DATA EVALUATION RECORD

1. Chemical: Permethrin

2. Test Material: Technical permethrin (FMC 33297, 95.7% ai)

3. Study Type: Fish full life cycle (egg to egg)

Species tested: Fathead minnow (*Pimephales promelas*)

4. Study ID: EG&G Bionomics, 1977. Chronic toxicity of FMC 33297 to the fathead minnow (*Pimephales promelas*). Study completed by: EG&G Bionomics Aquatic Toxicology Laboratory, 790 Main Street, Wareham, Mass. Submitted by: FMC Corporation Agricultural Chemical Division, Middleport, New York. Accession Number 096699.

5. Reviewed by: Thomas M. Armitage
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Date: 3-11-86

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7. Conclusions:

The authors report that this chronic full fish life cycle study yielded the following results:

Generally based on the reduced percentage survival of first and second generation fathead minnows exposed 30 days to mean measured concentrations $\leq$ .41 µg/L, the MATC of test material for this species is > 0.30 $\leq$ 0.41 µg/L.

1. No significant differences in hatch among egg groups initially exposed to different dose levels of test material were observed.

2. Mean measured concentrations of test material as high as 0.32 µg/L did not significantly affect the number of fish which survived to begin the spawning period on day 156.

3. Total lengths and weights of male and female fathead minnows which were measured at the termination of the spawning period (test day 246) did not differ significantly between controls and concentrations as high as .36 µg/L.

4. The number of spawns and eggs recovered from spawning chambers in all treatments was excellent.

5. The number of eggs per spawn, spawns per female and eggs per female were consistently high in all treatments and controls. These data indicate that concentrations as high as .41 µg/L do not adversely affect the spawning of fathead minnows.

6. Eggs spawned by fathead minnows exposed to concentrations as high as .41 µg/L hatched in percentages which did not differ significantly from control and solvent control.

7. The percentage of second generation fry which survived 30 days exposure to .41 µg/L was significantly reduced when compared to survival in lower concentrations and controls.

8. Total lengths and weights of 30-day-old second generation fry did not differ significantly among treatments and controls.

9. Exposure of minnows for 63 days resulted in whole body résidues which were generally 3000 times higher than measured concentrations in water.

10. Depuration in clean water was 90 percent complete after 14 days.

11. Accumulation in muscles and viscera was compared as were differences between males and females.

This study does not fulfill the guideline requirement for a full life cycle study because statistical analysis was not adequately documented, and raw data were not provided for EEB review. In addition, the use of an unacceptable solvent, DMSO, in large quantities was not justified. The study therefore only supplementally fulfills the guideline requirement for a full fish life cycle study.

8. Recommendations:

The study may be upgraded to core if raw data are supplied and indicate that the study conclusions are supported by statistically sound analysis. The use of relatively large quantities of an unacceptable solvent, DMSO, must also be justified. Other deviations from accepted protocol noted in section 14 must be addressed.

9. Background:

The study was submitted in support of registration of permethrin. It was reviewed by EEB in 1978 and identified as a "core chronic fish study" (O'Brien, January 17, 1978; Balcomb February 24, 1978). However, raw data were not subjected to analysis of variance to determine statistical significance. The study cannot fulfill the requirement of a full life cycle study until such analysis can be completed by EEB.

10. Discussion of Individual Test: N/A

11. Materials and Methods: (Portions below excerpted from submission.)

a. Test Animals

Fathead minnow eggs used to initiate the chronic test were obtained from brood stocks at the Aquatic Toxicology Laboratory, EG&G Bionomics. A group of 60 eggs was incubated in each duplicate aquarium and dead eggs were counted and removed daily until hatching was completed. Pecentage hatch (number of live fry/60 eggs x 100) was calculated for each duplicate. Two groups of twenty fry from each egg group were randomly selected and introduced into the fry chambers of each duplicate tank.

b. Test System, Dosage Form, Design

The system used in the exposure of fathead minnow fry and in the chronic test was equipped with a proportional diluter (Mount and Brungs, 1967) to provide intermittent introduction of the test material. The diluter was constructed with a dilution factor of 0.5 and delivered five concentrations of FMC 33297 and two controls to duplicate test aquaria. One control received only the diluent water and a second control received an amount of dimethyl sulfoxide (7.2 ug/L) equal to the amount added with the highest concentration of FMC 33297.

The nominal concentrations selected for the chronic test were 1.0, 0.50, 0.25, 0.13 and 0.063 ug/L. (Mean measured concentrations at Hatched)

At the time adult fish were terminated (day 246), all fish were sampled for FMC 33297 residues or used in a depuration study as described below. All samples were frozen and shipped to FMC Corporation for analysis.

