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AEHA

UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010-5422

PHASE 2
PRELIMINARY ASSESSMENT OF THE RELATIVE TOXICITY
OF THE CANDIDATE INSECT REPELLENT
1-(3-CYCLOHEXEN-1-YLCARBONYL)PIPERIDINE
AI3-35765
ACUTE TOXICITY
STUDY NO. 75-51-0234-90
AUGUST 1990

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Under the command jurisdiction of the U.S. Army Health Services Command, AEHA's mission is to support the worldwide preventive medicine programs of the Army and other Department of Defense and Federal agencies.

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This is accomplished through support in environmental quality, occupational and environmental health, toxicology, industrial hygiene, radiation and entomological sciences, pest management, and laboratory services. Various types of field services are provided upon request.

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

REPLY TO ATTENTION OF

HSHB-MO-T (40)

15 OCT 1990

MEMORANDUM FOR Executive Director, Armed Forces Pest Management Board, Forest Glen Section, Walter Reed Army Medical Center, Washington, DC 20307-5001

SUBJECT: Phase 2, Preliminary Assessment of the Relative Toxicity of the Candidate Insect Repellent 1-(3-Cyclohexen-1-ylcarbonyl)Piperidine, AI3-35765, Acute Toxicity, Study No. 75-51-0234-90, August 1990

Copies of the report with Executive Summary are enclosed.

FOR THE COMMANDER:

MAURICE H. WEEKS
Chief, Toxicology Division

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The compound AI3-35765 is comparatively non-toxic by ingestion and by dermal absorption. It has no potential for causing dermal sensitization and does not exhibit mutagenic activity in cell culture. No hazard exists from short-term inhalation of its saturated vapors. The technical grade may cause moderate, but reversible, injury to the eye.

Recommend conducting further entomological and toxicological studies with AI3-35765 to determine if candidate compound meets the requirements for an effective and safe full-spectra topical insect repellent.

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

REPLY TO ATTENTION OF

15 OCT 1990

EXECUTIVE SUMMARY
PHASE 2
PRELIMINARY ASSESSMENT OF THE RELATIVE TOXICITY OF THE CANDIDATE INSECT REPELLENT 1-(3-CYCLOHEXEN-1-YLCARBONYL)PIPERIDINE AI3-35765
ACUTE TOXICITY
STUDY NO. 75-51-0234-90
AUGUST 1990

1. Preliminary Assessment of the Relative Toxicity (PART) of the candidate insect repellent ID No. AI3-35765 was completed in August 1990. Report is enclosed.

2. ESSENTIAL FINDINGS. The compound AI3-35765 is comparatively non-toxic by ingestion and by dermal absorption. It has no potential for causing dermal sensitization and does not exhibit mutagenic activity in cell culture. No hazard exists from short-term inhalation of its saturated vapors. The technical grade may cause moderate, but reversible, injury to the eye.

3. RECOMMENDATION. Recommend conducting further entomological and toxicological studies with AI3-35765 to determine if candidate compound meets the requirements for an effective and safe full-spectra topical insect repellent.

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
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PRELIMINARY ASSESSMENT OF THE RELATIVE TOXICITY
OF THE CANDIDATE INSECT REPELLENT
1-(3-CYCLOHEXEN-1-YLCARBONYL)PIPERIDINE
AI3-35765
ACUTE TOXICITY
STUDY NO. 75-51-0234-90
AUGUST 1990

1. REFERENCES. See Appendix A.

2. AUTHORITY.

a. Memorandum of Understanding between the U.S. Army Health Services Command; the Department of the Army, Office of the Surgeon General; the Armed Forces Pest Management Board (AFPMB); and the U.S. Department of Agriculture, Agricultural Research, titled Biological and Toxicological Testing of Pesticides, effective 7 October 1987.

b. Letter, Armed Forces Pest Control Board, 29 November 1979, subject: Toxicological Testing of Candidate Repellent Compounds.

c. Letter, U.S. Department of Agriculture, Science and Educational Administration, Agricultural Research, Southern Region, Insects Affecting Man and Animals Research laboratory, Gainesville, Florida, 16 October 1979.

3. PURPOSE. Studies were conducted to obtain information concerning the potential health hazards associated with the use of 1-(3-cyclohexen-1-ylcarbonyl)piperidine, AI3-35765, as an insect repellent. The information from these studies will provide a basis for advising the AFPMB on the potential hazards associated with the use of this compound as a broad spectrum insecticide. Results from these studies will also provide guidance for further entomological testing of the subject insect repellent AI3-35765. The intent of this approach is to identify potential adverse human health effects that could be produced by acute exposure to the insecticide, and to determine the dose required to produce such effects.

Use of trademarked names does not imply endorsement by the U.S. Army but is intended only to assist in identification of a specific product.

