10-24-80

006315

[redacted]

SHAUGHNESSEY NO.

1

REVIEW NO.

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

EEB BRANCH REVIEW

DATE: IN 8/28/80 OUT 10/24/80

FILE OR REG. NO. 1729-REE, - REG

PETITION OR (EXP. PERMIT NO.)

DATE DIV. RECEIVED 8/20/80

DATE OF SUBMISSION 7/16/80

DATE SUBMISSION ACCEPTED

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

DATA ACCESSION NO(S). 243015

PRODUCT MGR. NO. A. Castillo (32)

(1729-REE) (1729-REG)

PRODUCT NAME(S) Spa Brom Mini-Pak; Spa Brom Feeder sticks

COMPANY NAME Great Lakes Chemical Corporation

SUBMISSION PURPOSE Incremental Risk Assessment for proposed conditional registration of uses in spas and hot tubs.

SHAUGHNESSEY NO.	CHEMICAL & FORMULATION	% A.I.
	(1729-REE and - REG:) Bromochloro-5,5-dimethylhydantoin	96.0%

Pesticide name: Bromochloro-5,5-Dimethylhydantoin
(See 100.2 below)

100 Pesticide Label Information

100.1 Pesticide Use

The pesticide is proposed for use in spas and hot tubs.

100.2 Formulation Information

Bromochloro-5,5-Dimethylhydantoin 96%

This name is used by Great Lakes Chemical Corp. to describe the active ingredient which is, [redacted]

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

100.3 Application Methods, Directions, Rates

Spa Brom Mini-Pak - "Place Spa Brom Mini-Pak(s) in the skimmer basket or put directly in the water by hanging it on a thermometer line near a return. Use one Mini-Pak per 300 gallons, or fraction, of water to be treated. Replace with new Mini-Pak(s) when the Spa Brom has been dissolved. Maintain active bromine residual between 1.0 and 5.0 ppm at all times."

Spa Brom Feeder Sticks - "Use one stick per 100 gallons, or fraction, of water to be treated. Replace with new sticks when the Spa Brom has been dissolved. Maintain active bromine residual between 1.0 and 5.0 ppm at all times."

100.4 Target Organisms

The product is a microbicide which is claimed to be effective against microbes including the following: Escherichia Coli, Streptococcus faecalis, and S. aureus.

100.5 Precautionary Labelling

"This product is toxic to fish. Prevent access of product or treated waters to lakes, streams, ponds, or public waters unless in accordance with an NPDES permit. For guidance contact the regional office of EPA."

101.1 Chemical Name

See 100.2 above.

101.4 Trade Names

Spa Brom Mini-Pak and Spa Brom Feeder Sticks.

103.2.3 Fish acute LC_{50}'s

Species	Cpd.*	# dosage levels/ # organisms/per level/ use of controls	96-hr. LC_{50} reported	Status**	Reviewer
Rainbow trout (Salmo gairdneri)	P	5/20/yes (neg.)	0.87 mg/l	Core	J. Felkel
"	D	5/20/yes (neg.)	12,700 mg/l	Suppl.	J. Felkel
Fathead minnow (Pimephales promelas)	P	5/20/yes (neg.)	2.25 mg/l	Core	J. Felkel
"	D	5/20/yes (neg.)	14,200 mg/l	Suppl.	J. Felkel

103.2.4 Aquatic Invertebrate LC_{50}

Species	Cpd.*	# dosage levels/ # organism per level/ use of controls	48-hr. LC_{50} reported	Status**	Reviewer
Daphnia magna	P	5/20/yes (neg.)	0.48 mg/l	Core	J. Felkel
"	D	5/20/yes (neg.)	6,100 mg/l	Suppl.	J. Felkel

103.4.1 Toxicity to Estuarine and Marine Animals

Species	Cpd.*	# dosage levels/ # organisms/per level/ use of controls	96-hr. LC_{50} reported	Status**	Reviewer
Juv. Sheepshead Minnow (Cyprinodon variegatus)	P	6/20/yes (neg.)	20 mg/l	Suppl.	J. Felkel
"	D	5/20/yes (neg.)	8,100 mg/l	Suppl.	J. Felkel
Grass Shrimp	P	7/20/yes (neg.)	13 mg/l	Suppl.	J. Felkel
(Palaemonetes pugio)	D	7/20/yes (neg.)	1,300 mg/l	Suppl.	J. Felkel
American oyster	P	7/20/yes (neg.)	_____	Suppl.	J. Felkel
(Crassostrea virginica)	D	5/20/yes (neg.)	13,300 mg/l	Suppl.	J. Felkel

* P = Bromochloro-5,5-Dimethylhydantoin (parent compound)
D = 5,5-Dimethylhydantoin (a degradation product)

** Status = Current status for a mfg.-use

104 Hazard Assessment

A hazard assessment is not conducted for this use pattern.

107 Conclusions

There is no incremental risk to fish or wildlife associated with the proposed registrations of these two products in spas and hot tubs as long as the proposed label regarding discharges is adhered to (see 100.5).

107.3 Environmental Hazards Labelling

See 100.5

107.4 Data Adequacy Conclusion

The studies on the three freshwater species (rainbow trout, fathead minnow, and Daphnia magna) with the parent compound satisfy guideline requirements for a manufacturing-use. The studies on the fathead minnow and Daphnia magna with the 5,5-Dimethylhydantoin degradation product would also satisfy guideline requirements if such tests with this compound are required in the future. If it can be shown that the aeration of the trout test vessels did not lower toxicant levels by more than 20% of what such levels would be without aeration, the trout study with the degradation product may also meet guideline requirements if this test is required in the future.

None of the studies on marine/estuarine species are required for a manufacturing - use or the proposed registrations. If such studies are required for future use-patterns, the tests with sheepshead minnow and grass shrimp may satisfy such requirements if the water temperature and dissolved oxygen variations beyond that permitted by accepted protocols can be adequately defended and if it can be verified that no aeration took place during the bioassays (or that aeration did not affect toxicant levels as noted above.) The studies on the American oyster could not satisfy a guideline requirement since this species is to be used in embryolarvae or shell deposition studies rather than the 96-hour LC_{50} studies submitted. If studies on acute toxicity to marine/estuarine organisms are required in the future, proposed subpart E guidelines also specify that a 96-hour LC_{50} on a species of crab and either a 48-hour EC_{50} for mollusc embryolarvae on a 96-hour EC_{50} for mollusc shell deposition be provided.

Depending on future use patterns, toxicity tests using a flow-through technique may be necessary. This is particularly true with salt water studies since salt water is particularly vulnerable to fouling.

107.7 Recommendations

Since there is no incremental risk to fish or wildlife associated with the proposed registration of Spa Brom Mini-Paks and Spa Brom Feeder Sticks in spas and hot tubs, as long as the proposed labels regarding discharges are adhered to (see 100.5), the Ecological Effects Branch concurs with these registrations.

