187848
RECORD NO.

014703
SHAUGHNESSY NO.

REVIEW NO.

EEB REVIEW

DATE: IN 11-6-87 OUT: 5-17-88

FILE OR REG. NO. 46506-1

PETITION OR EXP. NO.

DATE OF SUBMISSION 10-20-87

DATE RECEIVED BY HED 11-06-87

RD REQUESTED COMPLETION DATE 1-15-88

EEB ESTIMATED COMPLETION DATE 1-15-88

RD ACTION CODE/TYPE OF REVIEW 305

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

DATE ACCESSION NO (S). 403842-01 43842-01 thru-06

PRODUCT MANAGER NO. J. Kempter (32)

PRODUCT NAME (S) Sodium Hypochlorite

COMPANY NAME Bionox Corporation

SUBMISSION PURPOSE Data Submitted in response to Registration Standard

SHAUGHNESSEY NO.	CHEMICAL AND FORMULATION	% A.I.

100. Pesticide Name: Bionox

100.3 Submission Purpose

Submission of both fish and aquatic invertebrate studies in support of Bionox Registration Standard.

101 Chemical and Physical Properties

101.1 Chemical Name: Sodium Hypochlorite

101.2 Common Name: Bionox

103 Toxicological Properties

96-Hour LC_{50} for Bluegill Sunfish (Bionox A)
96-Hour LC_{50} for Rainbow Trout (Bionox A)
48-Hour LC_{50} for Daphnia magna (bionox A)
96-Hour LC_{50} for Bluegill Sunfish (Bionox A + B)
96-Hour LC_{50} for Rainbow Trout (Bionox A + B)
48-Hour LC_{50} for Daphnia magna (Bionox A + B)

103. Conclusions

A. Bluegill Sunfish (Bionox A -01)

This study does not fulfill the Guideline requirements for an acute static toxicity determination for a warm-water fish species due to the lack of critical data needed for the evaluation of this study and the use of an unacceptable dilution water. Bionox is considered slightly toxic to Bluegill Sunfish with an LC_{50} of 42.7 mg/L of test material.

B. Rainbow Trout (Bionox A -02)

This study does not fulfill the Guideline requirements for a coldwater static acute fish study due to numerous deviations from the Guidelines (lack of controls, inappropriate temperature range, etc.). Bionox is considered practically nontoxic under the conditions tested with an $LC_{50} > 100$ mg/L.

C. Daphnia Magna (Bionox A -03)

This study does not fulfill the Guideline requirements for an invertebrate species due to lack of critical data needed for the evaluation of this study. Bionox is considered slightly toxic to Daphnia magna with an LC_{50} of 49 ppm of test material.

2

D. Bluegill Sunfish (Bionox A + B -04)

This study does not fulfill the Guideline requirements for an acute static toxicity determination for a warmwater fish species due to lack of critical data needed for the evaluation of this study. The combination of Bionox A + B is considered slightly toxic to Bluegill Sunfish with a 96-hour LC_{50} of 91.4 ppm.

E. Rainbow Trout (Bionox A + B -05)

This study does not fulfill the Guideline requirements for a coldwater static acute fish study due to numerous deviations from the Guidelines (lack of controls, inappropriate temperature range, etc.). The combination of A + B Bionox in equal parts is considered practically nontoxic to Rainbow Trout with and LC_{50} > 100 mg/L.

F. Daphnia magna (Bionox A + B -06)

This study does not fulfill the Guideline requirements for an acute static toxicity determination for an invertebrate species due to lack of critical data needed for the evaluation of this study. The combination of A + B Bionox in equal parts is considered slightly toxic to Daphnia magna with an LC_{50} between 75.0 and 100 mg/L

All six of the above studies were considered as invalid/ supplemental for aquatic organisms due to lack of pertinent data such as:

- The percent of active ingredient of test material
- The weight, age, life stage of test organisms
- Source of the test organisms
- The culture and/ or holding history of test fish
- The survival of fish during the 48 hours prior to testing
- Acclimation and feeding regimes.
- The method of aasigning test organisms to test and control group
- The data provided do not mention a control, the Guidelines require that a control be included and that the survival in the control chamber (s) be reported.

