3-22-89

238457
RECORD NO.

014503
SHAUGHNESSEY NO.

REVIEW NO.

EEB REVIEW

DATE: IN 1-25-89 OUT 3-22-89

FILE OR REG. NO 31910-1

PETITION OR EXP. NO.

DATE OF SUBMISSION 1-13-89

DATE RECEIVED BY EFED 1-25-89

RD REQUESTED COMPLETION DATE 3-23-89

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RD ACTION CODE/TYPE OF REVIEW 660

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

DATA ACCESSION NO(S). 409667-01,409668-01, 409616-01

PRODUCT MANAGER NO. 1. Rossi (21)

PRODUCT NAME(S) Nabam

COMPANY NAME Nabam Task Force

SUBMISSION PURPOSE Submission of data in response to RS

| SHAUGHNESSEY NO. | CHEMICAL, & FORMULATION | % A.I. |
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Nabam Task Force Data Submission Dated 1/13/89

TO: Lois Rossi, PM 21
Fungicide-Herbicide Branch
Registration Division (H7505C)

FROM: Jim Ackerman, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

EEB has evaluated three estuarine organisms acute toxicity studies submitted to the Agency in response to the Nabam Registration Standard. The review results are indicated below and the data evaluation records are attached.

| Guideline Ref.No. | Test Type | Test Results | Toxicity Category | Study Status |
|---|---|---|---|---|
| 72-3 | mysid shrimp 96-hour LC50 | 0.17 ppm | Very Highly Toxic | Core |
| 72-3 | sheepshead minnow 96-hour LC50 | > 1100 ppm | Practically non-toxic | Core |
| 72-3 | oyster shell deposition 96-hour EC50 | 0.96 ppm | Very Highly Toxic | Core |

John Noles 3/22/89
John Noles, Biologist
Ecological Effects Branch

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Accession No. 409667-01

**DATA EVALUATION RECORD**

1. **CHEMICAL:** Nabam
Shaughnessey No. 014503.

2. **TEST MATERIAL:** 1) Nonlabelled material: Aquatreat DN 30 (Nabam: Disodium ethylene bisdithiocarbamate), a yellow-green liquid, 30.2% active ingredient.

2) Radiolabelled material: Nabam 14, Lot #10.5118, a white powder, 10.5 mCi/mmole (12.6 mCi/mmole as Nabam hexahydrate; a sodium salt, 20% NaCl).

3. **STUDY TYPE:** Estuarine Invertebrate Acute Static Test. Species Tested: Mysidopsis bahia.

4. **CITATION:** Surprenant, D.C. 1989. Acute Toxicity of Nabam/Aquatreat to Mysid Shrimp (Mysidopsis bahia) Under Flow-Through Conditions. SLS Report # 89-1-2901. Conducted by Springborn Life Sciences, Inc., Wareham, MA. Submitted by Alco Chemical Corporation, Chattanooga, TN. EPA Accession No. 409667-01.

5. **REVIEWED BY:**
Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

**Signature:** P. Kosalwat

**Date:** 3-15-89

6. **APPROVED BY:**
James R. Newman, Ph.D.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

**Signature:** James R Newman

**Date:** 3/16/89

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

**Signature:** H. T. Craven

**Date:** 3/22/89

John Noles 3/22/89

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an estuarine invertebrate acute flow-through test. With a 96-hour LC50 value of 0.17 mg a.i./L mean measured concentration, Nabam is considered highly toxic to Mysidopsis bahia. The NOEC was determined to be less than 0.085 mg a.i./L mean measured concentration. A more precise NOEC could not be determined due to mortalities of the test animals at all test levels.



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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The mysid shrimp (Mysidopsis bahia) used in the test were obtained from laboratory cultures maintained at Springborn Life Sciences, Inc. The culture water was prepared by filtering natural seawater collected from the Cape Cod Canal, Bourne, MA. The seawater was passed through a series of polypropylene core filters (20- and 5-micron) and then recirculated within an epoxy-lined concrete reservoir prior to use.

