

189889,189939,189940  
RECORD NO.

32501  
SHAUGHNESSEY NO.

REVIEW NO.

EEB REVIEW

DATE: IN 2-24-87 OUT 2-18-88

FILE OR REG. NO 3125-183, 3125-183, 3125-183 (3 Actions)

PETITION OR EXP. NO. \_\_\_\_\_

DATE OF SUBMISSION 1-27-87

DATE RECEIVED BY HED 2-19-87

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RD ACTION CODE/TYPE OF REVIEW 660

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

DATA ACCESSION NO(S). 400716-01, 400716-02, 400716-03

PRODUCT MANAGER NO. George Larocca (15)

PRODUCT NAME(S) Disulfoton (DI-SYSTON)

COMPANY NAME Mobay Chemical Corporation

SUBMISSION PURPOSE Submission of Data in Response To

Registration Standard

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

_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____



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

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Reg. No. 3125-183: Estuarine Organism Acute  
Toxicity Studies; EPA Accession No. 400716-01,  
400716-02, and 400716-03.  
Disulfoton

FROM: John Noles, Biologist  
Ecological Effects Branch  
Hazard Evaluation Division (TS-769C)

*John Noles*  
*2/1/88*

THRU: Otto Gutenson, Acting Head-Section 4  
Ecological Effects Branch  
Hazard Evaluation Division (TS-769C)

*Otto Gutenson*  
*2/4/88*

THRU: Henry T. Craven, Acting Chief  
Ecological Effects Branch  
Hazard Evaluation Division (TS-769C)

*Henry T. Craven*  
*2/18/88*

TO: George LaRocca, PM 15  
Insecticide-Rodenticide Branch  
Registration Division (TS-769C)

EEB has reviewed the following estuarine organism studies,  
submitted in response to the Disulfoton Registration Standard.  
The review results are indicated below:

<u>Guidance Ref No.</u>	<u>Species</u>	<u>Review Results</u>	<u>Toxicity Category</u>	<u>Review Status</u>
72-3	Sheepshead Minnow	96-hour LC <sub>50</sub> = 1.0 mg/l	Highly Toxic	Core
72-3	Mysid Shrimp	96-hour LC <sub>50</sub> = 0.10 mg/l	Very Highly Toxic	Core
72-3	Oyster	96-hour LC <sub>50</sub> = 0.72 mg/l	Very Highly Toxic	Supple- mental

The oyster study is subject to possible upgrade, pending the submission of information requested in the attached data evaluation record (i.e., documentation of the holding period, and raw data for the calculation of the EC<sub>50</sub> value).

A review of the FFR Science Chapter for additional data requirements for Disulfoton indicates that the fish early life stage and aquatic invertebrate life cycle (72-4) data requirement is in reserved status. The data requirement criteria for requiring such studies for Disulfoton is based upon the following rationale:

(1)  $LC_{50}/EC_{50} < 1 \text{ mg/l}$

(e.g., Bluegill  $LC_{50} = 0.039 \text{ ppm}$ ; glass shrimp  $LC_{50} = 0.0039$ ; mysid shrimp  $LC_{50}/EC_{50}$

(2)  $FEC > 0.01 LC_{50}/EC_{50}$

(e.g., Aquatic first cut FEC (see attachment) resulting from 1.0 to 8.0 lb/A of representative agricultural use patterns ranges from 6.1 ppb to 48.8 ppb which exceeds 0.01  $LC_{50}$  (3.9 ppb) of bluegills and is greater than the glass shrimp  $LC_{50}$ .

As a result, Guideline Requirement No. 72-4 must be satisfied with one fish early life stage study with one freshwater species and the aquatic invertebrate life cycle study must be satisfied with one freshwater species (Daphnia) and one estuarine species (mysid shrimp).

FFR requests the review results of reviewed environmental fate studies submitted in response to the Disulfoton Registration Standard. The results are necessary for FFR's hazard assessment purposes.

