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

CASWELL FILE

NOV 18 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: SAB Review of Acute Mammalian Toxicology Studies to Support the Registration of Neem Concentrate, a Biochemical Pesticide Containing Azadirachtin as the Technical Grade Active Ingredient (DP Barcode No.: D188533; Submission No.: S435999; I.D. No.: 011688-T; MRID Nos.: 425383-02 through -06)

TO: Willie Nelson/Phil Hutton (PM-18)
Insecticide-Rodenticide Branch
Registration Division (7505C)

FROM: J. Thomas McClintock, Ph.D., Microbiologist
Biological Pesticide Section
Science Analysis Branch
Health Effects Division (7509C)

JTMC 11/17/93

THROUGH: Roy D. Sjoblad, Ph.D., Section Head
Biological Pesticide Section
Science Analysis Branch
Health Effects Division (7509C)

RDS 11/17/93

ACTION REQUESTED: W. R. Grace and Company has submitted a registration application for Neem Concentrate TGAI, a biochemical product containing azadirachtin as the active ingredient. The product is intended for use as an insecticide. The acute mammalian toxicology studies, submitted by the registrant to support the application, were reviewed by the Oak Ridge National Laboratory. The Science Analysis Branch (SAB) of the Health Effects Division (HED) has performed a secondary review and has summarized the results below.

STUDY SUMMARIES:

152B-10. Acute Oral Toxicity Study in Rats (MRID No.: 425383-02). The data submitted by the registrant supports the conclusion that the acute oral LD_{50} of Neem Concentrate TGAI (4.5% azadirachtin) was greater than 5000 mg/kg in rats. All animals survived the study with the only noted clinical signs of toxicity were loss of abdominal and inguinal hair in one male rat. Upon necropsy the same rat had dark red mottled lungs.

Classification. CORE Guideline. Toxicity Category IV.

152B-11. Acute Dermal Toxicity Study in Rabbits (MRID No.: 425383-03). Using a single application/dose, the acute dermal LD_{50} of Neem Concentrate TGAI (4.5% azadirachtin) was determined to be greater than 2000 mg/kg in male and female rabbits. All treated animals gained weight during the course of the study. Slight to moderate erythema and edema were observed in all rabbits by Day 2 but was resolved by Day 10. One male and 1 female rabbit showed desquamation which was resolved by Day 11 of the study. Fecal staining and soft stools were observed in several rabbits. All signs of toxicity were resolved by Day 13.

Classification. CORE Guideline. Toxicity Category III.

152B-12. Acute Inhalation Toxicity Study. This study, which is required to satisfy the requirements under Series 152B, was not submitted or addressed by the registrant. Consequently, this data requirement remains outstanding. The registrant should either submit data to support this study or submit a waiver request using the appropriate scientific rationale as to the reason why such a study may not be warranted.

152B-13. Primary Eye Irritation Study in Rabbits (MRID No.: 425383-04). Although resolved by Day 7 of the study, the test material (0.1 ml of Neem Concentrate TGAI/4.5% azadirachtin) induced corneal opacity in 1 out of 6 eyes at 24 hr (Draize Group Mean Irritation Score - 11.17). Iritis was observed in 4 out of 6 rabbits within 1 hr post-instillation (Draize Group Irritation Score - 11.0) which was resolved within 3 days (Draize Group[Irritation Score - 5.33). Conjunctivitis was also observed in 6 out of 6 treated eyes but was resolved in all but 1 rabbit by Day 7 (Draize Group Irritation Score - 0.67) of the study.

Classification. CORE Guideline. Toxicity Category II.

152B-14. Primary Dermal Irritation Study in Rabbits (MRID No.: 425383-05). The test material (0.5 ml of Neem Concentrate TGAI/4.5% azadirachtin) produced very slight to well-defined erythema and very slight to no edema in 6 out of 6 test sites at 1 hr post-treatment. All dermal irritation was resolved by 72 hr. The test material was considered to be a slight irritant (Primary Irritation Index of 1.04).

Classification. CORE Guideline. Toxicity Category IV.

152B-15. Dermal Sensitization Study in Guinea Pigs (MRID No.:425383-06). Using the Buehler test to determine potential dermal sensitization, the test material did not induce delayed contact hypersensitivity in guinea pigs following induction (40% v/v and 100% v/v) and challenge (100% v/v). The irritation severity index was 0.0 (out of a possible 3) for 17/20 guinea pigs; whereas 3/20 treated animals had scores of 0.5 (out of a possible 3).

Classification. CORE Guideline. Not a dermal sensitizer.

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

152B-17. Mutagenicity Assays. The studies to detect potential genotoxicity (Ames Assay and Gene Mutation Assay/Mouse Lymphoma cells), are part of a separate package and are currently in review.

152B-18 - Immunotoxicity, 152B-20 - 90-Day Feeding (1 species) and 152B-23 - Developmental Toxicity Study (1 species). These studies were NOT addressed in this submission. The registrant must submit data to support the registration application or submit waiver requests with the appropriate scientific rationale as to why these studies would not be required for this product under the proposed use pattern.

EPA Reviewer:
Review Section

Signature: [signature]
Date: 11/2/93

EPA Mutagenicity Secondary Reviewer:
Review Section II, Toxicology Branch II

Signature: _____
Date: _____

Oak Ridge National Laboratory Reviewer:
Harold T. Borges, Ph.D., MT(ASCP)
Biomedical and Environmental Information Analysis Section
Health and Safety Research Division

Signature: _____
Date: _____

Oak Ridge National Laboratory, Secondary Reviewer:
Robert H. Ross, M.S., Group Leader
Biomedical and Environmental Information Analysis Section
Health and Safety Research Division

Signature: _____
Date: _____

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral Toxicity Study (81-1)

EPA IDENTIFICATION NUMBERS:

Tox. Chem. Number:
MRID Number: 425383-02

TEST MATERIAL: Neem Concentrate TGAI

SYNONYMS: None known or reported

SPONSOR: W.R. Grace and Company-Conn., Columbia, MD

STUDY NUMBER: SLS 3268.1

TESTING FACILITY: Springborn Laboratories, Inc., Spencerville, OH

TITLE OF REPORT: Acute Oral Toxicity Study in Rats with Neem Concentrate TGAI, Limit Test

AUTHOR: R.E. Rush, B.A.

STUDY COMPLETED: 10/9/92

CONCLUSION: The acute oral LD_{50} of Neem Concentrate TGAI was found to be greater than 5000 mg/kg in rats.

TOXICITY CATEGORY: IV

CLASSIFICATION: Core ~~Minimal~~. Evidence that the necropsy results were reviewed by a pathologist should be provided.