The mysid culture area received a regulated photoperiod of 16 hours of light and 8 hours of darkness. Light at the culture solution surface was at an intensity of 70-140 footcandles. Mysids were fed live brine shrimp nauplii twice daily and Hatchfry Encapsulon$^{R}$ three times weekly. Commercial aquarium heaters submerged in a water bath were used to maintain the culture solution temperatures at 25 $\pm$ 1°C.

B. **Test System:** The test system consisted of a modified continuous-flow serial diluter, a temperature-controlled water bath, and a set of 14 aquaria. The system was designed to provide 6 concentrations of the test material and a dilution water control. All treatment concentrations and the control were maintained in duplicate. Each glass test aquarium measured 39 x 20 x 25 cm with a self-starting siphon attached to the drain. The flow rate of exposure solutions to each aquarium was approximately 50 ml/minute which was equivalent to 6.5 volume additions per 24 hours.

Each aquarium contained two mysid retention chambers which housed the organisms during the exposure period. The retention chambers were constructed from glass Petri dishes, 10 cm in diameter to which 15 cm high Nitex$^{R}$ screen collars were attached. Test aquaria were impartially positioned in a water bath. The test solutions were not aerated and were maintained at 25 $\pm$ 1°C. Light intensity at the test area ranged from 50-

110 footcandles. The photoperiod was the same as provided in the culture area.

The dilution water used was from the same source as the culture water. A diluter stock solution of 359 mg a.i./mL were prepared by dissolving 16.3 mg of radiolabelled Nabam in 11.82 g of Aquatreat. A Sage Syringe Pump was calibrated to deliver 0.000694 ml/minute of the stock solution of Nabam to 250 ml/minute of dilution water to produce the highest diluter test concentration which was serially diluted (60% dilution factor) to produce the nominal test concentration range. The concentration of test material was measured as $^{14}$C-Nabam, which was converted to mg/L (combined test material).

C. Dosage: 96-hour acute flow-through LC50 test.

D. Design: Based on a preliminary test, the nominal concentrations selected for the definitive test were 0.078, 0.13, 0.22, 0.36, 0.60, and 1.0 mg a.i./L. Twenty mysids, $\leq$ 24 hours old, were impartially selected and distributed to each concentration and control (ten mysids per replicate). At any given time during the test, the maximum organism loading concentration was <3 mg of biomass per liter of solution. Mysids were fed live brine shrimp nauplii ad libitum twice daily.

Mortalities, biological observations, and observations of the physical characteristics of the test solutions were recorded at test initiation and at every subsequent 24-hour interval during the exposure period. Dead mysids were removed and discarded at each observation interval.

Dissolved oxygen concentration, pH, salinity, and temperature were measured once daily in each replicate of the treatment and control solutions. In addition, the test solution temperature was continuously monitored in one replicate of the highest treatment level throughout the study. Both replicates of the control and high, middle, and low test concentrations were sampled and analyzed for $^{14}$C-Nabam concentration prior to the initiation of the test. Also, single water samples were removed from both replicate test solutions of each treatment concentration and the control on days 0 and 4 for the analyses of $^{14}$C-Nabam.

E. **Statistics:** The mean measured concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate the median lethal concentrations (LC50) and 95% confidence intervals for each 24-hour interval of the exposure period by a computer program (Stephan, 1977, 1982).

12. **REPORTED RESULTS:** The diluter system which prepared and delivered the test solutions to the exposure aquaria functioned properly during the study. Throughout the exposure period, solutions were clear, colorless, and contained no visible signs of insoluble test material. Measured concentrations between replicate solutions and sampling intervals were generally consistent and produced the expected concentration gradient of the test material. The mean measured concentrations of the test solutions were between 82 and 109% of the nominal concentrations. During the 96-hour exposure period, dissolved oxygen concentrations of the test solutions ranged from 7.0 to 7.6 mg/L and pH's from 7.9 to 8.1. The temperature and salinity stayed at 24-25°C and 32 ppt, respectively.

