UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

008393

MAY 30 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Registration of Azadirachtin Biochemical Pesticide: SACB Review of Product Chemistry Data and Toxicity Studies (HED Project Nos. 1-0169, 1-0170, and 1-0171; I.D. Nos. 062552-R, 062552-G, and 062552-E; MRID Nos. 416264-01 through -21, 416269-01 through -19, 416263-01 through -13; Document Control Nos. D99348-A, -C through -L; Caswell No. 594A)

TO: Phil Hutton/Willie Nelson (PM-17)
Registration Division (H7505C)

FROM: J. Thomas McClintock, Ph.D., Microbiologist
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

THROUGH: Reto Engler, Ph.D., Chief
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

ACTION REQUESTED: Native Plants Incorporated (NPI) has submitted Product Chemistry/Identity data and a battery of Acute Toxicity Studies to support the registration of azadirachtin, a biochemical insecticide extracted from the seeds of the Neem tree. The Product Chemistry data was reviewed by the Chemistry Branch 2-Reregistration Support (CBRS); whereas the Acute Toxicity Studies were reviewed by the Dynamac Corporation. SACB has performed a secondary review and has summarized the results below.

STUDY SUMMARIES:

Product Identity/Chemistry

151A-10. Product Identity and Disclosure of Ingredients. The data/information submitted by NPI do not satisfy the requirements of 151A-10. In fact, due to the number of deficiencies SACB defers the Product Manager (PM) to the review provided by CBRS.

151A-11. Manufacturing Process. The submitted information is not adequate to fulfill the requirements of 151A-11. A description of beginning materials, other than Neem seeds, and all possible material which can be used in the various manufacturing processes presented by the registrant needs to be submitted. The description of the manufacturing process submitted allows for the use of various solvents. Consequently, all solvents must be specified. For specific information please refer to the CBRS response in the

Confidential Appendix (Pages 1 and 2).

151A-12. Discussion of the Formation of Unintentional Ingredients. CBRS was unable to adequately address the discussion of the formation of unintentional ingredients until additional information on the manufacturing process is submitted. The registrant must discuss the presence and/or the possibility of extraneous host residue or material in the product.

151A-13. Analysis of Samples. The information provided by the registrant does not adequately satisfy the data requirements for 151A-13. Since the registrant has stated the use of several solvents in the manufacturing process difficulties arise when attempting to define the technical material. If the submitter restricts the manufacturing process to the use of a single solvent at each point in the manufacturing process and the use of either whole or hulled Neem seeds the registrant would be required to analyze 10 batches (i.e. 2 lots of Neem seeds with varying concentrations x 5 batches/lot). For each analysis, the major impurities must be identified and quantified and this information should establish certified limits for each impurity. For specific details the Product Manager should refer to the CBRS response in the Confidential Appendix (Page 3-5).

151A-15. Certification of Ingredient Limits. The data requirements for 151A-15 have been partially satisfied. The certified limits for each impurity associated with the technical grade material must be designated. The theoretical mode of action also needs to be fully addressed. Assuming that azadirachtin acts as a feeding deterrent (mode of action) and that the spectrum of impurities in the material extracted from Neem seeds is complex CBRS would require that certified limits for the impurities be established at levels greater than 1.0% and not the required 0.1%. The PM should refer to the CBRS response in the Confidential Appendix (Page 5).

151A-16. Analytical Methods for Certified Limits. A description of the analytical methods was provided and adequately satisfies the requirements of 151A-16.

151A-17. Physical and Chemical Properties. The following properties were found to be deficient with respect to data/information: Density, solubility, and stability. The following data are also required for Azatin™ Technical: Vapor pressure and octanol-water partition coefficient. See the CBRS response in the Confidential Appendix for details (Pages 6-8).

Mammalian Toxicology

151B-10. Acute Oral Toxicity Study in Rats. Following the administration of a single oral dose of 1.0, 2.5 and 5.0 gm/kg of NPI 720 (technical grade material) the LD_{50} was determined to be 3540 mg/kg. Mortality was noted in the 2.5 gm/kg test group (1 male and 1 female) and 4 rats of each sex in the 5 gm/kg test

group. This study meets the minimum requirements set forth under Guideline 152B-10.

Classification. CORE Supplementary. Toxicity Category III. This study can be upgraded pending submission of the percent purity of the test material.

Limit Test. A limit test was conducted by administering 5 gm/kg of the test material in 1% carboxymethylcellulose to rats in a split dose. No animals died following exposure; all rats were lethargic at day of dosing with no other clinical signs of toxicity observed. Body weight gains were normal throughout the course of the study. The oral LD_{50} was determined to be greater than 5 gm/kg.

Classification. CORE Supplementary. Toxicity Category IV. This study could be upgraded pending submission of the purity of test material.

It should be noted that differences were observed in both oral studies (i.e. observed mortality). These differences should be discussed by the registrant since the Agency will classify the test material based on the most sensitive study (i.e. Toxicity Category III).

151B-11. Acute Dermal Toxicity Study in Rabbits. The test material when applied for 24 hr at a single dose of 2.0 gm/kg of NPI 720 (technical grade) to the shaved backs of rabbits caused dermal irritation (edema on Days 1 and 2 after exposure, erythema observed on Day 1, eschar formation in 1 animal on Days 3-5) which was resolved by Day 9.

Classification. CORE Supplementary. Toxicity Category III. This study can be upgraded pending submission of 1) the percent purity and the physical state of the test material and 2) individual animal data.

Limit Test. Using NPI 720; NPI-720-F/20-13 (formulated product) a limit test was performed at a dose level of 2.0 gm/kg. No deaths occurred during the course of the study and no signs of treatment-related systemic toxicity were observed. Al rabbits demonstrated signs of dermal irritation which included edema, erythema, eschar formation and flaking of the skin. By Day 9 and 10 edema and erythema were resolved, respectively. Eschar formation and flaking of the skin persisted throughout the course of the study in 4 to 5 rabbits. Body weight gains were normal in all animals.

Classification. CORE Supplementary. Toxicity Category III. This study could be upgraded pending submission of the purity of the test material.

152B-12. Acute Inhalation Toxicity Study in Rats. The data submitted by the registrant supports the conclusion that the acute inhalation LC_{50} was greater than 2.41 mg/L (average aerosol mass median aerodynamic diameter was 1.51 um with a standard deviation

of 1.83. Although below the 5.0 mg/L limit test dose for an acute inhalation study, the reported concentration was the maximum dose possible under the conditions of the study. No deaths occurred during the course of the study. Clinical signs of treatment-related toxicity included redness around the eyes, salivation, nasal congestion, wheezing and breathing through the mouth. All but one rat appeared normal by the end of the study. Weight gain was only observed between Days 8 and 15.

Classification. CORE Supplementary. Toxicity Category III. This study can be upgraded pending submission of 1) the percent purity of the test material and 2) individual body weight and clinical data.

152B-13. Primary Eye Irritation Study in Rabbits. Following instillation of approximately 0.1 gm of NPI 720 (technical grade), the undiluted test material, slight irritation of the iris and cornea was observed at 1 and 24 hr, respectively. At 1 hr post-instillation all animals had moderate to severe conjunctivitis which was resolved by 48 hr. At 1 hr post-instillation the maximum eye irritation score was obtained (15.3/110); by 24, 48, and 72 hr the scores were 6.2/110, 0.3/110 and 0/110, respectively.

Classification. CORE Supplementary. Toxicity Category III (Corneal involvement or irritation clearing in 7 days or less). This study can be upgraded pending submission of 1) the purity and physical state of the test material; 2) individual body weights; and 3) clinical signs of toxicity.

Using 0.1 ml aliquot of undiluted NPI-720-F/20-13 - NPI 720 (formulated product) the test material was placed into the conjunctival sac of the right eye of rabbits. No signs of systemic toxicity were observed; however, moderate to severe conjunctivitis was observed in all animals from 1 hr until 7 days post-instillation; moderate swelling and redness persisted through Day 14 in two rabbits and through Day 23 in one male rabbit. Although mild corneal opacity was observed in all treated eyes this condition was resolved by Day 17 in all rabbits. The maximum mean eye irritation score was 42.5/110 on Day 2; however by Day 21 the score dropped below 1.0/110.

Classification. CORE Supplementary. Toxicity Category II (Corneal involvement or irritation clearing in 8 to 21 days in 5/6 rabbits). This study could be upgraded pending submission of the purity of the test material.

152B-14. Primary Dermal Irritation Study in Rabbits. The test material (NPI 720; technical grade) when applied at a single dose (0.5 gm) to the shaved backs of rabbits did not cause dermal irritation after 4 hr of exposure. The dermal irritation score was zero for all treated rabbits at all examination times.

Classification. CORE Supplementary. Toxicity Category IV. Mild to slightly irritating. This study can be upgraded

pending submission of the purity and the nature of the test material (i.e. moistened versus dry), individual animal data, and clinical observations.

Following the application of 0.5 gm of the undiluted test material (NPI-720-F/20-13;NPI 720; formulated product) to the shaved backs of rabbits for 4 hr caused slight erythema in all rabbits within 24 hr. By Days 2 and 3 the condition developed in well-defined to severe erythema which was resolved in three rabbits by Day 7. Severe erythema with slight eschar formation continued in the remaining three rabbits. All signs of erythema and eschar were resolved by Day 14. The mean irritation score was 1.4/>5.0 with a primary dermal irritation index of 1.8/>5.0.

Classification. CORE Supplementary. Toxicity Category II (Mild irritant). This study could be upgraded pending submission of the purity of the test material, reporting of the individual dermal irritation data, and clinical observations.

152B-15. Dermal Sensitization Study in Guinea Pigs. A 25% (w/v) solution of NPI 720 (technical grade) elicited a slight dermal reaction in 2 out of 10 male guinea pigs at 24 hr post-challenge. The test material was considered a weak dermal sensitizer in guinea pigs.

Classification. CORE Supplementary. Toxicity Category - weak sensitizer. To upgrade this study, the PM is referred to the primary reviewer's comments for specific deficiencies.