A. MATERIALS

1. Test Material

Test material: Neem Concentrate, TGAI
Receipt: 3/18/92
Purity of material: 4.5% Azadirachtin, [redacted] other neem solids
Physical description: Brown, opaque viscous liquid
Active Ingredient: Azadirachtin
Inactive Ingredients: [redacted] and other neem solids
Lot number: 3/3/92
pH: 4.66 (measured at a 160/1 dilution in water)
Stability: Not reported. Springborn Laboratories, Inc., Study Number 3268.17, A Teratology Study in Rats with Neem Concentrate TGAI, lists the stability of the compound as greater than one year at 28°C.

2. Controls

Materials: Not needed.
Animals: Not reported.

3. Test Animals

Species: Rats
Strain: Sprague Dawley
Source: Charles River Laboratories, Inc., Portage, MI
Receipt Date: 5/21/92
Sex: Male and female
Numbers: 5 males and 5 females
Housing: The rats were ear tagged for identification, housed individually in suspended stainless steel cages, acclimated for a minimum of five days, and adjusted to a 12-hour light/dark cycle. The animal room temperature was controlled at 61-70°F with a relative humidity of 50 ± 10% during the study.
Age: Young adult
Weight: Male: 227-234 g; Female: 248-266 g
Feeding: Purina Certified Rodent Chow and fresh tap water were provided *ad libitum*.
Assignment: Because this was a limit test, five male and five female rats meeting acceptable body weight criteria were used for the one dose tested.
Location of raw data: All original data, specimens, and reports are archived at Springborn Laboratories, Inc., under Study Number 3268.1.

4. Exposure

Route of administration: Gavage
Dose level: 5000 mg/kg

INERT INGREDIENT INFORMATION IS NOT INCLUDED

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B. TEST PERFORMANCE

Neem Concentrate TGAI was administered to five male and five female rats as a single 5000 mg/kg gavage dose. Prior to dosing on Day 1, the animals were weighed and the administered dose calculated using a volume of 4.90 ml/kg body weight.

The rats were observed frequently for abnormal clinical signs of toxicity on the day of dosing and once daily thereafter throughout the duration of the study. Mortality checks were done twice daily. Body weights were recorded on Day -1, Day 1 (day of dosing), Day 8 and Day 15 (date of necropsy). Mean and standard deviations of body weights for rats included in the study were calculated using a MicroVax 3100. On day 15, the animals were asphyxiated with CO_2 and necropsied.

C. RESULTS AND STUDY AUTHOR'S CONCLUSIONS

One male rat had hair loss on the abdominal and left inguinal regions from day 3 through day 15 and at necropsy had dark red mottled lungs. No other clinical or pathological signs of toxicity were observed in any of the other treated rats. Body weight and mortality were unaffected by Neem Concentrate TGAI treatment.

Because no mortalities were observed in any of the rats during the 14 day observation period, the author concluded that under the conditions of the study, the acute dermal LD_{50} of Neem Concentrate TGAI was greater than 5000 mg/kg. oral LDO

D. REVIEWER'S COMMENTS

No rats died during the study and the only clinical signs of toxicity were loss of abdominal and inguinal hair in one male rat. The same rat had dark red mottled lungs at necropsy. No other rats developed clinical or pathological signs of Neem Concentrate TGAI toxicity during the study period. Based on this information, the author of the study correctly interpreted the results of the acute oral limit test. The greater than 5000 mg/kg acute oral LD_{50} of Neem Concentrate TGAI corresponds to Toxicity Category IV.

A discrepancy in the density of the test article was found by Springborn Laboratories, Inc. As measured by the laboratory, the density of the Neem Concentrate TGAI supplied by the sponsor was 1.02 g/ml. The sponsor had previously reported the density of the test article as 0.96 g/ml. After discussion with the sponsor, the animals were dosed according to the density obtained by Springborn Laboratories, Inc. The discrepancy in the density of the test compound between the sponsor and the testing laboratory is minor and likely does not impact the results, but may be indicative of evaporation of the test compound at some time prior to application. Additionally, it is not apparent whether the animal necropsies were done or reviewed by the consulting pathologist. The initials on the necropsy reports do not match any listed in Section V - Springborn Personnel Responsibilities - found on page 22 of the report. At a minimum, the necropsy results should be reviewed by the pathologist since "Scheduled necropsies shall be performed under the direct supervision of a qualified pathologist" (Pesticide Assessment Guidelines - Subdivision F Hazard Evaluation - Human & Domestic Animals - Revised Edition, U.S. EPA, PB86-108958, Nov. 1984, p. 26). Apart from these exceptions, the report was well written and all pertinent data associated with the study were provided.

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E. COMPLIANCE

A signed and dated Quality Assurance Unit Statement was provided.
A signed and dated Good Laboratory Practice (GLP) statement was provided.

F. CBI APPENDIX

None presented.

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EPA Reviewer:
Review Section

Signature: [signature]
Date: 11/1/93

EPA Mutagenicity Secondary Reviewer:
Review Section II, Toxicology Branch II

Signature: _____
Date: _____

Oak Ridge National Laboratory Reviewer:
Harold T. Borges, Ph.D., MT(ASCP)
Biomedical and Environmental Information Analysis Section
Health and Safety Research Division

Signature: _____
Date: _____

Oak Ridge National Laboratory, Secondary Reviewer:
Robert H. Ross, M.S., Group Leader
Biomedical and Environmental Information Analysis Section
Health and Safety Research Division

Signature: _____
Date: _____

DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Toxicity Study (81-2)

EPA IDENTIFICATION NUMBERS:

Tox. Chem. Number:
MRID Number: 425383-03

TEST MATERIAL: Neem Concentrate TGAI

SYNONYMS: None known or reported

SPONSOR: W.R. Grace and Company-Conn., Columbia, MD

STUDY NUMBER: SLS 3268.2

TESTING FACILITY: Springborn Laboratories, Inc., Spencerville, OH

TITLE OF REPORT: Acute Exposure Dermal Toxicity in Rabbits with Neem Concentrate TGAI, Limit Test

AUTHOR: R.E. Rush, B.A.

STUDY COMPLETED: 10/9/92

CONCLUSION: The acute dermal LD_{50} for Neem Concentrate TGAI was found to be greater than 2000 mg/kg in rabbits.

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TOXICITY CATEGORY: III

CLASSIFICATION: Core ~~Minimal~~ GUIDELINE Evidence that the necropsy results were reviewed by a pathologist must be provided.

A. MATERIALS

1. Test Material

Test material: Neem Concentrate, TGAI
Receipt: 3/18/92
Purity of material: 4.5% Azadirachtin [redacted] other neem solids
Physical description: Brown, opaque viscous liquid
Active Ingredient: Azadirachtin
Inactive Ingredients: [redacted] and other neem solids
Lot number: 3/3/92
pH: 4.66 (measured at a 160/1 dilution in water)
Stability: Not reported. Springborn Laboratories, Inc., Study Number 3268.17, A Teratology Study in Rats with Neem Concentrate TGAI, lists the stability of the compound as greater than one year at 28°C.

