

US EPA ARCHIVE DOCUMENT



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

8 FEB 1983

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

TO: W. Nelson, PM Team 17
Insecticides/Rodenticides Branch
RD (TS-767C)

THRU: Norman Cook, Section Head *N Cook*
Section #2, EEB (TS-769C)

THRU: Clayton Bushong, Chief *CB*
EEB (TS-769C)

SUBJECT: EEB Review of Aquatic Data Submitted for
Margosine-O, EPA Reg. No. 100 - 10, Due RD 2/10/83.

I have reviewed and validated the aquatic acute toxicity data on Margosine-O. All three studies are acceptable to support registration pending our review of the proposed label (not yet submitted), and a statement from the registrant concerning the percentage active ingredient in the technical grade of the active ingredient.

Also, I have made a correction on the avian acute oral study evaluation. Since the test material contained 0.3% Azadirachtin, the LD50 should be adjusted from greater than 16.64 g/kg to greater than 49.92 mg/kg. This would also change the statement of toxicity from "Azadirachtin is practically non-toxic to mallard ducks" to "Azadirachtin is from highly toxic to practically non-toxic to mallard ducks."

Douglas J. Urban
Douglas J. Urban
Wildlife Biologist
EEB, (TS-769C)

Attachments

EEB REVISIONED
STATEMENT

INDEX NO.

EEB BRANCH REVIEW

DATE: IN 12.13.82 OUT 8 FEB 1983

R REG. NO. 100-10

ON OR EXP. PERMIT NO. _____

F SUBMISSION 11.29.82

RECEIVED BY HED 12.10.82

REQUESTED COMPLETION DATE 2.10.83

ESTIMATED COMPLETION DATE 2.3.83

REASON CODE/TYPE OF REVIEW 420 / GENERAL CORRESPONDENCE

PRODUCT(S): I, D, H, F, N, R, S BIOLOGICAL

ACCESSION NO(S). _____

MANAGER NO. W. NELSON (R)

NAME(S) MARGOSINE - O

BY NAME VIKWOOD LTD

MISSION PURPOSE SUBMISSION OF DATA FOR REVIEW

INDEX NO. CHEMICAL, & FORMULATION

217183

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DATA EVALUATION RECORD

1. CHEMICAL: Azadirachtin (Neem tree extract)
2. FORMULATION: Margosine-O concentrate
3. CITATION: Spare, W.C. 1982. The Acute Toxicity of Margosine-O to Daphnia magna Straus. Biospherics Incorporated, 4928 Wyaconda Rd, Rockville, Md. 20852, Project Number: 82-E-424D, Test dates: 11/10/82-11/12/82, Submitted by Vikwood Ltd., 1221 A Superior Ave, Box 554, Seboygan, WI 53081, (Shaughnessy Number ? _____; Accession Number ? _____).
4. REVIEWED BY: Douglas J. Urban
Wildlife Biologist
5. DATE REVIEWED: 2/1/83
6. TEST TYPE: Aquatic Invertebrate Acute LC50
 - A. Test Species: Daphnia magna Straus. ✓
 - B. Test Material: Margosine-O, containing 0.3% Azadirachtin,
[REDACTED]
7. REPORTED RESULTS: The 48-hour LC50 of Margosine-O to Daphnia magna is 13 mg/l (C.L. 0 - 60 mg/l) The compound exerted an observable sublethal effect on the daphnids swimming behavior. The 48-hour NOEC is <10 mg/l and > 1 mg/l. The 48-hour LC50 of the reference toxicant (K₂Cr₂O₇) to Daphnia magna is 0.26 mg/l. This is well within the expected range (0.10-0.75 mg/l).
8. REVIEWER'S CONCLUSIONS: This study is scientifically sound and with an LC50 of 0.43 mg/l (C.L. 0.026-0.057 mg/l), Azadirachtin is very highly toxic to Daphnia magna. The study does fulfill the requirement for an LC50 to freshwater aquatic invertebrates.