# Attachment A

## EEC CALCULATION SHEET

### I. For foliar application

#### A. Runoff

$$\begin{array}{rcll} 1.0 & \text{lbs} & \times & 0.01 \\ 8.0 & & & (\% \text{ runoff}) \end{array} \times \begin{array}{l} 10 \text{ (A)} \\ \text{(from 10 A.} \\ \text{drainage basin)} \end{array} = \begin{array}{l} 0.1 \\ 0.0 \\ \text{(total runoff)} \end{array} \text{ lbs}$$

EEC of 1 lb a.i. direct application to 1 A. pond  
6-foot deep = 61 ppb.

$$\text{Therefore, EEC} = 61 \text{ ppb} \times \begin{array}{l} 0.1 \\ 0.3 \end{array} \text{ (lbs)} = \begin{array}{l} 6.1 \text{ ppb (from 1.0 lb/A)} \\ 48.8 \text{ ppb (from 8.0 lb/A)} \end{array}$$

\* No environmental data or product chemistry available. ~~1~~ 1% runoff used as a conservative estimate to show that EEC poses potential problems. A ~~5~~ 5% runoff would obviously result in a higher EEC.

### II. For aerial application

#### A. Runoff

$$\begin{array}{rcll} \text{lbs} & \times & 0.6 & \times 0.0 \\ & & \text{(appli.} & (\% \\ & & \text{efficiency)} & \text{runoff)} \end{array} \times \begin{array}{l} 10 \text{ (A)} \\ \text{(from 10 A.} \\ \text{drainage} \\ \text{basin)} \end{array} = \begin{array}{l} \text{lbs} \\ \text{(total} \\ \text{runoff} \end{array}$$

#### B. Drift

$$\begin{array}{rcll} \text{lbs} & \times & 0.0 & = \\ & & (\% \text{ runoff}) & \text{(total runoff)} \end{array}$$

$$\text{Total loading} = \begin{array}{l} \text{lb} \\ \text{(runoff)} \end{array} + \begin{array}{l} \text{lb} \\ \text{(drift)} \end{array} = \text{lbs}$$

$$\text{Therefore, EEC} = 61 \text{ ppb} \times \begin{array}{l} \text{lbs} \\ \text{(total} \\ \text{runoff)} \end{array} = \text{ppb.}$$

DATA EVALUATION RECORD

MRID 400716-03

1. CHEMICAL: Di-Syston (Di-Sulfoton technical)
2. TEST MATERIAL: Di-Syston Technical Grade; 97.8%  
active ingredient.  
Reference #79-R-225-40
3. STUDY TYPE: Acute Toxicity Test for Estuarine and Marine  
Organisms. Mollusc 96-Hour, Flow-Through Shell  
Deposition Study.  
Species Tested: Eastern Oyster, Crassostrea virginica
4. CITATION: Surprenant, D.C. 1986. Acute Toxicity of Di-Syston to  
Eastern Oysters (Crassostrea virginica), Bionomics Report  
#BW-86-7-2060. Prepared by Springborn Bionomics, Inc.,  
Wareham, Massachusetts. Submitted by Mobay Chemical  
Company, Stilwell, Kansas. Mobay Chemical Corporation  
Report Number 91346. MRID Number 400716-03.

5. REVIEWED BY:

G. Scott Ward  
Department Manager  
ESE

Signature: G. Scott Ward

Date: November 4, 1987

6. APPROVED BY:

James R. Newman, Ph.D.  
Project Manager/  
Principal Scientist  
KEN Engineering and  
Applied Sciences, Inc.

Signature:

Date:

*James R. Newman*  
*11/5/87*

Henry T. Craven  
Chief, EEB/HED  
USEPA

Signature:

Date:

*Henry T. Craven*  
*12/4/88*

7. CONCLUSIONS:

This study is scientifically sound but has been rated supplemental because it requires submission of supporting test data for proper verification. The 96-hour EC50 value for Crassostrea virginica exposed to Di-Syston under flow-through test conditions is 0.72 mg/L. Di-Syston is classified as highly toxic to Crassostrea virginica.

