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

AUG 3 1995

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Aquatic data review for MSMA (D182519)

FROM: Elizabeth M.K. Leovey, Chief for Daniel Rieder 8-3-95
Environmental Risk Characterization Branch
Environmental Fate and Effects Division (7507C)

TO: Barbara Briscoe, PM 51
Special Review and Reregistration Division (7508W)

The Environmental Risk Characterization Branch (ERCB) has completed the review of the data submitted in support of reregistration of MSMA, chemical number 013803. The following is a brief summary of the data reviewed:

Citation: Monosodium Methanearsonate - Acute Toxicity to Eastern Oyster (*Crassostrea virginica*) Under Flow-through Conditions. SLI Report No. 92-8-4367. **EPA MRID No. 424648-01.**

Conclusions: This study is scientifically sound and meets the guideline requirements for a mollusc shell deposition test. Based on mean measured concentrations, the 96 hour EC_{50} of MSMA (51%) is 160 mg a.i./L. MSMA is classified as practically nontoxic to eastern oysters. The NOEC is 49 mg a.i./L.

If there are any questions regarding this data review contact Renée Costello of my staff at 305-5294.

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DATA EVALUATION RECORD

1. **CHEMICAL:** MSMA.
Shaughnessey No. 013803.

2. **TEST MATERIAL:** Monosodium methanearsonate (MSMA) technical; CAS No. 2163-80-6; Notebook No. 20338-97-38; 50.4% active ingredient; a light green liquid.

3. **STUDY TYPE:** 72-3. Mollusc 96-Hour Flow-Through Shell Deposition Study. Species Tested: Eastern oyster (*Crassostrea virginica*).

4. **CITATION:** Dionne, E. 1992. Monosodium Methanearsonate - Acute Toxicity to Eastern Oyster (*Crassostrea virginica*) Under Flow-Through Conditions. SLI Report No. 92-8-4367. Performed by Springborn Laboratories, Inc., Wareham, MA. Submitted by MAA Research Task Force Three, c/o Luxembourg-Pamol, Inc., Memphis, TN. EPA MRID No. 424648-01.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: [signature]
Date: 11/17/92

6. **APPROVED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
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Date: 11/17/92

Renée Costello, Biologist, ERCB/EFED
~~Henry T. Craven, M.S.~~
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Date:

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7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a mollusc shell deposition test. Based on mean measured concentrations, the 96-hour EC_{50} of MSMA for eastern oysters was 160 mg ai/l. Therefore, MSMA is classified as practically non-toxic to eastern oysters. The NOEC was 49 mg ai/l.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Eastern oysters (*Crassostrea virginica*) were obtained from a commercial supplier in Pasadena, MD. The oysters were held in flowing seawater with a temperature of 20-22°C, a salinity of 7-31 parts per thousand (ppt), a pH of 7.2-7.9, and a dissolved oxygen concentration (DO) of 85-102% of saturation. The salinity was increased gradually from 7 to 31 ppt over the 15-day laboratory acclimation period. No mortalities occurred during the five days prior to testing. The oysters were inspected for parasites and maturity, were similar in age, and had a mean valve height of 37 ±4 mm.

During acclimation to the laboratory, the oysters were fed a supplemental diet of *Isochrysis galbana* and *Tetraselmis maculata*.

Seventy-two hours prior to testing, 3-5 mm of new peripheral shell growth was removed by grinding the shell with a grinding wheel. The oysters were held for three days and examined for signs of stress. Any oysters which appeared less than optimal were discarded. Immediately prior to test initiation, the outer edge of the shell was buffed by hand to remove any new shell growth.