2. Controls

Materials: Not needed.
Animals: Not reported.

3. Test Animals

Species: Rabbits
Strain: New Zealand White
Source: Mohican Valley Rabbitry, Loudonville, OH
Receipt Date: 5/13/92
Sex: Male and nulliparous female
Numbers: 5 males and 5 females
Housing: The rabbits were ear tagged for identification, housed individually in suspended stainless steel cages, acclimated for a minimum of five days, and adjusted to a 12-hour light/dark cycle. The animal room temperature was controlled at 61-70°F with a relative humidity of 50 ± 10% during the study. The animal room temperature exceeded (71 and 72°F) the acceptable range on two days of the study and the humidity (ranging from 65 to 89%) was out of range on eight days. According to the study author, the temperature and humidity excursions did not interfere with the study results.
Age: Adult
Weight: Male: 2.30-2.75 kg; Female: 2.46-2.61 kg
Feeding: Purina Certified Rabbit Chow and fresh tap water were provided *ad libitum*.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

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Assignment: Because this was a limit test, five male and five female rabbits meeting acceptable body weight criteria were used for the one dose tested.
Location of raw data: All original data, specimens, and reports are archived at Springborn Laboratories, Inc., under Study Number 3268.2.

4. Exposure

Route of administration: Dermal
Dose level: 2000 mg/kg

B. TEST PERFORMANCE

Depilation was done 24 hours prior to the test by shaving an area greater than 10% of the total body surface area on the dorsal trunk. Care was taken not to abrade the skin. On the day of dosing, Neem Concentrate TGAI, 2000 mg/kg, was applied uniformly over the shaved area of skin at a volume of 1.96 ml/kg. The test chemical was held in contact with the skin for 24 hours by covering the area of application with 4" × 8" gauze dressing that in turn was covered by plastic wrap secured with nonirritating tape. The test site was further covered with elastic wrap positioned around the trunk and secured with tape to ensure that the rabbits could not ingest the test substance. After the 24 hour exposure period, the wrappings were removed and residual Neem Concentrate TGAI removed with water and gauze. The rabbits were observed for toxicity and the data recorded throughout the 14-day observation period. The body weights of the rabbits were recorded on day 1, 8, and 15. The mean and standard deviation of body weights were calculated using a MicroVax 3000. All rabbits were sacrificed with sodium pentobarbital on day 15, necropsy done, and the results recorded.

C. RESULTS AND STUDY AUTHOR'S CONCLUSIONS

No effects on animal body weight (Table 1) were observed and no mortalities occurred during the study. Slight to moderate erythema and edema was observed in all rabbits by day 2 but resolved by the 10th day of the study. Desquamation was observed on one male and one female rabbit. While the severity of the desquamation was not reported the condition had resolved by day 11 in both rabbits. Skin thickening was found in 4/5 female rabbits, but only on the fourth day after dosing. Fecal staining and soft or mucoid stools were observed in several rabbits and one rabbit had a clear ocular discharge from the right eye during the study. All clinical indicators of toxicity were resolved by the 13th day of the study. At necropsy, multiple bilateral pinpoint depressed areas on the kidneys of one male rabbit and greenish-brown "tabs" on the medial lobe of the liver of one male and one female rabbit were observed. Periovarian cysts were found in 4/5 female rabbits. According to the author, these were not considered treatment related since the cysts are commonly found in New Zealand white rabbits.

Because no mortalities were observed in any of the rabbits during the 14 day observation period, the author concluded that under the conditions of the study, the acute dermal LD_{50} of Neem Concentrate TGAI was greater than 2000 mg/kg.

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D. REVIEWER'S COMMENTS

No rabbits died during the study and clinical signs of toxicity were limited to slight to moderate erythema and edema, loose stools and fecal staining, and an ocular discharge. All signs of toxicity resolved by the 12th day after treatment. Based on this information, the author of the study correctly interpreted the results of the acute dermal limit test. The greater than 2000 mg/kg acute dermal LD_{50} of Neem Concentrate TGAI corresponds to Toxicity Category III.

A discrepancy in the density of the test article was found by Springborn Laboratories, Inc. As measured by the laboratory, the density of the Neem Concentrate TGAI supplied by the sponsor was 1.02 g/ml. The sponsor had previously reported the density of the test article as 0.96 g/ml. After discussion with the sponsor, the animals were dosed according to the density obtained by Springborn Laboratories, Inc. The discrepancy in the density of the test compound between the sponsor and the testing laboratory is minor and likely does not impact the results, but may be indicative of evaporation of the test compound at some time prior to application. Additionally, it is not apparent whether the animal necropsies were done or reviewed by the consulting pathologist. The initials on the necropsy reports do not match any of those listed in Section V - Springborn Personnel Responsibilities - found on page 25 of the report. At a minimum, the necropsy results should be reviewed by the pathologist since "Scheduled necropsies shall be performed under the direct supervision of a qualified pathologist" (Pesticide Assessment Guidelines - Subdivision F Hazard Evaluation - Human & Domestic Animals - Revised Edition, U.S. EPA, PB86-108958, Nov. 1984, p. 26). Apart from these exceptions, the report was well written and all pertinent data associated with the study were provided.

E. COMPLIANCE

A signed and dated Quality Assurance Unit Statement was provided.
A signed and dated Good Laboratory Practice (GLP) statement was provided.

F. CBI APPENDIX

None presented.

TABLE 1. BODY WEIGHTS (g) OF RABBITS FOLLOWING ACUTE DERMAL EXPOSURE TO 2000 mg/kg NEEM CONCENTRATE TGAI[a]

Male Rabbit Number	Initial	Day 8	Day 15	Female Rabbit Number	Initial	Day 8	Day 15
3720	2345	2634	2932	3651	2605	2700	2974
3702	2466	3028	3296	3656	2461	2813	3088
3721	2300	2952	3251	3620	2589	2786	3163
3704	2746	3322	3578	3658	2603	2976	3156
3707	2350	3054	3391	3659	2523	2858	3147
Mean	2441	2998	3290		2556	2827	3106
S.D.	181.0	246.8	236.0		62.9	101.4	79.3

[a]Springborn Laboratories, Inc., (1992). Acute Exposure Dermal Toxicity in Rabbits with Neem Concentrate TGAI, Limit Test, Study No. SLS 3268.2, pages 16-17.