The mean measured concentrations tested and corresponding mortalities during the toxicity test are presented in Table 3 (attached). Table 4 (attached) summarizes the LC50 values, confidence intervals and states the no-observed-effect concentration (NOEC) through 96 hours.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 96-hour LC50 value and 95% confidence interval of Nabam for mysid shrimp were calculated by moving average angle analysis to be 0.16 (0.14-0.18) mg a.i./L mean measured concentrations. Based on criteria established by the U.S. EPA (1985), Nabam is considered highly toxic to Mysidopsis bahia. The NOEC was estimated as <0.085 mg a.i./L.

The data and final report were inspected by the Quality Assurance Unit of Springborn Bionomics, Inc. to assure compliance with the protocols, standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations.

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

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

o The test was conducted at 25°C, instead of 22°C as recommended by the SEP.



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o The dilution water used in the test had a salinity of 32 ppt, instead of 10-17 ppt recommended for euryhaline species.

o The flow rate of the diluter system provided only 3.7 volume additions per 24 hours (reported as 6.5 volume additions by the author). According to the SEP, flow rates should be 5 to 10 volume additions per 24 hours.

o There was no 15- to 30-minute transition period between light and dark photoperiod.

In addition to the above deviations, the author reported the dissolved oxygen concentration range of the test solutions during the test as 101-109% of saturation. According to the reviewer's calculation, DO range of 7.0-7.6 mg/L represents only 75-82% of saturation (using 9.3 mg/L = 100% of saturation at 24°C and 32 ppt salinity).

B. **Statistical Analysis:** The reviewer recalculated the 96-hour LC50 value using EPA's Toxanal computer program and obtained a slightly different result (attached). The difference was due to Abbott's correction by EPA's computer program for a data set with control mortalities. The recalculated LC50 value was 0.17 mg a.i./L with a 95% confidence interval of 0.15-0.19 mg a.i./L.

C. **Discussion/Results:** A 96-hour LC50 value of 0.17 mg a.i./L mean measured concentration, classifies Nabam as highly toxic to Mysidopsis bahia. The NOEC was determined to be less than 0.085 mg a.i./L mean measured concentration. A more precise NOEC value could not be determined due to mortalities of the test animals at all test levels.

D. **Adequacy of the Study:**

**(1) Classification:** Core.

**(2) Rationale:** Although the test procedures deviated from the guidelines, the reviewer does not believe that they significantly affected the toxicity results of this study.

**(3) Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes, March 10, 1989.



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NABAM

Page_____ is not included in this copy.

Pages 8 through 9 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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

____ The document is not responsive to the request.

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

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE
OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY,
THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

KOSALWAT NABAM/AQUATREAT MYSIDOPSIS BAHIA 03-10-89

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| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| .82 | 19 | 19 | 100 | 1.907348E-04 |
| .5 | 19 | 18 | 94.7368 | 3.814697E-03 |
| .3 | 19 | 19 | 100 | 1.907348E-04 |
| .23 | 19 | 10 | 52.6316 | 50 |
| .13 | 19 | 7 | 36.8421 | 17.96417 |
| .085 | 19 | 2 | 10.5263 | 3.643036E-02 |

THE BINOMIAL TEST SHOWS THAT .085 AND .3 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .2093078

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 3 | 7.669845E-02 | .1670876 | .1451347 | .1912578 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 3 | .1031295 | 1 | .125305 |

SLOPE = 4.097886
95 PERCENT CONFIDENCE LIMITS = 2.781899 AND 5.413872

LC50 = .1693369
95 PERCENT CONFIDENCE LIMITS = .1388672 AND .201711

LC10 = 8.295368E-02
95 PERCENT CONFIDENCE LIMITS = 5.351072E-02 AND .1066064

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Accession No. 409668-01

**DATA EVALUATION RECORD**

1. **CHEMICAL:** Nabam
Shaughnessey No. 014503.

2. **TEST MATERIAL:** 1) Nonlabelled material: Aquatreat DN 30 (Nabam: Disodium ethylene bisdithiocarbamate), a yellow-green liquid, 30.83% active ingredient.

