

- a. Letter, US Department of Agriculture - Agriculture Research, Southern Region, Insects Affecting Man and Animals Research Laboratory, Gainesville, Florida, 14 August 1984.
- b. Letter, US Department of Agriculture - Agriculture Research, Southern Region, Insects Affecting Man and Animals Research Laboratory, Gainesville, Florida, 28 August 1984.
- c. Letter, US Department of Agriculture - Agriculture Research, Northeastern Region, Beltsville Agricultural Research Center, Beltsville, MD, 26 October 1984.
- d. Letter, U.S. Department of Agriculture - Agriculture Research, Northeastern Region, Beltsville Agricultural Research Center, Beltsville, MD, 2 January 1985.
- e. 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 of Pesticides, effective 23 January 1979.

* 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.

2. REFERENCES.

a. Toxicology Division Standing Operating Procedures, US Army Environmental Hygiene Agency (USAEHA), 1982.

b. Toxicology Division, Topical Hazard Evaluation Program Procedural Guide, January 1982.

c. C.E. Schreck, N. Smith, T.P. McGovern, Repellency of Selected Compounds Against Two Species of Biting Midges (Diptera: Ceratopogonidae: Culicoides), Journal of Entomology, Vol. 16, pp. 524-527, 18 December 1979.

d. Letter, USAEHA, HSHB-LT/WP, 9 August 1984, subject: Topical Hazard Evaluation Program of Hexahydro-1-[(2-Methylcyclohexyl)carbonyl]-1-H-azepine (AI3-35770), US Department of Agriculture, Candidate Insect Repellent, Study Nos. 75-51-0366-84, 75-51-0435-84, and 75-51-0489-84, April 1982 - April 1984.

3. PURPOSE. The purpose of this program is to provide guidance for further testing of the candidate insect repellent AI3-35770 Hexahydro-1-[(2-Methylcyclohexyl)carbonyl]-1-H-azepine, US Department of Agriculture (USDA) Candidate Insect Repellent.

4. GENERAL.

a. Results of toxicity data developed with eight (8) different batches of this material have been reported by this agency (reference 2d). Summarizing these data, this chemical produced moderate-to-severe primary skin irritation when applied as the technical grade compound (Appendix A, Category IV) and was only slightly less irritating when applied unoccluded as a 25 percent (w/v) solution in ethanol (Appendix A, Category III) or when applied and washed off at 6-hours postapplication (Appendix A, Categories III & IV).

b. Candidate insect repellent AI3-35770 has been identified as number three (3) on a priority list of candidate insect repellents established by the Armed Forces Pest Management Board and the USDA. Further entomological testing at USDA's Gainesville, Florida laboratory has shown negligible primary skin irritation when tested on human skin (reference 2c). The apparent difference between irritation potentials on rabbit and human skin underscores the need for further studies on the mechanism and potential for skin irritation. These studies were indicated prior to the initiation of further testing with this compound.

5. MATERIALS ANED METHODS.

a. Testing was conducted using New Zealand White rabbits, Albino-Hartley guinea pigs from Hazelton-Dutchland Laboratories, Denver, Pennsylvania, and Sprague-Dawley rats from Charles River Laboratories, Wilmington, Massachusetts.

b. Samples tested in the present study were synthesized by Dr. Terrence P. McGovern, Organic Chemical Synthesis Laboratory, USDA, Beltsville, Maryland. The last letter in the chemical designation specifies a different batch or purification process. Samples tested were:

AI3-35770-eGj
AI3-35770-g
AI3-35770-h
AI3-35770-I

c. Primary dermal irritation studies in rabbits as described in reference 2b were conducted on samples AI3-35770-eGj and AI3-35770-h. The same procedure was used to study chemical AI3-35770-g on guinea pigs. The candidate insect repellents were tested for primary dermal irritation by application of 0.5 mL of the test compound to the intact and abraded skin of six rabbits (AI3-35770-eGj and AI3-35770-h) or six guinea pigs (AI3-35770-g). In the guinea pig, chemical AI3-70957-Ga (a known severe skin irritant in the rabbit) was applied in the same manner to act as a positive control. All substances were applied under an occlusive wrap for 24 hours, then removed. Application sites were scored for erythema and eschar formation and edema at postapplication times of 24 hours, 72 hours, 7 days, and (if lesions persisted) 14 days.

d. Chemical AI3-35770-g was also tested as a 25 percent (w/v) solution in 100 percent ethanol. These tests were similar to the above except no occlusive wrap was used. A 0.5 mL sample of the test solution was applied to the abraded (three sites) or unabraded (three sites) skin of six guinea pigs. A vehicle control was run identically using 0.5 mL of 100 percent ethanol per site.

e. Serial biopsy studies were conducted on New Zealand White rabbits and Albino-Hartley guinea pigs. Chemical AI3-35770-g was applied to rabbits' and guinea pigs' backs with an occlusive wrap for 24 hours. Biopsies were taken at postapplication times of 24 hours, 48 hours, 72 hours, 7 days, and 14 days. All sections were examined histologically.

f. The acute oral approximate lethal dose (ALD) was determined with sample AI3-35770-I on young mature female Sprague-Dawley rats as described in reference 2b.