152B-16. Hypersensitivity Incidents. All incidents must be reported to the Agency.

152B-17. Mutagenicity. NPI 720 (technical grade) was evaluated for the potential to cause gene mutations in the Salmonella typhimurium assay. There was no appreciable increase in mutant colonies of S. typhimurium strains TA1535, TA1538, TA98, or TA100 at any dose (5, 50, 500, 5000 µg/plate) with or without S-9 activation.

Classification. CORE Supplementary. Although the study was properly performed the study is incomplete. This study can be upgraded pending the submission of the purity and stability of the test material.

008393

EPA No.: 68D80056
DYNAMAC No.: 348-E
TASK No.: 3-48E
March 7, 1991

DATA EVALUATION RECORD

AZADIRACTIN (NPI 720)

Acute Oral Toxicity Study in Rats

STUDY IDENTIFICATION: Mega, W.M. Acute oral toxicity study of NPI-720-F/20-13 in rats. (Unpublished study No. L08289 performed by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated August 1990.) MRID No. 416264-21.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 3/6/91

1. CHEMICAL: Azadiractin; NPI 720.

2. TEST MATERIAL: NPI 720 was described as a brown liquid. The purity and lot number of the test material were not provided.

3. STUDY/ACTION TYPE: Acute oral toxicity study in rats.

4. STUDY IDENTIFICATION: Mega, W.M. Acute oral toxicity study of NPI-720-F/20-13 in rats. (Unpublished study No. L08289 performed by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated August 1990.) MRID No. 416264-21.

5. REVIEWED BY:

Mary E. Cerny, M.S.
Principal Reviewer
Dynamac Corporation

Signature: Mary E Cerny
Date: 3/6/91

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

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Date: 3/6/91

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L. McLellan
Date: 3-6-91

J. Thomas McClintock, Ph.D.
EPA Reviewer
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)

Signature:
Date: 3/12/91

Albin Kocialski, Ph.D.
EPA Section Head
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)

Signature: A. Kocialski
Date: 3/13/91

7. CONCLUSIONS:

Core Classification: CORE ~~Minimum~~ Supplementary. This study meets the minimum requirements set forth under Guideline 81-1 (152B-10) for an acute oral toxicity study in rats.

LD_{50}: 3540 mg/kg (males and females).

Toxicity Category: III.

8. SUMMARY: Groups of 10 CD rats (5/sex) (Charles River, Portage, MI), having mean weights between 156 and 185 g, were given a single oral dose of 1, 2.5, or 5 g NPI 720 (in 1% carboxymethyl cellulose)/kg in one 10-mL/kg volume. Animals were fasted for 20 hours before and 3 to 4 hours after dosing. Rats were observed for signs of toxicity and moribundity at 1 and 4 hours postdosing and daily, thereafter, for 14 days; animals were also checked twice a day (once a day on weekends and holidays) for mortality. Body weights were recorded prior to compound administration (fasted weight), 7 days after dosing, and at study termination. Surviving animals were killed on day 14; all rats were subjected to a gross necropsy, and tissues were discarded after examination. Mortality data were analyzed using probit analysis.

No animals in the 1-g/kg group died; however, two rats (one male, one female) in the 2.5-g/kg group and four rats of each sex in the 5-g/kg group died. All deaths occurred on day 1 or 2 of the observation period. Clinical signs in all groups of animals included lethargy and excessive salivation; some animals given the 2.5- or 5-g/kg dose of azadiractin also exhibited hunched posture and/or moribundity. Low-dose rats appeared normal within 2 to 3 days after dosing; all survivors in the mid- and high-dose groups appeared normal by day 4. Body weight gains were within normal limits for all survivors except one female in the 5-g/kg group that gained a total of only 30 g during the 14-day observation period and lost weight (10 g) between days 7 and 14 postdosing.

Autopsy results of all surviving rats were normal. Test material was found in the stomach and/or small intestine of all animals that died during the study; NPI 720 was also found in the esophagus of one high-dose male and one high-dose female. Animals in the mid- and high-dose groups had a red discharge from the mouth and red or blue/black fluid in the bladder. The two rats in the mid-dose group that died also had a red discharge from the nose, and a red discharge was observed on the vagina of one high-dose female rat. Two high-dose male rats and one mid-dose female rat had pale livers; two of these livers (one male, one female) were mottled. The acute oral LD_{50} for both males and females was determined to be 3540 mg/kg.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: The conduct and reporting of this study were adequate; one deficiency was found. The study author did not report the percent purity of the test material, which is used to calculate the amount of active ingredient administered and which can assist in determining whether contaminants may have contributed to the toxicity of the material. When administered in a single oral dose to rats, azadiractin was moderately toxic; the LD_{50}, 3450 mg/kg, was apparently the same for both males and females. Recovery of test material from the stomach and small intestine on days 1 and 2 after dosing (i.e., in all animals that died during the study) may indicate that absorption of NPI 720 was saturated; the persistence of some signs of toxicity may reflect this slow rate of absorption.

   A quality assurance statement, signed and dated August 22, 1990, and a statement of compliance with good laboratory practices (GLPs), signed and dated August 22 and 23, 1990, were provided.

10. CBI APPENDIX: APPENDIX A: Materials and Methods (CBI pp. 6-8); APPENDIX B: Protocol Deviation (CBI p. 17).

APPENDIX A

Materials and Methods
(CBI pp. 6-8)

Azadirachtin Toxicology Review #008393

Page _____ is not included in this copy.

Pages 11 through 13 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX B

Protocol Deviation
(CBI p. 17)

APPENDIX: PROTOCOL DEVIATION

008393

NOTICE OF DEVIATION FROM PROTOCOL

Date or period of deviation July 11 to August 2, 1990

Project No. L08289 - SN5 Test Articles NPI-720-F/20-13

Nature of deviation 10e. Food: Animals received uncertified Purina Rodent Chow 5001 instead of Certified Purina Rodent Chow 5002.

Reason for Deviation: Technician error. Deviation is not anticipated to significantly alter the results of the study.

Study Director: _____ Date: 8/13/90

Acknowledged by: _____ Date: 8/17/90

cc: Study Notebook

008393

EPA No.: 68D80056
DYNAMAC No.: 348-D
TASK No.: 3-48D
March 7, 1991

DATA EVALUATION RECORD

AZADIRACTIN (NPI 720)

Acute Oral Toxicity Study in Rats (Limit Test)

STUDY IDENTIFICATION: Furedi-Machacek, E. M. Acute oral toxicity study of NPI-720 in rats (limit-test). (Unpublished study No. L08270 prepared by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated July 1990.) MRID No. 416264-19.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir
Date: 3/6/91

016

1. CHEMICAL: Azadiractin; NPI 720.

2. TEST MATERIAL: The physical state and appearance, purity, and lot number of the test material were not described; however, on the basis of information in the materials and methods section of the report, the study reviewers assumed the test material was a solid. It was reported that NPI 720 is highly insoluble.

3. STUDY/ACTION TYPE: Acute oral toxicity study (limit test) in rats.

4. STUDY IDENTIFICATION: Furedi-Machacek, E. M. Acute oral toxicity study of NPI-720 in rats (limit-test). (Unpublished study No. L08270 prepared by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated July 1990.) MRID No. 416264-19.

5. REVIEWED BY:

Mary E. Cerny, M.S.
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Signature: Mary E Cerny
Date: 3/6/91

Margaret E. Brower, Ph.D.
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Date: 3/6/91

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L. McLellan
Date: 3-6-91

J. Thomas McClintock, Ph.D.
EPA Reviewer
Reregistration Section
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Coordination Branch
(H-7509C)

Signature:
Date:

Albin Kocialski, Ph.D.
EPA Section Head
Reregistration
Section
Science Analysis and
Coordination Branch
(H-7509C)

Signature: A. Kocialski
Date: 3/13/91

7. CONCLUSIONS:

Core Classification: CORE ~~Minimum~~ SUPPLEMENTARY. This study meets the minimum requirements set forth under Guideline 81-1 (152B-10) for an acute oral toxicity study (limit test) in rats.

LD_{50}: >5 g/kg (both sexes).

Toxicity Category: IV.

8. SUMMARY: Ten CD rats (five/sex; Charles River, Portage, MI) were given 5 g NPI 720/kg in two 10-mL/kg oral doses; the compound was delivered as a 250-mg/mL suspension of the test material in 1% carboxy-methylcellulose (CMC), and two doses were administered because of the compound's poor solubility. Rats were fasted 16 hours before and 4 hours after compound administration, and animals weighed between 147 and 195 g at the time of dosing. Animals were observed for mortality and signs of toxicity and moribundity at 1, 3, and 5 hours postdosing and daily thereafter for 14 days (except on weekends and holidays). All rats were weighed prior to fasting, after fasting but prior to compound administration, and on days 8 and 15 after dosing. Rats were killed at the end of the 14-day observation period and were subjected to a limited gross necropsy, which involved examination of all body surfaces and openings, as well as the brain, heart, lungs, spleen, liver, kidneys, stomach, intestinal tract, urinary bladder, adrenals, and gonads.

No animals died following exposure to the 5-g/kg oral dose of NPI 720. All rats were lethargic and had hunched posture on the day of dosing; one male rat also exhibited salivation. No other clinical signs of toxicity were observed. Body weight gains were normal for all animals, and no gross lesions were seen at necropsy. The study author concluded that the acute oral LD_{50} of NPI 720 for male and female rats is >5 g/kg.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: The reporting and conduct of this study were, in general, adequate; a few deficiencies were found. The purity of the test material was not reported; the study author stated that analytical characteristics of the compound (e.g., stability, homogeneity, and purity) are the sponsor's responsibility. However, providing the purity assists in determining whether signs of toxicity may be due to contaminants. The purity is also used to calculate the actual dose of active ingredient administered. In addition, because of azadiractin's poor solubility, determining the homogeneity and concentration of the dosing suspension (which was not done) is critical in delivering the correct dose. Administration of split, or two, doses was

acceptable because of the insolubility of the test material. On the basis of the data presented, the acute oral LD_{50} of NPI 720 (in suspension) in both male and female rats is >5 g/kg.