2) Radiolabelled material: Nabam 14, Lot #10.5118, a white powder, 10.5 mCi/mmole (12.6 mCi/mmole as Nabam hexahydrate; a sodium salt, 20% NaCl).

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

4. **CITATION:** Surprenant, D.C. 1989. Acute Toxicity of Nabam/Aquatreat to Eastern Oysters (Crassostrea virginica) Under Flow-Through Conditions. SLS Report # 88-12-2892. Conducted by Springborn Life Sciences, Inc., Wareham, MA. Submitted by Alco Chemical Corporation, Chattanooga, TN. EPA Accession No. 409668-01.

5. **REVIEWED BY:**
Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

**Signature:** P. Kosalwat

**Date:** 3-15-89

6. **APPROVED BY:**
James R. Newman, Ph.D.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

**Signature:** James R Newman

**Date:** 3/15/89

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

**Signature:** Henry T. Craven

**Date:** 3/22/89

John Noles
3/22/89

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a mollusc flow-through shell deposition test. With a 96-hour EC50 of 0.96 mg a.i./L mean measured concentration, Nabam is highly toxic to eastern oyster (Crassostrea virginica). The NOEC was determined to be 0.12 mg a.i./L.



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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Prespawned eastern oysters (Crassostrea virginica) were obtained from Aquacultural Research Corporation (ARC), Dennis, MA. At ARC, the oysters were held under conditions similar to the test conditions and in seawater from approximately the same source as that used in the test, during the fourteenth through two days prior to testing. These oysters were continuously fed a combination of marine algae. No mortality occurred during this 12-day holding period. Upon receipt at Springborn Life Sciences, Inc. (i.e., 48 hours prior to test initiation), they were carefully examined. If any oyster appeared unsuitable for testing due to the presence of boring sponges and/or mudworms, it was discarded.

The oysters were held prior to test initiation in a wooden epoxy-coated tray through which seawater was continuously pumped. They were of similar age and had a mean valve height of 37 ± 4 mm and a range of 32-50 mm. During the last 48 hours of acclimation and throughout the testing period, oysters were fed a supplementary diet of Isochrysis galbana, clone T-ISO, and Tetraselmis maculata. The water temperature was maintained at 20°C. The salinity was 32 parts per thousand (ppt); pH, 7.8-7.9; and dissolved oxygen concentration, 84-90.7% of saturation.

Twenty-four hours prior to testing, 3-5 millimeters of the new peripheral shell growth of each oyster were removed by grinding the shell to a blunt edge using a fine-grit grinding wheel. The test organisms were then held overnight, and carefully examined for any signs of stress which might have been caused by the removal of the shell. Immediately prior to the test initiation, the outer shell edge was buffed with an emery board to remove any new shell deposition.

B. **Test System:** A continuous flow serial diluter with a dilution factor of 0.60, a modification of the diluter described by Benoit et al. (1982), was used to deliver five test concentrations and a dilution water control to duplicate test aquaria. Each glass aquarium measured

20.5 x 39.5 x 26 cm and was equipped with a 13.5-cm side drain which maintained a test solution volume of approximately 11 liters. The flow of test solution to each aquarium was 50 ml/minute, which provided approximately 6.5 volume replacements every 24 hours. In addition, the contents of each aquarium were continuously circulated. The test solution was pumped from one end of the aquarium and returned through the other end using a Nylon impeller pump. Return water flowed through a perforated teflon tube, located along the entire length of the aquarium. The flow rate of the recirculating test solution was 1.75 L/minute or about 7 L/oyster/hour. Test solutions were not aerated.

The test aquaria rested in a temperature-controlled water bath. The water bath was designed to maintain a temperature at 20 $\pm$ 2°C during the test. A 16-hour light and 8-hour dark photoperiod at the test area was provided by fluorescent lights. Natural unfiltered seawater, collected from the Cape Cod Canal, Bourne, MA, was used as dilution and control water. The seawater used during the study had a salinity of 31-35 ppt and a pH of 7.5-7.9. A diluter stock solution containing 353 mg a.i./ml was prepared by dissolving 16.34 mg $^{14}$C-Nabam in a 9.94 ml of Aquatreat. The concentration of test material was measured in $^{14}$C-Nabam which was converted to mg/L.