James D. Felkel
Wildlife Biologist
EEB/HED

James D. Felkel 10/30/80

Raymond W. Matheny 10/3/80
Raymond W. Matheny, Head
Section #1, EEB/HED

Clayton Bushong 11/3/80
Clayton Bushong, Chief
EEB, HED

[redacted]

DATA EVALUATION RECORD

1. Chemical: Cited in report as 1-bromo-3-chloro-5, 5-dimethylhydantoin [redacted] (Shaughnessy #006315).
Reported by Great Lakes Chemical Co. (Dr. John Sands) as "Bromochloro-5, 5-dimethylhydantoin", [redacted]

2. Formulation: Report: Test material not labelled as to its percent active ingredient.
GLCC (Dr. Sands): 92.2% active ingredient.

3. Citation: Horne, J.D., et al. 1980. 96-hour static bioassays using two Great Lakes Chemical Corporation compounds with three marine and three freshwater species. NUS Corporation--Northern Environmental Services Div. (Pittsburgh, PA) and Southern Environmental Services Div. (Clear Lake, Texas). (Within Accession #243015).

4. Reviewed by: James D. Felkel
Wildlife Biologist
Ecological Effects Branch/HED

5. Date Reviewed: 9/4/80

6. Test Type: Fish acute 96-hour LC_{50}

A. Test Species: Rainbow Trout (Salmo gairdneri)
Fathead Minnows (Pimephales promelas)

7. Reported Results: The 96-hr. LC_{50} for the rainbow trout is reported to be 0.87 mg/l (95% C.L. of 0.76-1.00 mg/l) and 2.25 mg/l (2.05-2.48 mg/l) for the fathead minnow.

8. Reviewer's Conclusions: The studies appear to be scientifically sound and meet the intent of Proposed Guidelines for a manufacturing-use. The reported LC_{50} values indicate that the material tested is highly toxic to rainbow trout and moderately toxic to fathead minnows. Depending on future use patterns, a flow-through test may be advisable.

Materials/Methods Reported

A. Test Procedure

Dechlorinated Pittsburgh tap water (pretreatment included activated carbon filters and U.V. light) was used to prepare the test concentrations. The fish were purchased from commercial hatcheries. The trout ranged from 31-46 mm in length with an average weight of 0.84 g/fish and were acclimated to the test temperature of 10° (±1°) C. The juvenile fathead minnows ranged from 27-33 mm in length with an average weight of 0.29 g/fish and were acclimated to 17.5° (±1°) C and tested at 18°C. Food was withheld one day prior to, and during, all tests.

Rangefinder tests were conducted to estimate the upper and lower bounds of the concentration range expected to include the median lethal concentration and five concentrations spanning this range were selected for the definitive test.

All definitive treatment levels and controls were established in duplicate with 10 fish in each chamber. Test chambers were arranged randomly in temperature-controlled water baths and fish were randomly selected from the holding tank and placed two at a time in all chambers. Fish behavior and mortality were monitored daily with dead fish removed and preserved for length and weight measurements. Survivors were sacrificed and similarly measured after each bioassay. Water quality measurements included water temperature for all test concentrations every 24 hours; D.O. and pH for the high, middle, and low concentrations and controls at the start and finish of testing; plus the alkalinity, hardness, and conductivity for the highest concentrations and controls at the start of testing.

B. Statistical Analysis

The Litchfield-Wilcoxon method was used to fit a line to mortality data plotted on log-probit paper.

Discussion/Results Reported

1. Rainbow Trout

Test concentrations selected after rangefinder testing were 1, 1.3, 1.5, 1.7, and 2 mg/l. However, heavy mortality, including all fish dead in the upper three concentrations after 12 hours, necessitated the addition of three additional test levels: 0.3, 0.5, and 0.7 mg/l. All fish at the lowest two levels remained alive for the 96-hour period. Cumulative mortality was as follows:

Treatment Level (mg/l)	Time (hrs.) 24	48	72	96
1.5	20	20	20	20
1.3	18	19	19	19
1.0	16	17	17	17
0.7	2	3	4	4
0.5	0	0	0	0
Control	0	0	0	0

This table shows that the majority of deaths at all treatment levels occurred within the first 24 hours. The 96-hour LC_{50} was reported to be 0.87 mg/l (95% confidence limits of 0.76 and 1.00 mg/l).

2. Fathead Minnow

Definitive test concentrations selected after a rangefinder test were 3, 2.5, 2, 1.7, and 1 mg/l. Cumulative mortality was as follows:

Treatment Level (mg/l)	Time (hrs.) 24	48	72	96
3	20	20	20	20
2.5	13	13	13	13
2	7	7	7	7
1.7	0	0	0	0
1	0	0	0	0
Control	0	0	0	0

All mortality occurred within the first 24 hours. The 96-hour LC_{50} was reported to be 2.25 mg/l with 95% confidence limits of 2.48 and 2.05 mg/l.

3. Water Quality

At the start of testing with Rainbow Trout, D.O. was 10.5 mg/l at all test levels checked (ca. 93% saturation) and declined after 96 hours to levels ranging from 7.6 mg/l in the controls (ca. 62% saturation) to 9.8 mg/l at 1.0 mg/l test concentration (ca. 87% saturation). At the start of testing with fathead minnow, D.O. was 9.7 mg/l at all test levels checked (ca. 85% saturation) and declined after 96 hours to levels ranging from 5.2 mg/l (ca. 46% saturation) in the controls to 6.2 mg/l (ca. 54% saturation) at 1 mg/l test concentration.

pH was 6.7 - 6.8 at the start of all testing and declined slightly to 6.5 - 6.7 or remained constant by the end of the 96 hours in the test vessels. Conductivity level was 375 µmhos in the trout experiment and 225 with the minnow experiment. Alkalinity was 44 and 34 mg/l (as $CaCO_3$) in these two experiments, respectively, and hardness was 134 and 100 mg/l (as $CaCO_3$), respectively.

Reviewer's Evaluation

A. Test Procedure

Procedures generally followed the 1978 Proposed Guidelines (subpart E) and approved protocols (Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975). However, discrepancies include:

1) Fathead Minnows, with an average weight of 0.29 g/fish, were below the recommended range of 0.5-5g;

2) Food was withheld 1 day rather than 96 hours prior to testing;

3) The test temperature of 18°C for the fathead minnow was below the recommended 22 °C of the 1975 protocols; however, recent ASTM standard practices (E 729-80) permit a test temperature of 17 or 22°C.;

4) Information on storage of the test material was missing; and

5) Missing environmental conditions included the photoperiod/lighting and depth of solution in test chambers; and

6) D.O. was not measured every 48 hours in the control and the high, medium, and low toxicant concentrations. (These measurements were taken at the beginning and end of testing.)