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EPA Reviewer:
Review Section

Signature: [signature]
Date: 11/2/93

EPA Mutagenicity Secondary Reviewer:
Review Section II, Toxicology Branch II

Signature: _____
Date: _____

Oak Ridge National Laboratory Reviewer:
Harold T. Borges, Ph.D., MT(ASCP)
Biomedical and Environmental Information Analysis Section
Health and Safety Research Division

Signature: _____
Date: _____

Oak Ridge National Laboratory, Secondary Reviewer:
Robert H. Ross, M.S., Group Leader
Biomedical and Environmental Information Analysis Section
Health and Safety Research Division

Signature: _____
Date: _____

DATA EVALUATION REPORT

STUDY TYPE: Primary Eye Irritation Study in Rabbits (81-4)

EPA IDENTIFICATION NUMBERS:

Tox. Chem. Number:
MRID Number: 425383-04

TEST MATERIAL: Neem Concentrate TGAI

SYNONYMS: None known or reported

SPONSOR: W.R. Grace and Company-Conn., Columbia, MD

STUDY NUMBER: SLS 3268.3

TESTING FACILITY: Springborn Laboratories, Inc., Spencerville, OH

TITLE OF REPORT: Primary Eye Irritation Study in Rabbits with Neem Concentrate TGAI

AUTHOR: R.E. Rush, B.A.

STUDY COMPLETED: 10/9/92

CONCLUSION: Under the conditions of the test, Neem Concentrate TGAI was considered to be a mild irritant to the ocular tissue of the rabbit.

TOXICITY CATEGORY: II

CLASSIFICATION: Core - Guideline. The study is acceptable as presented.

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A. MATERIALS

1. Test Material

Test material: Neem Concentrate, TGAI
Receipt: 3/18/92
Purity of material: 4.5% Azadirachtin [redacted] other neem solids
Physical description: Brown, opaque viscous liquid
Active Ingredient: Azadirachtin
Inactive Ingredients: [redacted] and other neem solids
Lot number: 3/3/92
pH: 4.66 (measured at a 160/1 dilution in water)
Stability: Not reported. Springborn Laboratories, Inc., Study Number 3268.17, A Teratology Study in Rats with Neem Concentrate TGAI, lists the stability of the compound as greater than one year at 28°C.

2. Controls

Materials: Not needed.
Animals: Left eye

3. Test Animals

Species: Rabbit
Strain: New Zealand White
Source: Mohican Valley Rabbitry
Receipt Date: 5/6/92
Sex: Male and female
Numbers: 2 males and 4 nulliparous females
Housing: The rabbits were ear tagged for identification, housed individually in suspended stainless steel cages, acclimated for a minimum of five days, and adjusted to a 12-hour light/dark cycle. The animal room temperature was controlled at 61-70°F with a relative humidity of 50 ± 10% during the study. The animal room temperature exceeded (71°F) the acceptable range on one day of the study and the humidity (ranging from 65 to 89%) was out of range on six days. According to the study author, the temperature and humidity excursions did not interfere with the study results.
Age: Adult
Weight: Male: 2.53-2.61 kg; Female: 2.51-2.65 kg
Feeding: Purina Certified Rabbit Chow and fresh tap water were provided *ad libitum*.
Assignment: Male and female rabbits meeting the acceptable weight criteria
Location of raw data: All original data, specimens, and reports are archived at Springborn Laboratories, Inc., under Study Number 3268.3.

4. Exposure

Route of administration: Ocular
Dose level: 0.1 ml Neem Concentrate TGAI

B. TEST PERFORMANCE

The rabbits were weighed before the start of the study. Both eyes of each rabbit were examined macroscopically for ocular irritation using an auxiliary light source and for corneal defects with fluorescein dye. Rabbits having eye irritation, ocular defects or preexisting corneal lesions were not used in the study.

At least one hour after the ocular examination, 0.1 ml of Neem Concentrate TGAI was instilled into the conjunctival sac of the right eye of each rabbit and the eyelids were held together for approximately one second. The contralateral eye of each rabbit remained untreated and served as the control.

Both eyes of each rabbit were examined for irritation at 1, 24, 48, and 72 hours and up to 10 days after instillation using an auxiliary light source and a bimicroscopic slit-lamp. The results were scored according to the scheme shown in Table 1. After macroscopic observation at 24 hours postinstillation, fluorescein examination was repeated on all test and control eyes. Physiological saline, 50 ml, was used to rinse the fluorescein dye from the eyes and to remove residual test compound. If a positive fluorescein response was noted at 24 hours, the fluorescein exam was conducted on the affected eyes at each subsequent interval until a negative response was obtained. All animals were euthanized with sodium pentobarbital and discarded after completion of each animal's final scoring on the 10th day.

The ocular irritation scores were added for each rabbit and the mean irritation score of all rabbits in the study was calculated for each scoring interval according to the scheme shown in Table 2. Statistical analysis of the data was not done.

C. RESULTS AND STUDY AUTHOR'S CONCLUSIONS

Neem Concentrate TGAI induced corneal opacity in 1/6 eyes at the 24-hour scoring interval. The opacity was confirmed by a positive fluorescein dye retention. The corneal injury decreased and was completely resolved by day 7 of the study. Iritis was observed in 4/6 test eyes 1 hour postinstillation, but had resolved in all test eyes by 72 hours. Conjunctivitis, including redness, swelling, and discharge, was found in 6/6 test eyes 1 hour postinstillation. The condition diminished and was completely resolved in all test eyes by day 10 of the study. Additional ocular findings noted during the test period included sloughing (2/6 test eyes) and corneal neovascularization (1/6 test eyes). According to the author, Neem Concentrate TGAI was considered a mild irritant to the ocular tissue of rabbits under the conditions of the test. The ocular irritation data is summarized in Tables 3 and 4.

D. REVIEWER'S COMMENTS

Neem Concentrate TGAI induced corneal opacity in one rabbit, but the condition was totally resolved within 7 days. Iritis was found in 4/6 rabbits within one hour of treatment but the condition resolved within three days. Conjunctivitis was found in 6/6 test eyes shortly after instillation but was totally resolved in all but one rabbit within 7 days of treatment. Based on this data, the author of the study has correctly interpreted the results. Neem Concentrate TGAI should be placed in Toxicity Category II.

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A discrepancy in the density of the test article was found by Springborn Laboratories, Inc. As measured by the laboratory, the density of the Neem Concentrate TGAI supplied by the sponsor was 1.02 g/ml. The sponsor had previously reported the density of the test article as 0.96 g/ml. After discussion with the sponsor, the animals were dosed according to the density obtained by Springborn Laboratories, Inc. The discrepancy in the density of the test compound between the sponsor and the testing laboratory is minor and likely does not impact the results, but may be indicative of evaporation of the test compound at some time prior to application.