4. BACKGROUND.

a. In recent years there has been a substantial increase in the search for new and effective topical insect repellents and formulations of repellents. Factors that contribute to the need for these chemicals include (1) requirement of nonpersistent insecticides; (2) removal from commercial production of traditional insecticides due to regulatory action; (3) increased insect resistance to toxicants, and (4) reduction in the availability of suitable insect toxicants due to particular problems with their use, application, and production.

b. It is essential that the military have available safe and effective repellents for the control of insects in the event large-scale military deployment in the field occurs. Recognizing the need for effective toxicants, the U.S. Army through the AFPMB has a Memorandum of Understanding (MOU) with the U.S. Department of Agriculture (USDA) for the development of insect repellents (reference paragraph 2a). The MOU outlines procedures for biological testing and toxicologic evaluation of pesticides of interest to the Department of Defense. The USDA screens compounds for biological effectiveness using test procedures which do not involve direct contact of the compound with human skin. Tests may be made with mosquitoes, ticks, ectoparasites, chiggers, and other pest species potentially harmful to man. Compounds showing promise in preliminary entomologic tests are submitted to USAEHA for an initial toxic hazard screen called the "Topical Hazard Evaluation Program (THEP)." Following a favorable THEP evaluation, USDA is informed of the results for each compound and of USAEHA's recommendation for further testing. The USDA continues such efficacy tests as it considers appropriate and provides AFPMB with test data for promising compounds and reasons for considering that the Armed Forces may wish to use these materials. If the AFPMB concurs as to the promise and potential usefulness of the compounds, it will recommend that USAEHA proceed with further toxicological evaluation. The USDA will also furnish USAEHA with necessary amounts of material of the highest purity for toxicity testing.

c. One of the objectives of the USDA repellent research program is to develop a repellent that can replace the standard insecticide N,N-dimethyl-meta-toluamide (DEET) as a topical or clothing treatment either because it is more effective than DEET or because DEET would no longer be available.

d. The USDA has reported to AFPMB on a number of very promising insect repellent compounds for which there is a need for additional toxicological data (Appendix B). These compounds

represent a group of chemicals the USDA, Gainesville, Florida have been working on for the past several years in cooperation with the late Dr. Terry McGovern of the USDA Organic Chemicals Synthesis Laboratory, Beltsville, Maryland. They appear to have excellent potential in protecting against stable flies, deer flies, salt marsh mosquitoes, black flies, and biting midges. Further toxicological studies will be needed, however, if efforts are to continue in the development of this group as potential replacements for DEET.

e. One of the more promising of the candidate insect repellents is AI3-35765, 1-(3-cyclohexen-1-ylcarbonyl)piperidine.

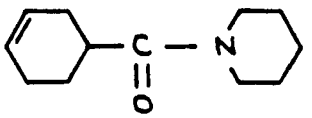
f. A preliminary THEP study recommended that this compound be approved for further testing (references 1 and 2). Additional toxicity data are being developed to provide a basis on which to develop safety information for handling large quantities of this material and its use as a general purpose topical insect repellent. The current report details results from acute animal studies and an in vitro Ames mutagenicity test. Additional reports will expand on further mutagenicity studies, subchronic dermal and oral studies as well as wildlife studies in avian and aquatic species.

5. MATERIALS.

a. Test Compound.

(1) The candidate insect repellent AI3-35765, 1-(3-cyclohexen-1-ylcarbonyl)piperidine, CAS 53736-58-0, $C_{12}H_{19}$-N-O, is a clear, yellow, slightly viscous liquid with a distinct musty odor. It's refractive index at 25 °C is 1.5110, molecular weight of 193, density of 1.034, and a boiling point of 113 °C. It is soluble in polar solvents (ether, acetone, alcohol, etc.), but only slightly soluble in water.

(2) The chemical was identified upon receipt using infrared (IR) spectrophotometry (Appendix C), and analytical procedures were developed by means of gas chromatography (GC). No significant impurities were found by these methods. The chemical structure of AI3-35765 is represented below:



All samples of AI3-35765 used in these studies were synthesized by the late Dr. Terrance P. McGovern, Organic Chemical Synthesis Laboratory, USDA, Beltsville, Maryland (reference 3).

*†b. Animals.

(1) Testing for primary skin irritation, photochemical skin irritation, and primary eye irritation were conducted using male New Zealand white rabbits obtained from Dutchland Laboratories, Denver, Pennsylvania. Male, albino Hartley guinea pigs, also from Dutchland Laboratories, were used for sensitization studies. Male and female Sprague-Dawley rats, selected from the USAEHA breeding colony whose source of animals was Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, were used for determination of oral toxicity, potential for enzyme induction, and an inhalation saturated vapor study.

(2) The rabbits and guinea pigs were housed individually in wire-bottom, stainless steel cages. Rats were group-housed with a maximum of five animals per cage in wire-bottom, stainless steel cages. Water and feed (Purina Rabbit Chow 5322, Purina Guinea Pig Ration 5026, and Purina Certified Rodent Chow 5026) were available ad libitum. The light/dark cycle was set at 12-hour intervals. Ambient temperatures were maintained at 21 to 25 °C with relative humidity between 40-60 percent. All studies were conducted in accordance with current standing operating procedures and Federal regulatory guidelines (reference 4 and 5).

c. Contract Studies. In vitro mutagenicity evaluation of the subject compound was performed by means of an Ames Salmonella/ Microsome Reverse Mutation Assay conducted by Litton Biometrics, Kensington, Maryland† under LBI Project No. 20988.

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* The studies reported herein were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

† 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.

