

**AN ASSESSMENT OF THE TOXICITY
OF THE DISPERSING AGENT
MOXIE 1500 SSE
TO *Artemia salina* AND *Fundulus heteroclitus***

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TABLE OF CONTENTS

	Page
1.0 Introduction	1
2.0 Methods	2
2.1 General Procedures	2
2.2 Test Material Preparation	2
2.2.1 <i>Artemia salina</i>	2
2.2.2 <i>Fundulus heteroclitus</i>	3
2.3 Test Conditions	3
2.3.1 <i>Artemia salina</i>	3
2.3.2 <i>Fundulus heteroclitus</i>	3
2.4 Analyses and Measurements	4
2.5 Calculations	4
2.6 Statistical Methods	4
3.0 Results	5
3.1 Results of <i>Artemia</i> Testing	5
3.2 Results of <i>Fundulus</i> Testing	5
4.0 Discussion	10
5.0 References	11

TABLES

Table 1 Summary of Results for <i>Artemia salina</i>	6
Table 2 Summary of Results for <i>Fundulus heteroclitus</i>	7
Table 2 Bioassay Procedure and Organism Data for <i>Artemia salina</i>	8
Table 3 Bioassay Procedure and Organism Data for <i>Fundulus heteroclitus</i>	9

APPENDICES

- A. Water Quality Data
- B. Analytical Data
- C. Raw Data

1.0
INTRODUCTION

The Moxie International Corporation requested the Bioassay Division of MEC Analytical Systems to conduct a Dispersant Toxicity Test with *Artemia salina* and *Fundulus heteroclitus*. The purpose of the study was to determine the acute toxicity of the petroleum dispersing agent, "Moxie 1500 SSE" and its effect on the toxicity of fuel oil No. 2 (supplied by the U.S. EPA) to two marine organisms. The *Artemia* study was initiated on March 2, 1990 and the *Fundulus* study was initiated on March 6, 1990. The following report presents the results of this study.

The study was conducted at the MEC Analytical Systems Bioassay Laboratory in Tiburon, California. The project was managed by Ms. Bridgette Hejmanek and was under the overall supervision of Dr. Kurt F. Kline, Bioassay Division Manager.

2.1. GENERAL PROCEDURES

Testing procedures and maintenance of all test organisms are described in 40 CFR Part 300 (1984).

2.2. TEST MATERIAL PREPARATION

The dilution water for all tests consisted of San Francisco Bay seawater diluted with bottled spring water to 20 ± 2 ppt. A copy of the analytical chemistry results for the seawater and the spring water is appended to this report (Appendix B). Preliminary range-finding tests were conducted on both species to determine the best concentrations to use for the definitive test. The No. 2 fuel oil was obtained from the USEPA (MEC # 890726). The chemical characteristics of this oil are appended to this report (Appendix B).

2.2.1 *Artemia salina*

The dispersant stock solution was prepared at 25,000 ppm by adding 25 ml of the test substance to one liter of dilution water in a blender and mixing for five seconds. This stock solution was used to prepare concentrations of 1563, 3125, 6250, 12,500, and 25,000 ppm. The oil stock solution was prepared by adding 1.0 ml of oil to 1 liter of dilution water and blending for 5 seconds. The 1:10 (dispersant to oil) stock solution was prepared by adding 1.0 ml oil and 0.1 ml of the dispersant to 1.1 liters of dilution water in a blender and mixing for 5 seconds. These stock solutions were used to prepare the following dilution concentration series: 6.25, 12.5, 25, 50, and 100 ppm.

The sodium dodecyl sulfate (SDS) reference toxicant stock solution was prepared by dissolving 1 g of SDS per liter of seawater to make a 1000 ppm solution. This stock solution was used to prepare the following concentration series: 6.25, 12.5, 25, 50, and 100 ppm.