A quality assurance statement, signed and dated July 10, 1990, and a statement of compliance with good laboratory practices (GLPs), signed and dated July 10 and 11, 1990, were provided.

10. CBI APPENDIX: Materials and Methods, CBI pp. 6-8.

APPENDIX

Materials and Methods
(CBI pp. 6-8)

Azadirachtin Toxicology Review. #008393

Page _____ is not included in this copy.

Pages 21 through 23 are not included in this copy.

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_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product quality control procedures.

_____ Identity of the source of product ingredients.

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_____ A draft product label.

_____ The product confidential statement of formula.

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_X_ FIFRA registration data.

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008393

EPA No.: 68D80056
DYNAMAC No.: 348-F
TASK No.: 3-48F
March 7, 1991

DATA EVALUATION RECORD

AZADIRACTIN (NPI 720)

Acute Dermal Toxicity Study in Rabbits (Limit Test)

STUDY IDENTIFICATION: Furedi-Machacek, E. M. Acute dermal toxicity study of NPI 720 in rabbits (limit-test). (Unpublished study No. L08270-03 performed by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated July 1990.) MRID No. 416264-22.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 3/6/91

1. CHEMICAL: Azadiractin; NPI 720.

2. TEST MATERIAL: The physical state and appearance, purity, and lot or batch number of the test material were not provided.

3. STUDY/ACTION TYPE: Acute dermal toxicity study (limit test) in rabbits.

4. STUDY IDENTIFICATION: Furedi-Machacek, E. M. Acute dermal toxicity study of NPI 720 in rabbits (limit-test). (Unpublished study No. L08270-03 performed by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated July 1990.) MRID No. 416264-22.

5. REVIEWED BY:

Mary E. Cerny, M.S.
Principal Reviewer
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Signature: Mary E. Cerny
Date: 3/6/91

Margaret E. Brower, Ph.D.
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6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L. McLellan for
Date: 2-6-91

J. Thomas McClintock, Ph.D.
EPA Reviewer
Reregistration Section
Science Analysis and
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(H-7509C)

Signature: [signature]
Date: 3/12/91

Albin Kocialski, Ph.D.
EPA Section Head
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)

Signature: A. Kocialski
Date: 3/12/91

7. CONCLUSIONS:

Core Classification: CORE Minimum. This study meets the minimum requirements set forth under Guideline 81-2 (152B-11) for an acute dermal toxicity study (limit test) in rabbits. This study could be upgraded provided that individual animal data are submitted.

LD_{50}: >2 g/kg for both male and female rabbits.

Toxicity Category: III.

8. SUMMARY: Undiluted test material was applied at a dose of 2 g/kg to the intact shaved and moistened backs of five male and five female New Zealand albino rabbits (Johnson Rabbit Ranch, Wilkinson, IN) weighing between 2.05 and 2.55 kg at the time of dosing. Rabbits were not restrained during the 24-hour exposure period and were housed individually throughout the study. At 24 hours before compound application, fur was clipped from the back of each animal, exposing an area of approximately 240 cm^2; care was taken to avoid abrading the skin. Following application of the test material, test areas were covered with surgical dressing coated with a plastic film; dressings were secured using a lint-free cloth and an elastic adhesive bandage, which prevented removal of the test material by the treated animals. The wrappings were removed 24 hours after the test material was applied; residual azadiractin was rinsed off using 0.9% saline. All animals were weighed immediately before dosing (day 0); these weights were used to calculate the appropriate amount of test material required for a 2-g/kg dose. Rabbits were also weighed on days 1 (after removal of wrappings), 8, and 15 (study termination). Animals were observed at 1, 2, and 4 hours after initial application and daily thereafter for 14 days for mortality, signs of dermal irritation (edema, erythema, and eschar formation), and clinical/systemic signs of toxicity. All rabbits were killed at the end of the 14-day observation period and were subjected to a limited gross necropsy, which included examination of the treatment site and of the external surfaces of the brain, heart, lungs, spleen, liver, kidneys, gastrointestinal tract, urinary bladder, and gonads.

No animals died in this study. Dermal irritation was present in most rabbits. Edema was observed in 2/10 and 4/10 animals on days 1 and 2 after exposure, respectively; erythema was first observed in 8/10 rabbits on day 1; and eschar formation was noted in one animal on days 3 through 5. Signs of irritation were resolved in all affected rabbits by day 9. Two male rabbits had diarrhea; in one animal, this condition persisted through day 12. The same two males lost weight during the study; weight loss was attributed to diarrhea and

was considered independent of treatment. All other animals gained weight throughout the study, but overall weight gains were somewhat low among the remaining three male rabbits (0.31 to 0.35 kg for the 14-day observation period) and in one female (0.21 kg). Weight gains were normal for the remaining four females. Gross necropsies were normal for three males and one female. One male had an enlarged spleen; another male and one female had an enlarged mesenteric lymph node. Clear cysts were found on the fallopian tube of four female rabbits; one of these animals also had a node on the ovary. All abnormal autopsy findings were considered incidental. The study author concluded that the dermal LD_{50} for NPI 720 in rats is >2 g/kg.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: The conduct and reporting of this study were, in general, acceptable; however, a few deficiencies were found. The study author failed to report the purity and describe the physical state (i.e., liquid or solid) of the test material; the liquid used to moisten the skin prior to application also was not described. The percent purity is used in calculating the amount of active ingredient the test animal is exposed to; percent purity can also indicate whether contaminants may have contributed to the toxicity of the test material. For solids in particular, a vehicle is often used as a moistener to ensure good contact with the skin; this vehicle may also influence dermal absorption, i.e., the ability of the test material to penetrate the skin. Another reporting deficiency in this study was the lack of individual animal data relating to clinical response to the test material; the group data presented did not allow for evaluation of sex-related differences in response or for monitoring the individual progression (or regression) of these responses. Consequently, the individual animal data should be provided. In addition, the rabbits were not restrained during the exposure period; however, the dressing used to secure the test material reportedly prevented removal of the test material, which can occur when animals are allowed to move freely. Finally, although not required in an acute dermal toxicity study, the degree of dermal irritation was not described; the resolution of all edema by day 6 and all erythema by day 9 and the presence of eschar formation in only one rabbit indicates that dermal irritation probably was not severe. As noted by the study author, the data suggest that the acute dermal LD_{50} of NPI 720 in both male and female rabbits is >2 g/kg, the maximum dose required as the limit test by EPA Guidelines (1984).

A quality assurance statement, signed and dated July 10, 1990, and a statement of compliance with good laboratory practices (GLPs), signed and dated July 10 and 11, 1990, were provided.

10. **CBI APPENDIX**: **APPENDIX A: Materials and Methods, CBI pp. 6-8; APPENDIX B: Protocol Deviation, CBI p. 15.**

APPENDIX A

Materials and Methods
(CBI pp. 6-8)

Azadirachtin Toxicology Review #008393

Page _____ is not included in this copy.

Pages 30 through 32 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action

__X__ FIFRA registration data.

_____ The document is a duplicate of page(s) _____

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX B

Protocol Deviation
(CBI p. 15)

008393

Appendix A: Protocol Deviation

NOTICE OF DEVIATION FROM PROTOCOL

ıte or period of deviation: April 24, 1990

roject No.: L08270, SN: 3 Test Article: NPI 720

ature of deviation

The lint-free cloth used to wrap the rabbits at dosing was not from Fisher Scientific.

eason of Deviation:

Cloth from Fisher Sci. was unavailable. This deviation is not anticipated to significantly alter the results of the study.

tudy Director: E. M. Fuscaldi-Madsack Date: 6-28-90

cknowledged by: William M. Mayer Date: 6-28-90

EPA No.: 68D80056
DYNAMAC No.: 348-G
TASK No.: 3-48G
March 7, 1991

DATA EVALUATION RECORD

AZADIRACTIN (NPI 720)

Acute Dermal Toxicity Study in Rabbits (Limit Test)

STUDY IDENTIFICATION: Mega, W. M. Acute dermal toxicity testing of NPI-720-F/20-13 in rabbits (limit-test). (Unpublished study No. L08289-02 performed by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated August 1990.) MRID No. 416264-23.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir
Date: 3/6/91

1. CHEMICAL: Azadiractin; NPI 720; NPI-720-F/20-13.

2. TEST MATERIAL: NPI-720-F/20-B was described as a brown liquid. The purity and lot number of the test material were not reported.

3. STUDY/ACTION TYPE: Acute dermal toxicity study (limit test) in rabbits.

4. STUDY IDENTIFICATION: Mega, W. M. Acute dermal toxicity testing of NPI-720-F/20-13 in rabbits (limit-test). (Unpublished study No. L08289-02 performed by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated August 1990.) MRID No. 416264-23.

5. REVIEWED BY:

Mary E. Cerny, M.S.
Principal Reviewer
Dynamac Corporation

Signature: Mary E Cerny
Date: 3/6/91

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: margaret brower
Date: 3/6/91

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L. McLellan for
Date: 3-6-91

J. Thomas McClintock, Ph.D.
EPA Reviewer
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)

Signature: J. Thomas McClintock
Date: 3/12/91

Albin Kocialski, Ph.D.
EPA Section Head
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)

Signature: A. Kocialski
Date: 3/14/91

7. CONCLUSIONS:

Core Classification: CORE ~~Minimum~~ SUPPLEMENTARY JTMC. This study meets the minimum requirements set forth under Guideline 81-2 (152B-11) for a dermal toxicity study in rabbits.

LD_{50}: >2 g/kg for both male and female rabbits.

Toxicity Category: III.