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6. METHODS.

a. Skin Irritation. The initial test in the THEP series is a skin irritation procedure designed to detect frank dermal toxic response. The test was conducted according to the method of Draize utilizing young adult rabbits. All hair was clipped from the backs of rabbits 24 hours prior to exposure. One-half milliliter (mL) of undiluted technical grade material was applied for a single 24-hour period under a porous gauze patch to intact and abraded skin of six rabbits. The patches were held in place with surgical adhesive tape, and the entire shaved areas was covered with an impervious self adherent elastic wrap of Coban®. After 24 hours, the wrap and patches were carefully removed; excess material was wiped from the skin; and the test areas were evaluated for irritation. Irritation evaluations were also performed at 48 and 72 hours and at 7 days. Scoring of irritation effects was based on the Draize method in which erythema and edema were evaluated on a grade of 0 to 4 for severity (reference Appendix D). Categorizing of the responses was based on the sum of the mean 24 and 72 hour scores (Appendix E).

b. Eye Irritation. The development of significant adverse effects from accidental ocular contact was evaluated by means of a Draize eye test. These irritation studies were performed by administering single 0.1 mL doses of technical grade chemical or 25 percent, 10 percent, and 1 percent propylene glycol solutions of AI3-35765 to one eye of each of six rabbits per group. The opposite eye was left untreated and served as a control. Eyes were examined for gross signs of irritation at 24, 48, 72 hours, and 7 days following instillation. Scoring of irritation effects was based on the Draize method in which the total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctiva (Appendix F). No gross pathology or histopathology was performed. Categorizing of the responses was based on the 24-hour evaluation (Appendix E).

c. Sensitization. Sensitization studies were performed to determine the potential of the candidate insect repellent for causing sensitization reactions in humans. Female Hartley guinea pigs weighing between 375 and 425 gms were used for all tests. The test procedure was based on the studies of Lansteiner and was conducted with 10 guinea pigs given intradermal (ID) injections

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of a 0.1 percent suspension (W/V) of the chemical in saline. The injections were given every other day for a total of 10 after which the animals were rested for 2 weeks and then challenged with a single ID injection of the compound at the same concentration. A positive control, dinitrochlorobenzene (DNCB), was run concurrently with the test substance. The skin responses were scored at 24 and 48 hours post challenge by the Draize method of scoring.

d. Acute Toxicity Studies. Acute toxicity studies were performed to determine the adverse effects occurring within a short period of time following a single dose of a substance. This type of study identifies the relative toxicity of a compound, investigates its mode of action and specific toxic effect, and determines the existence of species differences. The most frequently used acute toxicity test involves determination of the median lethal dose (LD_{50}) of the compound. The LD_{50} is defined as a statistically derived expression of a single dose of a material. In the present study, single doses of the compound were administered to male and female rats by gavage, intraperitoneal injection and to male rabbits by skin application. A 14-day observation period was used to observe death or clinical signs. Animals were weighed at 1, 3, 7, and 14 days after exposure. All survivors were euthanized at 14 days and submitted for gross necropsy. Calculation of the LD_{50} was performed by the Method of Bliss as described by Finney (reference 6).

e. Photochemical Skin Irritation. A photochemical skin irritation study was performed to determine the potential of the test compound to become chemically reactive as a result of exposure to sunlight, specifically, to ultraviolet (UV) irradiation. Studies were performed by administering a single 0.05 mL dose of a 25 percent (W/V) solution of AI3-35765 and a 10 percent (W/V) Oil of Bergamot solution (positive control) in 95 percent ethyl alcohol to the intact shaved skin of six rabbits. Five minutes after application, the rabbits were exposed to UV light (365 nm) for 30 minutes at a distance of 10-15 cm. Following UV exposure of the rabbits, 0.05 mL of test chemical, positive control, and diluent, were applied to additional skin areas to serve as unirradiated control sites. Application areas were evaluated for skin irritation at 24, 48, and 72 hours. Scoring of irritation responses was based on the Draize Method for erythema and edema (Appendix D).

f. In Vitro Mutagenicity Assay*. The AI3-35765 chemical repellent was evaluated for mutagenic activity in an Ames Salmonella/Microsome Plate assay. The Ames test was used with

Salmonella typimurium indicator strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100. The assays were conducted in duplicate in the presence and absence of metabolic activation. The assays were conducted at doses which had been selected on a preliminary toxicity test with the strain TA-100. For the actual assay, doses were selected with the highest doses exhibiting ≤90 percent toxicity and ranged over a series of six doses from .005 µL/plate to 10 µL/plate.

g. Saturated Vapor Inhalation Study.

(1) Saturated vapor inhalation studies are conducted to estimate the relative hazard of occasional handling of a chemical where exposure consists of inhaling vapors resulting from spilled liquid. This test measures hazard rather than quantitative toxicity because the amounts inhaled are governed by vapor pressure of the test compound.

(2) This study was conducted to determine the relative acute inhalation hazard from exposure to saturated vapors of the test substance. Groups of six male rats each were exposed for 8 hours to saturated vapors of AI3-35765 with dispersion bubblers containing compound held at 25 °C and 100 °C. A control group of six male rats were exposed for 8 hours to chamber air only. All animals were observed daily for signs and weighed on a regular schedule of 1, 3, 4, 7, and 14 days post exposure.

h. Enzyme Induction Studies.