E. COMPLIANCE

A signed and dated Quality Assurance Unit Statement was provided.
A signed and dated Good Laboratory Practice (GLP) statement was provided.

F. CBI APPENDIX

None presented.

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TABLE 1: OCULAR IRRITATION GRADING SYSTEM[a]

CORNEA	SCORE
(A) Opacity - Degree of density (area most dense taken for reading)	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	1*
Easily discernible translucent area, details of iris slightly obscured	2*
Opalescent (nacreous) area, no details of iris visible, size of pupil barely discernible	3*
Opaque cornea, iris not discernible through opacity	4*
(B) Area of cornea involved	
No ulceration or opacity	0
One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to a whole area	4
Score = A x B x 5	Total Maximum = 80

IRIS	
(A) Values	
Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris is still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2*
Score = A x 5	Total Maximum = 10

CONJUNCTIVAE	
(A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Blood vessels normal	0
Blood vessels definitely injected (hyperemic) above normal (slight erythema)	1
More diffuse, deeper crimson red, individual vessels not easily discernible (moderate erythema)	2*
Diffuse beefy red (marked erythema)	3*

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TABLE 1: OCULAR IRRITATION GRADING SYSTEM (continued)

CONJUNCTIVAE	SCORE
(B) Chemosis	
No swelling	0
Any swelling above normal (includes nictitating membrane, slightly swollen)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3*
Swelling with lids more than half closed	4*
(D) Discharge	
No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs and considerable area around the eye	3

Score = (A + B + C) x 2 Total Maximum = 20

* = Positive Response

[a]Draize, J.H., (1965). Appraisal of the safety of chemicals in foods, drugs, and cosmetics. The Association of Food and Drug Officials of the United States: 46-59.

TABLE 2. OCULAR EVALUATION CRITERIA[a]

Maximum Mean Total Score During First 4 Days	Maximum Mean Score on Day X	Persistence of Individual Scores	Descriptive Rating	Class
0.00 - 0.49	1 day = 0		Non-irritating	1
	1 day > 0		Practically Non-irritating	2
0.50 - 2.49	1 day = 0		Non-irritating	1
	1 day > 0		Practically Non-irritating	2
2.50 - 14.99	2 days = 0		Slight Irritant	3
	2 days > 0		Mild Irritant	4
15.00 - 24.99	3 days = 0		Mild Irritant	4
	3 days > 0		Moderate Irritant	5
25.00 - 49.99	7 days ≤ 20	> half of day 7 scores ≤ 10	Moderate Irritant	5
		> half of day 7 scores > 10, but no score > 20	Moderate Irritant	5
		> half of day 7 scores > 10, and any score > 20	Severe Irritant	6
	7 day > 20		Severe Irritant	6
50.00 - 79.99	7 day ≤ 40	> half of day 7 scores ≤ 30	Severe Irritant	6
		> half of day 7 scores > 30, but no score > 60	Severe Irritant	6
		> half of day 7 scores > 30, and any score > 60	Very Severe Irritant	7
	7 day > 40		Very Severe Irritant	7
80.00 - 99.99	7 day ≤ 80	> half of day 7 scores ≤ 60	Very Severe Irritant	7
		> half of day 7 scores > 60, but no score > 100	Very Severe Irritant	7
		> half of day 7 scores > 60, and any score > 100	Extremely Severe Irritant	8
	7 day > 80		Extremely Severe Irritant	8
100.00 - 110.0	7 day ≤ 80		Very Severe Irritant	7
	7 day > 80		Extremely Severe Irritant	8

[a]Kay, J.H. and Calandra, J.C., (1962). Interpretation of eye irritation tests, J. Soc. Cosmet. Chem. 13: 281-289.

TABLE 3. PRIMARY EYE IRRITATION TOTAL SCORES FOR NEEM CONCENTRATE TGAI[a]

Rabbit No./Sex	Initial Body Weight (kg)	Primary Eye Irritation Scores																	
		Cornea						Iris						Conjunctivae					
		Hour				Days		Hour				Days		Hours				Days	
		1	24	48	72	7	10	1	24	48	72	7	10	1	24	48	72	7	10
3606/M	2.529	0	0	0	0	0		5	0	0	0	0		8	6	6	4	0	
3616/M	2.612	0	0	0	0			5	0	0	0			8	4	4	0		
3635/F	2.647	0	20	20	10	0	0	5	5	5	0	0	0	8	12	8	4	4	0
3627/F	2.644	0	0	0	0	0		0	0	0	0	0		6	6	6	6	0	
3639/F	2.605	0	0	0	0	0		0	0	0	0	0		6	8	6	4	0	
3737/F	2.508	0	0	0	0	0		5	0	0	0	0		10	6	6	4	0	

Group Mean Irritation Scores					
Hour				Days	
1	24	48	72	7	10
11.00	11.17	10.17	5.33	0.67	0.00

[a]Springborn Laboratories, Inc., Primary Eye Irritation Study in Rabbits with Neem Concentrate TGAI, Study No. 3268.3, page 15.

TABLE 4. OCULAR IRRITATION SUMMARY FOR NEEM CONCENTRATE TGAI[a]

	Incidence					
	Hours				Days	
	1	24	48	72	7	10
Corneal Opacity	0/6	1/6	1/6	1/6	0/6	0/6
Iritis	4/6	1/6	1/6	0/6	0/6	0/6
Conjunctivae						
Redness	6/6	6/6	6/6	5/6	1/6	0/6
Chemosis	6/6	6/6	6/6	5/6	1/6	0/6
Discharge	6/6	2/6	0/6	0/6	0/6	0/6

[a]Springborn Laboratories, Inc.,Primary Eye Irritation Study in Rabbits with Neem Concentrate TGAI, Study No. 3268.3, page 16.

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EPA Reviewer:
Review Section

Signature: [signature]
Date: 4/3/93

EPA Mutagenicity Secondary Reviewer:
Review Section II, Toxicology Branch II

Signature: _____
Date: _____

Oak Ridge National Laboratory Reviewer:
Harold T. Borges, Ph.D., MT(ASCP)
Biomedical and Environmental Information Analysis Section
Health and Safety Research Division

Signature: _____
Date: _____

Oak Ridge National Laboratory, Secondary Reviewer:
Robert H. Ross, M.S., Group Leader
Biomedical and Environmental Information Analysis Section
Health and Safety Research Division

Signature: _____
Date: _____

DATA EVALUATION REPORT

STUDY TYPE: Primary Skin Irritation Study in Rabbits (81-5)

EPA IDENTIFICATION NUMBERS:

Tox. Chem. Number:
MRID Number: 425383-05

TEST MATERIAL: Neem Concentrate TGAI

SYNONYMS: None known or reported

SPONSOR: W.R. Grace and Company-Conn., Columbia, MD

STUDY NUMBER: SLS 3268.4

TESTING FACILITY: Springborn Laboratories, Inc., Spencerville, OH

TITLE OF REPORT: Primary Skin Irritation Study in Rabbits with Neem Concentrate TGAI

AUTHOR: R.E. Rush, B.A.

