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

003768

APR 20 1984

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA Reg.# 45167-1 Margosan-O (Neem Extract), used as an insect anti-feedant extract. Acc.# 252097
Caswell # 594A

TO: Willie Nelson (PM#17)
Registration Division (TS-767C)

FROM: Christine F. Chaisson, Ph.D. C.F. Chaisson 4/19/84
Head, Review Section No. 4
Toxicology Branch
Hazard Evaluation Division (TS-769C)

REVIEWED BY: William S. Woodrow, Ph.D. WSW 4/19/84
Toxicology Branch
Hazard Evaluation Division (TS-769C) Alt WSW 4/20/84

REGISTRANT: Vikwood Ltd., 1221A Superior Avenue
Sheboygan, WI 53081

ACTION REQUESTED:

Mr. Robert Larson of Vikwood Ltd., requests pesticide registration for MARGOSAN-O, an extract prepared from Indian Neem tree nuts reported to act as an anti-feedant to control Lepidopterous insects. The MARGOSAN-O active ingredient, azadirachtin is considered a "biorational" pesticide.

RECOMMENDATIONS:

1. The conditional registration of MARGOSAN-O to control certain Lepidopterous insects is toxicologically supported by the toxicity data reviewed in the present report, although another inhalation study must be provided for final registration.

2. Data reviewed:

   a. Acute oral Toxicity Evaluation of MARGOSAN-O, Rat.

      LD_{50} > 5 g/kg
      Toxicity Category IV
      Classification: Core Minimum Data

   b. Acute Dermal Toxicity Evaluation of MARGOSAN-O, Rabbit.

      LD_{50} > 2.08 g/kg
      Toxicity Category III
      Classification: Core Minimum Data

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c. Primary Skin Irritation Evaluation of MARGOSAN-O, Rabbit.

P.I. = 4.74, a moderately irritating substance.
Toxicity Category III
Classification: Core Minimum Data

d. Acute Inhalation Toxicity Evaluation of MARGOSAN-O, Rat.

The nominal LC_{50} > 43.9 mg/L
Toxicity Category - Not appropriate
Classification: Supplementary Data (The actual cloud concentration animals were exposed to was not determined.)

e. Primary Eye Irritation Evaluation of MARGOSAN-O, Rabbit.

The MMTS (maximum mean total score) for unwashed eyes was 4.33; for irrigated eyes, 3.33, which indicated a minimally irritating substance.
Toxicity Category III
Classification: Core Minimum Data.

f. Immune Response Evaluation of MARGOSAN-O, Rat.

Margosan-O does not elicit an immune response in the rat.
Classification: Core Minimum Data

g. Sensitization Evaluation of MARGOSAN-O, Guinea Pig.

Margosan-O is not a sensitizing agent.
Classification: Core Minimum Data.

h. Mutagenicity Evaluation of MARGOSAN-O using the Ames Test.

Margosan-O did not exhibit any mutagenic potential using the Ames test.
Classification: Acceptable Study.

3. The product signal word (CAUTION) is appropriate; however the precautinary statements should be revised to include: "Avoid contact with skin, eyes or clothing. In case of contact immediately flush eyes or skin with plenty of water."

4. The acute inhalation study should be repeated. The inhalation study using MARGOSAN-O reviewed in the present report did not determine the actual animal aerosol cloud exposure concentration (aerosol samples from the vicinity of the test animal heads during animal exposure should be used to measure the actual aerosol concentration).

5. A satisfactory acute inhalation study using MARGOSAN-O product is the only additional data requirement.

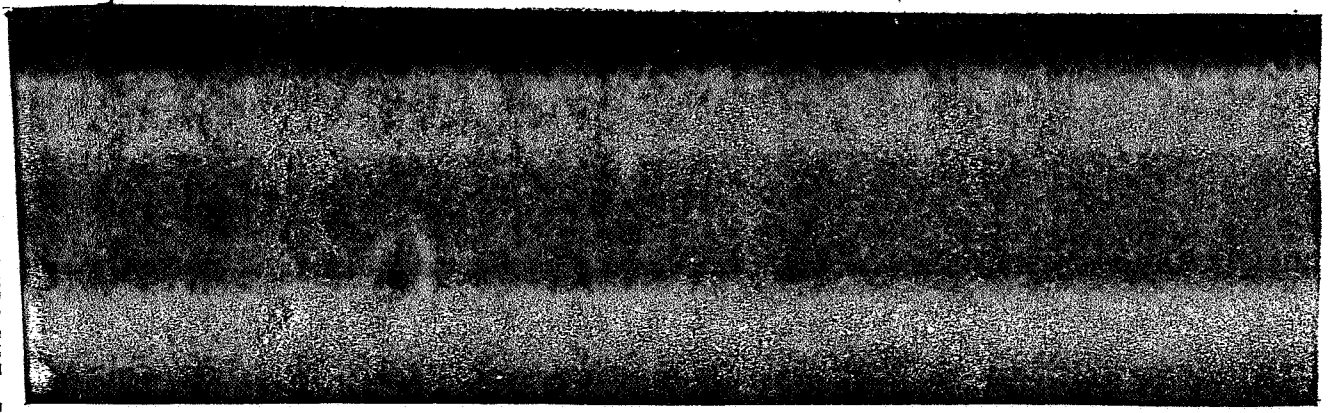
FORMULATION:

a. Product label

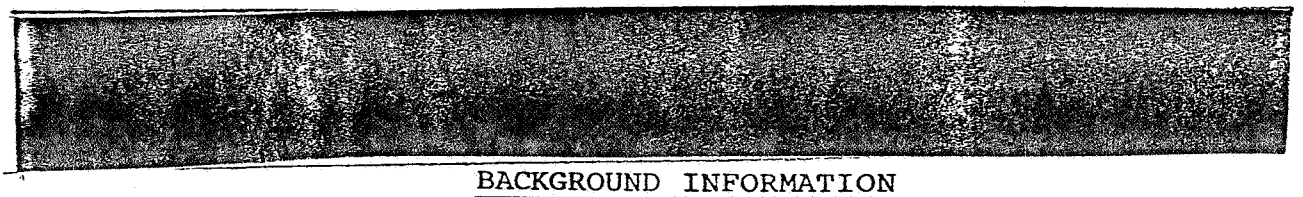
	by wt.
Active ingredient	
Azadirachtin	0.3%
Inert Ingredients	99.7%
	100.0

b. Detailed Formulation (Confidential)

Active ingredient



* Contains 3,000 ppm Azadirachtin, the active ingredient in the concentrate.



INERT INGREDIENT INFORMATION IS NOT INCLUDED

BACKGROUND INFORMATION

The toxic properties [redacted] documented, thus testing the MARGOSAN-O product in laboratory animals would make it difficult to distinguish between the active ingredient, azadirachtin, [redacted] Therefore,

Mr. Larson of Vikwood Ltd., was informed by Toxicology Branch that [redacted] content of MARGOSAN-O could be reduced for toxicity testing in animals which would more accurately reflect toxicity do to the product active ingredient (azadirachtin). Subsequently, MARGOSAN-O with an [redacted] was used in the animal toxicity tests contained in the present report.

At present, MARGOSAN-O has apparently demonstrated successful anti-feeding properties when tested using Gypsy moth larvae, leadminer (Liriomyza trifolii), Beet armyworm, Cabbage looper, Corn earworm, Fall armyworm, Colordo potato beetle, Bertha armyworm, Diamondback moth, Desert locust and Alfalfa looper.

DATA REVIEW:

1. Acute Oral Toxicity Evaluation of MARGOSAN-O, Rat.

Sponsor: Vikwood Ltd., 1221A Superior Avenue, Sheboygan, WI. Tester: Product Safety Labs. #T-2690, 12/16/82.

Test Material: Margoson-O concentrate, containing less [redacted] by volume. Density = 1.04 g/ml.

Following a 7 day quarantine period, 5 male and 5 female albino rats were each dosed by gavage with 5 g/kg. (actually 5.2 g/kg) of Margosan-O product. The rats had been fasted for 18 hours prior to treatment at termination of a 14 day observation period for mortality and illness, surviving rats were subjected to gross necropsies.

Results - No mortality. No signs of illness. The necropsies were unremarkable.

LD_{50} > 5 g/kg
Toxicity Category IV
Classification: Core Minimum Data

2. Acute Dermal Toxicity Evaluation of MARGOSAN-O, Rabbit.

Sponsor: Vikwood Ltd., Tester: Product Safety Labs. #T-2675, 12/30/82.

Test Material: Margosan-O product.

Two ml of test material contained on non-permeable patches were placed on shaved, abraded skin test sites on the trunks of each of 5 male and 5 female NZW rabbits. The patches were held in place with tape and occluded with elastic sleeves for a period of 24 hours; at which time the wrappings were removed. The rabbits were observed for 14 days: gross necropsies were performed on all survivors.

Results: No mortality, no illness; the gross necropsies were unremarkable.