(1) These studies were conducted to determine the potential for AI3-35765 to enhance or inhibit liver enzyme activity in the rat by means hexobarbital sleeping times performed after repeated sublethal intraperitoneal injections of the test compound. Five groups of 10 male rats each were injected intraperitoneally daily for 4 consecutive days. Groups received either the positive control sodium phenobarbital at 100 mg/kg/day, the solvent control corn oil at 1 mL/kg, two dosages of AI3-35765 in corn oil at 100 and 250 mg/kg/day or a sham control.

(2) On the fifth day, the sleeping times of all groups of rats were determined following an intraperitoneal injection of 220 mg/kg hexobarbital. Sleeping time for each rat was defined as the interval between the initial prostration caused by the hexobarbital and the time that the animal spontaneously righted itself twice after being placed on it's back. Sleeping times of each test group were compared with the sham control group and the times compared by the Student "t" test.

7. RESULTS.

a. Primary Dermal Irritation Studies. Primary dermal irritation studies in rabbits were conducted on several samples of AI3-35765. These technical grade samples caused no irritation or only slight to mild irritation responses at 24 hours on intact and abraded skin. Residual chemical scaling, but not irritation, was observed on the skin surface at 3- and 7-day post exposure. The USAEHA categories of I or II were assigned to these responses (Appendix E). The EPA hazard indicator index placed these skin responses in grade IV (Appendix G).

b. Eye Irritation. Technical grade material caused mild transitory irritation to conjunctiva and cornea of exposed rabbits. Responses ranged from 0 to 2 with a mode of 2 based on the Draize irritation grading scale. All injury had been resolved by 7 days following exposure. Washing the eyes for 1 minute following installation of the technical material did not diminish the irritation response. Results from lower concentrations of AI3-35765 in propylene glycol indicated a reduction in severity of responses at lower concentrations with a 1 percent solution causing no injury. A tabular presentation of the eye irritation data developed on the subject candidate insect repellent follows:

TABLE 1. EYE IRRITATION DATA

Condition of Application AI3-35765	Result (Responses)	USAEHA Category	EPA Category
Technical grade material	Mild irritation to the cornea and conjunctiva. Injury resolved in 7 days.	Cat E	Cat II
Technical grade material-eye washed for 1 min with tap water 30 sec after application	Moderate injury to the cornea and conjunctiva. Injury resolved in 7 days.	Cat E	Cat II
25 percent solution AI3-35765 in propylene glycol	Mild injury to the cornea and conjunctiva. Injury resolved in 7 days.	Cat D	Cat II
10 percent solution AI3-35765 in propylene glycol	Slight irritation to the cornea and conjunctiva. Injury resolved in 7 days.	Cat C	Cat II
1 percent solution AI3-35765 in propylene glycol	No injury to cornea very slight irritation of conjunctiva.	Cat A	Cat IV

c. Sensitization. The challenge dose of the candidate insect repellent did not produce a sensitization reaction. The positive control produced a strong sensitization reaction in all exposed animals.

d. Acute Studies.

(1) Tabular presentation of the oral and intraperitoneal median lethal values in male and female rats follows:

TABLE 2. AI3-35765, ACUTE LETHAL STUDIES IN RATS

Sex	Age	LD_{50} mg/kg (± 95% C.L.)	Slope (± SE)	Signs
Oral Administration (tech grade material)				
Male	3 weeks	1394 (321)	5.67 (1.47)	Ataxia at all dosages; tonic convulsions At lethal dosages.
	6 weeks	2043 (550)	4.50 (1.50)	Ataxia all dosages; tonic convulsions.
Female	3 weeks	1447 (321)	8.42 (1.85)	Ataxia all dosages; tonic convulsions.
	6 weeks	2256 (426)	7.95 (2.44)	Convulsions and bloody nasal discharge at all lethal dosages.
Oral Administration (corn oil solution)				
Male	3 weeks	2775 (373)	12.03 (4.29)	ibid.
	6 weeks	4517 (652)	12.18 (5.30)	ibid.
Female	3 weeks	2719 (401)	12.3 (4.03)	ibid.
	6 weeks	4003 (665)	12.3 (3.69)	ibid.

TABLE 2. AI3-35765, ACUTE LETHAL STUDIES IN RATS (Continued)

Sex	Age	LD_{50} mg/kg (± 95% C.L.)	Slope (± SE)	Signs
Intraperitoneal Administration (tech grade material)				
Male	3 weeks	491 (122)	4.33 (1.22)	Ataxia at all dosages; tonic convulsions At lethal dosages.
	6 weeks	468 (69)	11.65 (3.47)	Convulsions and bloody nasal discharge at all lethal dosages.
Female	3 weeks	507 (101)	5.93 (1.49)	Ataxia all dosages; tonic convulsions.
	6 weeks	480 (121)	5.50 (1.72)	Signs at lethal dosages were salivation and bloody nasal discharge.
Intraperitoneal Administration (corn oil solution)				
Male	3 weeks	505 (137)	5.22 (1.54)	ibid.
	6 weeks	477 (78)	10.12 (3.23)	ibid.
Female	3 weeks	435 (69)	9.72 (2.70)	ibid.
	6 weeks	574 (314)	4.64 (4.64)	ibid.

