

to-7-93
5-19-94

MRID Nos. 426998-02, 426998-03, and 427182-02

427182-02

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Methyl Anthranilate.
Shaughnessey Number: 128725.
- 2. **TEST MATERIAL:** Methyl anthranilate; CAS No. 134-20-3; Lot No. 271292387; purity of >98%; a clear liquid.
- 3. **STUDY TYPE:** 72-2. Freshwater Fish Acute Toxicity Test.
Species Tested: *Salmo salar*, *Oncorhynchus mykiss*, *Ictalurus punctatus*, *Lepomis macrochirus*.
- 4. **CITATION:** Clark, L. 1992. Acute Toxicity of Methyl Anthranilate to Fish: Rainbow Trout, Atlantic Salmon, Channel Catfish, and Bluegill Sunfish. Study No. QA-208. Study performed by USDA/APHIS/ADC/Denver Wildlife Research Center, Monell Chemical Senses Center, Philadelphia, PA. Submitted by USDA Denver Wildlife Research Center, Philadelphia, PA. EPA MRID Nos. 426998-02, 426998-03, and 427182-02.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 10/7/93 for RGM

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
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Signature: P. Kosalwat
Date: 10/7/93

Henry T. Craven, M.S.
Supervisor, EEB/EFED
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Signature:
Date:

- 7. **CONCLUSIONS:** These studies are ~~not~~ scientifically sound ~~and but~~ do not meet the guideline requirements for an acute toxicity study using freshwater fish. Due to degradation of methyl anthranilate in the test solutions, the actual concentrations to which the test fish were exposed are unknown. In the bluegill and catfish studies, the concentration of test material and dissolved oxygen decreased substantially from test initiation to test termination. No chemical analysis was conducted and dissolved oxygen was measured only at test initiation in the

Atlantic salmon and rainbow trout studies. The 96-hour LC₅₀ was 9.12 mg/l (based on measured concentrations) for bluegill, 16.23 mg/l (based on measured concentrations) for the catfish, 32.35 mg/l (nominal) for Atlantic salmon, and 22.91 mg/l (nominal) for rainbow trout. Based on the LC₅₀ values, methyl anthranilate would be classified as moderately toxic to bluegill, and slightly toxic to channel catfish, Atlantic salmon, and rainbow trout. The NOEC was 6 mg/l for Atlantic salmon, 5 mg/l for rainbow trout, 7 mg/l for channel catfish, and 7 mg/l for bluegill.

8. **RECOMMENDATIONS:**
9. **BACKGROUND:**
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.
11. **MATERIALS AND METHODS:**

A. **Test Animals:**

Atlantic salmon (*Salmo salar*) were obtained from the U.S. Fish and Wildlife Service in Cortland, NY. The salmon were held for 2 weeks in a holding tank with a pH of 7.1 and a temperature of 12.5°C. The fish had a mean weight of 0.3 ±0.21 g. Fish length was not measured.

Rainbow trout (*Oncorhynchus mykiss*) were obtained from the U.S. Fish and Wildlife Service in Cortland, NY. The trout were held for 2 weeks in a holding tank with a mean pH of 7.0 and a mean temperature of 12.6°C. The fish had a mean weight and length of 0.14 ±0.03 g and 35.5 ±0.44 mm, respectively.

Channel catfish (*Ictalurus punctatus*) were obtained from Delmarva Aquatics in Odessa, DE. The catfish were held for 2 weeks in a holding tank with a mean pH of 7.6 and a mean temperature of 22.6°C. The fish had a mean weight and length of 0.12 ±0.05 g and 21.24 ±0.15 mm, respectively.

Bluegill sunfish (*Lepomis macrochirus*) were obtained from Delmarva Aquatics in Odessa, DE. The catfish were held for 2 weeks in a holding tank with a mean pH of 7.6 and a mean temperature of 22.6°C. The fish had a mean weight and length of 0.62 ±0.02 g and 35.5 ±0.44 mm, respectively.

- B. **Test System:** The tests were conducted under static conditions in a circulating water bath to maintain the test temperature. The test vessels were 14-l glass tanks with 10 l of test solution at a solution depth of 30 cm. The test solutions were not aerated. The dilution water was cured water obtained from the laboratory which was filtered and treated prior to use.

For the Atlantic salmon study, the mean test temperature was 15.06°C. The photoperiod was 12 hours of light and 12 hours of darkness.

For the rainbow trout study, the mean test temperature was 9.6°C. The photoperiod was 12 hours of light and 12 hours of darkness.

For the catfish study, the test temperature was approximately 23°C and the mean temperature did not vary more than 1°C during the test. The photoperiod was 12 hours of light and 12 hours of darkness.