Minor internal discrepancies within the report include:

1) Page 16 of the text states that 1 trout died at 1.3 mg/l and 3 died at 1.0 mg/l of the test chemical, but Table 16 indicates 2 and 4 deaths, respectively; The submitted LC_{50} was confirmed using the data of Table 16; and

2) The description of water quality measurements taken on p.17 is inconsistent with the results in Table 22 in terms of the tests conducted. It is assumed that the tests conducted were those for which results were reported (Table 22).

B. Statistical Analysis

A probit analysis by this reviewer of the cumulative mortality data submitted in Tables 16 and 17 gave LC_{50} values and 95% confidence limits of 0.84 (0.76-0.91) mg/l for rainbow trout and 2.24 (2.11-2.38) mg/l for fathead minnow, closely approximating the submitted values.

C. Results/Discussion

The submitted results indicate that this compound is highly toxic to rainbow trout and moderately toxic to fathead minnows.

D. Conclusions

1. Category: Core

2. Rationale: While there were certain discrepancies from approved protocols as described above, the studies appear to be scientifically sound and meet the intent of the 1978 proposed subpart E guidelines for a manufacturing-use. Depending on future use patterns, a flow-through test may be advisable.

3. Repairability: N/A

[redacted]

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (P
1.5	20	20	100	9.53674
1.3	20	19	95.	2.0027
1	20	17	85.	.128841
.7	20	4	20.	.590897
.5	20	0	0	9.53674

THE BINOMIAL TEST SHOWS THAT .7 AND 1 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .823179

---------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	6.12738E-2	.829708	.756731	.909125

---------RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.111283	1	.674846

SLOPE = 10.7927
95 PERCENT CONFIDENCE LIMITS = 7.19235 AND 14.393

LC50 = .836785
95 PERCENT CONFIDENCE LIMITS = .760438 AND .913542

SRU 1.300 UNTS.

RUN COMPLETE.

PRODUCT INGREDIENT SOURCE INFORMATION IS NOT INCLUDED

```
9000 data 5
9001 data 3,2.5,2,1.7,1
9002 data 20,20,20,20,20
9003 data 20,13,7,0,0
run

 80/09/24. 14.33.24.
BASIC      PROGRAM   S79LC50
```

[redacted] Fathead minnow Acc. #243015

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
3	20	20	100	9.53674E-5
2.5	20	13	65.	13.1588
2	20	7	35.	13.1588
1.7	20	0	0	9.53674E-5
1	20	0	0	9.53674E-5

THE BINOMIAL TEST SHOWS THAT 1.7 AND 3 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 2.23607

--------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	4.90922E-2	2.22507	2.06362	2.42224

--------RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	.113443	1	.230874

SLOPE = 14.8701
95 PERCENT CONFIDENCE LIMITS = 9.86162 AND 19.8785

LC50 = 2.24066
95 PERCENT CONFIDENCE LIMITS = 2.10911 AND 2.38308

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RU 1.332 UNTS.

RUN COMPLETE.

PRODUCT INGREDIENT SOURCE INFORMATION IS NOT INCLUDED

[redacted]

DATA EVALUATION RECORD

1. Chemical: Cited in report as 1-bromo-3-chloro-5, 5-dimethylhydantoin [redacted] (Shaughnessy #006315). Reported by Great Lakes Chemical Co. (Dr. John Sands) as "Bromochloro-5,5-dimethylhydantoin", [redacted]

2. Formulation: Report: Test material not labelled as to its percent active ingredient.
GLCC (DR. Sands): 92.2% active ingredient.

3. Citation: Horne, J.D., et al. 1980. 96-hour static bioassays using two Great Lakes Chemcial Corporation compounds with three marine and three freshwater species. NUS Corporation--Northern Environmental Services Div. (Pittsburgh, PA) and Southern Environmental Services Div. (Clear Lake, Texas). (Within Accession #243015.)

4. Reviewed by: James. D. Felkel
Wildlife Biologist
Ecological Effects Branch/HED

5. Date Reviewd: 9/26/80

6. Test Type: Aquatic Invertebrate 48-hour LC_{50}

A. Test Species: Daphnia Magna

7. Reported Results: The 48-hour LC_{50} is reported to be 0.48 mg/l with 95% confidence limits of 0.53 mg/l and 0.42 mg/l.

8. Reviewer's Conclusions: The study is scientifically sound and fulfills the requirement for an aquatic invertebrate 48-hour LC_{50} for a manufacturing -use. The reported LC_{50} value indicates that the test material is highly toxic to daphnids. Depending on future use patterns, a flow-through test may be advisable.

Materials/Methods Reported

A. Test procedure

Dechlorinated Pittsburgh tap water (pretreatment included activated carbon filters and U.V. light) was used to prepare the test concetrations. The Daphnia cultures were purchased from Wards Natural Science Estab., Inc. on 2/14/80 and acclimated at 20° (±1°)C. First instars were used in all tests. Cultures were fed yeast solution every other day.

Rangefinder tests were conducted to estimate the upper and lower bounds of the concentration range expected to include the median lethal concentration and five concentrations spanning this range were selected for the definitive test.

All definitive treatment levels and controls were established in duplicate with 10 Daphnia magna individuals pipetted into each of the 250 ml test beakers containing a known concentration of test material. Water quality measurements taken at the start and finish of the experiment included:

1). Temperature (at all test levels); and

2. D.O. and pH. (at high, middle, and low concentrations and controls).

Parameters measured at the start of the experiment only, for the controls and highest test concentration, were alkalinity, hardness, and conductivity.

B. Statistical Analysis

The Litchfield- Wilcoxon method was used to fit a line to mortality data plotted on log-probit paper.

Discussion/Results Reported

After rangefinder testing, definitive test concentrations selected were 0.3, 0.4, 0.5, 0.7, and 1.0 mg/l. Cumulative mortality was as follows:

Treatment Level (mg/l)	Time (hrs.) 12	24	36	48
1.0	20	20	20	20
0.7	10	13	13	13
0.5	7	11	11	11
0.4	2	3	3	3
0.3	0	0	0	0
Control	0	0	0	0

The table shows that all mortality occurred within the first 24 hours. The 48-hour LC_{50} was reported to be 0.48 mg/l with confidence limits of 0.53 mg/l and 0.42 mg/l.

Reviewer's Evaluation

A. Test Procedures

Procedures generally followed the 1978 Proposed Guidelines (subpart E) and approved protocols (Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975). However, missing information included the method of storing the test material and the photoperiod/lighting conditions.

B. Statistical Analysis

Statistical analysis by this reviewer of the submitted mortality data indicates LC_{50} values of 0.54 mg/l using the probit and moving average methods, and 0.48 mg/l using the binomial test. These values closely approximate the submitted value.

C. Results/Discussion

The submitted results indicate that the compound is highly toxic to daphnids.

D. Conclusions

1. Category: Core

2. Rationale: The test appeared to satisfy the intent of the 1978 proposed guidelines (subpart E) for a manufacturing-use. Depending on future use patterns, however, a flow-through test may be advisable.