*John Niles*  
*2/1/88*

This study only partially fulfills the Guidelines requirement for an acute toxicity determination for a mollusc 96-hour flow-through shell deposition study because of certain departures from recommended protocol.

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

- A. Test Animals: Eastern oysters, Crassostrea virginica, were obtained from a commercial shellfish hatchery on Cape Cod, Massachusetts. Young oysters with a mean valve height of  $38 \pm 4$  mm were tested. Oysters were maintained at a salinity of 28 to 32 ‰ and temperature range of 15 to 20 °C.
- B. Test System: The test was conducted in a continuous flow serial diluter with a dilution factor of 0.56. The system was a modification of the diluter described by Benoit et al. The flow rate provided 6 volume replacements per day. The temperature was maintained by a water bath with immersion heaters at  $20 \pm 2$  °C. The photoperiod was 16 hours light and 8 hours dark.

The dilution water was natural seawater collected from the Cape Cod Canal near Bourne, Massachusetts. The salinity of the dilution water was 32 ‰ with a pH of 8.0.

- C. Dosage: 96-hour acute flow-through test.
- D. Design: Twenty oysters were tested per duplicate test aquaria (40 per test and control solutions). A control and nominal Di-Syston concentrations of 0.20, 0.35, 0.63, 1.1, and 2.0 mg/L were maintained. The mean measured test concentrations were 0.18, 0.23, 0.56, 0.50, and 0.94 mg/L. Test chambers maintained at approximately 18 liters of test solution with a through flow rate of 75 mL/minute/test aquarium.
- E. Statistics: A computer program developed at the testing laboratory was utilized to compute four linear regression curves based on least squares. Percentage reduction in growth data were transformed to probits and concentrations to logs. Both untransformed and transformed data were regressed. The regression line which provided the best fit of the untransformed or transformed data was selected based on the highest associated coefficient of determination. The regression equation was then applied to calculate the EC50 and its 95% confidence limits.

12. REPORTED RESULTS:

Mean Measured Concentration (mg/L)	Percentage Reduction*
Control	N/A
0.18	12
0.23	(+4)
0.50	21
0.56	21
0.94	75

\*Percentage reduction in new shell growth as compared to the seawater control. Treatments exhibiting greater mean shell growth as compared to the control are indicated with a plus sign.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

"The 96-hour EC50 and 95% confidence interval, calculated by linear regression, were 0.72 mg/L (0.55-1.0)."

The data were audited by the laboratory's Quality Assurance Unit to assure compliance with the protocols, standard operating procedures and pertinent EPA Good Laboratory Practice (GLP) Regulations. A GLP compliance statement was included and signed by the Quality Assurance Unit.

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

- A. Test Procedure: The test procedures were in general accordance with protocols recommended by the Guidelines, but deviated from them as follows:

There is a discrepancy in the holding period prior to test initiation. It is unclear whether the oysters were held only one day prior to testing or seven days. The author's statements were as follows: "Upon arrival at Bionomics, 24 hours prior to initiation of the test,..." and "No mortality occurred during the seven days prior to testing." In addition the Guidelines state that "After collection, molluscs must be held for at least ten days for observation and acclimation."

The test was conducted at a dilution of 56% rather than the recommended dilution of 60% or higher. A complete chemical characterization of the seawater was not supplied.

Test temperature was measured only daily compared to the recommended hourly measurements for both the acclimation and testing periods.