STUDY COMPLETED: 10/9/92

CONCLUSION: Under the conditions of the test, Neem Concentrate TGAI was considered to be a slight irritant (Primary Irritation Index = 1.04) to the dermal tissue of the rabbit.

TOXICITY CATEGORY: IV

CLASSIFICATION: Core - Guideline. The study was acceptable as presented.

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A. MATERIALS

1. Test Material

Test material: Neem Concentrate, TGAI
Receipt: 3/18/92
Purity of material: 4.5% Azadirachtin [redacted] other neem solids
Physical description: Brown, opaque viscous liquid
Active Ingredient: Azadirachtin
Inactive Ingredients: [redacted] and other neem solids
Lot number: 3/3/92
pH: 4.66 (measured at a 160/1 dilution in water)
Stability: Not reported. Springborn Laboratories, Inc., Study Number 3268.17, A Teratology Study in Rats with Neem Concentrate TGAI, lists the stability of the compound as greater than one year at 28°C.

2. Controls

Materials: Not needed.
Animals: Not reported

3. Test Animals

Species: Rabbit
Strain: New Zealand White
Source: Mohican Valley Rabbitry
Receipt Date: 5/13/92
Sex: Male and female
Numbers: 3 males and 3 nulliparous females
Housing: The rabbits were ear tagged for identification, housed individually in suspended stainless steel cages, acclimated for a minimum of five days, and adjusted to a 12-hour light/dark cycle. The animal room temperature was controlled at 61-70°F with a relative humidity of 50 ± 10% during the study.
Age: Young adult
Weight: Male - 2.10-2.32 kg; Female - 1.94-2.65 kg
Feeding: Purina Certified Rabbit Chow and fresh tap water were provided *ad libitum*.
Assignment: Male and female rabbits meeting the acceptable weight criteria
Location of raw data: All original data, specimens, and reports are archived at Springborn Laboratories, Inc., under Study Number 3268.4.

4. Exposure

Route of administration: Dermal
Dose level: 0.5 ml Neem Concentrate TGAI

INERT INGREDIENT INFORMATION IS NOT INCLUDED

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B. TEST PERFORMANCE

Twenty-four hours prior to dosing, the dorsal trunk of each rabbit was clipped. Abrasion to the skin was avoided. On the day of dosing, each rabbit was weighed and a test site on the exposed skin measuring 1" × 1" was chosen. A dose of 0.5 ml of the test compound was applied to the test site and immediately covered by a 1" × 1" gauze patch secured by nonirritating tape. The gauze was in turn covered by an elastic wrap placed around the trunk and secured by tape. After an exposure period of 4 hours, the elastic wrap and patch were removed and the test site wiped free of residual Neem Concentrate TGAI with gauze moistened in distilled water. The test site was examined for signs of erythema and edema at 1, 24, 48, and 72 hours after removal of the patch. Each site was graded according to the schemes shown in Table 1 and 2.

C. RESULTS AND STUDY AUTHOR'S CONCLUSIONS

Neem Concentrate TGAI produced very slight to well-defined erythema and very slight to no edema on 6/6 test sites one hour after treatment. Dermal irritation decreased throughout the study and was completely resolved by the 72-hour scoring point. Table 3 contains the dermal irritation scores recorded for the study. According to the author, Neem Concentrate TGAI was considered to be a slight irritant to the dermal tissue of the rabbit. The Primary Irritation Index calculated for the test was 1.04.

D. REVIEWER'S COMMENTS

Neem Concentrate TGAI produced slight erythema and edema in this study. Based on the data, the author of the study has correctly interpreted the results and the test compound should be classified as a slight irritant and placed in Toxicity Category ~~II~~ IV.

- A discrepancy in the density of the test article was found by Springborn Laboratories, Inc. As measured by the laboratory, the density of the Neem Concentrate TGAI supplied by the sponsor was 1.02 g/ml. The sponsor had previously reported the density of the test article as 0.96 g/ml. After discussion with the sponsor, the animals were dosed according to the density obtained by Springborn Laboratories, Inc. The discrepancy in the density of the test compound between the sponsor and the testing laboratory is minor and likely does not impact the results, but may be indicative of evaporation of the test compound at some time prior to application. Apart from this exception, the report was well written and all pertinent data associated with the study were provided.

E. COMPLIANCE

A signed and dated Quality Assurance Unit Statement was provided.
A signed and dated Good Laboratory Practice (GLP) statement was provided.

F. CBI APPENDIX

None presented.

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TABLE 1. DRAIZE[a] SCORING FOR DERMAL IRRITATION

Erythema and Eschar Formation (Most severely affected area graded):

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Edema Formation (Most severely affected area graded):

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

[a] Draize, J.H. 1959. The Appraisal of Chemicals in Foods, Drugs and Cosmetics, Association of Food and Drug Officials of the United States, Austin, Texas, pp. 36-45.

TABLE 2. DERMAL EVALUATION CRITERIA[b]

Primary Irritation Index	Irritation Rating
0.00	Nonirritant
0.01 - 1.99	Slight Irritant
2.00 - 5.00	Moderate Irritant
5.01 - 8.00	Severe Irritant

[b]Addendum 3 of Pesticide Assessment Guidelines - Dermal Irritation (U.S.) Environmental Protection Agency, Washington, D.C., January, 1988.

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TABLE 3. DERMAL IRRITATION SCORES[a]

Parameter[b]	Scoring Interval	Animal Number/Sex					
		3738/F	3714/M	3716/M	3696/M	3748/F	3739/F
Erythema	1 Hour	2	2	1	1	1	2
	24 Hours	2	2	1	1	1	1
	48 Hours	1	1	0	0	0	0
	72 Hours	0	0	0	0	0	0
Edema	1 Hour	1	1	1	1	0	1
	24 Hours	1	0	0	0	0	0
	48 Hours	0	0	0	0	0	0
	72 Hours	0	0	0	0	0	0
Total Score		7	6	3	3	2	4

[a]Springborn Laboratories, Inc., (1992). Primary Skin Irritation Study in Rabbits with Neem Concentrate TGAI, Study Number 3268.4, page 13.
[b]All test sites were noted to have a brown stain at patch removal on day 0.

$$\text{Primary Irritation Index} = \frac{\text{Total Erythema and Edema Scores for 1,24,48, and 72 hours}}{\text{24 (4 Scoring Intervals} \times \text{6 Sites)}} = 25/24 = 1.04$$ - Slight Irritant