3. Repairability: N/A

```
9001 data 1.0,.7,.5,.4,.3
9002 data 20,20,20,20,20
9003 data 20,13,11,3,0
run

 80/09/24. 14.37.29.
BASIC      PROGRAM    S79LC50
```

[redacted]/Daphnia magna Acc. #243015

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
1	20	20	100	9.53674E-5
.7	20	13	65.	13.1588
.5	20	11	55.	41.1901
.4	20	3	15.	.128841
.3	20	0	0	9.53674E-5

THE BINOMIAL TEST SHOWS THAT .4 AND 1 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .487282

---------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	5.44358E-2	.544681	.497148	.602398

---------RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.102371	1	.13592

SLOPE = 7.38204
95 PERCENT CONFIDENCE LIMITS = 5.02013 AND 9.74396

LC50 = .54575
95 PERCENT CONFIDENCE LIMITS = .492563 AND .609742

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SRU 1.314 UNTS.

RUN COMPLETE.

PRODUCT INGREDIENT SOURCE INFORMATION IS NOT INCLUDED

[redacted]

DATA EVALUATION RECORD

1. Chemical: Cited in report as 1-bromo-3-chloro-5, 5-dimethylhydantoin [redacted] (Shaughnessy #006315.)
Reported by Great Lakes Chemical Co. (Dr. John Sands) as "Bromochloro-5, 5-diemthylhydantoin", [redacted]

2. Formulation: Report: Test material not labelled as to its percent active ingredient.
GLCC (Dr. Sands): 92.2% active ingredient.

3. Citation: Horne, J.D., et al. 1980. 96-hour static bioassys using two Great Lakes Chemical Corportation compounds with three marine and three freshwater species. NUS Corporation--Northern Environmental Services Div. (Pittsburgh, PA) and Southern Environmental Services Div. (Clear Lake, Texas). (Within Accession #243015).

4. Reviewed by: James D. Felkel
Wildlife Biologist
Ecological Effects Branch/HED

5. Date Reviewed: 10/16/80

6. Test Type: Acute toxicity (96-hour) to estuarine and marine organisms

A. Test Species: Juvnile sheepshead minnow (Cyprinodon variegatus) Grass shrimp (Palaemonetes pugio) American Oyster (Crassostrea virginica)

7. Reported Results:

The 96-hour LC_{50} for sheepshead minnow is 20 mg/l (95% C.I. = 17-23 mgl/) and 13 mg/l (95% C.I. = 10.1-16.6 mg/l) for the grass shrimp. No LC_{50} was calculable for the American oyster.

8. Reviewer's Conclusions:

The studies appear to be scientifically sound. However, further information cited in the Repairability section below is needed before the tests on the sheepshead minnow and grass shrimp could meet proposed subpart E guideline requirements. The test on the American oyster cannot meet the intent of the guidelines. The submitted results indicate that the material tested is "slightly toxic" to the minnows and shrimps, following EPA-accepted toxicity category criteria. Depending on future use patterns, flow-through tests may be advisable.

Materials/Methods Reported

A. Test procedure

A synthetic seasalt ("Biocrystals Marinemix") was dissolved in deionized water to produce water with a salinity of 20 (±2) ppt (0/00). This water was used in the culture/acclimation tanks, the test tanks, and to prepare the stock solutions.

The sheepshead minnows were spawned and reared at the NUS laboratory. They were 29 (±2) days old, averaged 0.05 g/ individual, and ranged from 8-22mm in length at the start of testing. The grass shrimp were collected locally in Texas and acclimated to a final temperature of 22°C and 20 ppt (0/00) salinity. They averaged 0.18g/ individual and ranged from 17-38 mm in length (rostrum to telson). The osyters were purchased from a commerical lease-bed operator. The average wet-tissue weight of the oyster was 3.8 g/individual.

Rangefinder tests were conducted to determine the appropriate definitive test concentrations for all species. Six test levels were selected for the tests with sheepshead minnows (200, 112, 64, 36, 20, and 11.2 mg/l) seven for the grass shrimp (52, 36, 20, 11.2, 6.4, 3.6, and 2.0 mg/l), and seven for the American oysters (640, 200, 112, 64, 36, 20, and 11.2 mg/l.) Each definitive bioassay included a control and all treatment levels were established in duplicate. The minnows and shrimps were tested in 3 l of test solution in 1-gal widemouth jars with 10 organisms/container. The oysters were tested in 10-gal aquaria containing 30 l of test solution and 10 organisms/container. Test containers were arranged randomly in the test area and organisms were assigned randomly to the containers.

Behavior and mortality were checked daily. Temperature, D.O., and salinity were measured daily for all test solutions.

B. Statistical Analysis

The Litchfield-Wilcoxon method was used to fit a line to mortality data plotted on log-probit paper.

Discussion/Results Reported

A. Cumulative Mortality

Cumulative mortality (number of indivduals out of 20 at each test level) was as follows:

1. Sheepshead Minnow

Treatment Level (mg/l)	Time (hrs.) 24	48	72	96
200	20	20	20	20
112	20	20	20	20
64	20	20	20	20
36	20	20	20	20
20	4	10	10	10
11.2	0	0	0	0
Control	0	0	0	0

The LC_{50} is reported to be 20 mg/l (95% confidence interval of 17-23 mg/l.

2. Grass Shrimp

Treatment Level (mg/l)	Time (hrs.) 24	48	72	96
52	20	20	20	20
36	20	20	20	20
20	7	11	13	14
11.2	2	7	9	9
6.4	0	0	0	0
3.6	0	0	0	0
2.0	0	0	0	0
Control	0	0	0	1

The LC_{50} is reported to be 13 mg/l (95% C.I. of 10.1 - 16.6 mg/l)

The one control mortality resulted from the shrimp escaping the test vessel.

3. American Oyster

Treatment Level (mg/l)	Time (hrs.) 24	48	72	96
640	0	1	2	2
200	0	0	0	2
112	0	0	1	1
64	0	0	0	1
36	0	1	1	2
20	0	0	1	2
11.2	0	0	0	0
Control	0	0	0	0

Since greater than 50% mortality did not occur at any treatment level, an LC_{50} value could not be calculated.

B. Water Quality

1. Sheepshead Minnow

Water temperature was 22-23°C in all vessels at the start of testing, rose to 24°C in all vessels after 24 hours, rose to 25°C after 48-72 hours in all vessels for which measurements were taken (i.e., those still containing live fish), and then returned to 24°C after 96 hours in these latter vessels.

Dissolved Oxygen was 6.5-7.4 mg/l at the start of testing and declined to a low of 4.0 mg/l at 48 hours in the 20 mg/l test vessel. Salinity was constant at 21 ppt (0/00) except in the 200 mg/l vessel where it rose to 22 ppt (0/00).

2. Grass Shrimp

Water temperature was 19-20°C throughout the test. D.O. at the start of testing was 7.9-8.0 mg/l in all vessels and declined to a low of 2.5 mg/l after 96 hours in the 2 mg/l test vessel. Salinity was constant at 20 ppt (0/00).