8. SUMMARY: Undiluted test material at a dose level of 2 g/kg was applied to the intact shaved skin on the backs of five male and five female New Zealand albino rabbits (Johnson Rabbit, Wilkinson, IN) weighing between 2.09 and 3.01 kg. Approximately 24 hours before compound application, backs were shaved to expose an area of approximately 240 cm^2; care was taken to avoid abrading the skin. The skin was then examined for any abnormalities. The treated test sites were covered with surgical dressing coated with a plastic film, and this dressing was secured by a lint-free cloth and an elastic adhesive bandage, which prevented removal of the test material by the unrestrained animals. Dressings were removed after 24 hours, and the application sites were rinsed with 0.9% saline to remove residual test material. Animals were observed for clinical signs of toxicity at 1, 2, and 4 hours after dosing and at least once per day thereafter for 14 days; animals were also observed daily for 14 days for mortality (per Protocol Deviations, CBI p. 14). Treated test sites were also observed for signs of progressing and regressing dermal irritation, including edema; erythema; eschar formation; sensitivity to touch; superficial flaking, cracking, and bleeding of the skin; partial or total hair regrowth; new skin growth or repair; and residual test material. Body weights were recorded before dosing (day 0); these weights were used for dose calculations. Weights were also taken on days 7 and 14 (study termination). Survivors were killed at the end of the 14-day observation period. A limited gross necropsy was performed, which included examination of the treatment site and of the external surfaces of the brain, heart, kidneys, gastrointestinal tract, bladder, and gonads.

No deaths occurred during this study, and no signs of systemic toxicity were observed. All rabbits exhibited some signs of dermal irritation; all females and most males had edema, erythema, eschar formation, sensitivity to touch, and flaking of the skin. The skin of one male also cracked and bled on days 7 through 10. Edema and erythema were resolved within 10 and 9 days after dosing in males and females, respectively. Flaking of the skin and eschar formation in four to five rabbits of each sex and touch sensitivity in all females and one male persisted through study termination. Reversal of

dermal reaction (i.e., skin repair) was apparent in most females by day 7 and in most males by day 10. Residual test material was present at the application sites throughout the study and was found on the skin of four female and two male rabbits on day 14. Body weights and body weight gains were within normal limits for all animals. Autopsy findings included a pale kidney in one female rabbit and mottled lungs in one male and one female rabbit. It was concluded that the acute dermal LD_{50} of NPI 720 in rabbits was >2 g/kg.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: The reporting and conduct of this study were adequate, but a few minor deficiencies were found. The study author failed to report the purity of the test material, which is used to calculate the amount of active ingredient administered, and which can assist in determining whether contaminants may have contributed to the toxicity of the test compound. In addition, despite the washing of the application sites with saline at the end of the 24-hour exposure period, azadiractin was still present on the skin of six animals (four females, two males) at study termination. The persistence and severity of the dermal reaction to azadiractin may have resulted from the residual test material. Although not required in an acute dermal toxicity study, dermal irritation, which in this case appeared severe, was not scored; as a result, the actual degree of dermal irritation could not be evaluated. Finally, the protocol deviation stated that mortality and clinical signs of toxicity were monitored daily but did not state whether observations were also made at 1, 2, and 4 hours postdosing, as outlined in the materials and methods; this should be clarified. An LD_{50} value of >2 g/kg was identified for both male and female rabbits; this is the maximum dose required as the limit test by EPA Guidelines (1984).

   A quality assurance statement, signed and dated August 21, 1990, and a statement of compliance with good laboratory practices (GLPs), signed and dated August 21 and 23, 1990, were provided.

10. CBI APPENDIX: APPENDIX A: Materials and Methods, CBI pp. 6-8; APPENDIX B: Protocol Deviation, CBI p. 14.

APPENDIX A

Materials and Methods
(CBI pp. 6-8)

Azadirachtin Toxicology Review #008393

Page _____ is not included in this copy.

Pages 40 through 43 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action

X FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX B

Protocol Deviation
(CBI p. 14)

008393

EPA No.: 68D80056
DYNAMAC No.: 348-H
TASK No.: 3-48H
March 7, 1991

DATA EVALUATION RECORD

AZADIRACTIN (NPI 720)

Acute Inhalation Toxicity Study in Rats

STUDY IDENTIFICATION: Ledbetter, A. Acute inhalation toxicity study of NPI-720-F in rats. (Unpublished study No. L08270-L06-1 performed by IIT Research Institute, Chicago, IL, for Native Plants, Inc., Salt Lake City, UT; dated July 26, 1990.) MRID No. 416264-24.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: [signature]

Date: 3/6/91

045

1. CHEMICAL: Azadiractin; NPI-720-F; NPI 720.

2. TEST MATERIAL: The test material (lot No. 13) was described as a thick, brown viscous liquid. The percent purity of the material was not reported.

3. STUDY/ACTION TYPE: Acute inhalation study in rats.

4. STUDY IDENTIFICATION: Ledbetter, A. Acute inhalation toxicity study of NPI-720-F in rats. (Unpublished study No. L08270-L06-1 performed by IIT Research Institute, Chicago, IL, for Native Plants, Inc., Salt Lake City, UT; dated July 26, 1990.) MRID No. 416264-24.

5. REVIEWED BY:

Mary E. Cerny, M.S.
Principal Reviewer
Dynamac Corporation

Signature: Mary E Cerny
Date: March 6 '91

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: margaret brower
Date: march 6, 1991

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L. McLellan for
Date: 3-6-91

J. Thomas McClintock, Ph.D.
EPA Reviewer
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)

Signature:
Date:

Albin Kocialski, Ph.D.
EPA Section Head
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)

Signature: A Kocialski
Date: 3/14/91

7. CONCLUSIONS:

Core Classification: CORE ~~Minimum~~. This study meets the minimum requirements set forth under Guideline 81-3 (152B-12) for an acute inhalation study in rats. This study could be upgraded pending submission of individual animal data.

LC_{50}: >2.41 mg/L (males and females).

Toxicity Category: III.

8. SUMMARY: A group of five male and five female Sprague-Dawley rats (Charles River Laboratories, Portage, MI) were exposed via whole-body inhalation to an aerosol of the test material for 4 hours. Mean body weights at the time of exposure were 258 g (males) and 199 g (females). Control rats (i.e., animals exposed to only air) were not included in this study.

The test atmosphere was generated by a modified gravity-fed aerosol generator; to accommodate the viscosity of the test material, a venturi effect was created in which test material was drawn into an aerosol generator placed above a 0.5-m^3 stainless steel inhalation chamber. (Prior to conducting the actual inhalation study, a 4-hour preliminary test was conducted to verify that the desired test atmosphere could be maintained.) The flow rate of the test material was not reported; the flow rate of the carrier gas (compressed air) was 69 L/min. The aerosol concentration was analyzed gravimetrically 10 times during the 4-hour exposure; the aerosol analyzed was drawn from the breathing zone of the rats. Aerosol particle size was determined twice during the exposure period, using a Mercer Cascade Impactor.

Because of the density of the aerosol in the inhalation chamber, animals could not be observed during the exposure period. All animals were observed immediately after exposure, 1.5 hours after removal from the inhalation chamber, and at least once each day thereafter for 14 days for mortality and clinical signs of toxicity. Body weights were recorded on days 1, 8, and 15. Fourteen days after exposure, animals were killed, subjected to a gross necropsy, and discarded.

The mean aerosol concentration (± standard deviation) was 2.41 ± 0.15 mg NPI 720/mL; because of the viscosity of the test material, this was the maximum obtainable concentration under the conditions of the study. The average aerosol mass median aerodynamic diameter (MMAD) was 1.51 µm, with a geometric standard deviation of 1.83. All animals survived to study termination. All rats were covered with the test material at the end of the exposure period; animals were cleaned to prevent ingestion of azadiractin via grooming. Clinical signs

redness around the nose, discoloration around the mouth, and wet and discolored inguinal fur. Other clinical signs of toxicity included redness around the eyes, salivation, rales (two females only), nasal congestion, wheezing, and breathing through the mouth (one male only). All but one rat (sex not reported) appeared normal by the end of the observation period. Four males and one female lost weight (6 to 26 g) during the first week after exposure; all rats gained weight between day 8 and day 15 (study termination), although the body weight of one male remained below its prestudy level. No abnormal findings were noted at autopsy. Based on the results, the 4-hour LC_{50} for NPI in rats is >2.41 mg/L.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: The conduct and reporting of this study were adequate, but a few deficiencies were found. The study author did not report the percent purity of the test material, which is used to calculate the amount of active ingredient administered and which assists in determining whether contaminants may have contributed to the toxicity of the test material. Although the MMAD was reported, the distribution of aerodynamic diameters of the aerosol was not described. Diameter distribution indicates respirability of particles, i.e., ≤5 µm for rats, and can indicate homogeneity of the test atmosphere if measured over time. A reporting deficiency was the lack of time-course individual body weight and clinical data; text in the study report provided incomplete information on these parameters. Consequently, this data should be submitted to the Agency. Despite the viscosity of the test material, a consistent aerosol concentration was maintained during the 4-hour exposure period. On the basis of the results, the 4-hour LC_{50} for azadiractin in rats is >2.41 mg/L; although below the 5-mg/L limit test dose for an acute inhalation study (EPA Guidelines, 1984), the 2.41-mg/L level was the maximum dose possible under the conditions of the study.

   A quality assurance statement, signed and dated July 27, 1990, and a statement of compliance with good laboratory practices (GLPs), signed and dated July 26 and August 2, 1990, were provided.

10. CBI APPENDIX: APPENDIX A: Materials and Methods, CBI pp. 15-19; APPENDIX B: Protocol Deviation, CBI p. 20.

APPENDIX A

Materials and Methods
(CBI pp. 15-19)

Azadirachtin Toxicology Review #008393

Page _____ is not included in this copy.