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EPA Reviewer:
Review Section

Signature: Thomas M Chitlock
Date: 11/4/93

EPA Mutagenicity Secondary Reviewer:
Review Section II, Toxicology Branch II

Signature: _____
Date: _____

Oak Ridge National Laboratory Reviewer:
Harold T. Borges, Ph.D., MT(ASCP)
Biomedical and Environmental Information Analysis Section
Health and Safety Research Division

Signature: _____
Date: _____

Oak Ridge National Laboratory, Secondary Reviewer:
Robert H. Ross, M.S., Group Leader
Biomedical and Environmental Information Analysis Section
Health and Safety Research Division

Signature: _____
Date: _____

DATA EVALUATION REPORT

STUDY TYPE: Delayed Hypersensitivity in Guinea Pigs (81-6)

EPA IDENTIFICATION NUMBERS:

Tox. Chem. Number:
MRID Number: 425383-06

TEST MATERIAL: Neem Concentrate TGAI

SYNONYMS: None known or reported

SPONSOR: W.R. Grace and Company-Conn., Columbia, MD

STUDY NUMBER: PH 424-SI-003-92

TESTING FACILITY: Pharmakon Research International, Inc., Waverly, PA

TITLE OF REPORT: Delayed Contact Hypersensitivity in Guinea Pigs (Ritz and Buehler 1980) with Neem Concentrate TGAI

AUTHOR: S.E. Armondi, LAT

STUDY COMPLETED: 10/28/92

CONCLUSION: Under the conditions of the test, Neem Concentrate TGAI did not induce delayed contact hypersensitivity in guinea pigs induced at 40% (v/v) and 100% and challenged at 100%.

CLASSIFICATION: Core - Guideline. The study was acceptable as presented.

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A. MATERIALS

1. Test Material

Test material: Neem Concentrate, TGAI
Receipt: 6/19/92
Purity of material: 4.5% Azadirachtin (per sponsor's certificate of analysis)
Physical description: Dark brown liquid
Active Ingredient: Azadirachtin
Inactive Ingredients: Not reported
Lot number: 3/3/92
pH: Not reported
Stability: According to the study author, the identity, purity, strength, and stability of the test article were the responsibility of the Sponsor, and no apparent change in the physical state of the test compound was noted during storage. Springborn Laboratories, Inc., Study Number 3268.17, A Teratology Study in Rats with Neem Concentrate TGAI, lists the stability of the compound as greater than one year at 28°C.

2. Controls

Materials: 0.3% 1-Chloro-2,4-dinitrochlorobenzene (DNCB) (Aldrich Chemical Co., Milwaukee, WI) in 80% ethanol - Positive Control; 80% ethanol - Vehicle Control
Animals: Positive control - 5 male guinea pigs; Vehicle control - 10 male guinea pigs; Test Article - 20 male guinea pigs

3. Test Animals

Species: Guinea pig
Strain: Hartley-derived albino
Source: BuckberG Lab Animals, Tomkins Cove, NY
Receipt Date: July 6, 1992; July 13, 1992, and August 10, 1992
Sex: Male
Numbers: Dose-Range Finding-Studies - 6 male guinea pigs; Positive control - 5 male guinea pigs; Vehicle control - 10 male guinea pigs; Test Article - 20 male guinea pigs
Housing: The guinea pigs were housed individually in stainless steel wire mesh cages, acclimated for a minimum of five days, and adjusted to a 12-hour light/dark cycle. The guinea pigs were ear-tagged for the definitive study and cage cards marked with the Study Number, animal number, dose level and sex were used for both the definitive and range-finding studies. The room temperature was controlled at 22°C ± 3°C with a relative humidity of 50% ± 20%. The temperature and humidity were maintained within the specified ranges throughout the study period with the exception of day 6 when the humidity was 80%. According to the study author, this excursion did not adversely affect the validity of the study.
Age: 4 - 6 weeks
Weight: Dose-range-finding study, 396 to 435 g; Definitive study, 328 to 385 g
Feeding: Purina Certified Guinea Pig Diet® or Purina Guinea Pig Diet® and fresh tap water were provided *ad libitum*.

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Assignment: Based on health and body weight
Location of raw data: All raw data, final reports, protocol and study documentation are maintained by Pharmakon Research International, Inc. The reference notebook is 1682, pages 1 - 101.

4. Exposure

Route of administration: Dermal
Dose level: 0.3 mL of 40% in 80% ethanol or 100 % Neem Concentrate TGAI

B. TEST PERFORMANCE

Dose-Range-Finding Study: The study was conducted to determine the irritation potential of Neem Concentrate TGAI. Four male guinea pigs were exposed to three different concentrations (1.0, 10.0, and 50% v/v test compound in 80% ethanol) and the neat compound. For the study, the animal was placed in a restrainer and the dorsal area equivalent to approximately 20% of the total body surface on both sides of each guinea pig was clipped. Each of four concentrations of Neem Concentrate TGAI (0.3 mL/site) were applied to four patches and the patches placed on the shaved skin at four separate sites. All patches were covered with a rubber dental dam pulled tightly around the animal and fastened to the bottom of the restrainer with binder clips. After 6 hours, the dental dam and patches were removed and the guinea pig returned to its cage. Primary irritation responses were graded 24 hours later. The highest non-irritating concentration of the test compound was defined as that concentration that induced responses not exceeding two + and two 0 grades in the group of four guinea pigs (Table 1 contains the scoring system). One of four guinea pigs had dermal irritation scores of 2 and two had scores of 0.5 at sites treated with 50% and 100% test compound. Because of the irritation observed and discussions with the sponsor, the study was repeated with two male guinea pigs exposed to concentrations of 10, 20, 30, and 40% v/v in 80% ethanol Neem Concentrate TGAI similar to that described above. One of these guinea pigs had a score of 0.5 for the 30% and 40% concentrations of the test compound. Based on the dose-range-finding studies, 40% Neem Concentrate TGAI in 80% ethanol was used for sensitization study.

Sensitization Study: Three groups of guinea pigs were used in this portion of the study. The Positive Control Group was composed of 5 male guinea pigs; the Neem Concentrate TGAI Group was composed of 20 male guinea pigs; and 10 male guinea pigs were in the Negative Control Group. Twenty-four hours prior to induction or challenge applications, the dorsal area of all animals in the study was clipped free of hair. The clipped area, approximately 5 × 10 cm, comprised roughly 10% of the total body surface of the animal. During the induction phase of the study, guinea pigs in the Positive Control Group, the Neem Concentrate TGAI Group and the Negative Control Group were placed in a restraining unit and a patch applied to the shaved portion of the skin on the left flank. Each patch contained 0.3 mL of either 0.3% DCNB for the Positive Control Group; 80% ethanol for the Negative Control Group or 40% Neem Concentrate TGAI in 80% ethanol for the test compound group. The patch was covered by a rubber dental dam that in turn was pulled tight over the patch and secured to the bottom of the restrainer with binder clips. The patches remained in contact with the skin for 6 hours before being removed. The induction site was examined for erythema immediately after treatment, as well as 24 and 48 hours postinduction and scored as described in Table 1. Based on the irritation results recorded 24 and 48 hours after the first induction and after consultation with the study sponsor, the concentration of Neem Concentrate TGAI was increased to 100% for the

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remaining two inductions. The processes of clipping the hair, applying the patch for 6 hours, and scoring for erythema were done at weekly intervals for a total of three applications.