For the bluegill study, the test temperature was approximately 23°C and the mean temperature did not vary more than 1°C during the test. The photoperiod was 12 hours of light and 12 hours of darkness.

The test solutions were prepared by transferring appropriate amounts of test material to a volumetric flask. Dilution water was added to the flask. The solution was sonicated for 30 minutes and stirred for 30 minutes. Stock solutions were serially diluted with the dilution water to prepare test solutions.

- C. **Dosage:** Ninety-six-hour static tests. For the Atlantic salmon study, five nominal test concentrations (1, 6, 13, 25, and 50 ppm) were selected. For the rainbow trout study, seven nominal test concentrations (1, 5, 10, 15, 20, 25, and 50 ppm) were selected. For the catfish and studies, six nominal concentrations (5, 10, 20, 40, 50, and 100 ppm) were selected. In addition, a dilution water control was included in each study.

- D. **Design:** Five salmon were added, without bias, to each of four test vessels per treatment. The biomass loading rate was 0.152 g/l.

Ten trout were added, without bias, to each of two test vessels per treatment. The biomass loading rate was <0.8 g/l.

For the catfish and bluegill studies, ten fish were added to each of four test vessels per treatment. The biomass loading rate was 0.12 g/l for catfish and 0.06 g/l for bluegill. The mean mass of fish within each treatment was equal.

For each test, pH and dissolved oxygen concentration (DO) were measured at test initiation and test termination. In addition, temperature in four test tanks was measured every 30 minutes throughout the test period.

For the catfish and bluegill studies, the nominal concentrations were verified using high pressure liquid chromatography.

- E. **Statistics:** Dose response curves and confidence intervals were generated using logit and probit procedures.

12. **REPORTED RESULTS:** Atlantic Salmon Test. At test initiation, the test solutions had a pH of 7.0 and a DO of 11.2 mg/l. These parameters were not measured at any other time during the test. The temperature did not vary more than 1.5°C during the study. Based on nominal concentrations, the 96-hour LC₅₀ was 32.35 ppm. The mobility and color of the fish in the 1 and 6 ppm concentrations were similar to that of the controls throughout the test. The NOEL was 6.0 ppm.

Rainbow Trout Test. At test initiation, the test solutions had a mean pH of 7.3 and a mean DO of 9.9 mg/l. "DO and pH were not monitored at the end of the 96 hr test. Given that DO was well above guideline levels, we did not expect that this would effect our interpretations of the mortality data." The temperature did not vary more than 1°C during the study. Based on nominal concentrations, the 96-hour LC₅₀ was 29.91 ppm with a 95% confidence interval of 21.6-24.4 ppm. The NOEL was 5 ppm.

Channel Catfish Test. The test solutions at test initiation and test termination had a mean pH of 7.8 and 7.5, and a mean DO of 7.2 and 4.4 mg/l, respectively. "The DO content of test vessels decreased significantly from 0 to 96 hr." The mean temperature did not vary more than 1°C during the study. "Some tanks showed dramatic signs of methyl anthranilate loss. The tanks with the greatest loss of methyl anthranilate were those that also had large die off of fish (i.e., high concentrations of MA both killed the

fish on the short term and were more readily degraded over a longer term)." In addition, the author reported that some of the surviving fish showed signs of oxygen stress. Mortality and exposure concentrations (based on the highest measurable quantity of test material) are presented in Table 3 (attached). Based on the highest measurable quantity of methyl anthranilate, the 96-hour LC₅₀ was 16.23 ppm with a 95% confidence interval of 11.6-22.5 ppm. The slope of the dose-response curve was 3.8. The NOEL was 7 ppm.

Bluegill Sunfish Test. "On the average, the DO content of test vessels decreased significantly from 0 to 96 hr." The test solutions at test initiation and test termination had a mean pH of 7.4 and 7.2, and a mean DO of 7.2 and 2.4 mg/l, respectively. The mean temperature did not vary more than 1°C during the study. Mortality and mean measured concentrations are presented in Table 2 (attached). The LC₅₀ for the first 24-hours was 19.8 ppm, but fish at the lower-mid concentrations were still and apparently oxygen stressed." The 96-hour LC₅₀ was 9.12 ppm with a 95% confidence interval of 8.0-10.5 ppm. The NOEL was 7 ppm. The slope of the dose-response curve was 9.4.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions were presented by the author.

A GLP compliance statement and a quality assurance statement were included in the report indicating the tests were performed according to Good Laboratory Practice Standards (40 CFR).

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

- A. **Test Procedure:** The test procedures were generally in accordance with the SEP, except for the following deviations:

Raw mortality data for the rainbow trout and Atlantic salmon studies were not included in the report.