Pages 50 through 54 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX B

Protocol Deviation
(CBI p. 20)

008393

PROTOCOL DEVIATION
Number 1

IITRI Project No.:L08270

Study Number: L06-1

Study Title: Acute Inhalation Toxicity Study of NPI-720-F in Rat:

Effective Date: 4/27/90

Nature and Reason of Deviation:

Section 8d. Test Article Storage: The test article was received o 4/27/90 and placed in a refrigerator in the origina plastic container. Refrigerating the test article has n effect on it and the reason for transferring the tes article to glass containers was to prevent the plascti containers from becoming brittle. The test article wa removed from the refrigerator approximately four hour prior to the start of the exposure on 4/30/90 and allowe to warm to room temperature. The test article was place in a glass reservoir during the exposure.

Approval:

Study Director: Allen Ledbetter Date: 6/6/9

Allen Ledbetter

EPA No.: 68D80056
DYNAMAC No.: 348-I
TASK No.: 3-48I
March 7, 1991

008393

DATA EVALUATION RECORD

AZADIRACTIN (NPI 720)

Primary Eye Irritation Study in Rabbits

STUDY IDENTIFICATION: Furedi-Machacek, E. M. Primary eye irritation study of NPI 720 in rabbits. (Unpublished study No. L08270-06, performed by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated July 1990.) MRID No. 416264-25.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: _____

Date: 3-11-91

057

1. CHEMICAL: Azadiractin; NPI 720.

2. TEST MATERIAL: The physical state and appearance, purity, and lot or batch number of the test material were not reported.

3. STUDY/ACTION TYPE: Primary eye irritation study in rabbits.

4. STUDY IDENTIFICATION: Furedi-Machacek, E. M. Primary eye irritation study of NPI 720 in rabbits. (Unpublished study No. L08270-06, performed by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated July 1990.) MRID No. 416264-25.

5. REVIEWED BY:

Mary E. Cerny, M.S.
Principal Reviewer
Dynamac Corporation
Signature: Mary E Cerny
Date: 3/6/91

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation
Signature: margaret Brower
Date: 3/6/91

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation
Signature: William L McClellan for
Date: 3-6-91

J. Thomas McClintock, Ph.D.
EPA Reviewer
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)
Signature:
Date:

Albin Kocialski, Ph.D.
EPA Section Head
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)
Signature: A. Kocialski
Date: 3/15/91

7. CONCLUSIONS:

Core Classification: CORE ~~Minimum~~ SUPPLEMENTARY. This study meets the minimum requirements set forth under Guideline 81-4 (152B-13) for a primary eye irritation study in rabbits. This study could be upgraded pending submission of individual animal data.

Primary Eye Irritation Index: 15.3/110 (1 hour postinstillation); 6.2/110 (24 hours); 0.3/110 (48 hours); 0/110 (72 hours). A ranking system based on this index was not provided.

Toxicity Category: III. Corneal involvement or irritation clearing in 7 days or less.

8. SUMMARY: Approximately 0.1 g of undiluted test material was placed into the conjunctival sac of the right eye of each of three male and three female New Zealand albino rabbits (Johnson Rabbit Ranch, Wilkinson, IN) that weighed between 2.38 and 2.82 kg at the time of dosing; the other eye was left untreated and served as the control for each animal. The lids of all treated eyes were closed for about 2 seconds following application of the article; it was not reported whether the eyes were washed after compound administration. (Prior to dosing, the rabbits' eyes were examined using fluorescein to eliminate animals with corneal lesions.) Eyes were examined at 1, 24, 48, and 72 hours after compound instillation; fluorescein and ultraviolet light were used for the 1-hour examination interval. Eye irritation was scored using the method of Draize, and a primary eye irritation index was calculated based on a maximum score of 110. Determination of whether the test material was an irritant was based on a set of evaluation criteria described in the Appendix of this report. All rabbits were observed daily for 3 days after dosing for mortality, morbidity, physical appearance, and behavior. Animals were killed 3 days after dosing and discarded.

No deaths occurred during the study. Irritation of the cornea and iris was minimal: slight iritis was observed in one male and one female at 1 hour after instillation of azadiractin, and slight corneal opacity was noted in another female at 24 hours. All animals had moderate to severe conjunctivitis at the 1-hour postinstillation observation; this condition lessened in severity within 24 hours and was essentially resolved within 48 hours after compound administration. The maximum eye irritation score -- 15.3/110 -- was recorded 1 hour after exposure; at 24, 48, and 72 hours, the scores were 6.2/110, 0.3/110, and 0/110, respectively. The study author concluded that azadiractin was a nonirritant to the eye.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: The conduct and reporting of this study were adequate, but a few deficiencies were found. The study author failed to describe the physical state, pH, and purity of the test material. The physical state of the test material can influence how the material is treated prior to application (e.g., grinding a solid to a powder before instillation) and how it is applied to the eye (e.g., apply undiluted; vehicle necessary). Review of the materials and methods section indicates that the test material was a solid. The pH can influence the degree of damage that may occur following exposure; the reaction observed suggests that the azadiractin formulation used was neither a strong acid nor a strong base. The percent purity is used to calculate the amount of active ingredient to which an animal is exposed; the purity can also indicate whether contaminants may have played a role in the toxicity of the test material. Finally, the study author did not provide individual body weights or any information on clinical signs of toxicity (which, presumably, were monitored, as described in the materials and methods section of the study). These data should be submitted to the Agency. On the basis of the study author's evaluation criteria, azadiractin was not an irritant to the eyes of rabbits; however, based on the toxicity category used to describe the results of this study, aza-diractin can be considered a slight to moderate eye irritant that caused moderate to severe conjunctivitis that was resolved within 3 days after exposure.

A quality assurance statement, signed and dated July 10, 1990, and a statement of compliance with good laboratory practices, signed and dated July 10 and 11, 1990, were included.

10. CBI APPENDIX: Materials and Methods, CBI pp. 6-8.

APPENDIX

Materials and Methods
(CBI pp. 6-8)

Azadirachtin Toxicology Review #008393

Page _____ is not included in this copy.

Pages 62 through 64 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

EPA No.: 68D80056
DYNAMAC No.: 348-J
TASK No.: 3-48J
March 7, 1991

DATA EVALUATION RECORD

AZADIRACTIN (NPI 720)

Primary Eye Irritation Study in Rabbits

STUDY IDENTIFICATION: Mega, W. M. Primary eye irritation testing of NPI-720-F/20-13 in rabbits. (Unpublished study No. L08289-04 performed by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated August 1990.) MRID No. 416264-26.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: [signature]
Date: 3-7-91

1. CHEMICAL: Azadiractin; NPI-720-F/20-13; NPI 720.

2. TEST MATERIAL: The test material was described as a brown liquid. The purity, pH, and lot or batch number of the material were not reported.

3. STUDY/ACTION TYPE: Primary eye irritation study in rabbits.

4. STUDY IDENTIFICATION: Mega, W. M. Primary eye irritation testing of NPI-720-F/20-13 in rabbits. (Unpublished study No. L08289-04 performed by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated August 1990.) MRID No. 416264-26.

5. REVIEWED BY:

Mary E. Cerny, M.S.
Principal Reviewer
Dynamac Corporation
Signature: Mary E Cerny
Date: 3/6/91

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation
Signature: margaret E Brower
Date: 3/6/91

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation
Signature: William L. McLellan for
Date: 3-6-91

J. Thomas McClintock, Ph.D.
EPA Reviewer
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)
Signature: J. Thomas McClintock
Date: 3/12/91

Albin Kocialski, Ph.D.
EPA Section Head
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)
Signature: A Kocialski
Date: 3/15/91

7. **CONCLUSIONS:**

Core Classification: CORE ~~Minimum~~ SUPPLEMENTARY. This study meets the minimum requirements set forth under Guideline 81-4 (152B-13) for a primary eye irritation study in rabbits.

Primary Eye Irritation Index: 42.5/110 at 48 hours (maximum); 13.8/110 at 7 days; 1.0/110 at 17 days; 0.7/110 at 21 days. A ranking system based on this index was not described.

Toxicity Category: II. Corneal involvement or irritation clearing in 8 to 21 days in 5/6 animals.

8. SUMMARY: A 0.1-mL aliquot of undiluted test material was placed into the conjunctival sac of the right eye of each of two male and four female New Zealand albino rabbits (Johnson Rabbit Ranch, Wilkinson, IN) weighing between 1.79 and 2.76 kg; the untreated left eye served as the control.

Lids of all treated eyes were held together for approximately 2 seconds following compound administration; it was not reported whether the eyes were rinsed after dosing. Prior to study initiation, the rabbits' eyes were examined using fluorescein and ultraviolet light to exclude animals with ocular lesions. Treated eyes were examined at 1, 24, 48, and 72 hours and at 4, 7, 10, 14, 17, 21, and 23 days postdosing; fluorescein and ultraviolet light aided in the 1-hour examination. Ocular irritation was scored using the method of Draize, and a primary eye irritation index was calculated based on a total maximum score of 110. Irritation potential of azadiractin was also assessed for the 24-, 48-, and 72-hour evaluations using a set of criteria described in the Appendix of this report. All rabbits were weighed on days 0 (i.e., before dosing), 7, and 14. In addition, animals were observed daily for the 23-day postexposure period for mortality, moribundity, and changes in physical appearance and behavior. Animals were killed on day 23 and discarded.