Four surviving males in the high concentration (treatment 1) were sampled individually to determine the amount of FMC 33297 which was adsorbed to the skin. Two fish were dipped in methanol and then methylene chloride prior to freezing the whole fish, and the solvents, as separate samples. The remaining two fish were frozen without solvent immersion.

In the remaining treatments, 3 males and 6 females were transferred to aquaria receiving diluent water for a depuration study. On days 3, 7 and 14 after transfer, a sample of one male and a sample of two females were removed as whole fish. The remaining males and females from each treatment not used in the depuration study

were sampled individually for residues of FMC 33297. Fish from treatment 2 were dissected into viscera and eviscerated body portions for separate analysis. Male portions were from individual fish while female portions were combined from two fish. Males and females from the remaining treatments were analyzed individually as whole fish.

Residue Analysis of Fish Tissue

At various times during the chronic test, fathead minnows and eggs were sampled to determine accumulation and depuration of FMC 33297 residues.

Extra fish which were removed on day 63 were pooled from duplicate aquaria at each treatment and sampled as whole fish. These analyses were performed by Bionomics' analytical chemistry personnel.

On test day 156, extra fish removed were pooled by treatment and divided into two samples of whole fish per treatment. One group of samples was analyzed by Bionomics' analytical chemistry personnel and the other group was shipped frozen to FMC Corporation, Richmond, California for analysis. In addition, six fish were removed from nominal concentrations of 0.38 and 0.19 ug/L on day 156, and were transferred to aquaria receiving only diluent water for a depuration study. Two fish were removed from each aquarium on days 14, 28, and 42 after transfer and shipped frozen to FMC Corporation for analysis.

At the end of 30 days exposure, individual groups of second generation fry were pooled as whole fish and frozen. After all groups had completed 30-day exposures they were shipped to FMC Corporation for analysis.

On test day 63 (60 days post-hatch), fish from the two fry groups in each aquarium were pooled and 15 individuals were impartially selected to continue exposure in the spawning chamber. While in the spawning chambers, fish were fed twice daily with frozen brine shrimp supplemented with trout food granules. All tanks were siphoned weekly to remove particles and brushed at intervals of 8 to 10 weeks to remove a film of algae and diatoms which adhered to the glass.

On test day 156, most fish had well-developed secondary sexual characteristics. At this time, the surviving fish in each aquarium were sexed and their number reduced to a ratio as close to three males and seven females as was possible.

When spawning began, eggs were removed from the underside of spawning tiles after 1:00 p.m. each day. The number of eggs in each spawn was counted and groups of fifty eggs from a maximum of ten spawns in each aquarium were incubated for hatchability determinations.

Twenty fry which hatched from two of the earlier spawnings in each aquarium were placed in the respective fry chambers and reared for 30 days as described previously for first generation fish. At the end of 30 days each group was photographed for total length determinations and then weighed wet. Fry groups were transferred from control to treatment 2 and from treatment 2 to control in order to obtain further information on the critical life state (newly hatched fry). Exposure of adult fish was terminated on day 246 when spawning activity had ceased in most aquaria. At this time each fish was individually weighed, measured and examined for sex and degree of sexual maturity.

The photoperiod followed a recommended schedule (U.S. EPA, 1971) and was adjusted on the first day and fifteenth day of each month beginning with the day length of December 1 on the first day of the test. Illumination was provided by a combination of Durotest (Optima FS) and wide spectrum Gro Lux fluorescent lights. These lights were affixed centrally 64 cm above the surface of the water in the aquaria. The entire exposure system was screened with black polyethylene curtains to prevent disturbance of the fish and to minimize the effect of laboratory lighting on the intended photoperiod.

Temperature and dissolved oxygen concentrations were measured daily using a YSI dissolved oxygen meter with a combinatiqqqelectrode-polarographic probe. All aquaria were qqqqked once each week on a rotating basis. Total hardness, alkalinity and pH were measured in each concentration of FMC 33297 and controls to detect possible alterations in these parameters due to the test material.

Water from each duplicate aquarium was sampled eight times during a 10-day period immediately prior to the initiation of the chronic study to monitor concentration of the test material in water. During the chronic test, water from each duplicate aquarium was sampled weekly for analysis of FMC 33297.