2.2.2 *Fundulus heteroclitus*

Two liters of 20 ppt dilution water was added to each test container. The appropriate amount of test material was then added and the test container was mixed on a rotary shaking table for 5 minutes at 160-180 rpm. The test concentrations for the oil alone and the 1:10 dispersant to oil mixture were 188, 315, 750, 1500, and 3000 ppm. The dispersant itself was tested at 6250, 12,500, 25,000, 50,000, and 100,000 ppm.

The SDS reference toxicant stock solution was 1000 ppm. The concentrations tested were 1.6, 3.1, 6.25, 12.5, and 25 ppm.

2.3 TEST CONDITIONS

2.3.1 *Artemia salina*

Nauplii approximately 24 hours old were collected from culture containers which consisted of 5-gallon plastic carboys which were filled with seawater and aerated. These systems are prepared to generate 24 old nauplii. Twenty nauplii were randomly placed in each of 5 chambers per concentration. A total of 100 *Artemia* were tested at each concentration. The test chambers consisted of 100 X 50 mm crystallizing dishes.

The photoperiod was 16 hours light and 8 hours dark. The test containers were illuminated from below by placing them on large light tables. Daily observation of the exposed organisms were made to monitor mortality and abnormal behavior. Immobility of test organisms was noted. Organisms were not fed during testing.

2.3.2 *Fundulus heteroclitus*

Juvenile *Fundulus* which were approximately 1.0-1.5 inches were obtained from Aquatic Research Organisms, Hampton, New Hampshire, at least 10 days prior to test initiation. Two fish were randomly placed in each of 5 chambers per concentration. A total of 10 fish were tested at each concentration. The test chambers consisted of 1-gallon wide mouth glass jars which had been washed according to MEC SOP No. A-022.

The photoperiod was 16 hours light and 8 hours dark. All test chambers were aerated at 100 ± 15 bubbles/minute with 1 ml pipettes. Daily observations of the exposed organisms

were made to monitor mortality and abnormal behavior. Organisms were not fed during testing. At the conclusion of the test, at least 10 fish were weighed (wet weight) and measured (standard length).

2.4. ANALYSES AND MEASUREMENTS

Temperature, pH, dissolved oxygen, and salinity were measured daily in one replicate of each test concentration.

2.5. CALCULATIONS

$$\text{Percent Mortality} = \frac{\text{Total \# dead organisms} \times 100}{\text{Total organisms added}}$$

2.6. STATISTICAL METHODS

The LC₅₀ and its confidence limits were calculated using the Probit method. If the Probit Method was not applicable (less than two concentrations with partial mortalities) the Moving Average or Binomial Method was used.

The water quality parameters were acceptable. The pH of all solutions was 8.0 ± 0.2 units on Day 0 and varied thereafter. Tables 1 and 2 present a summary of the results. Tables 3 and 4 summarize the test conditions. The tables in Appendix A present more detailed data.

3.1 RESULTS OF *Artemia* TESTS

The controls had 100% survival, exceeding the criteria in the guidelines (EPA, 1984). The dispersant had a 48-hour LC_{50} of 19,518 ppm. The oil had a 48-hour LC_{50} of 64.8 ppm and the 1:10 dispersant to oil mixture had an LC_{50} of 50.8 ppm. There was no increase in toxicity between the oil and the 1:10 dispersant to oil mixture. The SDS reference toxicant showed a 48-hour LC_{50} of 7.96 ppm.

3.2 RESULTS OF THE *Fundulus* TESTS

The controls had 100% survival, meeting the criteria in the guidelines (EPA, 1984). The dispersant had a 96-hour LC_{50} of 31,455 ppm. The oil had an LC_{50} of 188 ppm and the 1:10 dispersant to oil mixture had an LC_{50} of 188 ppm. There was no significant difference between the LC_{50} 's for the oil and the 1:10 mixture. The 96-hour LC_{50} for the SDS was 9.5 ppm.