(2) The acute dermal median lethal dosage of AI3-35765 in white female New Zealand rabbits was 1983 mg/kg with a slope of 3.48 for the dose response curve. Major signs were dilated pupils and convulsions preceding death. This LD_{50} study used impervious plastic wrap to cover the application site. The plastic material reacted with the AI3-35765 compound causing formation of an unknown sticky material. Repeating the dermal study using rubber dam and at a limit dosage of 2000 mg/kg showed no signs of material reaction or toxic signs in exposed animals. The dermal LD_{50} under these conditions was > 2000 mg/kg.

e. Photochemical Skin Irritation. No photochemical skin irritation resulted from irradiation of a single application of a 25 percent ethanol solution of AI3-37565.

f. In Vitro Mutagenicity Plate Assay. The test material, AI3-35765, did not exhibit genetic activity in the Ames assay.

g. Saturated Vapor Inhalation Study. Rats exposed for 8 hours to vapors from AI3-35765 showed no toxic signs over a 2-week observation period. No changes were observed in the organ-to-body weight ratios of lungs, liver, kidneys, spleen, heart, testes, thymus, and brain from exposed rats compared to controls.

h. Enzyme Induction Studies. The mean sleeping times with standard deviation from each treatment group are shown in Table 3. No decrease in sleeping time, compared to the Sham control, was demonstrated by treatment with AI3-35765 at 100 or 250 mg/kg/day. However, the higher dosage of AI3-35765 produced some effect on the hepatic microsomal enzyme system, causing a significant increase in the sleeping times of the rats treated at 250 mg/kg day.

TABLE 3. ENZYME INDUCTION

Treatment	Mean Hexobarbital Sleeping Time (Min ± SE)
Phenobarbital 100 mg/kg	32.2* ± 14.3
Solvent Control (corn oil - 1 ml/kg)	68.5 ± 19.7
AI3-35765 100 mg/kg	73.5 ± 16.0
250 mg/kg	101.7* ± 30.0
Sham Control	70.9 ± 16.6

*Significantly different from the shame control at the 0.05 level of probability.

8. DISCUSSION.

a. The purpose of these studies conducted under the aegis of the Topical Hazard Evaluation Program (THEP) was to investigate relevant health endpoints of candidate insect repellents. Data from these short term toxicological studies are used to recommend the course of further entomological and toxicological evaluations.

b. A review of the results from the acute studies show that AI3-35765 is relatively non-toxic by ingestion and by dermal absorption. The compound has no potential for causing sensitization, and does not exhibit mutagenic activity in cell

culture. No hazard exists from short-term inhalation of its saturated vapors. However, the technical grade material as well as dilutions as low as 10 percent in propylene glycol may cause moderate, but reversible, injury to the eye. Caution should be employed when handling quantities of the compound because of the potential for eye irritation.

c. The toxic responses in animals caused by administration of the piperidine compound AI3-35765 can be placed in perspective by comparison with responses from other well known compounds. The compounds used for comparison were parathion, malathion, Sevin, DEET, aspirin and ibuprofen. The data on these six compounds were obtained from the RTECS file from the National Library of Medicine and are shown in Table 4.

TABLE 4. ACUTE TOXICITY RESPONSES OF VARIOUS COMPOUNDS

	Rabbit Skin and Eye Irritation	Rat LD_{50} (mg/kg) Oral	Rat LD_{50} (mg/kg) Intraperitoneal	Rabbit LD_{50} (mg/kg) Dermal
AI3-35765	Slight Mild	2256	480	>2000
Parathion	Not reported	2	2	15
Malathion	Not reported	290	250	4100
Sevin	Severe Mild	230	64	Not reported
DEET	Moderate	1950	Not	3180
Aspirin	Not reported	200	340	Not reported
Ibuprofen	Not reported	636	626	Not reported

The comparisons demonstrate that AI3-35765 presents relatively little toxic hazard from acute exposures. Therefore, AI3-35765 must be considered a prime candidate for continued development as a topical insect repellent with these outstanding toxicological attributes.

9. *RECOMMENDATION.*

a. The current range finding toxicity studies conducted under the THEP program support recommendation for conducting further extensive entomological and toxicological studies with AI3-35765 .

b. Avoid eye contact with subject candidate insect repellent AI3-35765.

Maurice H. Weeks

MAURICE H. WEEKS
Chief, Toxicology Division

APPENDIX A

REFERENCES

1. Report, Topical Hazard Evaluation Program of Candidate Insect Repellents AI3-35765 and AI3-35765R, Study Nos. 75-51-0837-79 and 75-51-0116-79, 17 August 1979, ADA073181, USAEHA, APG.

2. Report, Phase 1 Dermal Penetration and Distribution of ^{14}C-labeled 1-(3-cyclohexen-1-ylcarbonyl)piperidine, AI3-35765 Study No. 75-51-0234-84, July 1984, USAEHA, APG.