A flow rate of 75 ml/minute/test aquarium was utilized providing a flow of 225 ml/oyster/hour. This rate is only about 4% of the rate set forth in test protocols recommended in the Guidelines (approximately 5 liter/oyster/hour) and only one quarter of the minimum rate set forth in EPA's Environmental Effects Guidelines for oyster shell deposition studies referenced by the author. To offset the reduced flow the test facility supplemented the water with the alga, Isochrysis galbana Parke at a density of  $1 \times 10^5$  cells/ml, and utilized pumps to recirculate test solutions at a flow rate of 5 L/oyster/hour. No mention was made of the number of checks on algal numbers or additions made to test aquaria. It should be noted however, that a flow-through test, as defined by ASTM (1980), consists of a "test solution that flows through the test chamber on a once-through basis throughout the test" and not on a recirculated flow.

These procedural modifications do not appear to have affected the validity of the test. Since the concentrations were measured and the EC50 value was calculated from those measured concentrations, the results of the study are probably an accurate indication of the toxicity of the test material. Supportive documentation is requested, as shown below.

- B. Statistical Analysis: The reviewer does not have access to the computer program utilized by the author to determine the EC50 value. Raw data was not provided, therefore the EC50 value could not be validated. Simple graphical interpolation, however, does provide a very similar EC50 value (i.e., 0.74 mg/L).
- C. Discussion/Results: The 96-hour EC50 value of 0.72 mg/L for Eastern oysters, Crassostrea virginica, classifies Di-Syston as highly toxic. The toxicity test was conducted at a salinity of 32 ‰ and temperature of 21 to 23°C.
- D. Adequacy of the Study:

Classification: Supplemental

Rationale: This study is classified as supplemental because of departures from recommended protocol as described in section 14.A. Data required to validate this study include documentation of the holding period, feeding frequency during testing, and raw data for calculation of the EC50 value.



DER MRID 400716-03  
Page Five

Repairability: Submission of supporting raw data from the study  
are required to support scientific validity of the study.

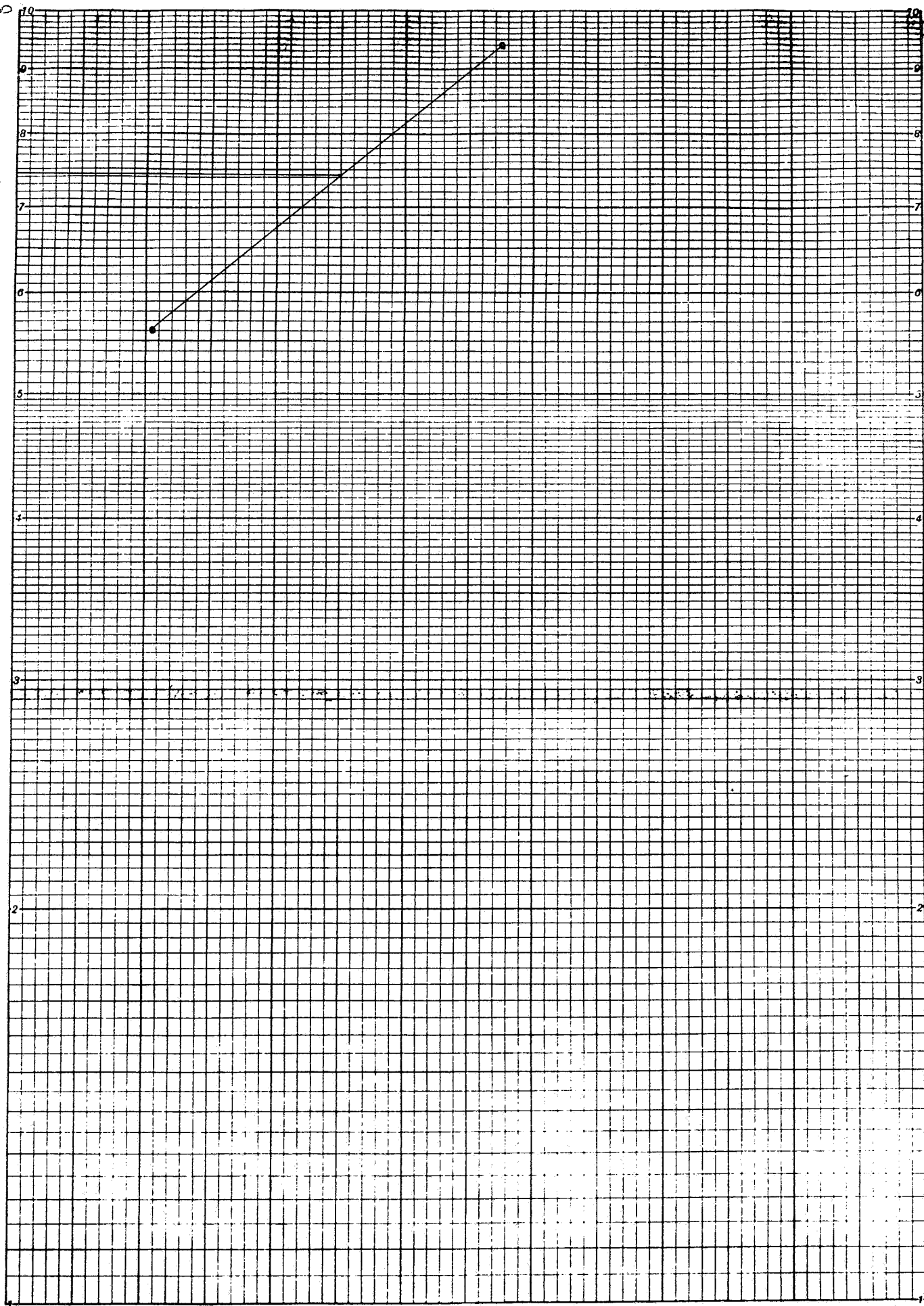
15. COMPLETION OF ONE-LINER FOR STUDY: Yes, October 27, 1987