Methods and Materials The pertinent facts concerning the test practices follow:

- Stock cultures of daphnia are maintained at $20 \pm 2^\circ\text{C}$;
- Test Temperature = 22°C ;
- Photoperiod: 16 hr light/8 hr dark;
- ph: 7.3-8.0;
- Age of Daphnia: less than 20 hours;
- No solvent used;
- 5 daphnia/250 ml/ beaker;
- 20 daphnia/test concentration;
- Range finding Test Run:
0.1, 1, 10, 100, 1000 mg/l.

Statistical Analysis

The LC50 and 95% C.L. at 24 and 48 hours were calculated using the binomial and moving average methods. The Stephan's (1979) program was referenced.

Results % Mortality

	<u>Control</u>	<u>10</u>	<u>18</u>	<u>33</u>	<u>60</u>	<u>100</u>	(ppm)		<u>24 hour</u>	<u>48 hour</u>
								LC50	33	13
								C.L.	0-60	0-60
24 hour	0	40	45	50	100	100				
48 hour	0	40	65	70	100	100				

The pH and DO levels ranged from 7.3 to 8.0 and 77 to 97% raturation, respectively.

REVIEWER'S EVALUATION

A. Test Procedure The test procedure generally followed Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975.

B. Statistical Analysis

See attached data sheets A. The statistical analysis verified the reported results.

C. Discussion/Results

See data sheet B. Margosine-O actually contains 0.3% active ingredient (See Telephone report 1/27/83), not 95% as assumed by the laboratory. The results of the bioassay were adjusted to 0.3% a.i. and the LC50 recalculated: $LC50=0.043$ mg/l (C.L. 0.026 to 0.057 mg/l) by probit.

Conclusions

1. Category: Core, if the technical grade of the active ingredient is the same as Margosine-O with 0.3% Azadirachtin as the active component.
2. Rationale: N/A
3. Repairability: N/A

* *****

Q	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
100	20	20	100	9.53674E-05
60	20	20	100	9.53674E-05
33	20	14	70	5.76592
18	20	13	65	13.1588
10	20	8	40	25.1722

THE BINOMIAL TEST SHOWS THAT 0 AND 60 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 12.6363

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
1	1.61325	12.6363	0 +INFINITY

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
10	.170988	1	.23257

SLOPE = 2.57825
 95 PERCENT CONFIDENCE LIMITS = 1.51212 AND 3.64437

LC50 = 13.687
 95 PERCENT CONFIDENCE LIMITS = 8.58131 AND 18.2122

LC10 = 4.40277
 95 PERCENT CONFIDENCE LIMITS = 1.37071 AND 7.38938

	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.32	20	20	100	9.53674E-05
.19	20	20	100	9.53674E-05
.1	20	14	70	5.76592
.06	20	13	65	13.1588
.03	20	8	40	25.1722

THE BINOMIAL TEST SHOWS THAT 0 AND .19 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .0395327

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
1	1.61325	.0395327	0 +INFINITY

RESULTS CALCULATED USING THE PROBIT METHOD


ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.169587	1	.281595

SLOPE = 2.56157
 95 PERCENT CONFIDENCE LIMITS = 1.50669 AND 3.61645

LC50 = .0427166
 95 PERCENT CONFIDENCE LIMITS = .0266698 AND .0569974

LC10 = .0136397
 95 PERCENT CONFIDENCE LIMITS = 4.23123E-03 AND .0229787

DATA EVALUATION RECORD

1. CHEMICAL: Azadirachtin (Neem tree extract)
2. FORMULATION: Margosine-O concentrate
3. CITATION: Spare, W.C. 1982. The Acute Toxicity of Margosine-O to Rainbow Trout (Salmo gairdneri). Biospherics Incorporated, 4928 Wyaconda Rd, Rockville, Md. 20852, Project Number: 82-E-424 R, Test dates: 9/21/82-9/25/82, Submitted by Vikwood Ltd., 1221 A Superior Ave, Box 554, Seboygan, WI 53081, (Shaughnessy Number ? ; Accession Number ?)
4. REVIEWED BY: Douglas J. Urban
Wildlife Biologist
5. DATE REVIEWED: 2/2/83
6. TEST TYPE: Fish Acute LC50
 - A. Test Species: Rainbow Trout
 - B. Test Material: Margosine-O, containing 0.3% Azadirachtin