3. Schreck, C.E., T.P. McGovern and N. Smith, Repellency of Selected Esters and Arnides of Alicyclic Acids Against the Stable Fly, Stomoxys Calcitrans (Diptera: Muscidae). J. Med. Entomol. 14: 580-591 (1978).

4. Title 40, Code of Federal Regulations (CFR), Part 160, Good Laboratory Practice Standards, 7 January 1988.

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Phase 2, Toxicological Study No. 75-51-0234-90, Aug 90

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, 1981 rev, Part 58, Good Laboratory Practice for Nonclinical Laboratories 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 Fisher

TIMOTHY FISHER
Chief, Analytical Quality Assurance Office

APPENDIX B

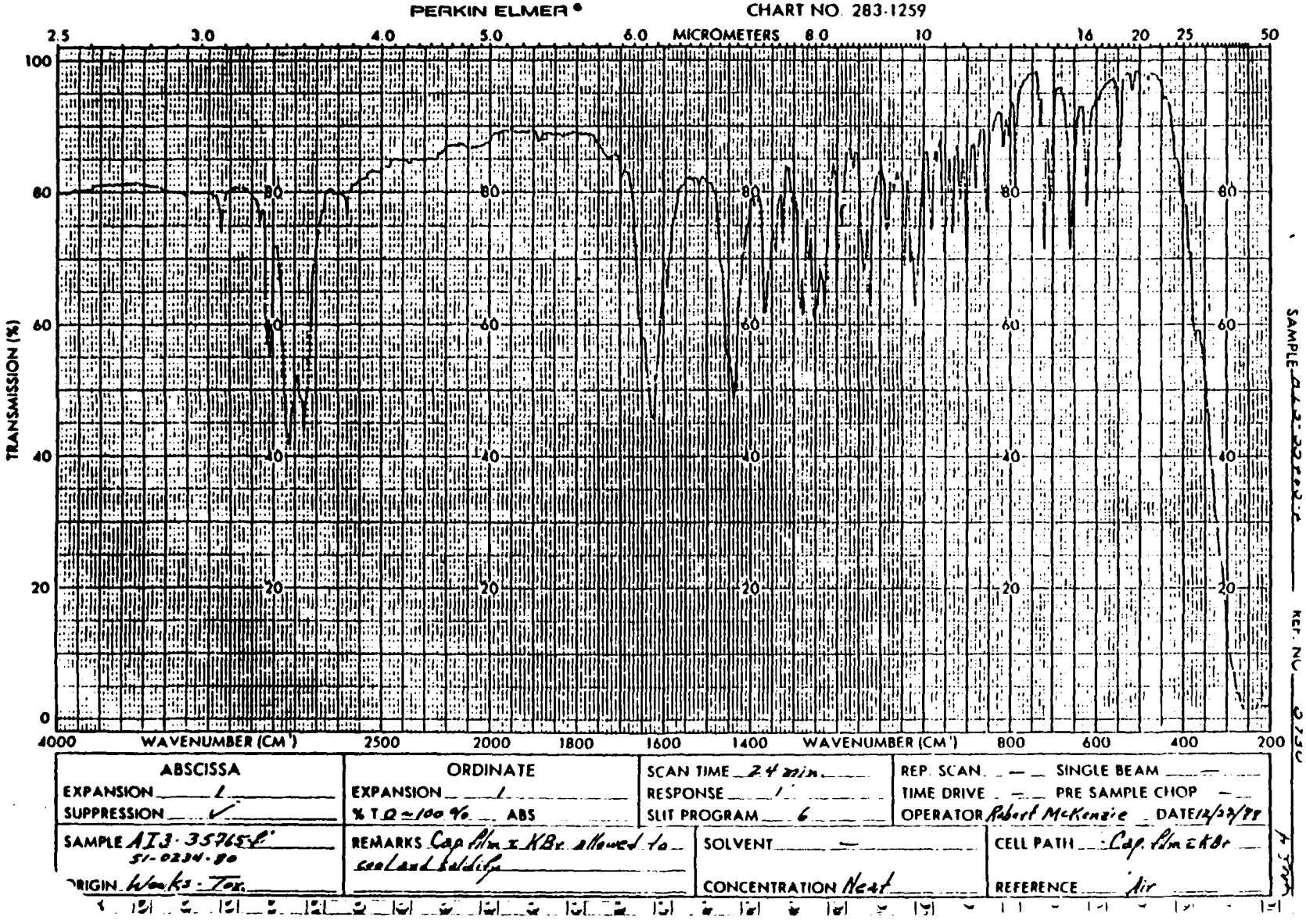
REPELLENT COMPOUNDS WITH THEIR RELATIVE EFFECTIVENESS* COMPARED TO DEET

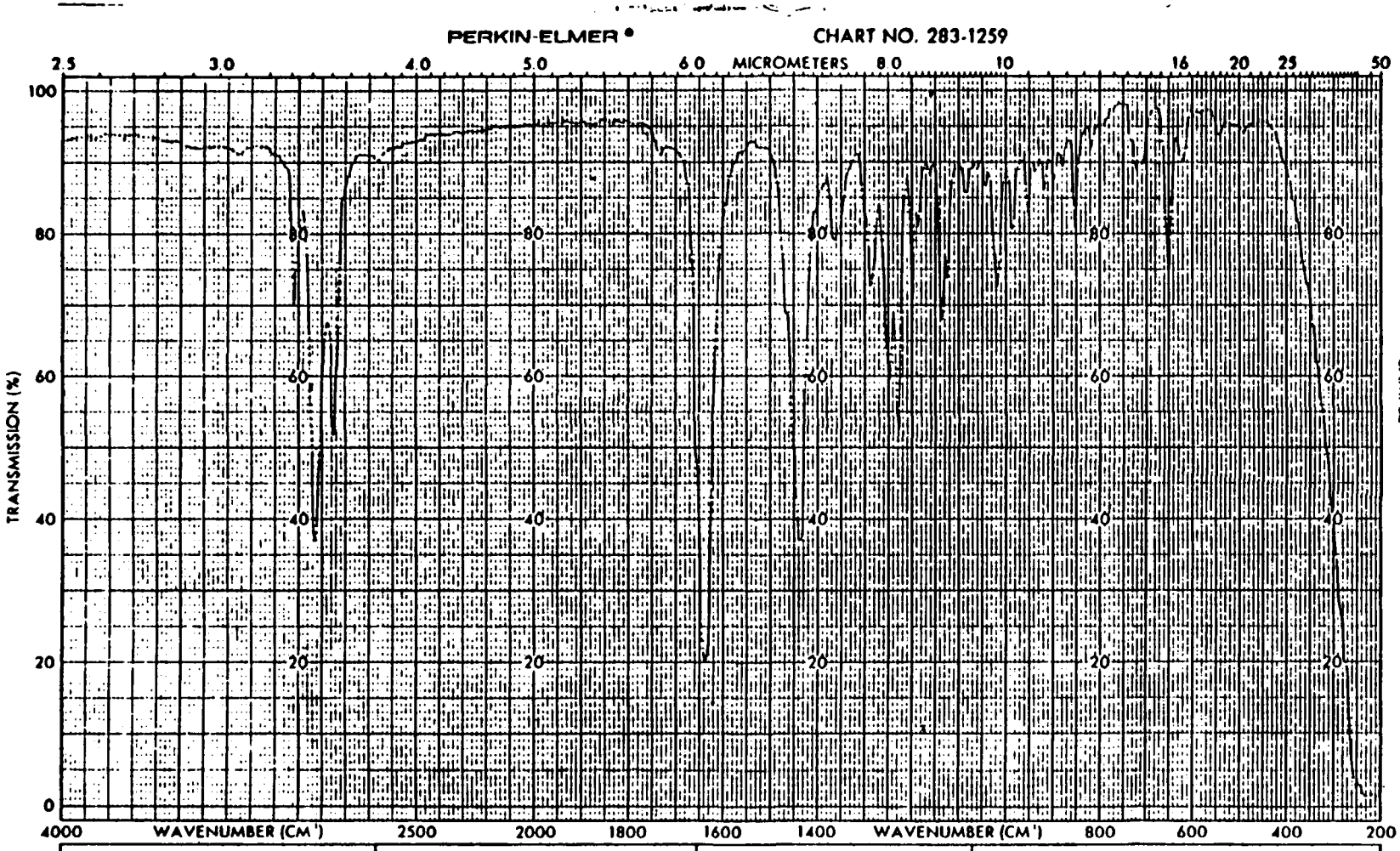