Measured Concentration (mg/L)

1.00

0.74

0.50



PERCENTAGE REDUCTION

DATA EVALUATION RECORD

MRID 400716-02

1. CHEMICAL: Di-Syston (Di-Sulfoton technical)
2. TEST MATERIAL: Technical Grade, 97.8%, Reference #79R-225-40
3. STUDY TYPE: Acute Toxicity Test for Estuarine Fish, 96-Hour Flow Through. Species tested: Sheepshead minnow, Cyprinodon variegatus
4. CITATION: Surprenant, Donald C. 1986. Acute Toxicity of Di-Syston to Sheepshead Minnow (Cyprinodon variegatus) Under Flow-Through Conditions; Bionomics Report #BW-86-7-2091. Prepared by Springborn Bionomics, Inc., Wareham, Massachusetts. Submitted by Mobay Chemical Corporation, Stilwell, Kansas. Mobay Chemical Corporation Report Number 91345. MRID Number 400716-02.

5. REVIEWED BY:

C. Steve Manning  
Aquatic Toxicologist  
ESE

Signature:

Date:

*C. Steve Manning*  
*11/4/87*

6. APPROVED BY:

James R. Newman, Ph.D.  
Project Manager/  
Principal Scientist  
KEN Engineering and Applied  
Sciences, Inc.

Signature:

Date:

*James R. Newman*  
*11/5/87*

Henry T. Craven  
Chief, EEB/HED  
USEPA

Signature:

Date:

*Henry T. Craven*  
*2/4/88*

7. CONCLUSIONS:

This study is scientifically sound. The 96-hour LC50 value for Cyprinodon variegatus exposed to Di-Syston under flow-through test conditions is 1.0 milligrams per liter (mg/L). Di-Syston is classified as moderately toxic to Cyprinodon variegatus. This study fulfills the Guidelines requirement for an acute toxicity determination for an estuarine fish species.

*John Nales*  
*2/11/88*

8. RECOMMENDATIONS: N/A

9. BACKGROUND: N/A

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: Cyprinodon variegatus (Bionomics Lot #86A37) were obtained from a local commercial supplier. Fish were held a minimum of 14 days prior to testing and demonstrated no mortality in the 48 hours prior to testing. Average wet weight was 0.2 g and mean standard length was 23 mm (range 15 to 30 mm). All fish were fed a dry commercial pelleted food, ad libitum, daily.