7. REPORTED RESULTS: The 96-hour LC50 of Margosine-O to Rainbow Trout is 8.8 mg/l (C.L. 5-12 mg/l). The 96-hour NOEC is 5 mg/l.
8. REVIEWER'S CONCLUSIONS: This study is scientifically sound and with an LC50 of 0.032 mg/l (C.L. 0.02-0.04 mg/l), Azadirachtin is very highly toxic to Rainbow Trout. The study does fulfill the requirement for an LC50 to coldwater fish.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Methods and Materials The pertinent facts concerning the test practices follow:

- Test temperature: 13°C;
- Photoperiod: 16 hr L/8 hr. D;
- ph 7.3-8.0;
- no solvent used;
- Age of fish, approx 2 months;
- Mean weight: 0.61 g (0.43-0.81g);
- 10 fish/test concentration; *10 fish / ~~10~~ 19 L glass carboys (15 L water)*;
- Biological loading: 0.41g/l
- A range finding test was run: 0.1, 1, 10, 100, 1000 mg/l.

Statistical Analysis

The LC50 and 95% C.L. at 24, 48, 72, and 96 hours were calculated using the binomial method. The Stephan's (1979) program was referenced.

Results

Hours	% Mortality						LC50	$\frac{24hr}{18}$	$\frac{48hr}{11}$	$\frac{72hr}{10}$	$\frac{96hr}{8.8}$
	Control	3	5	8	12	20 (ppm)					
24	0	0	0	0	0	70	C.L. (12-inf)	(8-20)	(8-12)	(5-12)	
48	0	0	0	0	70	100					
72	0	0	0	0	100	100					
96	0	0	0	30	100	100					

The pH and DO levels ranged from 7.1 to 7.6 and 40 to 90% saturation respectively.

VERIFIER'S EVALUATION

- Test Procedure The test procedure generally followed Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975.
- Statistical Analysis
See attached data sheets A. The statistical analysis verified the reported results.
- Discussion/Results
See data sheet B. margosine-O actually contains 0.3% active ingredient (see Telephone report 1/27/83), not 95% as assumed by the laboratory. The results of the bioassay were adjusted to 0.3% a.i. and the LC50 recalculated: LC50=0.032 mg/l (C.L. 0.02 to 0.04 mg/l) by binomial.

The low DO levels are not considered significant factors concerning the acceptability of the test. Low levels were recorded in the control and treatment groups where no mortality was reported, as well as in the treatment groups with mortality.

Conclusions

1. Category: Core, if the technical grade of the active is declared to contain 0.3% Azadiractin.
2. Rationale: N/A
3. Repairability: N/A

DOUGLAS J. URBAN MARGOSINE-O RAINBOW TROUT 96 HOUR LC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
20	10	10	100	.0976563
12	10	10	100	.0976563
8	10	3	30	17.1875
5	10	0	0	.0976563
3	10	0	0	.0976563

A.

THE BINOMIAL TEST SHOWS THAT 5 AND 12 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 8.77581

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

DOUGLAS J. URBAN MARGOSINE-O RAINBOW TROUT 96 HOUR LC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.06	10	10	100	.0976563
.04	10	10	100	.0976563
.03	10	3	30	17.1875
.02	10	0	0	.0976563
.009	10	0	0	.0976563

B.