Priority	Code & Name	*Aedes aegypti*	*Aedes taeniorhynchus*	*Anopheles quadrimaculatus*	*Stomoxys calcitrans*	*Chrysops atlanticus*	Black Flies	*(Culicoides)* Sand Flies
1	AI3-35765 1-(3-Cyclohexen-1-ylcarbonyl)pieridine	<	>	=	>	>	>	>
2	AI3-36326 *N*,*N*-dipropylcyclohexanecarboxamide	<	>	<	>	>	<	>
3	AI3-35770 Hexahydro-1-[(2-methylcyclohexyl)carbonyl]-1*H*-azepine	<	=	<	>	>	=	>
4	AI3-35766 1-(3-Cyclohexen-1-ylcarbonyl)hexahydro-1*H*-azepine	<	<	=	>	=	>	
5	AI3-301180 *p*-Isopropyl-*N*,*N*-dimethylbenzamide	<	=	=	=	=	<	>
6	AI3-36328 1-[(6-Methyl-3-cyclohexen-1-yl)carbonyl]pyrrolidine	<	=	<	=	=	=	
7	AI3-36325 1-(Cyclohexylcarbonyl)hexahydro-1*H*-azepine	<	=	<	<	=	=	
8	AI3-35768 1-[(2-Methylcyclohexyl)carbonyl]pyrrolidine	=	<	=	=		=	

*, >, <, = indicates statistically significant differences of greater than, less than, and equal.