Fourteen days after the third induction treatment, the animals in each respective test and positive control group were challenged using the same treatment procedure described above, with the exception that the challenge patch was placed at a naive site on the left side. Animals in the negative control group were challenged with 80% ethanol on the left flank and challenged only with the test compound on the right flank. All guinea pigs were observed for local and systemic effects. Twenty-four hours after challenge, a depilatory cream was applied at the site of patch application. The cream remained on the site for a maximum of 30 minutes before being thoroughly washed off. At least 2 hours later, the site was scored for erythema (24-hour score) and scored again 24 hours (48-hour score) later. After the 48-hour score was recorded, the guinea pigs were weighed and then killed by CO_2 inhalation.

C. RESULTS AND STUDY AUTHOR'S CONCLUSIONS

The results of the challenge are shown in Table 2. Twenty-four hours after challenge, 17/20 guinea pigs in the test compound treatment group had irritation severity index scores of 0.0 and 3/20 had scores of 0.5. Forty-eight hours post-challenge, all irritation severity scores for guinea pigs challenged with Neem Concentrate TGAI were 0.0. The guinea pig irritation severity index was 3.0 and 2.8 at 24 and 48 hours, respectively, in the Positive Control Group and was 0.0 at both 24 and 48 hours in the Negative Control Group. Based on these results under the conditions of the study, the author concluded that Neem Concentrate TGAI, induced at 40% (v/v) and 100% and challenged at 100%, does not cause delayed contact hypersensitivity in guinea pigs.

D. REVIEWER'S COMMENTS

Based on the data presented by Pharmakon Research International, Inc., Study Number Ph 424-SI-003-92, the author has correctly interpreted the results and Neem Concentrate TGAI does not promote hypersensitivity in guinea pigs when induced at 40% and 100% and challenged at 100%.

It should be noted that Table 1, Evaluation of Dermal Irritation (Erythema), for Study Number PH 424-SI-003-92 was not present in the report.

E. COMPLIANCE

A signed and dated Quality Assurance Unit Statement was provided.
A signed and dated Good Laboratory Practice (GLP) statement was provided.

F. CBI APPENDIX

None presented.

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TABLE 1. DERMAL IRRITATION (ERYTHEMA) SCORING SYSTEM

0 = No reaction
+ = Slightly patchy erythema
1 = Slightly or confluent or moderate patchy erythema or area
2 = Moderate erythema
3 = Severe erythema with/without edema

Scores ≥ 1 in the test group indicate sensitization. If scores ≥ 1 are seen on negative control animals, the reactions of the test article group animals that exceed the most severe negative control reactions are considered to be positive scores. INCIDENCE is reported as the number of positive animals in each group divided by the total number of animals tested in that group. SEVERITY is reported as the sum of the test grades divided by the total number of animals tested in a given group determined for both 24 and 48 hours. Grades + are equal to 0.5 for calculation of severity indices.

All average grades are rounded off to the nearest tenth of a unit. A minimum of two out of five positive control animals must be shown to have elicited a positive reaction (scores > 1) to the positive material to validate the test system for the particular compound.

TABLE 2. INCIDENCE AND SEVERITY OF RESPONSES AFTER NEEM CONCENTRATE TGAI CHALLENGE[a]

Group	Challenge at Naive Site			
	24 Hours		48 Hours	
	Incidence	Severity[b]	Incidence	Severity
Neem Concentrate TGAI	0/20[c]	0.1	0/20	0.0
DNCB Positive Control (0.3%)	5/5	3.0	5/5	2.8
Negative Control Group (Induced with 80% ethanol, challenged with 80% ethanol)	0/10	0.0	0/10	0.0
Negative Control Group (induced with 80% ethanol challenged with Neem Concentrate TGAI)	0/10	0.1	0/10	0.0

[a]Pharmakon Research International, Inc., Delayed Contact Hypersensitivity in Guinea Pigs (Ritz and Buehler, 1980) with Neem Concentrate TGAI, PH 424-SI-003-92, page 26.
[b]Severity = the sum of the test grades divided by the total number of animals tested and rounded to the nearest 0.1. Grades of + = 0.5 for the calculation of the severity indices.
[c]Animals were graded positive if the grade was one or higher; 3/20 hamsters scored 0.5. If grades of one or greater were seen on negative control animals, the reactions of the test compound group animals that exceeded the most severe negative control reactions were considered positive scores.

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Tox Chem No. _____ File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
ACUTE ORAL/RAT Springborn LAB., INC. SLS No. 3268.1 10/9/92	4.5% AZADIRACHTIN (Neem Conc. TECH) + [redacted] other Neem SOLIDS	42538-02	LD_{50} > 5000 mg/kg	GUIDELINE	IV
Acute Dermal/Rabbits SPRINGBORN LAB., INC. SLS No. 3268.2 10/9/92	" (Same as above)	42538-03	LD_{50} > 2000 mg/kg in ♂ & ♀ rabbits. Slight to moderate erythema & edema; resolved by Day 10.	GUIDELINE	III
Primary Eye Irritation/Rabbits SPRINGBORN LAB, INC. SLS No. 3268.3 10/9/92	"	42538-04	Test material induced corneal opacity (Draize - Group Mean score 11.17) @ 24 hr in 1 of 6 eyes. (Resolved by DAY 7). Iritis observed in 4 out of 6 rabbits @ 1 hr (DRAIZE Score 11) which was resolved by DAY 3	Guideline	II
Primary Dermal Irrit. in Rabbits SPRINGBORN LAB., INC. SLS No. 3268.4 10/9/92	"	42538-05	very slight to well-defined erythema and very slight to no edema in 6/6 test sites @ 1 hr post-treatment	GUIDELINE	IV
DERMAL SENSITIZATION IN Guinea Pigs Pharmakon Research Internat. Waverly, PA	"	42538-06	Using the Buehler test the material did not induce delayed contact hypersensitivity - INDUCTION (40% v/v & 100% v/v);		

UNDER "INGREDIENT" INFORMATION IS NOT INCLUDED