Exposure

After 30 and 60 days of post-hatch exposure, fry groups were transferred onto a translucent millimeter

grid and photographed for determination of total lengths. Percentage survival was also recorded at these intervals. During the initial 30 days of exposure, fry were fed brine shrimp nauplii four times daily. During the 30- to 60-day exposure interval, minnows were fed three times daily with frozen brine shrimp.

The diluter was equipped with a mechanical injector (manufactured by George Frasier, Duluth, MN) and a 50 mL glass syringe with a stainless steel needle. The injector pumped microliter quantities of the FMC 33297 stock solution to the mixing container during each diluter cycle. The average number of cycles during the test was approximately 320/day. A one liter volume was delivered to each 40 liter aquarium during each cycle which provided a flow rate equal to 8 volume replacements per day in each aquarium.

Each glass aquarium measured 90 x 30 x 30 cm and approximately one-third of the area was subdivided by a stainless steel screen to provide space for two fry chambers (30 x 12 x 25 cm). The remaining area served as the spawning chamber. The water depth in each aquarium was 15 cm, maintained by a standpipe drain.

Spawning sites for adult fathead minnows were made from halved 7.5 cm sections of 10 cm diameter cement-asbestos drain tile. Five spawning sites were placed in each spawning chamber with the concave surface facing downward. Egg groups were incubated in cups made from 5 cm diameter glass jars with Nytex 40 mesh screen bottoms. The egg cups were oscillated in the test water by a rocker arm apparatus driven by a 2 rpm motor.

A constant temperature of 25 $\pm$ 1 °C was maintained in the aquaria by resting them in water baths. Circulating water in the baths was heated by immersion coils connected to a relay which was controlled by a mercury column themoregulator.

c. Statistics

Means of measured biological parameters from duplicate aquaria were subjected to analysis of variance (completely randomized block design, P = 0.05). Data for percentage survival and percentage hatch were transformed to arc sin $\sqrt{\text{percentage}}$ prior to analysis. When treatment effects were indicated, the means of these parameters were compared to control using Dunnett's procedure

(Steel and Torrie, 1960). When a treatment mean was significantly different from the control mean (P = 0.05), that treatment was considered an effect level.

12. Reported Results:

Reported results are noted in section 7 above.

13. Study Author's Conclusions/QA Measures:

Conclusions noted in section 7 above. No QA measures were indicated.

14. Reviewer's Discussion:

a. Test Procedures: The following deviations from accepted protocol were noted:

(1) DMSO should not be used as a solvent.

(2) The maximum calculated concentration of solvent (7.2 mg/L) exceeds the maximum recommended concentration (1/20 the concentration of toxicant).

(3) Materials used to construct the test system were not identified clearly.

(4) Test system temperature should be monitored continuously. No indication of this was provided.

(5) No data were provided to indicate temperature range during the study.

(6) Operation of the diluter must be checked daily either through measurement of the toxicant in the chamber or directly. No indication of this was provided.

(7) Eggs or larvae used to initiate the study must come from at least three different females. This must be verified.

(8) Prior to selection of 15 fish to continue exposure in each spawning chamber, the number of deformed fish discarded from each tank must be noted. This was not addressed in the report.

b. Statistical Analysis: Subjecting the means of measured biological parameters from two duplicate aquaria at each dose level to analysis of variance does not appear to be an appropriate method of statistical analysis.

This implies that only two observations per dose level were subjected to ANOVA. Larger sample sizes of at least four observations per dose level, are required to provide statistically sound analysis of variance. Results of statistical analysis of the raw data, not simply reported means per replicate, should be submitted to EEB along with the actual data for statistical analysis.

Analysis of percentage hatch and survival data by ANOVA does not provide a statistically sound conclusion because only two observations per dose level (two replicates) were available for analysis. However, visual inspection of the percent hatch and survival data supports the author's conclusion. No further statistical analysis would appear to be necessary.

c. Discussion/Results: Upon the basis of percentage survival and percentage hatchability data, the MATC of test material for the fathead minnow would appear to be $> 0.30 \leq 0.41$. It is not possible, however, to assess the effect of chronic exposure upon length and weight of fathead minnows. This is because only the mean values of length and weight within each of two replicates per exposure level were statistically analyzed and presented to EEB. It is not possible to develop conclusions with respect to bioaccumulation because the effect of the solvent DMSO is not clear. Inspection of the data indicate that concentrations of test material as high as .41 ug/L did not appear to affect egg production or spawning behavior. Because of the limited number of replicates it is not possible to subject these data to statistical analysis.

d. Adequacy of Study:

(1) Classification: Supplemental

(2) Rationale: The results of length and weight measurement cannot be statistically validated without raw data. Deviations from accepted protocol listed in section 14 above must be addressed.