THE BINOMIAL TEST SHOWS THAT .02 AND .04 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .0320363

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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DATA EVALUATION RECORD

1. CHEMICAL: Azadirachtin (Neem tree extract)
2. FORMULATION: Margosine-O concentrate
3. CITATION: Spare, W.C. 1982. The Acute Toxicity of Margosine-O to Bluegill Sunfish (Lepomis macrochirus) Biospherics Incorporated, 4928 Wyaconda Rd, Rockville, Md. 20852, Project Number: 82-E-424 BG, Test dates: 9/21/82-9/25/82, Submitted by Vikwood Ltd., 1221 A Superior Ave, Box 554, Seboygan, WI 53081, (Shaughnessy Number ? _____; Accession Number ? _____)
4. REVIEWED BY: Douglas J. Urban
Wildlife Biologist
5. DATE REVIEWED: 2/2/83
6. TEST TYPE: Fish Acute LC50
 - A. Test Species: Bluegill sunfish
 - B. Test Material: Margosine-O, containing, 0.3% Azadirachter
[REDACTED]
7. REPORTED RESULTS: The 96-hour LC50 of Margosine-O to Bluegill Sunfish is 37 mg/l (C.L. 20-60 mg/l). The 96-hour NOEC is 20 mg/l.
8. REVIEWER'S CONCLUSIONS: This study is scientifically sound and with an LC50 of 0.12 mg/l (C.L. 0.06-0.19 mg/l), Azadirachtin is highly toxic to Bluegill Sunfish. The study does fulfill the requirement for an LC50 to warmwater fish.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Methods and Materials The pertinent facts concerning the test practices follow:

- Test temperature: 20°C;
- Photoperiod: 16 hr L/8 hr D;
- Ph 7.6;
- No solvent used;
- Age of fish: 6-7 months old;
- Mean weight: 0.80 g(0.40-1.66 g);
- Mean length: 40.5 mm (34.5-54.7 mm);
- 10 fish/19 l glass carboys (15 l water);
- 10 fish/test concentration;
- Biological loading: 0.54 g/l
- A range finding test was run: 0,1,1,10,100,1000 mg/l.

Statistical Analysis

The LC50 and 95% C.L. at 24, 48, 72, and 96 hours were calculated using the binomial method. The Stephen's (1979) program was referenced.

Results

Hours	<u>% Mortality</u>						LC50	24hr 41	48 hr 37	72 hr 37	96 hr 37
	Control	12	20	35	60	100 (ppm)					
24	0	0	0	20	100	100	C.L. (20-60)	(20-60)	(20-60)	(20-60)	
48	0	0	0	40	100	100					
72	0	0	0	40	100	100					
96	0	0	0	40	100	100					

The pH and DO levels ranged from 6.8 to 7.6 and 11 to >100% saturation, respectively.

REVIEWER'S EVALUATION

A. Test Procedure The test procedure generally followed Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975.

B. Statistical Analysis

See attached data sheets A. The statistical analysis verified the reported results.

C. Discussion/Results

See data sheet B. margosine-O actually contains 0.3% active ingredient (See Telephone report 1/27/83), not 95% as assumed by the laboratory. The results of the bioassay were adjusted to 0.3% a.i. and the LC50 recalculated: LC50 = 0.12 mg/l (C.L. 0.06 to 0.19 mg/l) by binomial.

The low DO levels are not considered significant factors concerning the acceptability of the test. Low levels were recorded in the treatment groups where no mortality was reported, as well as in the treatment groups with mortality.

Conclusions

1. Category: Core, if the technical grade of the active is declared to contain 0.3% Azadiractin.
2. Rationale: N/A
3. Repairability: N/A

DOUGLAS J. URBAN MARGOSINE-O BLUEGILL SUNFISH 96 HOUR LC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
100	10	10	100	.0976563
60	10	10	100	.0976563
35	10	4	40	37.6953
20	10	0	0	.0976563
12	10	0	0	.0976563

A.

THE BINOMIAL TEST SHOWS THAT 20 AND 60 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 37.4758

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

DOUGLAS J. URBAN MARGOSINE-O BLUEGILL SUNFISH 96 HOUR LC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.32	10	10	100	.0976563
.19	10	10	100	.0976563
.11	10	4	40	37.6953
.06	10	0	0	.0976563
.04	10	0	0	.0976563

B.

THE BINOMIAL TEST SHOWS THAT .06 AND .19 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .117894

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.
