2-17-83

028201
SHAUGHNESSEY NO.

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REVIEW NO.

EEB BRANCH REVIEW

DATE: IN 11/10/82 OUT 2/17/83

FILE OR REG. NO. 707-109

PETITION OR EXP. PERMIT NO.

DATE OF SUBMISSION 11/3/82

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RD REQUESTED COMPLETION DATE 3/1/83

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RD ACTION CODE/TYPE OF REVIEW 400/Data Submission (other)

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

DATA ACCESSION NO (S).

PRODUCT MANAGER NO. R. Taylor (25)

PRODUCT NAME (S) STAM M-4

COMPANY NAME Rohm & Haas Company

SUBMISSION PURPOSE Submission of fish embryo-larvae studies to clarify previous research and questions raised by EEB

SHAUGHNESSEY NO.	CHEMICAL, & FORMULATION	% A.I.
	Technical Propanil	

STAM M-4

100.1 Pesticide Use

STAM M-4 is a technical compound used to formulate several herbicides.

103.4.2 Embryo-larvae and Life Cycle Studies

Attached are two reviews of embryo-larvae studies, one using STAM M-4 dissolved in water, the other using soil treated with STAM M-4.

104.4 Adequacy of the Toxicity Data

An earlier embryo-larvae study had been judged Core in an EEB review (Leitze 1979). That study found that the effect/no effect level of STAM M-4 to fathead minnow embryos and larvae was between 0.4 and 0.6 ppb. The registrant objected to our use of that study, claiming that at that time, STAM M-4 contained impurities that drastically increased its toxicity.

The two new studies were submitted by the registrant in the hope that EEB would use their results in making our hazard evaluations. Neither study can support a hazard evaluation. Neither study found a no-effect level. The soil study did not contain a data table mentioned in the text of the review, and this table was crucial to our ability to evaluate the test. Both studies tended to show that STAM M-4 was very highly toxic to the embryos and larvae of developing fish.

104.5 Additional Data Required

A valid embryo-larvae test, that shows a no-effect level, is necessary for the completion of several of our hazard evaluations. The submitted studies cannot be upgraded to support those evaluations.

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DATA EVALUATION

1. CHEMICAL: Stam M-4

2. FORMULATION: Technical Material - (Lot 9287 - 85.4% active ingredient by actual assay, and Lot 3143 - 98.7% active ingredient by actual assay)

3. CITATION: E G & G Bionomics, Inc. (1980) The Toxicity of STAM to Fathead Minnows (Pimephales promelas) Embryos and Larvae, When Applied to Sediments at Levels Comparable to and in Excess of Normal Field Usage - Report #BW-80-7-707, submitted to EPA 11/4/82 by Rohm & Haas Company, Inc., Spring House, Pennsylvania

4. REVIEWED BY: Thomas B. Johnston
Biologist, EEB/HED

5. REVIEW DATE: February 8-10, 1983

6. TEST TYPE: Embryo-larvae test - Exposure to treated soil

7. REPORTED RESULTS: All larvae exposed to STAM-treated soil died. Percentage hatch of eggs exposed to soil that had been treated with 134 ug/sq.cm (equivalent to 12 lbs per acre) of STAM while moist was significantly lower than that of the controls. Percentage hatch of eggs exposed to soil that had received the same level of treatment while dry was not significantly lower than that of the controls. Percentage hatch was not significantly affected at levels of application lower than 134 ug/sq.cm. Percentage hatch of embryos incubated in the soil control was comparable to that of the control containing no soil.

8. REVIEWERS'S CONCLUSIONS: The study (as given) is not scientifically sound, and does not fulfill any listed USEPA guidelines requirement. Table 2, which contained the critical data on egg hatch rates and larval mortality, was inexplicably left out of the report. Without that table, the results cannot be evaluated.