3. American Oyster

Water temperature ranged from 22-24°C during the test. D.O. at the start of testing was 7.0 - 7.2 mg/l and declined to a low of 2.7 mg/l at 48 hours in the control group. Salinity ranged from 20-22 ppt.

III. Reviewer's Evaluation

A. Test Procedure

Discrepancies from accepted protocols (Stephan, 1975) include:

1) sheeshead minnows averaged 0.05 g/individual rather than 0.5 - 5 g and the standard length of the largest fish was more than twice that of the shortest;

2) oysters are generally used in embryolarvae or shell deposition studies rather than adult 96-hour LC_{50} studies; and

3) information was not provided on the photoperiod.

B. Statistical Analysis

An analysis by this reviewer of the cumulative mortality data submitted confirmed the two LC_{50} values reported and the fact that a reliable LC_{50} value could not be calculated for the American oyster. However, it should be noted that the LC_{50} value for the sheepshead minnow is more approximate than for the grass shrimp since there were less than two concentrations at which the percent dead was between 0 and 100.

C. Results/Discussion

Discrepancies from accepted protocols (Stephan, 1975) include:

1) with the sheepshead minnow and American oyster, water temperature occasionally varied by more than $1^{o}C$ from the selected temperature (maximum of $3^{o}C$ above starting temperature with the minnows and $2^{o}C$ with the oysters);

2) D.O. was less than 60% saturation during at least part of the lst 48 hours of testing with

   a.) the sheepshead minnow test solution in the 20 mg/l test vessel;

   b.) the grass shrimp test solutions in the 11.2, 6.4, 3.6, 2.0 mg/kg, and control vessels; and

   c.) the American oyster test solution in the 11.2 mg/l and control test vessels.

3) D.O. was less than 40% saturation during at least part of the 2nd 48 hours of testing with the grass shrimps test solutions in the 3.6, 2.0 mg/l and the control vessels.

4) on several occasions, such as with the sheepshead minnow 20 mg/l and control vessels, the grass shrimp 20 mg/l and 11.2 mg/l vessels, and the oyster 11.2 mg/l and control vessels, D.O. increased from the 48-hour sample to the 96-hour sample. The cause of this increase should be clarified since aeration during the tests is strictly prohibited; and

5) a reliable LC_{50} was not calculable for the American oyster.

The results indicate that the material tested is "slightly toxic" to sheepshead minnows and grass shrimps, following EPA-accepted toxicity category criteria..

D. Conclusions

1. Category: Supplemental

2. Rationale: Estuarine and marine organism toxicity tests are required to support the registration of a formulated product where such product is intended for direct application to the marine on estuarine environment or if it may be expected to enter this environment in significant concentrations because of its use or mobility pattern. Since such studies are not a requirement for the proposed registrations (1729-22 and 1729-23), they could not technically be considered "Core " (i.e., satisfying an existing guideline requirement).

Also, there were some discrepancies from accepted protocols (see Reviewer's Evaluation of procedures and results above). In particular, it should be clarified whether there was any aeration during the tests, and if not, what the cause of the D.O. pattern referred to in III. C. 4. above was. The acute toxicity results submitted for the American oyster were not suitable for calculation of a reliable LC_{50} value; this species is not generally acceptable for such tests and is more suited for embryolarvae and shell deposition studies.

3. Repairability: a.) Sheepshead minnow and grass shrimp tests - If the temperature and D.O. pattern discrepancies can be adequately defended and if it can be verified that no aeration took place during the bioassays, then these tests may be suitable for Core status. [If acute toxicity tests on estuarine and marine organisms are required in the future, subpart E guidelines specify that a 96-hour LC_{50} on a species of crab and either a 48-hour EC_{50} for mollusc embryolarvae or a 96-hour EC_{50} mollusc shell deposition also be provided (using the technical grade of the active ingredient). Flow-through tests may also be required since salt water is particularly vulnerable to fouling in static tests and since the test material (in minnow test) appears to be active for a very short period.]

b.) American oyster acute toxicity test is not repairable for the reasons noted above.

640,320,200,112,64,36,20,11.2
1,1,1,1,1,2,0

13.09.37.
PROGRAM S79LC50

[redacted]/Crassotrea virginica Acc. 243015; Shaugh. #006315/[redacted]

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
640	20	2	10.	2.01225E-2
200	20	2	10.	2.01225E-2
112	20	1	5.	2.00272E-3
64	20	1	5.	2.00272E-3
36	20	2	10.	2.01225E-2
20	20	2	10.	2.01225E-2
11.2	20	0	0	9.53674E-5

THIS DATA SET DOES NOT MEET THE CRITERIA ESTABLISHED BY THE COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS BECAUSE NO PERCENT DEAD IS GREATER THAN 65 PERCENT.

NEITHER THE BINOMIAL TEST NOR THE MOVING AVERAGE METHOD CAN GIVE ANY RESULTS FOR THIS DATA SET. EITHER THE HIGHEST CONCENTRATION KILLED LESS THAN 50 PERCENT OR THE LOWEST KILLED MORE THAN 50. IF THE PROBIT SLOPE IS NEGATIVE, ENTER DATA AGAIN USING NUMBER ALIVE INSTEAD OF NUMBER DEAD.

---------RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	5.58841	1	.743748

SLOPE = .235321
95 PERCENT CONFIDENCE LIMITS =-.320974 AND .791617

LC50 = 1.31570E+8
95 PERCENT CONFIDENCE LIMITS = 5065.44 AND +INFINITY
****

SRU 1.261 UNTS.

RUN COMPLETE.

ILLEGAL PARAMETER.

PRODUCT INGREDIENT SOURCE INFORMATION IS NOT INCLUDED

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

```
data 7
data 52,36,20,11.2,6.4,3.6,2.0
data 20,20,20,20,20,20,20
data 20,20,14,9,0,0,0

run

 80/09/25. 13.00.37.
BASIC      PROGRAM    S79LC50
```

[redacted]/Palaemonetes pugio Acc. #243015; Shaugh. #006315[redacted]

****

CONC. (mg/l)	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
52	20	20	100	9.53674E-5
36	20	20	100	9.53674E-5
20	20	14	70.	5.76591
11.2	20	9	45.	41.1901
6.4	20	0	0	9.53674E-5
3.6	20	0	0	9.53674E-5
2	20	0	0	9.53674E-5

THE BINOMIAL TEST SHOWS THAT 6.4 AND 36 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 12.549

---------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
6	2.67807E-2	12.8789	10.8127	15.407

---------RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	.109279	1	.531515

SLOPE = 5.27602
95 PERCENT CONFIDENCE LIMITS = 3.5319 AND 7.02014

LC50 = 13.8465
95 PERCENT CONFIDENCE LIMITS = 11.6506 AND 16.45

****

SRU 1.297 UNTS.

RUN COMPLETE.