B. Test System: A 0.5-liter proportional diluter (Mount and Brungs, 1967) in conjunction with a mechanical injector (Lenke et al, 1978) was utilized. Flow-rate provided an average daily volume addition of 4.8. Dissolved oxygen concentrations remained above 60% saturation and temperature was 22°. The seawater used during the study had a salinity of 31 parts per thousand (ppt) and a pH range of 7.7 to 7.9.

C. Dosage: 96-hour acute flow-through test.

D. Design: Twenty fish (10/duplicate) were exposed to each treatment and control. Nominal test concentrations were 2.5, 1.6, 1.0, 0.68 and 0.44 mg/l. A control and solvent control were conducted concomitantly. The maximum concentration of solvent was 14 microliter acetone/ liter of dilution water. The mean measured concentrations were: 2.1, 0.63, 0.41, 0.28, and 0.20 mg/L Di-Syston.

E. Statistics: Moving average angle analysis was conducted to determine LC50 values (Stephen, 1977).

12. REPORTED RESULTS:

Mean Measured Concentration (mg/L)	Cumulative Mortality (%)			
	<u>24 Hour</u>	<u>48-Hour</u>	<u>72-Hour</u>	<u>96-Hour</u>
Control	0	0	0	0
Solvent Control	0	0	0	0
0.20	0*	0*	10*	20*
0.28	0*	5*	10*	15*
0.41	0*	5*	10*	25*
0.63	5*	5*	5*	10*
2.1	20*	60*	80*	95*

\* Fish exhibited various types of abnormal behavior.

The NOEC (no observed effect concentration) was reported as being less than 0.20 mg/L or the lowest test concentration due to fish demonstrating signs of poisoning and/or significant mortality at all exposure levels. No control mortality was exhibited. The 96-hour LC50 was 1.0 mg/L with 95 percent confidence limits of 0.79 and 1.6 mg/L.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The highest exposure concentration was 84% of nominal, but all other concentrations were 40 to 45% of nominal. This was explained by the author as due to increased residency of undissolved compound not available to other exposure levels. This increased residency time occurred as a result of the test substance being out-of-solution in the mixing chamber and flowing over the water's surface through the chemical cells to the highest concentration test chamber. The presence of undissolved material in the highest concentration allowed time for additional material to enter into solution.

The data were audited by the laboratory's Quality Assurance Unit to assure compliance with the protocols, standard operating procedures and pertinent EPA Good Laboratory Practice (GLP) Regulations. A GLP compliance statement was included and signed by the Quality Assurance Unit.

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

- A. Test Procedure: With the exceptions of temperature not being measured as often as recommended by the protocol and test specimens being less than the recommended size, this study followed the protocol adequately.
- B. Statistical Analysis: The reviewer used the computer program by Stephan (1977) and confirmed values reported by the author. However, based on the concentration response, the reviewer believes that the moving average method was an inappropriate selection and that binomial probability should have been utilized. The moving average method provides confidence limits that are too close together for data sets that are not monotonic and thus is inappropriate for these data. No slope for the concentration-response curve was reported by the author.
- C. Discussion/Results: Acceptable results were achieved, although difficulties with maintaining a good serial dilution complicated the results of this exposure by the production of an, at best, roughly linear response. This is the result of working with a test compound at levels approaching saturation. This was considered when reviewed.
- D. Adequacy of the Study:
  - (1) Classification: Core
  - (2) Rationale: N/A
  - (3) Repairability: N/A