C. **Dosage:** Ninety-six-hour EC50 shell deposition test.

D. **Design:** Based on a preliminary, range-finding test, five nominal concentrations (i.e., 0.13, 0.21, 0.36, 0.59, and 0.99 mg a. i./L) were selected for the definitive test. The test was initiated by impartially selecting and placing 15 oysters in each test aquarium (30 per treatment). The oysters were evenly spaced with their valve openings facing toward the flow from the teflon circulator tube. During the exposure, the oysters received supplemental feeding of algae (*Isochrysis galbana* and *Tetraselmis maculata*). One hundred and thirty-five milliliters of a concentrated algal suspension of $10^7$ cells/ml were added to each test aquarium three times daily. This feeding regime resulted in an algal density in excess of $10^5$ cells/ml in each aquarium.

Biological observations were made daily during the exposure to detect any mortality of oysters and to record any visible abnormalities, such as excessive mucus production or a failure siphon and feed, as

evidenced by a lack of feces and pseudofeces production. After 96 hours of exposure, the oysters were removed and the new shell growth was measured microscopically to 0.1 mm using a calibrated micrometer.

During the exposure, the pH, temperature, salinity, and dissolved oxygen concentration were measured daily in each replicate aquarium. In addition, the temperature was monitored continuously in one test aquarium. Water samples from each replicate aquarium including the controls were analyzed for $^{14}$C-Nabam concentrations on days 0 and 4.

E. **Statistics:** The mean measured concentrations and the biological results were used to statistically estimate a median effect concentration (EC50) and the 95% confidence interval. The EC50 was the estimated concentration of test material in seawater which reduced shell deposition (growth) of exposed oysters by 50% of the growth measured in control oysters. Since during this study, insufficient effects (i.e., $\geq$ 50% reduction in shell deposition) were observed, the EC50 value was empirically estimated as being greater than the highest test concentration.

The no-observed-effect concentration (NOEC) was determined by subjecting the shell growth data to Bartlett's test for homogeneity of variance, analysis of variance and William's test. The highest test concentration causing no significant reduction of shell growth was identified as the NOEC.

12. **REPORTED RESULTS:** The water quality of the exposure solutions was unaffected by test concentrations of Nabam and was satisfactory for the survival and growth of the test organisms. Daily measurements of each replicate solution established that the pH ranged from 7.5 to 7.9 and the dissolved oxygen concentration ranged from 7.3 to 8.1 mg/L. The salinity, measured daily in each solution, ranged from 31 to 35 ppt. Continuous temperature monitoring in one test aquarium demonstrated that the test solution temperature ranged from 20-21°C during the exposure period. The diluter which prepared and delivered the test solutions to the exposure aquaria functioned properly during the 24-hour period prior to test initiation and throughout the 96-hour study period. Mean measured concentrations of Nabam ranged from 78 to 119% of the nominal concentrations.

Throughout the 96-hour exposure, oysters in all exposure solutions and the controls appeared to be feeding and

siphoning normally and no mortality occurred. At test termination, the mean shell growth of oysters exposed to concentrations ≥ 0.25 mg a.i./L Nabam ranged from 1.5 to 2.1 mm which was significantly ($p = 0.05$) reduced as compared to the growth (2.5 mm) of the control organisms (Table 3 and Figure 2, attached). The 96-hour EC50 value was estimated to be greater than the highest test concentration (i.e., 0.98 mg a.i./L Nabam) and the NOEC was 0.12 mg a.i./L.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The data generated during this study (preliminary and definitive testing) established that the 96-hour EC50 for Nabam and Eastern oysters was >0.98 mg a.i./L and <3.9 mg a.i./L. The NOEC was determined to be 0.12 mg a.i./L. Based on EPA (1985) criteria, Nabam would be classified as moderately toxic to Eastern oysters (Crassostrea virginica).

The data and final report were inspected by the Quality Assurance Unit of Springborn Bionomics, Inc. to assure compliance with the protocols, standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations.