According to the author, the amount of test material in the bluegill and catfish studies decreased dramatically. Therefore, the actual concentrations to which the test organisms were exposed are unknown.

In the bluegill test, there is a discrepancy between raw data (MRID No. 426998-03) and the data summary (MRID No. 426998-02). Raw data showed that six nominal concentrations (5, 10, 20, 40, 50, and 100 ppm) were

used. However, the summary showed 10 mean measured concentrations (4, 8, 15, 20, 30, 35, 45, 70, 82, and 92 ppm).

The DO must be measured at test initiation and every 48 hours thereafter. For the bluegill and catfish studies, the DO was measured only at test initiation and test termination. For the salmon and trout studies, the DO was measured only at test initiation.

During the test period, the mean DO decreased from 83% to 28% of saturation in the bluegill study and from 83% to 50% of saturation in the catfish study. For an acute toxicity test, the DO must remain between 60% and 100% of saturation during the first 48 hours and between 40% and 100% of saturation during the last 48 hours.

Atlantic salmon (*Salmo salar*) is not a recommended test species and no justification for use of this species was given.

The photoperiod was 12 hours light and 12 hours dark. A photoperiod of 16 hours light and 8 hours dark with a 15- to 30-minute transition period between light and dark is recommended.

- B. **Statistical Analysis:** For the bluegill and catfish studies, the reviewer calculated the 96-hour LC₅₀ values using EPA's Toxanal computer program and obtained results similar to those of the author (printouts, attached).
- C. **Discussion/Results:** Analytical data were not submitted. The test material was reported to be "subject to microbial attack." In addition, "microbial degradation can be rapid, and is sometimes correlated with substantial reductions in dissolved oxygen." The concentration of DO and test material in the bluegill and catfish studies decreased substantially from test initiation to test termination. For the rainbow trout and Atlantic salmon studies, the DO content at test termination and the concentration of test material in solution were not measured. The author suggests "the recovery of equilibrium and mobility at specific concentrations towards the end of the test (e.g. the trout and salmon tests) may reflect loss of MA in the static system as a function of time, thus allowing the fish to recover." Given the substantial decrease in

test material and DO in the bluegill and catfish studies, it is likely that there was a similar reduction of test material and DO in the salmon and rainbow trout studies. A flow-through system would have been more appropriate for studies conducted with this test material.

These studies are ~~not~~ scientifically sound and ^{not} do not meet the guideline requirements for an acute toxicity study using freshwater fish. The 96-hour LC₅₀ was 9.12 mg/l (based on mean measured concentrations) for bluegill, 16.23 mg/l (based on the highest measured concentrations) for the catfish, 32.35 mg/l (nominal) for Atlantic salmon, and 22.91 mg/l (nominal) for rainbow trout. Therefore, based on the LC₅₀ values, methyl anthranilate would be classified as moderately toxic to bluegill and slightly toxic to channel catfish, Atlantic salmon, and rainbow trout. The NOEC was 6 mg/l for Atlantic salmon, 5 mg/l for rainbow trout, 7 mg/l for channel catfish, and 7 mg/l for bluegill. *OK 5/19/94*

D. Adequacy of the Study:

- (1) **Classification:** ~~Invalid.~~ *Supplemental*
- (2) **Rationale:** Due to degradation of methyl anthranilate in the test solutions, the actual concentrations to which the test fish were exposed are unknown. The test material was reported to have decreased substantially during the catfish and bluegill studies. No chemical analysis was conducted in the salmon and trout tests. In addition, DO decreased substantially during these tests.
- (3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes; 28 September 1993.

Table 2. Mortality summary for bluegill sunfish. QA208.

ID	PPM	Mortality				
		N	H96	H72	H48	H24
r8	92.3768	10	10	10	10	10
r14	92.0887	10	10	10	10	10
r15	82.8842	10	10	10	10	10
l7	70.4704	10	10	10	10	10
r3	45.3035	10	10	10	10	10
l6	36.4167	10	10	10	10	10
r2	35.3476	10	10	10	10	10
r13	35.0822	10	10	10	10	8
l9	30.5011	10	10	10	10	7
r12	22.3769	10	10	10	10	10
r7	21.8212	10	10	10	10	0
r16	19.1724	10	10	10	10	0
r5	18.0866	10	10	10	10	10
l3	16.5518	10	10	10	10	0
l12	14.8895	10	10	10	10	10
l8	7.9016	10	10	10	10	6
l5	7.5098	10	2	2	2	0
l1	4.5587	10	3	3	3	0
r11	4.5272	10	0	0	0	0
r10	4.2864	10	0	0	0	0
r1	4.2712	10	0	0	0	0
l10	4.1981	10	0	0	0	0
l2	4.1810	10	0	0	0	0
r6	0.0458	10	0	0	0	0
L4,R4,L11,	0.0000	40	0	0	0	0