PRODUCT INGREDIENT SOURCE INFORMATION IS NOT INCLUDED

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

...11.2,64,36,20,11.2
...0,20,20,20
...20,20,20,20,10,0
...20,20,20,20,20,20

09/25. 12.52.05.
PROGRAM S79LC50

[redacted] / Juv. Sheepshead minnow Acc. #243015; Shaugh. #006315[redacted]

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
200	20	20	100	9.53674E-5
112	20	20	100	9.53674E-5
64	20	20	100	9.53674E-5
36	20	20	100	9.53674E-5
20	20	10	50	58.8099
11.2	20	0	0	9.53674E-5

THE BINOMIAL TEST SHOWS THAT 11.2 AND 36 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 20.

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.

****

SRU 1.285 UNTS.

RUN COMPLETE.

PRODUCT INGREDIENT SOURCE INFORMATION IS NOT INCLUDED

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

DATA EVALUATION RECORD

1. Chemical: 5,5-dimethylhydantoin (No Shaughnessy number available)

2. Formulation: Test material not labelled as to purity or percent active ingredient. GLCC (Dr. John Sands) reports a.i. to be a minimum of 97%.

3. Citation: Horne, J.D., et al. 1980. 96-hour static bioassays using two Great Lakes Chemical Corporation compounds with three marine and three freshwater species. NUS Corporation--Northern Environmental Services Div. (Pittsburgh, PA) and Southern Environmental Services Div. (Clear Lake, Texas.) (Within Accession #243015.)

4. Reviewed by: James D. Felkel
   Wildlife Biologist
   Ecological Effects Branch/HED

5. Date Reviewed: 10/23/80

6. Test Type: 96-hour fish and 48-hour aquatic invertebrate LC_{50}

   A. Test Species: Rainbow trout (Salmo gairdneri)
   Fathead minnows (Pimephales promelas)
   Daphnia magna

7. Reported Results: LC_{50} values are 12.7 g/l for rainbow trout, 14.2 g/l for fathead minnow, and 6.2 g/l for Daphnia magna.

8. Reviewer's Conclusions: The reported results indicate that the test material is practically non-toxic to the species tested. The tests appear to be scientifically sound and should acute toxicity tests be required in the future for these species with this degradation product as the test material, the tests with the fathead minnow and Daphnia magna would satisfy such a requirement. Further information regarding the aeration, as noted under Repairability below, would be needed before the trout study would satisfy such a requirement. Depending on future use proposals, flow-through testing may also be advisable.

I. Materials/Methods Reported

A. Test Procedures

Dechlorinated Pittsburgh tap water (pretreatment included activated carbon filters and U.V. light) was used to prepare the test concentrations. The fish were purchased from commercial hatcheries and the Daphnia magna from Wards Natural Science Estab., Inc. The trout ranged from 32-58 mm with an average weight of 2.18 g/fish. The minnows were 21-31 mm in length with an average weight of 0.51 g/individual. The fish were not fed one day prior to, or during, testing.

Rangefinder tests were conducted to estimate the upper and lower bounds of the concentration range expected to include the median lethal concentration and five concentrations spanning this range were selected for the definitive test.

All definitive treatment levels and controls were established in duplicate with 10 organisms in each chamber. Test chambers were arranged randomly in temperature-controlled water baths and fish were randomly selected from the holding tank and placed two at a time in all chambers. Fish behavior and mortality were monitored daily with dead fish removed and preserved for length and weight measurements. Survivors were sacrificed and measured after each bioassay.

Water quality measurements in the tests included (1) temperature for all test concentrations every 24 hours for the fish test vessels and at the start and end of testing for the Daphnia containers; (2) D.O., pH, alkalinity, hardness, and conductivity for the control and high, middle, and low fish test concentrations (D.O. and pH every 24 hours and the last three parameters at the start and end of testing) and; (3) D.O. and pH for the control and high, middle, and low Daphnia test concentrations at the start and end of testing and alkalinity, hardness, and conductivity for the control and high Daphnia test concentration at the start of testing.

It is reported that "gentle aeration" was required in the test with rainbow trout from the 48-hr. period to the end of the test and that this was the only freshwater test in which this was done.

Initial test temperatures were 10.0-10.9°C for the trout, 17.3-17.5°C for the minnows, and 20°C for the Daphnia. Treatment levels were 25, 15, 12.5, 10, and 5 g/l for the trout; 15, 10, 7, 5, and 3 g/l for the Daphnia; and 20, 15, 12.5, 10 and 5 g/l for the minnows.

B. Statistical Analysis

The Litchfield-Wilcoxon method was used to fit a line to mortality data plotted on log-probit paper.

II. Discussion/Results Reported

A. Rainbow trout

Cumulative mortality was as follows:

treatment level (g/l)	time (hrs.)			
	24	48	72	96
25	9	17	20	20
15	0	3	11	17
12.5	0	0	1	8
10	0	0	0	2
5	0	0	0	0
Control	0	0	0	0

An LC_{50} of 12.7 g/l (95% C.I. = 11.3-14.2 g/l) is reported.

D.O. levels dropped from a starting level of 10.4-10.6 mg/l to 2.2-5.5 after 48 hours and "gentle aeration" was conducted. Other parameters remained relatively constant.

B. Fathead minnow

Cumulative mortality was as follows:

treatment level (g/l)	time (hrs.)			
	24	48	72	96
20	20	20	20	20
15	11	13	14	14
12.5	3	3	3	3
10	0	0	0	0
5	0	0	0	0
Control	0	0	0	0

An LC_{50} of 14.2 g/l (C.I. = 13.2-15.3 g/l) is reported. Water quality parameters were within acceptable limits.

C. Daphnia magna

Cumulative mortality was as follows:

treatment level (g/l)	time (hrs.)			
	12	24	36	48
15	20	20	20	20
10	9	12	17	20
7	0	5	9	11
5	0	1	6	7
3	0	0	0	0
Control	0	0	0	0

An LC_{50} of 6.2 g/l (95% C.I. = 5.3-7.3 g/l) is reported. Water quality parameters were within acceptable limits.

III. Reviewer's Evaluation

A. Test Procedures

Procedures generally followed the 1978 proposed subpart E guidelines and approved protocols (Stephen, 1975). The principal discrepancy was an overloading of the trout test vessels (1.36 g/l). This resulted in the low D.O. levels and necessitated aeration. Aeration is not permitted by accepted protocols unless it can be shown that it did not reduce toxicant levels (through volatization) by more than 20% from the levels found in non-aerated vessels. Other discrepancies included:

1) food was withheld 1 day rather than 96 hours prior to testing;

2) test temperature for the fathead minnow was below that of the 1975 protocols but consistent with recently-published ASTM standard practices (E 729-80);

3) the test tempertures for the trout varied by slightly more than $1^{o}C$ in some vessels;

4) information on the storage of the test material was missing; and

5) missing environmental conditions included the photoperiod and depth of solution in the test chambers.