B. **Test System:** A continuous-flow serial diluter with a dilution factor of 60% was used to deliver 5 MSMA concentrations and a dilution water control to the test vessels. Twelve aquaria, two replicate aquaria per concentration, were randomly positioned in a temperature-controlled water bath set to maintain 20 ±2°C. Each glass aquarium (60 x 30 x 30 cm) was equipped with a 10 cm standpipe and had a total test solution volume of approximately 18 l. The flow to each aquarium (75 ml/minute) provided six volume replacements every 24 hours. Recirculation of the test solution was provided in each individual aquarium to give a flow rate of about 5 l/oyster/hour. During the exposure, the oysters received supplemental feedings of 180 ml of algal suspension (*I. galbana* and *T. maculata*, 10^7 cells/ml) per aquarium three times daily. Overhead fluorescent lighting was maintained on a 16-hour light photoperiod and sudden transitions from light to dark were avoided.

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Natural, unfiltered seawater was used as dilution water. The seawater was pumped from Cape Cod Canal, Bourne, MA, into a large fiberglass holding tank before distribution to the diluter. The salinity and pH of the seawater were 31 ppt and 7.9-8.0, respectively.

A peristaltic pump delivered 0.097 ml/minute of the test material (773.29 mg ai/ml) directly into the chemical mixing chamber which also received 375 ml/minute of seawater. This resulted in a solution which was equivalent to the highest nominal test concentration of 200 mg ai/l. A portion of this solution was serially diluted to produce the lower concentrations. The calibration of the diluter system was confirmed prior to test initiation and at termination.

C. **Dosage:** Ninety-six-hour flow-through toxicity test. Based on a preliminary test, five nominal concentrations (26, 43, 72, 120, and 200 mg ai/l) and a dilution water control were chosen for the definitive test.

D. **Design:** Twenty oysters were impartially distributed to each aquarium for a total of 40 oysters per concentration or control. Oysters were placed equidistant from each other with their valves facing towards the flow of water from the recirculator.

The pH, temperature, salinity, and DO of the test solutions were measured in each replicate aquarium every 24 hours. Temperature was also monitored continuously in replicate A of the dilution water control. The diluter function was checked twice daily during the test.

Every 24 hours, the oysters and test solutions were observed for visible abnormalities and solution characteristics. After 96 hours, new shell growth was measured microscopically to the nearest 0.1 mm using a calibrated micrometer.

Water samples were removed from each replicate of all solutions on day 0 and day 4 for analysis of MSMA by gas chromatography. The samples were frozen and sent to Pharmacology and Toxicology Research Laboratory East, Inc., Richmond, KY.

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E. **Statistics:** The 96-hour EC_{50} value was determined by linear regression of response (percent reduction of shell growth as compared with the control) vs. mean measured exposure concentration over the range of test concentrations. Various mathematical manipulations (logarithm and probit transformations) were used on the concentration and response data to get the linear regression with the highest coefficient of determination (r^2). The 95% confidence interval (C.I.) was determined using the method of inverse prediction. The growth data were analyzed for homogeneity of variance and the no-observed-effect concentration (NOEC) was determined using William's test.

12. **REPORTED RESULTS:** The mean measured concentrations were 29, 49, 82, 130, and 210 mg ai/l (Table 2, attached), which averaged 111% of nominal.

No mortality occurred during the test. Oyster shell deposition decreased with increasing MSMA concentration (Table 3, attached). The mean shell growth of the control oysters was 2.4 mm. The 96-hour EC_{50} of MSMA for eastern oysters was 160 mg ai/l. The slope of the dose-response curve was 5.09. The NOEC was 49 mg ai/l. The EC_{50} classifies MSMA as practically non-toxic to eastern oysters.

The temperature during the test was 20-22°C. The pH was 7.3-8.0, and the DO ranged from 4.8 to 7.5 mg/l or 65 to 101% of saturation. The salinity was 31-32 ppt.

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

Good laboratory practice and Quality Assurance Unit statements were included in the report indicating compliance to EPA Good Laboratory Practice Standards (GLPs) with the following exception: stability, characterization and verification of the test substance identity and maintenance of records on the test substance are the responsibility of the study sponsor. Additionally, routine water contaminant screening analyses were conducted by an independent laboratory which did not collect data in accordance with GLPs.