All rabbits survived the duration of the study. Weight ranges increased during the study; weights were between 1.79 and 2.76 kg on day 0, between 2.09 and 2.94 kg on day 7, and between 2.36 and 3.17 kg on day 14. No signs of systemic toxicity were reported, and the only clinical signs of toxicity were at the application site. Moderate to severe conjunctivitis was observed in all animals from 1 hour until 7 days after compound instillation; slight to moderate swelling and redness persisted through day 14 in two rabbits (male No. 418, female No. 422) and through day 23 in one male (animal No. 418). The cornea of all treated rats appeared normal at the 1-hour scoring. Within 24 hours, however, mild corneal opacity throughout the entire corneum was observed in all treated eyes; this condition was

resolved in all rabbits by day 17. Moderate to severe iritis was observed in one male rabbit (i.e., animal No. 418, described above) on days 2 through 10; pannus developed in this rabbit on day 3 and was present at study termination. Slight iritis was also observed in one female rabbit on day 10. The maximum mean eye irritation score was 42.5/110 on day 2; the mean irritation score dropped below 1.0/110 on day 21. The study author concluded that undiluted NPI-720-F/20-13 was an irritant to the eye.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: The conduct and reporting of this study were, in general, adequate; a few deficiencies were found. The study author did not report the purity of the test material, which is used to determine the amount of active ingredient administered and to assess whether contaminants may have contributed to the toxicity of the material. The study author also did not provide the pH of the test material, which can give an indication of the corrosive nature of a substance, particularly if that substance is a strong acid or base. Although not necessarily a deficiency, per se, the use of two males and four females, rather than three rabbits of each sex, resulted in a somewhat unbalanced assessment of the toxicity of azadiractin to the eye, particularly because one male (i.e., half of the male population in this study) seemed to be affected more severely than the other animals. Overall, the animals' weight ranges increased throughout the study; however, individual weights were not recorded, making it impossible to confirm that all animals gained weight consistently throughout the study. As noted by the study author, this formulation of azadiractin acted as an irritant to the eye, but with the exception of the one male described above, conjunctivitis and corneal involvement cleared within 21 days after compound administration.

   A quality assurance statement, signed and dated August 22, 1990, and a statement of compliance with good laboratory practices (GLPs), signed and dated August 22 and 23, 1990, were provided.

10. CBI APPENDIX: Materials and Methods, CBI pp. 6-9.

APPENDIX

Materials and Methods
(CBI pp. 6-9)

Azadirachtin Toxicology Review # 008393

Page _____ is not included in this copy.

Pages 70 through 73 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action

_X_ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

EPA No.: 68D80056
DYNAMAC No.: 348-K
TASK No.: 3-48K
March 7, 1991

008393

DATA EVALUATION RECORD

AZADIRACTIN (NPI 720)

Primary Dermal Irritation Study in Rabbits

STUDY IDENTIFICATION: Furedi-Machacek, E. M. Primary dermal irritation testing of NPI 720 in rabbits. (Unpublished study No. L08270-05 performed by IIT Research Institute, Chicago, IL, for-- NPI, Salt Lake City, UT; dated August 1990.) MRID No. 416264-27.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: [signature]

Date: 3/6/91

074

1. CHEMICAL: Azadiractin; NPI 720.

2. TEST MATERIAL: The test material was described as a gold-colored granular solid. The purity, pH, and lot or batch number of the test substance were not reported.

3. STUDY/ACTION TYPE: Primary dermal irritation study in rabbits.

4. STUDY IDENTIFICATION: Furedi-Machacek, E. M. Primary dermal irritation testing of NPI 720 in rabbits. (Unpublished study No. L08270-05 performed by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated August 1990.) MRID No. 416264-27.

5. REVIEWED BY:

Mary E. Cerny, M.S.
Principal Reviewer
Dynamac Corporation

Signature: Mary E Cerny
Date: March 6, 1991

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: margaret brower
Date: march 6, 1991

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L. McLellan for
Date: 3-6-91

J. Thomas McClintock, Ph.D.
EPA Reviewer
Reregistration Section
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(H-7509C)

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Date: [illegible]

Albin Kocialski, Ph.D.
EPA Section Head
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)

Signature: A Kocialski
Date: 3/15/91

7. CONCLUSIONS:

Core Classification: CORE Supplementary. This study was (152B-14) judged supplementary because (1) the effectiveness of the dry test material (rather than moistened material, as recommended by EPA) in producing the correct dermal effect is questioned, and (2) guidelines for examination of treated animals were not followed with respect to clinical signs of toxicity.

Primary Dermal Irritation Index: 0 (nonirritant).

Toxicity Category: IV. Mild or slight irritation (no irritation or slight erythema).

8. SUMMARY: At 24 hours before application of the test substance, fur was clipped from the backs of three male and three female New Zealand albino rabbits (Johnson Rabbit Ranch, Wilkinson, IN), exposing an area of approximately 240 cm^2. At the time of dosing, 0.5 g of undiluted test material was applied to each test site and then covered with a 2.5-inch by 2.5-inch adhesive dressing. The midsection of each animal was wrapped in a lint-free cloth and elastic adhesive bandage, which prevented removal of the test material while allowing animals to move about freely. All dressings and wrappings were removed 4 hours after compound application, and treated skin was subsequently rinsed with 0.9% saline to remove residual test material. Each application site was examined for dermal irritation (i.e., edema, erythema, and/or eschar formation) and corrosivity (i.e., ulceration and/or necrosis) at 30 to 60 minutes and 24, 48, and 72 hours after removal of the dressings. Dermal irritation was scored using the method of Draize, and a primary dermal irritation index was calculated by averaging the 24- and 72-hour dermal irritation scores for a maximum total score of 8. The test material was considered a (1) nonirritant if the primary dermal irritation index was 0.0; (2) negligible irritant if the index was >0.0 to 0.5; (3) mild irritant if the index was >0.5 to 2.0; (4) moderate irritant if the index was >2.0 to 5.0; and (5) severe irritant if the index was >5.0. Animals were weighed at study initiation and termination, and all rabbits were observed daily for 3 days for mortality.

No deaths occurred during the study. Weights of rabbits were between 3.15 and 3.45 kg on the day of dosing and between 3.27 and 3.56 kg at study termination. The irritation score was 0 (zero) for all rabbits at all examination times. The primary dermal irritation score was also 0 (zero). No signs of dermal corrosivity were observed in any animal. The study author concluded that NPI 720 was a nonirritant when applied to the skin of rabbits.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: Several deficiencies were found in this study. The pH and purity of the test material were not given. The pH can indicate the degree of corrosivity of a substance, particularly for strong acids and bases; since no dermal reaction to NPI 720 was reported, the pH of the test material is not essential to this study. The purity is used to determine the amount of active ingredient administered and to assess the degree to which contaminants may have contributed to the toxicity of the test material; unlike pH, the percent purity is required for all reports. The test substance was described as a granular solid; EPA Guidelines (1984) state that such materials should be moistened with water or another vehicle to ensure good contact with the skin. It is not clear whether application of the test material "as is" had an influence on the reported lack of reaction; the study reviewers believe the results may have been compromised because of this oversight. Animals were not observed for clinical or systemic signs of toxicity because of an inadvertent technician error (CBI p. 18); as noted by the study author, this protocol deviation probably did not significantly alter the results of the study. Nevertheless, the study author failed to follow EPA Guidelines (1984) regarding animal observation in a primary dermal irritation study. The study author also failed to provide individual animal data per EPA Guidelines (1984). The data indicate that dermally applied NPI 720 is a nonirritant in rabbits.

   A quality assurance statement, signed and dated August 3, 1990, and a statement of compliance with good laboratory practices, signed and dated August 3 and 9, 1990, were provided.

10. CBI APPENDIX: APPENDIX A: Materials and Methods, CBI pp. 6-8; APPENDIX B: Protocol Deviations, CBI pp. 15-18.

APPENDIX A

Materials and Methods
(CBI pp. 6-8)

Azadirachtin Toxicology Review # 008393

Page _____ is not included in this copy.

Pages 79 through 81 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action

X FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX B

Protocol Deviations
(CBI pp. 15-18)

Azadirachtin Toxicology Review # 008393

Page _____ is not included in this copy.

Pages 83 through 86 are not included in this copy.

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_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product quality control procedures.

_____ Identity of the source of product ingredients.

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_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action

__X__ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

008393

EPA No.: 68D80056
DYNAMAC No.: 348-L
TASK No.: 3-48L
March 7, 1991

DATA EVALUATION RECORD

AZADIRACTIN (NPI 720)

Primary Dermal Irritation Study in Rabbits

STUDY IDENTIFICATION: Mega, W. M. Primary dermal irritation test of NPI-720-F/20-13 in rabbits. (Unpublished study No. L08-89-03 performed by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated August 1990.) MRID No. 416264-28.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: _____

Date: _____

087

1. CHEMICAL: Azadiractin; NPI-720-F/20-13; NPI 720.

2. TEST MATERIAL: The test material was described as a brown liquid. The purity, pH, and lot or batch number of the test substance were not reported.

3. STUDY/ACTION TYPE: Primary dermal irritation study in rabbits.

4. STUDY IDENTIFICATION: Mega, W. M. Primary dermal irritation test of NPI-720-F/20-13 in rabbits. (Unpublished study No. L08289-03 performed by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated August 1990.) MRID No. 416264-28.

5. REVIEWED BY:

Mary E. Cerny, M.S.
Principal Reviewer
Dynamac Corporation

Signature: [signature]
Date: 3/6/91

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: 3/6/91

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L. McLellan for
Date: 3-6-91

J. Thomas McClintock, Ph.D.
EPA Reviewer
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)

Signature: [signature]
Date: 3/[illegible]/91

Albin Kocialski, Ph.D.
EPA Section Head
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)

Signature: A Kocialski
Date: 3/15/91

CTM 008393

7. CONCLUSIONS:

Core Classification: CORE ~~Minimum~~ SUPPLEMENTARY. This study meets the minimum requirements under Guideline 81-5 (152B-14) for a primary dermal irritation study in rabbits. This study could be upgraded pending submission of individual animal data.

Primary Dermal Irritation Index: 1.8, mild irritant.

Toxicity Category: II. Chosen by the study reviewers because the moderate erythema at 72 hours developed into severe erythema by day 7 in half of the test animals.