B. Statistical Analysis

Probit analyses of the submitted data by this reviewer gave LC_{50} values closely approximating the submitted values (analyses attached).

C. Results/Discussion

The submitted results indicate that this degradation is practically non-toxic to the species tested.

D. Conclusions

1. Category: Supplemental

2. Rationale: Guidelines do not require fish and wildlife testing on degradation products for the present registrations (1729-122 and 1729-123). Aeration in the test with rainbow trout, unless it can be shown that this would not have affected toxicant levels available to the fish by more than 20%, would prevent this study from meeting "Core" status.

3. Repairability: If 96-hour acute toxicity studies with an aquatic invertebrate and a warmwater fish species are required

for this degradation product in the future, these tests would meet "Core" status. Only if it can be shown that the aeration of the trout test vessels did not substantially effect toxicity, as noted above, would the trout meet "Core" status if this test is required in the future. Depending on future use patterns, a flow-through test may also be advisable.

,12.5,10,5
,20,20,20
,12,8.2,0

13.16.02.
PROGRAM S79LC50

5,5-Dimethylhydantoin/Rainbow trout Acc. #243015;

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
25	20	20	100	9.53674E-5
15	20	17	85.	.128841
12.5	20	8	40.	25.1722
10	20	2	10.	2.01225E-2
5	20	0	0	9.53674E-5

THE BINOMIAL TEST SHOWS THAT 10 AND 15 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 12.9826

--------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.112353	12.7795	11.4769	14.0927

--------RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.189957	1	.935389

SLOPE = 13.2322
95 PERCENT CONFIDENCE LIMITS = 7.46504 AND 18.9993

LC50 = 12.7575
95 PERCENT CONFIDENCE LIMITS = 11.8959 AND 13.7326

SRU 1.324 UNTS.

RUN COMPLETE.

data 15,10,7,5,3
data 20,20,11,7,0
run

80/09/25. 13.24.10.
BASIC PROGRAM S79LC50

5,5-Dimethylhydantoin/Daphnia magna 48-hr. Bioassay; Acc.#243015;
****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
15	20	20	100	9.53674E-5
10	20	20	100	9.53674E-5
7	20	11	55.	41.1901
5	20	7	35.	13.1588
3	20	0	0	9.53674E-5

THE BINOMIAL TEST SHOWS THAT 3 AND 10 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 6.44038

---------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	5.39519E-2	5.97328	5.38581	6.62557

---------RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.136787	1	.274481

SLOPE = 7.934
95 PERCENT CONFIDENCE LIMITS = 4.99963 AND 10.8684

LC50 = 6.05161
95 PERCENT CONFIDENCE LIMITS = 5.354 AND 6.76519
****

SRU 1.334 UNTS.

RUN COMPLETE.