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

A. **Test Procedure:** The test procedures generally followed the SEP guidelines, except for the following deviations:

o There was no 15- to 30-minute transition period between light and dark photoperiod.

o In this study, the flow rate of recirculating test solution was about 7 L/oyster/hour. According to the protocols recommended by the SEP (APHA, 1981 and Anonymous, 1976), each oyster should receive a minimum of 5 L of once-through, flow-through test solution per hour. However, the test is considered acceptable because a supplemental diet was added.

The author stated on page 22 of the report that "analyses of the exposure solutions during the in-life portion of the study established consistent measured concentrations." In Table 2 (attached), the measured concentrations at 96 hours were substantially lower than those measured at 0 hour (ranging from 42 to 80% of the 0-hour values).

B. **Statistical Analysis:** A 96-hour EC50 value was estimated using the probit analysis. The printout is attached. The NOEC value could not be verified due to lack of raw data on shell growth. However, the lowest

concentration tested (0.12 mg a.i./L) showed no inhibition in shell growth when compared to the control. Therefore, 0.12 mg a.i./L will be used as an NOEC value in this study.

C. **Discussion/Results:** It is incomprehensible why 0.99 mg a.i./L of Nabam was chosen as the highest test concentration. The preliminary test showed that 96%, 96%, 83%, 42%, and 29% reduction in oyster shell growth occurred at test concentrations of 98, 20, 3.9, 0.78, and 0.16 mg a.i./L, respectively. A concentration close to 3.9 mg a.i./L should have been used as the highest concentration in the definitive test.

The author established that by using a dilution ratio of 60%, a concentration range (5 levels) which included both an EC50 value and an NOEC value could not be obtained. In the reviewer's opinion, more test levels (if feasible) should have been included. Furthermore, the main purpose of the acute test was to estimate an EC50 value. Therefore, when the determination of both values was not possible in one test, the emphasis should have been placed on determining an EC50, not an NOEC.

Since the highest response in this study (i.e., 40% inhibition) was very close to the median response, an extrapolative EC50 was obtained using probit analysis. With an estimated 96-hour EC50 value of 0.96 mg a.i/L mean measured concentration, Nabam is considered highly toxic to eastern oyster (Crassostrea virginica). The NOEC value obtained from this study was 0.12 mg a.i./L.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** This test is in the border-line since the highest response was less than but close to 50%. However, the test is considered acceptable because the extrapolation of EC50 value resulted in a conservative value (i.e., less than the highest concentration tested).

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes, March 14, 1989.

NABAM

Page_____ is not included in this copy.

Pages 17 through 19 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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

____ The document is not responsive to the request.

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

KOSALWAT NABAM CRASSOSTREA VIRGINICA 03-14-89

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| CONC. | NUMBER EXPOSED | NUMBER ~~DEAD~~ | PERCENT ~~DEAD~~ INHIBITION | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| .98 | 100 | 40 | 40 | 0 |
| .49 | 100 | 40 | 40 | 0 |
| .28 | 100 | 36 | 36 | 0 |
| .25 | 100 | 16 | 16 | 0 |
| .12 | 100 | 0 | 0 | 0 |

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

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 0

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 5 | 2.044739 | 10.24429 | 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 = 1.532334
95 PERCENT CONFIDENCE LIMITS =-.6588173 AND 3.723486

EC50 = .9558574
95 PERCENT CONFIDENCE LIMITS = .3615057 AND +INFINITY

LC10 = .1417726
95 PERCENT CONFIDENCE LIMITS = 0 AND .373506

**********

Accession No. 409616-01

**DATA EVALUATION RECORD**

1. **CHEMICAL:** Nabam
Shaughnessey No. 014503

2. **TEST MATERIAL:** 1) Aquatreat DN-30, 30.83% active ingredient as Nabam (disodium ethylene bisdithiocarbamate), Lot #11788-KBB, a yellow-green colored liquid.

2) Nabam Synthesis C-14, 36 uCi/mmol as Nabam hexahydrate, lot #3688, a solid white powder.