8. SUMMARY: A 0.5-mL aliquot of undiluted test material was applied for 4 hours to the clipped back of three male and three female New Zealand albino rabbits (Johnson Rabbit Ranch, Wilkinson, IN) weighing between 2.28 and 2.66 kg. Approximately 24 hours before study initiation, fur was clipped from the back, exposing an area of 240 cm^2. Azadiractin was applied to the right side of each test site and covered with a 2.5- by 2.5-inch adhesive dressing. The midsection of each rabbit was then wrapped in a lint-free cloth and an elastic adhesive bandage, which prevented removal of the test substance while allowing rabbits to move about freely. All wrappings were removed 4 hours after application, and the test sites were subsequently rinsed with 0.9% saline. Treated skin was examined for dermal irritation (i.e., edema, erythema, and/or eschar formation) and corrosivity (i.e., ulceration and/or necrosis) at 30 to 60 minutes and 1, 2, 3, 7, 10, and 14 days after exposures ended. Dermal irritation was scored according to the Draize method, and a mean primary dermal irritation index was calculated by taking the average of the 24- and 72-hour irritation scores, for a maximum of 8.0. The dermal irritation potential of azadiractin was assessed as follows: the test article was considered a nonirritant if the primary dermal irritation score was 0.0; a negligible irritant, >0.0 to 0.5; a mild irritant, >0.5 to 2.0; a moderate irritant, >2.0 to 5.0; a severe irritant, >5.0. Animals were observed daily for 14 days for mortality. Body weights were recorded on days 1 (day of dosing), 7, and 14 (study termination). Rabbits were killed at the end of the 14-day observation period and discarded.

All rabbits survived, and animals gained weight consistently throughout the study. Very slight edema was present in one animal immediately after exposure was terminated and in three rabbits at both the 48- and 72-hour postapplication observations. Very slight erythema, observed in all six rabbits within 24 hours, developed into well-defined to severe erythema on days 2 and 3; the condition was resolved by day 7 for three rabbits but worsened in three (severe erythema with

possible slight eschar formation). All signs of erythema and eschar were resolved by day 14. No signs of dermal corrosivity were seen in any animal. The mean irritation score (i.e., mean edema score plus mean erythema score) for all time points was 1.4, and the primary dermal irritation index (as described in the previous paragraph) was 1.8. The study author concluded that azadiractin liquid was a mild dermal irritant in rabbits.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: The conduct and reporting of this study were, in general, adequate, although a few deficiencies were found. The study author did not report the purity or pH of the test material. Percent purity is used to calculate the amount of active ingredient administered and is used to determine whether contaminants may have contributed to the toxicity of the test material. Although not required, reporting of the pH may indicate the potential dermal corrosivity of a substance, particularly if that substance is a strong acid or base. Two other deficiencies in this report were the failure to report individual dermal irritation/corrosivity data and the failure to monitor animals daily for clinical/systemic signs of toxicity; without this information, it is difficult to evaluate the progression and regression of the responses seen, particularly by sex. These data should be provided to the Agency. Although the study author rated the test azadiractin as a mild irritant, the study reviewers have rated this material as a moderate irritant because of the presence of severe erythema in three rabbits on day 7 postapplication.

   A quality assurance statement, signed and dated August 22, 1990, and a statement of compliance with good laboratory practices, signed and dated August 21 and 23, 1990, were provided.

10. CBI APPENDIX: Materials and Methods, CBI pp. 6-8.

APPENDIX

Materials and Methods
(CBI pp. 6-8)

Azadirachtin Toxicology Review #008393

Page _____ is not included in this copy.

Pages 92 through 94 are not included in this copy.

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_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action

X FIFRA registration data.

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EPA No.: 68D80056
DYNAMAC No.: 348-A
TASK No.: 3-48A
March 7, 1991

008393

DATA EVALUATION RECORD

AZADIRACTIN (NPI 720)

Dermal Sensitization Study in Guinea Pigs (Buehler Test)

STUDY IDENTIFICATION: Sherwood, R. L. Dermal sensitization study of NPI 720 in guinea pigs using the modified Buehler method. (Unpublished study No. L08257 performed by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated March 6, 1990.) MRID No. 416264-19.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: [signature]

Date: 3/6/91

05

008393

1. CHEMICAL: Azadiractin; NPI 720.

2. TEST MATERIAL: The test material (lot No. 10) was described as a tan crystalline powder. The purity of this substance was not reported.

3. STUDY/ACTION TYPE: Dermal sensitization study (152B-15) in guinea pigs (Buehler test).

4. STUDY IDENTIFICATION: Sherwood, R. L. Dermal sensitization study of NPI 720 in guinea pigs using the modified Buehler method. (Unpublished study No. L08257 performed by IIT Research Institute, Chicago, IL, for NPI, Salt Lake City, UT; dated March 6, 1990.) MRID No. 416264-19.

5. REVIEWED BY:

Mary E. Cerny, M.S.
Principal Reviewer
Dynamac Corporation

Signature: Mary E. Cerny
Date: 3/6/91

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret Brower
Date: 3/6/91

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L. McClellan for
Date: 8-6-91

J. Thomas McClintock, Ph.D.
EPA Reviewer
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)

Signature: J. Thomas McClintock
Date: 3/21/91

Albin Kocialski, Ph.D.
EPA Section Head
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)

Signature: A. Kocialski
Date: 3/12/91

7. CONCLUSIONS:

Core Classification: CORE Supplementary. This study was judged supplementary because of the following deletion from and modification to the standard Buehler test: (1) no positive controls were used; and (2) inappropriate methods were used to rank the sensitization potential of azadiractin.

Skin Sensitization Potential: A 25% (w/v) solution of NPI 720 in ethanol elicited a slight dermal reaction in only 2/10 male guinea pigs at 24 hours after challenge and is considered to be a weak dermal sensitizer in these animals.

8. SUMMARY: The results of preliminary range-finding tests, conducted to choose dose levels for the induction and challenge phases of the definitive study, were reported by the study author (CBI p. A-3); a description of these tests was not provided, however. In the preliminary tests, animals (presumably male guinea pigs) received dermal applications of 5, 10, 25, or 50% (w/v) NPI 720 in ethanol or 0.05, 0.1, 0.5, or 1.0% (w/v) NPI 720 in ethanol. Based on the results, a 25% solution of the test material was chosen for the induction phase of the definitive study; a 0.5% solution, intended to produce a negative or minimal erythema reaction in control animals, was used in the challenge phase.

At 1 day prior to test material application in the definitive study, the backs of groups of 10 adult male Hartley guinea pigs (Murphy Breeding Laboratories, Plainfield, IN) were clipped free of hair. For the induction phase of the study, 0.3-mL doses of 25% NPI 720 in ethanol (w/v) were applied to the upper-left quadrant of the back once/week for 3 weeks. For each application, the test material was held in place for 6 hours with an elastic adhesive bandage wrapped around the midsection of each animal. A group of 10 control guinea pigs was handled similarly with the exception that they were exposed only to the vehicle (ethanol). Two weeks after application of the third induction dose, a (1) challenge dose of 0.3 mL of a 0.5% (w/v) formulation of NPI 720 in ethanol (treated animals) or (2) a 0.3-mL dose of ethanol (control animals) was applied to the lower-left quadrant of the back. No positive controls were used. The test material or vehicle was held in place for 6 hours as described above. Test sites were scored for erythema, according to the Draize method, at approximately 24 and 48 hours after application of the first induction dose and the challenge dose. Scoring was facilitated via depilitation with Neet Hair Remover at 2 hours prior to the 24-hour postapplication examinations. A reaction with a Draize erythema score of 2 or greater was considered positive. A two-factor log-linear model was used to assess the effect of

treatment (treated vs. control) and time of scoring (24 vs. 48 hr) on reaction ($p \leq 0.05$). All animals were observed daily for mortality and signs of morbidity. Body weights were recorded weekly.

No deaths occurred, and mean body weight data indicated that treated and control guinea pigs gained similar amounts of weight during the study. During the induction phase, very slight to well-defined erythema was observed in all treated animals at the 24-hour scoring and in 8/10 test animals at the 48-hour scoring. However, a positive erythema reaction (i.e., a score ≥ 2) was observed in only two of the previously treated guinea pigs during the challenge phase of the study; very slight erythema (i.e., a score of 1) was observed in 6/10 and 3/10 control guinea pigs at the 24- and 48-hour scorings, respectively. Statistical analysis of dermal irritation scores indicated that neither treatment nor time of scoring had a significant ($p \leq 0.05$) effect on erythema reaction. The study author concluded that NPI 720 did not induce dermal sensitization in guinea pigs.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: There were several technical problems with this study. Only males were used, and the study author did not explain why females were excluded; however, because the use of both males and females is not necessarily required in the Buehler test, the omission of an explanation may be considered a reporting, rather than a technical, deficiency. No positive controls were used. The use of a positive control (e.g., DNCB) is an integral part of the Buehler test; it demonstrates that the test is working properly. In another significant deviation from the Buehler test, the study author failed to use the incidence of severity (i.e., the percent of challenged animals exhibiting at least mild erythema, as compared with vehicle or negative controls) for ranking the sensitization potential (e.g., as weak, mild, moderate) of the test material. This ranking is usually based on a response/reaction scale of 1 to 3 rather than on the Draize scale, which ranks dermal reactions on a scale of 0 to 4. In addition, in contrast with the standard protocol for the Buehler test, a 3-week, rather than a 2-week, induction phase was followed. The purity of the test material, was not reported; purity is used to determine the amount of active ingredient administered and to assess whether contaminants may have contributed to the toxicity of the test substance. Another minor deficiency was the reporting of only mean, rather than individual, body weights. The data indicate that the formulation of azadiractin used in this study was a weak dermal sensitizer in male guinea pigs.

A quality assurance and GLP (good laboratory practice) statement, signed and dated March 6 and 26, 1990, was included.

10. CBI APPENDIX: Materials and Methods, CBI pp. 4-6.

APPENDIX

Materials and Methods
(CBI pp. 4-6)

Azadirachtin Toxicology Review #008393

Page _____ is not included in this copy.

Pages 101 through 103 are not included in this copy.

The material not included contains the following type of information:

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_____ Description of product quality control procedures.