~~000010~~
8

Table 3. Mortality data for channel catfish. QA 208

ID	HPLC	N	MORTALITY			
			H96	H72	H48	H24
R15	86.891	10	10	10	10	10
L7	85.458	10	10	10	10	10
R14	84.067	10	10	10	10	10
R8	82.852	10	10	10	10	10
R3	45.651	10	10	10	10	10
R12	45.462	10	10	10	10	10
R7	42.559	10	10	10	10	10
R13	40.612	10	10	10	10	10
R2	33.913	10	10	10	9	2
R16	19.702	10	0	0	0	0
R5	16.885	10	0	0	0	0
L9	16.055	10	9	9	9	9
R6	9.012	10	1	0	0	0
R11	8.035	10	1	0	0	0
L12	7.863	10	6	6	6	6
L3	7.019	10	0	0	0	0
L5	6.162	10	0	0	0	0
R10	2.495	10	0	0	0	0
R1	1.862	10	0	0	0	0
R4	0.322	10	0	0	0	0
R9	0.252	10	0	0	0	0
L8	0.138	10	0	0	0	0
L1	0.101	10	0	0	0	0
L10	0.083	10	0	0	0	0
L11	0.000	10	0	0	0	0
L6	0.000	10	0	0	0	0
L2	0.000	10	0	0	0	0
L4	0.000	10	0	0	0	0

~~000011~~

ROSEMARY GRAHAM MORA MA CHANNEL CATFISH

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
43	10	10	100	9.765625E-02
41	10	10	100	9.765625E-02
34	10	10	100	9.765625E-02
20	10	0	0	9.765625E-02
17	20	9	45	41.19014
9	10	1	10	1.074219
8	20	7	35	13.1588
7	10	0	0	9.765625E-02
6	10	0	0	9.765625E-02
3	10	0	0	9.765625E-02
2	10	0	0	9.765625E-02
.3	20	0	0	9.536742E-05
.1	30	0	0	9.313227E-08

THE BINOMIAL TEST SHOWS THAT 7 AND 34 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 26.07681

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
12	5.487956E-02	21.60402	16.36293	29.79915

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.2967115	2.771	1.331449E-03

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

SLOPE = 3.591352
 95 PERCENT CONFIDENCE LIMITS = 1.635098 AND 5.547605

LC50 = 17.17484
 95 PERCENT CONFIDENCE LIMITS = 11.83151 AND 27.35937

LC10 = 7.607909
 95 PERCENT CONFIDENCE LIMITS = 2.856561 AND 11.1857

ROSEMARY GRAHAM MORA BLUEGILL MA

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
92	20	20	100	9.536742E-05
82	10	10	100	9.765625E-02
70	10	10	100	9.765625E-02
45	10	10	100	9.765625E-02
34	30	30	100	9.313227E-08
30	10	10	100	9.765625E-02
20	40	40	100	0
15	20	20	100	0
8	20	5	25	0
4	60	0	0	0
.05	10	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 9.473942

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.

Ecological Effects Branch One-Liner Data Entry Form

Chemical Methyl Anthranilate Shaughnessy No. 128725 Pesticide Use

AQUATIC VERTEBRATE TOX.	% AI	LC ₅₀ (95%CL) SLOPE	HRS/TYPE	NOEC	STUDY/REVIEW DATES	MRID/CATEGORY	LAB	RC
1. <i>Salmo salar</i>	>98	32.35 (ND) mg/l#	96 hr static	6 mg/l#	1992/1993	426998-02	USDA	RGM
2. <i>Oncorhynchus mykiss</i>	>98	22.91 (21.6-24.4) mg/l#	96 hr static	5 mg/l#	1992/1993	426998-02	USDA	RGM
3. <i>Ictalurus punctatus</i>	>98	16.23 (11.6-22.5) mg/l* 3.8	96 hr static	7 mg/l*	1992/1993	426998-02 427182-02	USDA	RGM
4. <i>Lepomis macrochirus</i>	>98	9.12 (8.0-10.5) mg/l* 9.4	96 hr static	below- 10.7 mg/l*	1992/ 1993	426998-02 426998-03	USDA	RGM
5.								
6.								
7.								

COMMENTS: * = Results based on measured concentrations. # = Results based on nominal concentrations. Studies performed by USDA/APHIS/ADC/Denver Wildlife Research Center, and Monell Chemical Senses Center.