3. **STUDY TYPE:** Estuarine Fish Acute Flow-Through Test.
Species test: Cyprinodon variegatus.

4. **CITATION:** Surprenant, D.C. 1988. Acute Toxicity of Nabam/Aquatreat to Sheepshead Minnow (Cyprinodon variegatus) under Flow-Through Conditions. Conducted by Springborn Life Sciences, Inc., Wareham, Massachusetts. S.L.S. Report #88-12-2890. Submitted by Alco Chemical Corporation, Chattanooga, TN. Accession #409616-01.

5. **REVIEWED BY:**

Joseph Aufmuth
Ecologist
KBN Engineering and
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6. **APPROVED BY:**

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7. **Conclusions:** This study is scientifically sound and fulfills the guideline requirements for an estuarine fish acute test. With a 96-hour LC50 value of greater than 1100 mg A.I./L, mean measured concentration, Nabam/Aquatreat is considered practically non-toxic to sheepshead minnow (Cyprinodon variegatus). The NOEL was determined to be 1100 mg A.I./L, the highest concentration tested.



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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The Sheepshead minnow (Cyprinodon variegatus), obtained from a commercial supplier in New York, were held in a 500-L fiberglass tank under a photoperiod of 16 hours light and 8 hours darkness. A closed loop recirculating filtration system provided natural seawater with a salinity range of 31-36 parts per thousand, a pH range of 7.6-7.7, and a dissolved oxygen concentration range of 89-97% of saturation. Test fish were maintained under these conditions for a minimum of 14 days prior to test initiation. The temperature range in the holding tank was 21-23°C during this period.

The fish were fed a dry commercially pelleted food, ad libitum, daily except during the 48 hours prior to testing. There was no mortality of the test population during this two day period. The mean weight of the test fish population was 0.38 g (range 0.20-0.83 g) and the mean total length was 25 mm (range 21-23 mm).

B. **Test System:** The test was conducted using an exposure system consisting of a continuous flow serial diluter, a temperature controlled water bath and a set of 14 aquaria. Each glass aquarium measured 39 x 20 x 25 centimeters with a 14.5 cm high stand pipe that maintained a constant test water volume of 11 liters. Test aquaria were impartially positioned in a water bath containing recirculating water. The water temperature was maintained at 22 $\pm$ 1°C. A photoperiod of 16 hours light and 8 hours dark with an average light intensity of 33 (range 20-40) footcandles was maintained. The diluter was constructed to deliver 50 ml of solution per minute to each replicate test aquarium. This solution delivery rate provided approximately 6.5 volume replacements per aquarium every 24 hours.

The stock solution was prepared by dissolving 837.63 mg of radiolabeled Nabam (1191 mg as Nabam Hexahydrate) in 2997 ml of Aquatreat (1053 g as active ingredient, specific gravity of 1.14 g/ml, 30.83% A.I.) resulting in a stock solution of 351 mg A.I./ml. The stock solution

was diluted with seawater to provide a high treatment concentration of 950 mg A.I./L (nominal) which was diluted (65% dilution factor) to provide the desired concentration range. The concentration of the material was measured as $^{14}$C-Nabam which was converted to mg/L.

The test system provided six concentrations of test materials and a dilution water (seawater) control. All treatment concentrations and controls were maintained in duplicate. The exposure system was allowed to equilibrate for a minimum of 48 hours before initiation. The dilution water was prepared by filtering natural seawater through a series of polypropylene core filters (20- and 5-micron) and then recirculated within an epoxy-lined concrete reservoir. The filtered water was pumped through PVC pipe, an activated carbon filter and a polypropylene heat exchanger system. Test dilution water quality was monitored through the maintenance of continuous cultures of mysids (Mysidopsis bahia).

C. **Dosage:** Ninety-six-hour acute flow-through LC50 test.

D. **Design:** Based on a preliminary test, the nominal concentrations selected for the definitive test were 110, 170, 260, 400, 620, and 950 mg A.I./L. Ten sheepshead minnow were impartially selected and distributed to each replicate aquarium. The maximum organism loading concentration was 0.053 g of biomass per liter of flowing test solution per day.