bye

```
)000 data 5
)001 data 20,15,12.5,10,5
)003 data 20,14,3,0,0
:un

 80/09/25. 13.19.35.
)ASIC      PROGRAM    S79LC50



;,5-Dimethylhydantoin/Fathead minnow 96-hr. LC50  Acc.#243015;
:**********************************************************************
:ONC. (g/l)      NUMBER          NUMBER          PERCENT         BINOMIAL
                 EXPOSED         DEAD            DEAD            PROB.(PERCENT)
 20               20              20              100             9.53674E-5
 15               20              14              70.             5.76591
 12.5             20              3               15.             .128841
 10               20              0               0               9.53674E-5
 5                20              0               0               9.53674E-5

'HE BINOMIAL TEST SHOWS THAT  12.5  AND  20  CAN BE
ISED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
:ONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL
.SSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.


.N APPROXIMATE LC50 FOR THIS SET OF DATA IS  14.0765


--------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
;PAN             G               LC50            95 PERCENT CONFIDENCE LIMITS
3                6.23526E-2      14.2861         13.4176        15.307


--------RESULTS CALCULATED USING THE PROBIT METHOD
TERATIONS        G               H               GOODNESS OF FIT PROBABILITY
8                .2253           1               .996363

;LOPE  =         20.4134
5 PERCENT CONFIDENCE LIMITS = 10.724    AND    30.1028

.C50  =          14.1019
5 PERCENT CONFIDENCE LIMITS  =   13.3841    AND    14.9903
**********************************************************************


;RU       1.324 UNTS.

:UN COMPLETE.
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DATA EVALUATION RECORD

1. Chemical: 5,5-dimethylhydantoin (No Shaughnessy number available)

2. Formulation: Test material not labelled as to purity or percent active ingredient. GLCC (Dr. John Sands) reports a.i. to be a minimum of 97%.

3. Citation: Horne, J.D., et al. 1980. 96-hour static bioassays using two Great Lakes Chemical Corporation compounds with three marine and three freshwater species. NUS Corporation--Northern Environmental Services Div. (Pittsburgh, PA) and Southern Environmental Services Div. (Clear Lake, Texas.) (Within Accession #243015.)

4. Reviewed by: James D. Felkel
   Wildlife Biologist
   Ecological Effects Branch/HED

5. Date Reviewed: 10/21/80

6. Test Type: Acute toxicity to estuarine and marine organisms

   A. Test Species: Juvenile sheepshead minnow (Cyprinodon variegatus)
   Grass shrimp (Palaemonetes pugio)
   American oyster (Crassostrea virginica)

7. Reported Results: The LC_{50} for sheepshead minnow is 8,100 mg/l, 1,300 mg/l for grass shrimp, and 13,300 mg/l for American oyster.

8. Reviewer's Conclusions: The reported results indicate that this degradation product is practically non-toxic to the species tested. While the studies may be scientifically sound, further information cited in the Repairability section below is needed before the studies on sheepshead minnow and grass shrimp could satisfy a potential requirement for this type of test. Flow-through tests may be advisable. The test on the American oyster could not satisfy a potential requirement for an acute toxicity test with this material.

I. Materials/Methods Reported

A. Test Procedures

A synthetic seasalt ("Biocrystals Marinemix") was dissolved in deionized water with a salinity of 20(±2) ppt (o/oo). This water was used in the culture/acclimation tanks, the test tanks, and to prepare the stock solutions.

The sheepshead minnows were spawned and reared at the NUS laboratory. They were 45(±2) days old, averaged 0.13 g/individual, and ranged from 10-26 mm in length at the start of testing. The grass shrimp were collected locally in Texas and acclimated to a final temperature of 22°C and 20 ppt (o/oo) salinity. They averaged 0.18 g/individual and ranged from 17-38 mm in length. The oysters were purchased from a commercial lease-bed operator. The average wet-tissue weight of the oysters was 3.8 g/individual.

Rangefinder tests were conducted to determine the appropriate definitive test concentrations for all species. Five test levels were selected for the tests with sheepshead minnows (10, 6.5, 4.2, 2.7, and 1.8 g/l), seven for the grass shrimp (10, 6.5, 4.2, 2.7, 1.8, 1.17, and 0.76 g/l), and five for the American oysters (20, 17, 14.5, 12.25, and 10.4 g/l). Each definitive bioassay included a control and all treatment levels were established in duplicate. The minnows and shrimp were tested in 3 l of the test solution in 1-gal. jars with 10 organisms/container. Test containers were arranged randomly in the test area and organisms were assigned randomly to the containers.

Behavior and mortality were checked daily. Temperature, D.O., and salinity were measured daily for all test solutions.

B. Statistical Analysis

The Litchfield-Wilcoxon method was used to fit a line to mortality data plotted on log-probit paper for the shrimp and oyster data and the straight-line interpolation method described in "Standard Methods, 14th ed." (APHA, 1975) was used for the minnow data.

II. Discussion/Results Reported

A. Sheepshead Minnow

100% mortality occurred at the 10 g/l level with no mortality at or below the 6.5 g/l test level. The submitted LC_{50} for this species is 8.1 g/l. 95% confidence limits could not be determined using the analytical procedure employed.

B. Grass shrimp

100% mortality occurred at the highest four test levels after 96 hours and partial mortalities occurred at the lower three levels

during the same period. The submitted LC_{50} is 1.3 g/l (95% C.I. = 1.1-1.6 g/l).

C. American oyster

Partial mortalities occurred at all concentration levels tested. The submitted LC_{50} is 13.3 g/l (95% C.I. = 10.3-17.5 g/l).

D. Water Quality

Tables of water quality data submitted indicate that temperature rose from 20°C at the start of testing to a high of 26°C after 24 hours in all the sheepshead minnow test vessels while salinity levels ranged from 20 ppt (o/oo) at the start to 22-31 ppt (o/oo) by the end of testing. D.O. levels declined from 7.2-7.6 mg/l at the start to 2.2-3.9 mg/l after 24 hours in the minnow test vessels before generally increasing as the test progressed. Similar variation in these parameters occurred in the grass shrimp test vessels while water quality was considerably more constant in the oyster bioassays.

III. Reviewer's Evaluation

A. Test procedure

Discrepancies from accepted protocols (Stephen, 1975) include:

1) sheepshead minnows averaged 0.13 g/individual rather than 0.5-5g and the standard length of the largest fish was more than twice that of the shortest;

2) oysters are generally used in embryolarvae or shell deposition studies rather than in adult 96-hr. LC_{50} studies; and

3) information was not provided on the photoperiod.

B. Statistical Analysis

An analysis by this reviewer of the cumulative mortality data submitted provided LC_{50} values that generally approximated the submitted values except for the 95% C.I. for the oyster LC_{50}. For the sheepshead minnow data, it was necessary to rely on the binomial test since where 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 (analyses attached).

C. Results/Discussion

Discrepancies from accepted protocols (Stephen, 1975) include:

1) water temperature varied substantially more than 1°C from the initial temperature in all test vessels;

2) dissolved oxygen was under 60% saturation at some point during the lst 48 hours in all the sheepshead minnow and grass shrimp test vessels; and

3) in many of the minnow and shrimp test vessels, D.O. increased sharply from the 24-hour check to the later checks in the bioassys; while this is not a discrepancy per se, aeration during the tests, if it occurred, would be.

D. Conclusions

1. Category: Supplemental

2. Rationale: At present, neither marine/estuarine organism toxicity testing nor tests on degradation products, such as the material tested here, are required for the present registration proposals and thus "Core" status (satisfying an existing requirement) could not apply.

   Also, therewere substantial discrepancies from accepted protocols regarding water temperatue and D.O. and it also is not clear that aeration was not conducted. The American oyster is not an acceptable species for the type of acute toxicity test conducted.

3. Repairability: Although this degradate product appears to be relatively non-toxic, the water temperature and D.O. patterns must be explained and adequately defended and it must be verified whether aeration took place during the tests. If this is done and if tests on degradation products are required for marine/estuarine species in the future, the tests on sheepshead minnow and grass shrimp may satisfy such a requirement. The test on the American oyster would not be repairable for the reasons cited earlier. Flow-through tests are generally recommended for studies using salt water since salt water is particularly vulnerable to fouling.

COMMAND.

data 5
data 10,6.5,4.2,2.7,1.8
data 20,20,20,20,20
data 20,0,0,0,0
run

80/09/24. 14.40.34.
BASIC PROGRAM S79LC50

5,5-Dimethylhydantoin/Sheepshead Minnow***Acc.#243015**********

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCEN
10	20	20	100	9.53674E-5
6.5	20	0	0	9.53674E-5
4.2	20	0	0	9.53674E-5
2.7	20	0	0	9.53674E-5
1.8	20	0	0	9.53674E-5

THE BINOMIAL TEST SHOWS THAT 6.5 AND 10 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 8.06226

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.
****

SRU 1.308 UNTS.

RUN COMPLETE.

run

80/09/25. 12.43.53.
BASIC PROGRAM S79LC50

5,5-Dimethylhydantoin/Crassostrea virginica) Acc. #243015;

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
20	20	11	55.	41.1901
17	20	15	75	2.06947
14.5	20	15	75	2.06947
12.25	20	8	40.	25.1722
10.4	20	4	20.	.590897

THE BINOMIAL TEST SHOWS THAT 10.4 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 12.839

---------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.299595	12.8431	11.1723	14.304

---------RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	3.73209	3.20942	2.20054E-2

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

SLOPE = 3.86461
95 PERCENT CONFIDENCE LIMITS =-3.60128 AND 11.3305

LC50 = 13.8122
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

****

SRU 1.336 UNTS.

RUN COMPLETE.

```
TERMINAL:   37, P 33/TTY
USERS, TYPE*WRITEUP(NOTE)* 80/09/25.
RECOVER /SYSTEM: basic,old,s791c50
READY.
9000 data 7
9001 data 10,6.5,4.2,2.7,1.8,1.17,0.76
9001 data 10,6.5,4.2,2.7,1.8,1.17,0.76
9002 data 20,20,20,20,20,20,20
9003 data 20,20,20,20,16,7,3
run

 80/09/25. 12.33.02.
BASIC     PROGRAM   S79LC50
```

5,5-Dimethylhydantoin/Grass Shrimp Acc. #743015

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
10	20	20	100	9.53674E-5
6.5	20	20	100	9.53674E-5
4.2	20	20	100	9.53674E-5
2.7	20	20	100	9.53674E-5
1.8	20	16	80.	.590897
1.17	20	7	35.	13.1588
.76	20	3	15.	.128841

THE BINOMIAL TEST SHOWS THAT .76 AND 1.8 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.34439

---------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMI	
3	8.50121E-2	1.27142	1.0807	1.45953

---------RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.119289	1	.896154

SLOPE = 5.8511
95 PERCENT CONFIDENCE LIMITS = 3.83023 AND 7.87197

LC50 = 1.25593
95 PERCENT CONFIDENCE LIMITS = 1.07766 AND 1.44501

****

SRU 1.317 UNTS.

RUN COMPLETE.