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, October 29, 1987

DATA EVALUATION RECORD

MRID 400716-01

1. CHEMICAL: Di-Syston (Di-Sulfoton technical)
2. TEST MATERIAL: Di-Syston Technical Grade; 97.8% active ingredient.  
Reference #79R-225-40
3. STUDY TYPE: Acute Toxicity Test for Shrimp. Flow-Through.  
Species Tested: Mysid Shrimp, Mysidopsis bahia
4. CITATION: Surprenant, D.C. 1986. Acute Toxicity of Di-Syston to Mysid Shrimp (Mysidopsis bahia) Under Flow-Through Conditions, Bionomics Report #BW-86-7-2112. Prepared by Springborn Bionomics, Inc., Wareham, Massachusetts. Submitted by Mobay Chemical Company, Stilwell, Kansas. Mobay Chemical Corporation Report Number 91344. MRID Number 400716-01.

5. REVIEWED BY:

G. Scott Ward  
Department Manager  
ESE

Signature: G. Scott Ward

Date: November 4, 1987

6. APPROVED BY:

James R. Newman, Ph.D.  
Project Manager/  
Principal Scientist  
KEN Engineering and  
Applied Sciences, Inc.

Signature:

Date:

Henry T. Craven  
Chief, EEB/HED  
USEPA

Signature:

Date:

7. CONCLUSIONS:

This study is scientifically sound. The 96-hour LC50 value for Mysidopsis bahia exposed for 96 hours to Di-Syston under flow-through test conditions is 0.10 mg/L. Di-Syston is classified as very highly toxic to Mysidopsis bahia.

This study fulfills the Guidelines requirement for an acute toxicity determination for an estuarine or marine shrimp.

8. RECOMMENDATIONS: N/A

9. BACKGROUND: N/A

10. DISCUSSION OF INDIVIDUAL TEST: N/A

11. MATERIALS AND METHODS:

- A. Test Animals: Mysidopsis bahia were obtained from an in-house mysid culture maintained at Springborn Bionomics, Inc. Post-larvae ( $\leq 24$  hours old) were selected for testing.
- B. Test System: The test was conducted in an intermittent-flow proportional diluter described by Mount and Brungs (1967) using a mechanical toxicant injector system described by Lemke et al. (1978) to dispense the stock solution and solvent in the test system. The flow rate provided 4.8 volume additions per day. The temperature was maintained by a water bath with immersion heaters at  $25 \pm 1^\circ\text{C}$ . The photoperiod was 16 hours light and 8 hours dark.

The dilution water was natural seawater collected from the Cape Cod Canal near Bourne, Massachusetts. The water was filtered to exclude particulates greater than 5 micrometers and then passed through an activated carbon filter prior to use. The salinity of the dilution water was 32 ‰ with a pH of 7.9 to 8.0.
- C. Dosage: 96-hour acute flow-through test.
- D. Design: Five mysids were tested per screened retention chamber, 10 per duplicate, and a total of 20 per each test and control solution. A control, solvent control (acetone), and nominal Di-Syston concentrations of 0.089, 0.14, 0.21, 0.32, and 0.50 mg/L were maintained. The mean measured test concentrations were 0.058, 0.094, 0.14, 0.21, and 0.44 mg/L.
- E. Statistics: The computer program developed by Stephan et al. was used to calculate the  $\text{IC}_{50}$  values. The 96-hour  $\text{IC}_{50}$  value was calculated by probit analyses.



12. REPORTED RESULTS:

Mean Measured Concentration (mg/L)	Percent Mortality			
	24 Hr	48 Hr	74 Hr	96 Hr
Control	0	5	5	5
Sol. Control	0	0	0	0
0.058	0*	5*	20*	20*
0.094	10*	25*	45*	50*
0.14	15*	50*	55*	65*
0.21	30*	45*	75*	80*
0.44	95*	100*	100*	100*

\* "All surviving mysids were lethargic and swimming erratically."  
 "The 96-hour LC50 for mysid shrimp exposed to Di-Syston was calculated to be 0.10 mg/L with a 95% confidence interval of 0.080 to 0.13 mg/L." "...the no discernible effect concentration for mysid shrimp and Di-Syston was determined to be <0.058 mg/L, the lowest concentration tested."