All aquaria were examined after 0, 24, 48, 72, and 96 hours of exposure for mortalities, biological observations of the sheapshead minnow and the physical characteristics of the test solutions. Mortalities were recorded and removed every 24 hours. Dissolved oxygen concentration, temperature, salinity, and pH were measured once daily in each replicate of each treatment level and control throughout the exposure. Test solution temperature was also continuously monitored in one replicate of the control solution throughout the study. The control and the high, middle,and low test concentrations were analyzed for $^{14}$C-Nabam concentrations prior to the start of the definitive exposure. During the definitive study, single water samples were removed from both replicate test solutions of each treatment concentration and the dilution control on test days 0 and 4 for analysis of 0$^{14}$C-Nabam.

E. **Statistics:** The mean measured concentrations tested (day 0 and 4 analysis) and the corresponding mortality

data derived from the toxicity test were used to estimate the median lethal concentrations (LC50) and 95% confidence intervals at each 24 hour interval of the exposure period. Since insufficient mortality occurred at all treatment levels of Nabam tested, LC50 values were empirically estimated as being greater than the highest mean measured concentration tested.

12. **REPORTED RESULTS:** The water quality parameters measured during the test remained within acceptable ranges for the survival of sheepshead minnow. Table 1 (attached) shows the pH, dissolved oxygen concentration, and temperature measured during the 96-hour exposure period. The diluter functioned properly throughout the 96-hour exposure period.

Table 2 (attached) presents the results of the analysis of the test solutions for Nabam concentrations based on measurements of $^{14}$C-Nabam concentrations. Measured concentrations were consistent between replicate solutions. The mean measured concentrations were 92, 150, 260, 440, 930, and 1100 mg A.I./L Nabam; representing 84, 88, 100, 110, 150, and 116% of the nominal concentrations, respectfully.

Table 3 (attached) summarizes the concentrations tested, the corresponding cumulative mortalities and the observations made during the toxicity test. Under flow-through conditions no effects or mortalities were observed at any mean concentration of Nabam tested. The 96-hour LC50 value for sheeepshead minnow exposed to Nabam was empirically estimated to be >1100 mg A.I./L, the highest tested concentration.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Nabam was practically non toxic to sheepshead minnow (Cyprinodon variegatus). The No Observed Effect Concentration was 1100 mg A.I./L.

The data were audited to assure compliance with the protocols, standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations. The final report was signed by the Quality Assurance Unit of Springborn Life Sciences, Inc.

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

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

o The age of the fish was not reported. It was not known if all the fish were from the same year class. In addition, some of the test fish were smaller than the recommended size of 0.5-5.0 grams. The mean length of 25 mm was larger than the reported range of 21-23 millimeters.

o The fish were tested and acclimated with water containing 31-36 parts per thousand of salinity instead of 10-17 parts per thousand as recommended for euryhaline fish species.

o During the 14-day holding period, the fish were acclimated to a pH of 7.6-7.7 and not the test p.H. of 8.0-8.1.

o No calibration of the continuous diluter was mentioned.

o There was no 15- to 30- minute transition period between light and dark photoperiod.

o Dissolved oxygen concentrations fell as low as 46% of saturation during the first 48 hours. The SEP states that the D.O. level during the first 48 hours must be between 60% and 100% of saturation.

B. **Statistical Analysis:** Since no mortality occurred, the reviewer agrees with the empirically calculated LC50.

C. **Discussion/Results:** A 96-hour LC50 value of > 1100 mg A.I./L classifies Nabam/Aquatreat as practically non-toxic to Sheepshead minnow. The No Observed Effect Level (NOEL) was > 1100 mg A.I./L.

D. **Adequacy of the Study:**

**(1) Classification** : Core.

**2)Rational:** Although the test procedures deviated from the guidelines, the reviewer does not believe they significantly affected the toxicity results of this study.

**(3) Repairability:** N/A

15. **COMPLETION OF ONE-LINER:** Yes, February 22, 1989.

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