MATERIALS/METHODS

Perry clay soil obtained from a rice growing area 5 miles east of Mer Rouge, La was added wet to aquariums (approx. volume 20 liters) to a depth of 2.5 cm in each aquarium. The soil was allowed to dry completely for 9 days. The aquaria were then separated into four test groups, with two aquariums per group:

Group 1: STAM Lot 9287 (85.4% ai) applied at 67.25 ug/sq.cm. (6 lbs/acre) to soil moistened with 200 ml of dilution water prior to spraying,

Group 2: STAM Lot 9287 applied to moistened soil at 134 ug/sq.cm. (12 lbs/acre),

Group 3: STAM Lot 3143 (98.7% ai) applied to moistened soil at 134 ug/sq.cm.,

Group 4: STAM Lot 3143 applied to dried soil at 134 ug/sq.cm.

For each group, the appropriate amount of STAM was dissolved in 50 ml acetone and sprayed on the soil with a hand-held pump sprayer.

Two aquaria containing untreated soil, and two containing no soil nor pesticide, were run as controls.

Deionized, reconstituted well water (15 liters per aquarium) was added to each aquarium 24 hrs after the application of STAM to the soil. Sixty embryos less than 48 hours old were impartially distributed to each of 12 incubation cups, one of which was suspended in each of the test aquaria. Dead embryos were counted daily and removed, until hatching was complete.

To initiate the 10-day larval exposure, 40 larvae from each incubation cup were transferred to the respective aquarium upon completion of hatching. Larvae were fed brine shrimp twice daily. The number of live larvae was estimated on days 3 and 7 post-hatch, and at the test's termination. (Note that both series of tests were terminated early because all the larvae exposed to STAM died.)

STATISTICAL ANALYSES

Percentage hatch was calculated as the number of live larvae after hatching was complete, divided by the 60 embryos at the start of the test. Percentage hatch of the embryos and survival of the larvae were subjected to analysis of variance (Steel and Torrie 1960). If treatment effects were indicated, the means of the treatments were compared to the controls using Dunnett's procedure (Steel and Torrie 1960). If a treatment mean was significantly ($p<0.05$) less than the controls, then that treatment was considered to have adversely affected the test organisms.

RESULTS

Exposure to the soil that was sprayed with 134 ug/sq.cm. STAM technical Lot 9287 while moist significantly reduced the hatch of the minnow embryos. (The amount of the reduction was reported in a table left out of the report.) Exposure to all other STAM treatments, including the one where 134 ug/sq.cm. was sprayed on dry soil, had no significant adverse effect on the hatching rates of the minnow embryos. Percentage hatch of embryos in the soil controls was comparable to that of the control containing no soil.

All larvae exposed to treatments containing STAM died during the 10-day planned exposure period. In some cases, all larvae were dead within three days. (These mortality data were also contained in a table left out of the report.)

REVIEWER'S CONCLUSIONS:

Validation Category: Invalid

Category Rationale: Without raw mortality data and raw data on the hatch rates of the larvae, the test cannot be properly reviewed.

Category Repairability: This test appears scientifically sound overall. Only the lack of the data table (referred to as Table 2 in the report) prevents the completion of this review.

DATA EVALUATION

1. CHEMICAL: Stam M-4

2. FORMULATION: Technical material - active ingredient = 85.4%
TD #79-240, Lot #9287

3. CITATION: E G & G Bionomics, Inc. (1980) The Toxicity of Stam Technical (TD-79-240) to Fathead Minnow (Pimephales promelas) Eggs and Fry; submitted to EPA 11/4/82 by Rohm & Haas Company, Pa.

4. REVIEWED BY: Thomas B. Johnston
Biologist, EEB/HED

5. REVIEW DATE: Feb. 3-8, 1983

6. TEST TYPE: Embryo-larvae test

7. REPORTED RESULTS: The lowest concentration tested, 0.024 mg/l, significantly reduced the survival of fathead minnow fry exposed for 11 days. The percentage hatch of fathead minnow eggs was not significantly affected by exposure to 0.073 mg/l from egg age of 48 hrs to the time of hatching. Eggs exposed to 0.15 mg/l showed significantly lower rates of hatching.