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The 96-hour LC50 for mysid shrimp (*Mysidopsis bahia*) exposed to Di-Syston under flow-through test conditions and based upon mean measured concentrations (0 and 96 hour measurements) was 0.10 mg/L with 95% confidence limits of 0.080 and 0.13 mg/L.

The data were audited by the laboratory's Quality Assurance Unit to assure compliance with the protocols, standard operating procedures and pertinent EPA Good Laboratory Practice (GLP) Regulations. A GLP compliance statement was included and signed by the Quality Assurance Unit.

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

A. Test Procedure: The test procedures were in accordance with protocols recommended by the Guidelines, but deviated from the Guidelines as follows:

The test mysids being less than 24 hours old were not held for the 10 days required. In addition, due to the fact the mysids were <24 hours old, no survival data is available for the 48-hours prior to test initiation. Although the test was conducted in a temperature-regulated water bath, temperature was not monitored at 6-hour intervals as recommended. Tests were conducted at 3°C higher than the 22°C recommended.

A complete chemical characterization of the dilution water was not supplied.

These procedural modifications did not affected the validity of the test. The test concentrations were measured, and the LC50 values were calculated from those measured concentrations, thus the results of the study are an accurate indication of the toxicity of the test material.

- B. Statistical Analysis: The reviewer used the computer program developed by Stephan et al. to calculate the LC50 values. These calculations are attached. The program does indicate that the selection of probit analyses, as done by the author, for reporting the 96-hour LC50 is not recommended. However, the use of the moving average method provides a similar LC50 and 95 percent confidence limits (0.10 mg/L with limits of 0.07 and 0.13 mg/L). The report did not specify the slope of the toxicity curve as required by the Guidelines. The value calculated by Stephan's program was 3.28.
- C. Discussion/Results: The 96-hour LC50 value of 0.10 mg/L for Mysidopsis bahia classifies Di-Syston as very highly toxic. The toxicity test was conducted at a salinity of 32 ‰ and temperature of 25°C.
- D. Adequacy of the Study:
- Classification: Core  
Rationale: N/A  
Repairability: N/A

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, October 27, 1987

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (%)
.44	20	20	100	9.536743E-05
.21	20	16	80	.5908966
.14	20	13	65	13.1588
9.399999E-02	20	10	50	58.80986
.058	20	4	20	.5908966

THE BINOMIAL TEST SHOWS THAT .058 AND .21 CAN BE  
 USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT  
 CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL  
 ASSOCIATED WITH THESE LIMITS IS 98.81821 PERCENT.  
 AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 9.400001E-02

>>>>>>>RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	.2663066	.1020486	7.255796E-02 .1316449

>>>>>>>RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.123816	.3198986	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

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

SLOPE = 3.28014  
 95 PERCENT CONFIDENCE LIMITS = 2.125941 AND 4.434339

LC50 = .1027988  
 95 PERCENT CONFIDENCE LIMITS = 8.183576E-02 AND .1241037  
 LC1 = 2.007386E-02  
 95 PERCENT CONFIDENCE LIMITS = 7.453682E-03 AND .0327462

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (%)
2.1	20	19	95	2.002716E-03
.63	20	2	10	2.012253E-02
.41	20	5	25	2.069473
.28	20	3	15	.1288414
.2	20	4	20	.5908966

THE BINOMIAL TEST SHOWS THAT .63 AND 2.1 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS 99.97788 PERCENT. AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.090928

>>>>>>>RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.1592308	1.052027	.7930153	1.574971

>>>>>>>RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	1.670101	4.411568	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

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

SLOPE = 2.253292  
95 PERCENT CONFIDENCE LIMITS = -.658691 AND 5.165274

LC50 = .8233643  
95 PERCENT CONFIDENCE LIMITS = 0 AND + INFINITY  
LC1 = 7.637897E-02  
95 PERCENT CONFIDENCE LIMITS = 0 AND .281608