LD_{50} > 2.0 ml (2.08 g)/kg
Toxicity Category III
Classification: Core Minimum Data

3. Primary Skin Irritation Evaluation of MARGOSAN-O, Rabbit.

Sponsor: Vikwood Ltd., Tester: Product Safety Labs. #T-2658, 12/13/82.

Test Material: Margosan-O product (the same material used in experiment #1 above).

Five-tenths ml of undiluted test material was placed under two, 2.5 cm^2 gauze patches on 1 intact and 1 abraded, shaved skin test sites on the backs of 6 (sex not given) NZW rabbits. The patches were secured in place with tape, and the entire rabbit trunks were occluded with rubberized elastic cloth. After a product contact period of 24 hours, the wrappings were removed and test sites washed. Skin irritation at test sites was evaluated and scored at 24 and 72 hours and at 4 and 7 days, according to Draize (EPA 40 CFR 183).

Results: The P.I. Score = 4.74; which indicates a moderately irritating substance.

Toxicity Category III
Classification: Core Minimum Data

4. Acute Inhalation Toxicity Evaluation of MARGOSAN-O, Rat.

Sponsor: Vikwood, Ltd., Tester: Product Safety Labs. #T-3069, 5/3/83.

Test Material: Margosan-O concentrate (the same material used in #1 above).

Five male and 5 female Wistar derived rats were exposed for a period of 4 hours to an aerosol cloud concentration determined by dividing the total weight of material expended by the number of liters of air passed through the exposure chamber (nominal concentration). Treated animals were observed (presumeably for mortality and illness) for 14 days; at termination, survivors were subjected to gross necropsies.

Results: No mortality, no signs of illness. The necropsy results were unremarkable. The nominal concentration was determined to be 43.9 mg/liter.

LC_{50} (nominal concentration) > 43.9 MG/L.
Toxicity Category - Not appropriate.
Classification: Supplementary Data (The actual cloud concentration rats were exposed to was not determined).

5. Primary Eye Irritation Evaluation of MARGOSAN-O, Rabbit.

Sponsor: Vikwood Ltd., Tester: Product Safety Labs. #T-2659, 12/16/82.

Test Material: Margosan-O product.

One-tenth ml of test material was placed on everted lids of one eye of each of 9 NZW rabbits; the upper and lower eye lids were then held together for 1 second. Untreaed eyes served as cotrols. The treated eyes of 3 rabbits were irrigated with 20 ml of water 30 seconds after test material instillation; the remaining 6 treated eyes were not irrigated. Rabbit eyes were observed and scored for lesions at 24, 48 and 72 hours, and at 4 and 7 days post-treatment according to Draize, et al, J. Pharmacol. Exp. Ther. 83: 377-390, 1944.

Results: The MMTS (maximum mean total score) for unwashed eyes was 4.33, and the MMTS for irrigated eyes was 3.33:

MMTS	Classification
0.0 - 0.5	Non-irritating
0.6 - 2.5	Practically non-irritating
2.6 - 15.0	Minimally irritating
15.1 - 25.0	Mildly irritating
25.1 - 50.0	Moderately irritating
50.1 - 80.0	Severely irritating
80.1 - 100.0	Extremely irritating
100.1 - 110.0	Maximally irritating

The test material was found to be minimally irritating to rabbit eyes.

Toxicity Category III
Classification: Core Minimum Data

6. Immune Response Evaluation of MARGOSAN-O, Rat.

Sponsor: Vikwood Ltd., Tester: Product Safety Labs. #T-3277, 9/23/83.

Test Material: Margosan-O product.

Nine male and nine female Sprague-Dawley rats were divided into two groups; 6 males and 6 females in one test group, and 3 male and 3 female rats in an untreated control group, following a one-week laboratory acclimation period.

The control and test rats were anesthetized and bled by cardiac puncture; all rats were weighed, and the test rats (6 male and 6 female) were injected I.P. with 0.5 ml of test material at initiation of the experiment.

Fourteen days after injecting the test animals with Margosan-O, control and test animals were again weighed, and blood samples were again collected for analyses.

The pre-test and terminal blood samples were analyzed for the following parameters:

Parameter	Unit
Red Blood Cells	$10^6/mm^3$
White Blood Cells	$10^3/mm^3$
Hemglobin	g/dl
Hematocrit	%
MCH	PG
MCV	FL
MCHC	%
Polymorphonuclear Cells	%
Bands	%
Lymphocytes	%
Monocytes	%
Eosinophils	%
Basophils	%
Protein	g/dl

MCH = mean corpuscular hemoglobin
MCV = mean corpuscular volume
MCHC= mean corpuscular hemoglobin conc.
g/dl= g/deciliter

Albumin		g/dl
1		g/dl
2		g/dl
		g/dl
		g/dl
Albumin/Globulin		

NOTE: The above list is quoted from the tester's report.