8. REVIEWER'S CONCLUSIONS: The study is scientifically sound, but does not fulfill the USEPA guidelines requirements for an embryo-larvae test. Significant detrimental effects were found at all levels tested, so no determination of a no-effect level was possible. The experiment was abandoned long before its scheduled 30-day time duration because of the effects found at the lowest levels tested. With an 11-day MATC of somewhere less than 0.024 mg/l, Stam M-4 technical is very highly toxic to fathead minnow eggs and fry.

Materials/Methods

Methods used generally followed USEPA guidelines. Eggs were placed in a proportional diluter system within 48 hrs after laying and fertilization were completed. The system was maintained at 25° C, and triethylene glycol was used as a solvent. Control and solvent control aquaria were run concurrently. The test was designed to last 30 days, and the suriving fry were to have been weighed and measured, but significant mortality had occurred at all test levels by Day 11, and the test was abandoned.

Statistical Analyses

The percentage hatch of eggs and the survival of fry after 11 days exposure were subjected to analysis of variance (Steel and Torrie 1960) complete random block design, p=0.05 set as level for rejection of the null hypothesis (significance).

Results

Mean Measured Concentrations (ppm)	Replicate	Percentage Hatch	Survival (%) of Fry at Day 11
0.26	A	23	0
	B	43	0
0.15	A	63	0
	B	53	0
0.073	A	77	0
	B	87	0
0.048	A	85	18
	B	80	12
0.024	A	88	68
	B	97	72
Solvent Control	A	92	85
	B	90	82
Control	A	92	78
	B	95	80

Conclusions:

Validation Category: Supplemental

Category Rationale:

No "no-effect" level was found, nor could one be estimated from the results. In a prior fish embryo-larvae study, fry mortality increased dramatically just after 11 days. Therefore, the mortality level shown at 0.024 mg/l in this study cannot be expected to be the maximum mortality level that would result from exposure to that concentration. Nor could this lowest concentration (0.024 mg/l) be reasonably believed to be the lowest concentration at which adverse effects would be noted.

Some information from the study can be cautiously used in hazard evaluations. Exposure of 48-hr old fathead minnow eggs to 0.15 mg/l Stam M-4 can result in reduced hatch rates. The reductions are statistically significant ($p=0.05$), and may be biologically significant as well, since the rate in this test was reduced around 33%, relative to the controls. The fact that exposure to 0.024 mg/l for 11 days can significantly reduce fry survival can also be used.

The exposure to Stam M-4 must apparently be prolonged in order to produce toxic effects among fathead minnow fry. Exposing fry to up to 10 mg/l (nominal concentration) for 48 hrs produced some toxic symptoms, but no fatalities. Prolonging the exposure to 96 hrs killed all the test fish at that concentration, and produced an LC_{50} of 3.97 mg/l. It is unclear whether exposure of the organisms at the egg stage is necessary to increase the toxicity to the very high levels shown in the embryo-larvae test, or whether the toxicity would be apparent even if the organisms were only exposed for longer periods at the fry stage.

Category Repairability:

The test cannot be repaired to Core. As noted above, some of the information contained in the study can be cautiously used in hazard evaluations.

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE
OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY,
THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

JOHNSTON STAM M-4 96-HR LC50 W/FATHEAD MINNOW FRY
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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
10	19.5	19.5	100	1.3487E-04
6	19.5	13.5	69.2308	2.24747
3.6	19.5	1.5	7.6923	.0026974
2.2	19.5	7.5	38.4615	12.7026
1.3	19.5	1.5	7.6923	.0026974
.78	19.5	1.5	7.6923	.0026974
.46	19.5	.5	2.5641	1.3487E-04

THE BINOMIAL TEST SHOWS THAT 1.3 AND 6 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 5.1866

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.0740541	4.11849	3.4257	5.05911

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.577687	3.69818	2.38925E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.56604
95 PERCENT CONFIDENCE LIMITS = .615704 AND 4.51638

LC50 = 3.97006
95 PERCENT CONFIDENCE LIMITS = 1.93474 AND 14.1007

LC10 = 1.2702
95 PERCENT CONFIDENCE LIMITS = .0511926 AND 2.41422
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