(3) Repair: This study may be upgraded to core if raw length and weight data are subjected to statistical analysis and provided to EEB. Deviations from accepted protocol listed above in section 14a must also be explained or justified.

15. Completion of One-Liner for Study: One-liner completed February 28, 1986.

16. CBI Appendix: N/A

Table 4 -- Measured concentrations of FMC 33297 (total cis and trans isomers) in water during the initial days of the chronic test and during the remaining portion of the test after nominal concentrations were increased.

Treatment level	Days 0-35					Days 36-246				
	Nominal concentration (µg/ℓ)	Measured concentration (µg/ℓ)				Nominal concentration (µg/ℓ)	Measured concentration (µg/ℓ)			
		Mean ± S.D.	Range	N[a]	N_1[b]		Mean ± S.D.	Range	N	N_1
1	1.0	0.41 ± 0.12	0.20-0.60	9	0	1.5	0.76 ± 0.38	0.31-2.4	39	0
2	0.50	0.14 ± 0.061	0.070-0.25	10	0	0.75	0.39 ± 0.26	0.14-1.7	57	0
3	0.25	0.092 ± 0.050	0.040-0.16	7	2[c]	0.38	0.27 ± 0.21	0.040-0.98	53	4[c]
4	0.13	<0.032 ± 0.022	<0.011-0.059	5	2	0.19	0.17 ± 0.19	0.021-1.0	47	
5	0.063	<0.023 ± 0.023	<0.010-0.068	6	5	0.094	<0.096 ± 0.11	<0.011-0.42	45	23

[a] Total number of observations used to compute mean.

[b] Number of observations in which concentration of one or both isomers was below detectable limit.

[c] Observations below minimum detectable limit were not included in mean when their incidence was low in relation to observations in detectable range.

Table 5 -- Measured concentrations of cis isomer FMC 33297 in water during the initial 35 days of the chronic test and during the remaining portion of the test after nominal concentrations were increased.

Treatment level	Days 0-35					Days 36-246				
	Nominal concentration (µg/ℓ)	Measured concentration (µg/ℓ)				Nominal concentration (µg/ℓ)	Measured concentration (µg/ℓ)			
		Mean ± S.D.	Range	N[a]	N_1[b]		Mean ± S.D.	Range	N	N_1
1	0.40	0.22 ± 0.062	0.11-0.31	9	0	0.60	0.49 ± 0.30	0.14-1.8	59	0
2	0.20	0.087 ± 0.034	0.059-0.16	10	0	0.30	0.23 ± 0.23	0.059-1.5	57	0
3	0.10	0.051 ± 0.033	0.029-0.12	8	1[c]	0.15	0.18 ± 0.19	0.020-0.82	57	0
4	0.050	<0.023 ± 0.018	<0.005-0.046	5	2	0.075	0.14 ± 0.17	0.010-0.87	52	1[c]
5	0.025	<0.014 ± 0.017	<0.005-0.051	7	5	0.038	0.083 ± 0.10	0.007-0.39	45	4[c]

a
Total number of observations used to compute the mean.

b
Number of observations in which concentration was below detectable limit.

c
Observations below minimum detectable limit not included in mean.

Table 6 -- Measured concentrations of trans isomer FMC 33297 in water during the initial 35 days of the chronic test and during the remaining portion of the test after nominal concentrations were increased.

Treatment level	Days 0-35					Days 36-246				
	Nominal concentration (µg/ℓ)	Measured concentration (µg/ℓ)				Nominal concentration (µg/ℓ)	Measured concentration (µg/ℓ)			
		Mean ± S.D.	Range	N[a]	N_1[b]		Mean ± S.D.	Range	N	
1	0.60	0.19 ± 0.061	0.088-0.29	9	0	0.90	0.47 ± 0.13	0.16-0.68	59	
2	0.30	0.057 ± 0.032	0.011-0.12	10	0	0.45	0.16-0.074	0.011-0.38	58	
3	0.15	0.029 ± 0.023	0.011-0.077	8	1[c]	0.23	0.084-0.042	0.022-0.18	54	4[c]
4	0.075	<0.008 ± 0.005	<0.005-0.023	8	4	0.11	0.036-0.029	0.0056-0.14	51	8[c]
5	0.038	<0.007 ± 0.003	<0.005-0.017	9	8	0.056	<0.014 ± 0.015	<0.0056-0.079	51	26

[a] Number of observations used to compute the mean.

[b] Number of observations below detectable limit.

[c] Observations below minimum detectable limit not included in mean.