APPENDIX C

SPECTROPHOTOMETRY READOUTS


PERKIN ELMER
CHART NO. 283-1259
2.5
3.0
4.0
5.0
6.0
MICROMETERS
8.0
10
16
20
25
50
100
80
60
40
20
0
TRANSMISSION (%)
4000
WAVENUMBER (CM')
2500
2000
1800
1600
1400
WAVENUMBER (CM')
800
600
400
200
ABSCISSA
EXPANSION 1
SUPPRESSION ✓
ORDINATE
EXPANSION 1
%T.Q ≈ 100 % ABS
SCAN TIME 24 min.
RESPONSE 1
SLIT PROGRAM 6
REP. SCAN — SINGLE BEAM —
TIME DRIVE — PRE SAMPLE CHOP —
OPERATOR Robert McKenzie DATE 12/27/78
SAMPLE AI3-35765-F
51-0234-80
ORIGIN Weeks-Fox
REMARKS Cap film x KBr allowed to cool and solidify
SOLVENT —
CONCENTRATION Neat
CELL PATH Cap. film KBr
REFERENCE Air



SAMPLE AI3-35265-2 REF. NO. 5722

ABSCISSA	ORDINATE	SCAN TIME 24 min	REP. SCAN — SINGLE BEAM —
EXPANSION 1	EXPANSION 1	RESPONSE 1	TIME DRIVE — PRE SAMPLE CHOP —
SUPPRESSION V	%T 0-100% ABS —	SLIT PROGRAM 6	OPERATOR Robert M McKenzie DATE 12/22/77

SAMPLE AI3-35765-2 SI-0234-80	REMARKS Cap film KBr mull	SOLVENT —	CELL PATH Cap film KBr
ORIGIN Weeks - Tox.		CONCENTRATION Neat	REFERENCE Air

APPENDIX D

EVALUATION OF SKIN REACTIONS*

Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate-to-severe erythema	3
Severe erythema (beet redness to slight eschar formation)	4
Edema Formation	
No edema	0
Very slight (barely perceptible)	1
Slight edema (edges or area well defined by definite raising)	2
Moderate edema (edges raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

* An individual irritation score is equal to the sum of the scores for edema formation and erythema and eschar formation.

APPENDIX E

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.

CATEGORY II - Compounds producing mild primary irritation of the intact skin and the skin surrounding an abrasion.

CATEGORY III - Compounds producing moderate primary irritation of the intact skin and the skin surrounding an abrasion.

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.

CATEGORY V - Compounds impossible to classify because of straining of the skin or other masking effects owing to physical properties of the compound.

EYE CATEGORIES:

A. Compounds noninjurious to the eye.

B. Compounds producing mild injury to the cornea.

C. Compounds producing mild injury to the cornea, and in addition some injury to the conjunctiva.

D. Compounds producing moderate injury to the cornea.

E. Compounds producing moderate injury to the cornea, and in addition producing some injury to the conjunctiva.

F. Compounds producing severe injury to the cornea and to the conjunctiva.

APPENDIX F

SCALE FOR SCORING OCULAR LESIONS

1. Cornea

a. Opacity-degree of density (most dense area taken for reading)

No opacity.....0
Scattered or diffuse area, details of iris clearly visible.....1
Easily discernible translucent areas, details of iris slightly obscured.....2
Opalescent areas, no details of iris visible, size of pupil barely discernible.....3
Opaque, iris invisible.....4

b. Area of cornea involved

One quarter (or less) but not zero.....1
Greater than one quarter but less than one half.....2
Greater than one half but less than three quarters.....3
Greater than three quarters up to whole area.....4

Score = (a) x (b) x (5) = Total max score = 80

2. Iris

Values

Normal.....0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive).....1
No reaction to light, hemorrhage, gross destruction (any or all of these).....2

Score = (a) x 5 Total max score = 10

3. Conjunctivae

a. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal.....0
Vessels definitely injected above normal.....1

More diffuse, deeper crimson red, individual vessels not easily discernible.....2
Diffuse beefyred.....3

b. Chemosis

No swelling.....0
Any swelling above normal (included nictitating membrane).....1
Obvious swelling with partial eversion of lids.....2
Swelling with lids about half closed.....3
Swelling with lids about half closed to completely closed.....4

c. Discharge

No discharge.....0
Any amount different from normal (does not include small amounts observed with moistening of the lids and hairs just adjacent to lids.....2
Discharge with moistening of the lids and hairs, and considerable area around the eye.....3

Score (a + b + c) x 2 Total max score = 20

The individual numerical scores for each eye to which a given compound has been applied are added together and then divided by the number of eyes used to obtain the score.

APPENDIX G

TABLE. EPA HAZARD INDICATORS

Hazard Indicators	Toxicity Categories I	II	III	IV
Oral LD_{50}	Up to and including 50 mg/kg	From 50 thru 500 mg/kg	From 500 thru 5000 mg/kg	Greater than 5000 mg/kg
Dermal LD_{50}	Up to and including 200 mg/kg	From 200 thru 2000 mg/kg	From 2000 thru 20,000 mg/kg	Greater than 20,000 mg/kg
Eye effects	Corrosive, corneal opacity not reversible within 7 days	Corneal opacity reversible within 7 days; irritation persisting for 7 days	No corneal opacity; irritation reversible within 7 days	No irritation
Skin effects	Corrosive	Severe irritation at 72 hours	Moderate irritation at 72 hours	Mild or slight irritation at 72 hours