Quoted from the tester's report:

"The values obtained for each test animal on each parameter on Day 0 and on Day 14 were combined within sex groups and a mean and standard deviation calculated. Control animal values were also combined to provide similar data.

In addition, body weights on Day 0 and Day 14 were combined for each of the 4 groups and standard deviation calculated.

Mean differences between Days 0 and 14 were then determined for each parameter. Individual and mean differences were determined for body weights."

Results:

No differences in weight or weight gains were observed between test and control rats.

The only hematologic parameter found different between 0 and 14 day value determinations was a very small, but statistically significant difference in MCV (mean corpuscular volume).

Electrophoretic pattern of blood sera indicated no differences between pre-test and 14 day values for protein and albumin; however, $\alpha 1$, $\alpha 2$, and γ globulin fractions showed small, but statistically significant differences.

The only statistically significant differences between 0 and 14 day differential WBC (white blood cell) counts was that determined for polymorphonuclear cells.

Statistical comparisons to determine significant differences in the actual changes in blood values between control and treated rats indicated no differences; the only significant difference was found for MCV-the mean corpuscular volume.

Conclusions - The study results indicate that MARGOSAN-O does not elicit an immune response in the rat; the rats collective immune defense mechanisms did not recognize a 0.5 ml I.P. dose of MARGOSAN-O as harmful foreign material.

7. Sensitization Evaluation of MARGOSAN-O in the Guinea Pig.

Sponsor: Vikwood Ltd., Tester: Product Safety Labs. #T-2877, 1/31/83.

Test Material: Margosan-O product.

One-half ml of test material was placed under 2.2 cm^2 gauze patches on each of the shaved backs of 10 male Hartley strain guinea pigs. the patches were held in place for 6 hour periods with adhesive tape.

This exposure procedure was repeated on alternate days for a total of nine sensitizing doses; 24 hours following each exposure, observations were made of the diameter, height and color of test site reactions. Fourteen days after the last sensitizing dose, a challenge dose was applied to each test site in the manner described above. Test site reactions were scored as follows:

D = diameter of wheal
H = height (0-4)
C = color (redness, 0-4)

The tester explained that the amount of test material (concentration) applied had been previously determined as the concentration causing minimal or no irritation; the actual amount applied/sensitization or challenge dose was 0.25 ml Margosan-O + 0.25 ml water.

Results: No wheals, erythema or edema was observed; either during sensitization or post-challenge.

Conclusions: MARGOSAN-O is not a sensitizing agent.

Classification: Core Minimum Data

8. Mutagenicity Evaluation of MARGOSAN-O using the Ames Salmonella/microsome assay.

Sponsor: Vikwood Ltd., Tester: Product Safety Labs. #T-2463, 9/30/82.

Test Material: MARGOSAN-O product.

Five strains of Salmonella typhimurium were employed:

TA 1535;
TA 1537;
TA 1538;
TA 98, and;
TA 100

These bacteria were exposed to 0, 106.8, 1,068, 10,680, 53,400, or 106,800 ug of test material per plate (each concentration was replicated in triplicate, with and without S-9 microsomal activation).

The method used for preparation of arachlor induced rat liver microsomes and for plate incorporation evaluation of the test material was described by Ames et al (1975: Mutation Research, 31, 347-364).

Potential mutagenicity of the test material was assessed by ability to induce revertant mutations from histidine dependence to histidene independence.

The various test concentrations were absorbed onto filter paper discs, following dissolution of test material in dimethyl sulfoxide.

Postive Control Chemicals:

4 - nitroquinoline-1-oxide-for strains TA-1535, TA-1538, TA-98, TA-100

9 (5) amino acridine monohydrochloride strain TA-1537

2-amino anthracene + S-9 liver microsomes for strains TA-1535, TA-1538, TA-98, TA-100

Bengo (a) pyrene and S-9 liver microsomes for TA-98, TA-100

1 amino pyrene and S-9 liver microsomes for TA-1537, TA-1538, TA-98 and TA-100

The positive control agents were tested by plate incorporation tests for mutagenic evaluation.

Results:

1. No differences were observed between the number of histidine revertant mutants for control and test material at all concentrations tested for any of the Salmonella mutant strains tested.

2. Positive controls all showed increased histidine reversion to prototroply for all Salmonella strains tested.

Conclusions: Margosan-O did not display any mutagenic potential when evaluated using the Ames test.

Classification: Acceptable Study.