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

A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended by the guidelines with the following deviation:

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The flow rate of the "recirculating" test solution was about 5 l/oyster/hour. According to the protocols recommended by the SEP, each oyster should receive a minimum of 5 l of "once-through" flow-through test solution per hour. However, the above method is considered acceptable because a supplemental algal diet was provided.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to determine the EC_{50} for oyster shell deposition and obtained results similar to the author's. The NOEC value was determined using two-way analysis of variance coupled with Dunnett's test and a less conservative value was obtained (see attached printouts). Therefore, the author's NOEC of 49 mg ai/l is accepted.

C. **Discussion/Results:** Although the purity of the test material was reported to be 50.4%, the reviewer believes that the remainder of the material was water, as is the case for many acidic compounds. If inert ingredients other than water were present, a formulation control should have been included in the study design.

This study is scientifically sound and meets the guideline requirements for a mollusc shell deposition test. Based on mean measured concentrations, the 96-hour EC_{50} of MSMA for eastern oysters was 160 mg ai/l. Therefore, MSMA is classified as practically non-toxic to eastern oysters. The NOEC was 49 mg ai/l.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 10-29-92.

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Page_____ is not included in this copy.

Pages 7 through 8 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Analysis of Variance File: OYS Date: 10-16-1992

FILTER: None

N's, means and standard deviations based on dependent variable: HEIGHT

* Indicates statistics are collapsed over this factor

Factors: T	R	N	Mean	S.D.
*	*	240	1.9608	1.2061
1	*	40	2.3550	1.1368
2	*	40	2.3925	0.8468
3	*	40	2.6050	1.0593
4	*	40	2.2925	0.9037
5	*	40	1.8775	0.9582
6	*	40	0.2425	0.3522
*	1	120	2.0442	1.2354
*	2	120	1.8775	1.1753
1	1	20	2.5950	1.0303
1	2	20	2.1150	1.2123
2	1	20	2.2200	0.9785
2	2	20	2.5650	0.6722
3	1	20	2.6200	0.9919
3	2	20	2.5900	1.1484
4	1	20	2.6250	1.0114
4	2	20	1.9600	0.6484
5	1	20	1.9850	0.9494
5	2	20	1.7700	0.9793
6	1	20	0.2200	0.2628
6	2	20	0.2650	0.4295

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Fmax for testing homogeneity of between subjects variances: 21.28

Number of variances= 12 df per variance= 19.

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Analysis of Variance Dependent variable: HEIGHT

Source	df	SS (H)	MSS	F	P
Between Subjects	239	347.6720			
T (TRT)	5	153.0508	30.6102	37.479	0.0000
R (REP)	1	1.6667	1.6667	2.041	0.1545
TR	5	6.7413	1.3483	1.651	0.1463
Subj w Groups	228	186.2132	0.8167		

Analysis of Variance File: OYS Date: 10-16-1992

FILTER: None

Post-hoc tests for factor T (TRT)

Level	Conc mg ai/l	Mean	Level	Conc mg ai/l	Mean
1	control	2.355	6	210	0.243
2	29	2.392			
3	49	2.605			
4	82	2.293			
5	130	1.878			

Comparison	Dunnett
1 < 2	
1 < 3	
1 > 4	
1 > 5	
1 > 6	0.0100
2 < 3	N.A.
2 > 4	N.A.
2 > 5	N.A.
2 > 6	N.A.
3 > 4	N.A.
3 > 5	N.A.
3 > 6	N.A.
4 > 5	N.A.
4 > 6	N.A.
5 > 6	N.A.

NOEC = 130 mg ai/l

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

MOSSLER MSMA CRASSOSTREA VIRGINICA 11-3-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
210	100	90	90	0
130	100	20	20	0
82	100	3	3	0
49	100	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 158.2686

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	1.714531E-02	155.3013	146.917	165.1589

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.5798383	3.480644	3.078771E-02

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

SLOPE = 8.496371
95 PERCENT CONFIDENCE LIMITS = 2.02663 AND 14.96611

LC50 = 154.3282
95 PERCENT CONFIDENCE LIMITS = 113.191 AND 221.2768

LC10 = 109.388
95 PERCENT CONFIDENCE LIMITS = 35.39982 AND 137.4778

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