6. RESULTS.

a. Primary Dermal Irritation Studies. Chemicals AI3-35770-eGj and AI3-35770-h produced moderate to severe primary irritation of rabbit skin. Initial erythema and edema formation led to the development of a leathery plaque over the application site between 72 hours and 7 days post treatment. These patches sloughed at 10-14 days to leave a healing, epithelializing fibrosed thickened area. This finding is consistent with the results from previous studies (reference 2d). These compounds were assigned a USAEHA skin irritation category of IV (Appendix A).

b. Second Species Study of Primary Dermal Irritation.

(1) The positive control (AI3-70957-Ga) produced severe primary dermal irritation (Appendix A, Category IV) of guinea pig skin demonstrating that guinea pigs react to primary dermal irritants. The vehicle control (100 percent ethanol) produced no significant irritation (Appendix A, Category I).

(2) Chemical AI3-35770-g produced moderate-to-severe primary dermal irritation when applied as technical grade material to guinea pig skin. The effect was markedly less severe than that observed in rabbits even though both species exhibited a USAEHA Category IV (Appendix A) effect.

(3) The 25 percent (w/v) solution in ethanol produced only a mild irritation (Appendix A, Category II) when applied to intact and abraded guinea pig skin, suggesting that the irritant response may be dose related to some degree. The severity of response in rabbits (Appendix A, Category III), as reported in reference 2d, suggests that rabbits may be more sensitive as a species to this candidate repellent than are guinea pigs.

c. Serial Biopsy Studies.

(1) Chemical AI3-35770-g was applied as noted in paragraph 6a to the intact skin of two (2) rabbits (five sites per rabbit) and three (3) guinea pigs (two sites per guinea pig). Full thickness skin biopsies were taken, under general anesthesia at 24 hours, 48 hours, 72 hours, 7 days and 14-days postapplication. At each of these times, a biopsy was taken from each rabbit (two) and one biopsy was taken from one guinea pig. The biopsies were examined histologically.

(2) Histopathology consisted of early dermal edema with necrosis of the epidermis and polynuclear cell infiltration of the dermis. With time the necrotic tissue sloughed, hyperplasia of the epidermis occurred, and the dermis was thickened by material compatible with collagen. The lesion was similar in both rabbits and guinea pigs but was much more severe in rabbits. Normal guinea pig skin has a thicker epidermis which may have contributed to the difference in severity.

d. The 14-day oral ALD was determined to be 2200 mg/kg. Major signs during the first 24 hours following dosing were tonic convulsions, salivation, and rear leg weakness. All signs disappeared within 48 hours and no gross lesions were recognized on necropsy.

7. DISCUSSION.

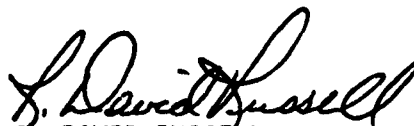
a. Although previous testing of this compound resulted in a recommendation for disapproval for further testing (reference 2d), further data produced by USDA indicated a potential for safe human use which was

Phase 1, Study No. 75-51-0435-85, Aug 84 - Jun 85

unexpected in light of earlier primary dermal irritation studies done on rabbits. This coupled with the high priority placed on this compound dictated the need for further study of the primary dermal irritation to include studies conducted in a second species. The data reported here suggest that species differences in dermal irritation response to this repellent may be significant, and that the rabbit may be more sensitive than other species to the primary dermal irritant effects of this compound.

b. The studies reported here were monitored by the Analytical Quality Assurance Office (Appendix B).

8. RECOMMENDATIONS. Recommend that chemical AI3-35770 be tentatively approved for further testing as a candidate insect repellent. This testing should include human patch testing to further evaluate this compound's potential for primary dermal irritation in man. Further recommend that this compound not be used near mucous membranes (e.g., near eyes or mouth).

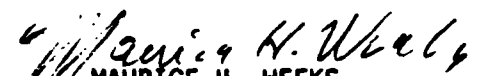


R. DAVID RUSSELL

CPT, VC

Laboratory Animal Veterinary Officer
Toxicology Division

APPROVED:



MAURICE H. WEEKS
Chief, Toxicology Division

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 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 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 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. (INTERPRETATION: Not suitable for use on humans.)

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.

(2) Title 21, Code of Federal Regulations, 1984 rev, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

b. Facilities were inspected during its operational phase to insure 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

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