TABLE 1
SUMMARY OF RESULTS

<i>Artemia salina</i> Concentration (ppm)	% Mortality	48-Hour LC50 (ppm)	95% Confidence Limits (ppm)
Control	0		
SDS		7.96	7.25-8.78
1.6	2		
3.1	5		
6.25	9		
12.5	92		
25	100		
Oil		64.8*	54.1-81.2
6.25	6		
12.5	4		
25	21		
50	47		
100	62		
Dispersant		19,518	15,935-25,457
1563	1		
3125	7		
6250	22		
12500	42		
25000	52		
1:10		50.8*	46.1-56.0
6.25	2		
12.5	7		
25	6		
50	58		
100	81		

*Not significantly different.

TABLE 2
SUMMARY OF RESULTS

<i>Fundulus heteroclitus</i> Concentration (ppm)	% Mortality	96 hr LC50 (ppm)	95% Confidence Limits (ppm)
Control	0		
SDS		9.5	6.25-12.5
1.6	0		
3.1	0		
6.25	0		
12.5	90		
25	100		
Oil		188*	0-3000
188	50		
375	80		
750	90		
1500	80		
3000	100		
Dispersant		31,455	12,500-50,000
6250	0		
12500	0		
25000	18.2		
50000	100		
100000	100		
1:10		188*	0-1500
188	50		
375	60		
750	70		
1500	100		
3000	100		

*Not significantly different from one another.

TABLE 3

**BIOASSAY PROCEDURE AND ORGANISM DATA
FOR *Artemia salina***

<u>Parameter</u>	<u>Data</u>
Test Type	Acute/Static
Duration	2 days (48 hours)
Test Photoperiod	16 hour light : 8 hour dark
Start Date	3/2/90
Completion Date	3/4/90
Control Water	Spring and San Francisco Bay seawater mixture
Test Temperature	20 ± 2°C
Organisms per Container	20
Test Chamber/Exposure Volume	100 X 50 mm crystallizing dish/ 100 ml
No. of Test Containers	5 for each concentration and control
Sample Storage Conditions	4°C in the dark
Treatment Problems	None
Organism	
Test Species	<i>Artemia salina</i>
Source	In house stock; Argentemia Gold from San Francisco Bay
Age	Approximately 24 hours old
Acclimation Water	20 ppt seawater
Acclimation Temperature	20 ± 2°C
Acclimation Photoperiod	16 hour light : 8 hour dark

TABLE 4

**BIOASSAY PROCEDURE AND ORGANISM DATA
FOR *Fundulus heteroclitus***

<u>Parameter</u>	<u>Data</u>
Test Type	Acute/Static
Duration	4 days (96 hours)
Test Photoperiod	16 hour light : 8 hour dark
Start Date	3/6/90
Completion Date	3/10/90
Control Water	Spring and San Francisco Bay seawater mixture
Test Temperature	20 ± 2°C
Organisms per Container	2
Test Chamber/Exposure Volume	1-gallon jars/ 2 L
No. of Test Containers	5 for each concentration and control
Sample Storage Conditions	4°C in the dark
Treatment Problems	None
Organism	
Test Species	<i>Fundulus heteroclitus</i>
Source	Aquatic Research Organisms, Hampton, NH
Age	Juveniles, 1.0 - 1.5 inches
Acclimation Time	> 10 days
Acclimation Water	20 ppt seawater
Acclimation Temperature	20 ± 2°C
Acclimation Photoperiod	16 hours light : 8 hours dark

4.0 DISCUSSION

The toxicity of the dispersant to both *Fundulus* and *Artemia* appears to be low based upon the results of our test program. The addition of the dispersant to oil did not significantly increase the toxicity of oil to either species.

The reference toxicant test data yielded typical toxicity values and showed no significant sensitivity for the species tested.

5.0 REFERENCES

- EPA. 1984. National oil and hazardous substances pollution contingency plan; Final rule. 40 CFR Part 300; Published 7-18-84
- Garner, W. and M. Barge. Proposed FIFRA Good laboratory practice standards. Prepared for USEPA / Washington, D.C.