_____ Identity of the source of product ingredients.

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_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action

_X_ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

008393

EPA No.: 68D80056
DYNAMAC No.: 348-C
TASK No.: 3-48C
February 28, 1991

DATA EVALUATION RECORD

AZADIRACHTIN

Mutagenicity--Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 2/27/91

008393

Guideline Series 84: **MUTAGENICITY**
EPA No.: 68D80056
DYNAMAC No.: 348-C
TASK No.: 3-48C
February 28, 1991

DATA EVALUATION RECORD

AZADIRACHTIN

Mutagenicity--Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 2-27-91

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 2-27-91

APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L. McKellen for
Date: 2-27-91

J. Thomas McClintock, Ph.D.
EPA Reviewer
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(H-7509C)

Signature: J. Thomas McClintock
Date: 3/15/91

Albin Kocialski, Ph.D.
EPA Section Head
Reregistration Section
Science Analysis and
Coordination Branch
(H-7509C)

Signature: A. Kocialski
Date: 3/15/91

DATA EVALUATION RECORD

Tox. Chem. No.:
EPA File Symbol:

CHEMICAL: Azadirachtin.

STUDY TYPE: Salmonella/mammalian activation gene mutation assay.

ACCESSION OR MRID NUMBER: 416264-21.

SYNONYM/CAS NUMBER: NPI-720.

SPONSOR: NPI, Salt Lake City, UT.

TESTING FACILITY: IIT Research Institute, Chicago, IL.

TITLE OF REPORT: Ames Salmonella Mammalian Microsomal Test of Test Article No. NPI-720.

AUTHOR: P.W. Barbera.

STUDY NUMBER: L08270-07.

REPORT ISSUED: May 30, 1990.

CONCLUSIONS - Executive Summary: NPI-720 was evaluated for the potential to cause gene mutations in the Salmonella typhimurium/mammalian microsome plate incorporation mutagenicity assay. The five [illegible]activated or five S9-activated doses, which ranged from 5 to [illegible]000 µg/plate, were neither cytotoxic nor mutagenic in S. typhimurium TA1535, TA1537, TA1538, TA98, or TA100 in a well-controlled study.

[illegible]hough the assay was properly conducted and NPI-720 was tested to [illegible] adequate level with no evidence of a mutagenic effect, the study [illegible] incomplete. No information on the purity or stability of the test material was furnished. Additionally, analytical data to verify actual concentrations used in the assay were not provided. We conclude, therefore, that the study does not fully satisfy Guideline requirements for genetic effects, Category I, Gene Mutations.

CORE SUPPLEMENTARY JTM

Study Classification: ~~Unacceptable~~. The study can be upgraded pending submission of the information (test material).

A. MATERIALS:

1. Test Material:

Name:	NPI-720 (Azadirachtin as the active ingredient).
Description:	Yellowish-brown granular crystal.
Batch/Lot No.:	13.
Purity:	Not reported.
Contaminants:	The report stated that the test material contained [redacted] was not reported.
Solvent used:	Dimethylsulfoxide (DMSO).
Other comments:	The test material was stored at room temperature, protected from light. NPI-720 was found to form a medium-brownish solution at 50 mg/mL in DMSO. Solutions of the test material were prepared immediately prior to use. No information on test material stability was provided.

2. Control Materials:
Negative: Culture medium
Solvent/final concentration: DMSO/100 µL/plate
Positive: Nonactivation:
Sodium azide 10 µg/plate TA100, TA1535
2-Nitrofluorene 10 µg/plate TA98, TA1538
9-Aminoacridine 100 µg/plate TA1537
Other:

Activation:
2-Aminoanthracene (2-anthramine) 10 µg/plate all strains.

3. Activation: S9 derived from male Sprague-Dawley

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The S9 liver homogenate (Lot 2008) was purchased from Organon Teknika-Cappel, West Chester, PA, and was characterized for its ability to metabolize benzo[a]pyrene to a mutagenic form using S. typhimurium strains TA98 and TA100. In addition, analytical determinations indicated that the S9 liver homogenate contained 38.5 mg protein/mL; alkoxyphenoxazone dealkylase activity was reported to be 5419 pmol/min/mg protein.

The S9 mix composition was as follows:

Concentration/Component	Volume
4 µM NADP/5 µM glucose-6-phosphate	0.45 mL
8 µM MgCl/33 µM KCl/100 µM sodium phosphate buffer (pH 7.4)	0.45 mL
S9 homogenate	0.10 mL

4. Test Organism Used: S. typhimurium strains

_____ TA97	x TA98	x TA100	_____ TA102	_____ TA104
x TA1535	x TA1537	x TA1538; list any others:		

5. Test Compound Concentrations Used:

a. Preliminary cytotoxicity assay: Five concentrations (5, 50, 500, 1000, and 5000 µg/plate) were evaluated in all tester strains in both the presence and absence of

S9 activation. Single plates were prepared per dose per strain per condition.

b. Mutation assay: As above for the preliminary cytotoxicity assay with the exception that triplicate plates were prepared per dose per strain per condition, and negative, solvent, and positive controls were included.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay: ___x___ Standard plate test
_____ Pre-incubation (__) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (describe).

a. Protocol:

1) Plating procedures: In general, similar procedures were used for the preliminary cytotoxicity and the mutation assays. To tubes containing 2-mL volumes of molten top agar, 100 µL of a 16-hour broth culture of the appropriate tester strain, 100 µL of the appropriate test material dose, solvent, or positive controls, and 0.5 mL of phosphate buffer were added. For the S9-activated test, 0.5 mL of the S9 cofactor mix replaced the 0.5 mL of the phosphate buffer used in the nonactivated series; tester strains and test and control solutions were added as described. The contents of the tubes were mixed, poured over Vogel-Bonner minimal medium E, and incubated at 37°C for 2 days. At the end of incubation, plates were scored for revertant colonies; means and standard deviations were determined for the mutation assay.

2) Sterility controls: Sterility tests were performed on the test material, S9 mix components, top agar, minimal medium, phosphate buffer, and the solvent as described for the mutation assay.

b. Evaluation criteria:

1) Assay validity: The assay was considered valid if the following criteria were met: (1) there was no evidence of microbial contamination; (2) the

the spontaneous revertants and the solvent control values of each strain fell within the reporting laboratory's expected ranges; and (3) the number of revertants for the nonactivated and S9-activated positive controls were within the expected range of the reporting laboratory.

2) Positive response: The test material was considered positive if a ≥2-fold increase in mean revertant colonies of any tester strain at one or more doses was observed and if the increase was accompanied by a dose-response.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: NPI-720 was not cytotoxic at any assayed level (5, 50, 500, 1000, or 5000 ug/plate +/-S9) in any tester strain. In addition, there was no indication that the test material was insoluble at any concentration. Based on these results, the doses evaluated in the preliminary cytotoxicity assay were tested in the mutation assay.

2. Mutation Assay: Representative results from the mutation assay are presented in Table 1. In agreement with the preliminary assay findings, NPI-720 was not cytotoxic at any dose with or without S9 activation. Similarly, there was no evidence of a mutagenic response in any tester strain at any dose level either in the presence or absence of S9 acti- vation.

   In contrast to the uniformly negative test material results, all strains responded to the appropriate nonactivated or S9-activated positive controls.

   Based on the overall findings, the study authors concluded that NPI-720 was not mutagenic in this microbial test system.

D. REVIEWER'S DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and that the study authors interpreted the data correctly. NPI-720 was tested up to an acceptable high dose for soluble noncytotoxic compounds, and did not induce a mutagenic response under nonactivated or S9-activated conditions.

TABLE 1. Representative Results of the Salmonella typhimurium Mutagenicity Assay with NPI-720

Substance	S9 Activation	Dose/plate	Revertants per Plate of Bacterial Tester Strain[a]				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
Culture medium	-	--	14 ± 1.5	7 ± 0.6	21 ± 2.1	20 ± 2.0	157 ± 7.2
	+	--	15 ± 1.2	9 ± 1.5	22 ± 2.0	21 ± 1.7	175 ± 7.8
Solvent Control							
Dimethylsulfoxide	-	100 µL	14 ± 1.0	7 ± 1.2	20 ± 2.0	17 ± 1.0	161 ± 12.7
	+	100 µL	15 ± 1.0	8 ± 1.5	22 ± 3.0	20 ± 2.1	178 ± 7.8
Positive Control							
Sodium azide	-	10 µg	957 ± 56.9	--	--	--	940 ± 59.9
2-Nitrofluorene	-	10 µg	--	--	649 ± 44.7	594 ± 34.1	--
9-Aminoacridine	-	100 µg	--	2159 ± 182.7	--	--	--
2-Anthramine	+	10 µg	386 ± 39.9	780 ± 14.2	1833 ± 94.5	1726 ± 76.2	1950 ± 151.2
Test Material							
NPI-720	-	5000 µg[b]	16 ± 1.5	7 ± 1.2	22 ± 3.8	18 ± 2.0	178 ± 10.3
	+	5000 µg[b]	23 ± 4.5	8 ± 1.5	23 ± 2.0	22 ± 1.5	186 ± 9.8

[a] Means and standard deviations of counts from triplicate plates.

[b] Results for lower doses (5, 50, 500, and 1000 µg/plate +/-S9) did not suggest a mutagenic effect.

We further assess that the presence of [redacted] as a contaminant did not interfere with the negative conclusion or outcome of the study.

Although the results clearly showed that NPI-720 was not mutagenic in S. typhimurium, the study is incomplete. Critical information on the purity and stability of the test material was missing. In addition, analytical data to support actual concentrations used in the assay were not reported. The study is, therefore, classified as unacceptable, but can be upgraded pending submission of the information (test material).

E. QUALITY ASSURANCE MEASURES: A quality assurance statement was signed and dated May 30, 1990.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 8-14; Appendix B, Protocol, CBI pp. 23-28.

Manufacturing Process Information is not Included

APPENDIX A

Materials and Methods
(CBI pp. 8-14)

Azadirachtin Toxicology Review #008393

Page _____ is not included in this copy.

Pages 114 through 120 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action

__X__ FIFRA registration data.

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APPENDIX B

Protocol
(CBI pp. 23-28)