Curtis E. Laird 5-17-88
Curtis E. Laird, Fishery Biologist
Ecological Effects Branch
Hazard Evaluation Division (TS-769C)

Norman J. Cook 5-18-88
Norman J. Cook, Head-Section 2
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James W. Akerman, Chief
Ecological Effects Branch
Hazard Evaluation Division (TS-769C)

DATA EVALUATION RECORD
Accession Number 403842-01

1. **CHEMICAL:** BIONOX A

2. **TEST MATERIAL:** BIONOX A, Lot Number 342861, strength 4251 parts per million (ppm). Percent Active Ingredient not provided.

3. **STUDY TYPE:** Static Acute Freshwater Fish.
Species Tested: Bluegill Sunfish, Lepomis macrochirus.

4. **CITATION:** Terrell, Y. and B. Till. 1987. The Acute Toxicity Bioassay of BIONOX A 342861 on Bluegill Sunfish. American Standards Biosciences Corp. Project Number 87-366. Prepared by American Standards Biosciences Corp., Reading, Pennsylvania. Submitted by Metrex Research Corporation, Parker, Colorado. Accession Number 403842-01.

5. **REVIEWED BY:**

Isabel C. Johnson, M.S.
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Signature:
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6. **APPROVED BY:**

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W Cook
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Date:

7. **CONCLUSIONS:** This study does not fulfill the Guidelines requirement for an acute static toxicity determination for a warmwater fish species due to the lack of critical data needed for the evaluation of this study and the use of an unacceptable dilution water. Bionox is considered slightly toxic to Bluegill Sunfish with a 96-hour LC_{50} of 42.7 mg/L of test material.

8. RECOMMENDATIONS: The registrant should conduct another fish study following 72-1 Guidelines which will include items listed under 14A below.

9. BACKGROUND: This study was submitted in response to Bionox Registration Standard.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Bluegill Sunfish, Lepomis macrochirus. No other information was provided on the test fish.

B. Test System: Glass aquaria of 20 liters capacity were used. The test solution was 10 liters (approximately 10.5-centimeter depth). Tap water from the City of Reading was used. Tap water was left standing for 24 hours to dechlorinate naturally. No other treatments were reported. Water characteristics included: pH of 7.8; alkalinity of 94 milligrams per liter (mg/L) as calcium carbonate ($CaCO_3$); and hardness of 132 mg/L as $CaCO_3$. Lighting was 14 hours light: 10 hours darkness. Water temperature was 20 ± 2 degrees Celsius (^oC).

C. Dosage: Static acute test.

D. Design: A range-finding and a definitive test were conducted. Both tests were conducted for 96 hours. The range-finding test concentrations were 0.1, 1.0, 10.0, and 100.0 mg/L of the test material. The range-finding test was not replicated, and 5 fish were tested per concentration. The definitive test concentrations were: 24.0, 32.0, 42.0, 56.0, 75.0, and 100 mg/L. This test was replicated and 20 fish were tested per concentration. The reported loading was 1 fish per liter of water, but the individual fish weight or age was not provided. The tests were examined at 6, 24, 48, 72, and 96 hours.

E. Statistics: The estimation of Median Tolerance Limits for each day and the 95 percent confidence limits was calculated using the J.T. Litchfield, Jr. and F.W. Wilcoxon Method.

12. REPORTED RESULTS: The range-finding results indicated that the 96-hour LC_{50} was between 10 an 100 mg/L. During the definitive test "no mortality was observed at 24.0 mg/L, while 25, 45, 75, and 100 percent mortalities were exhibited at 42.0,6.0, 75.0, and 100 mg/L, respectively."

Concentration (mg/L)	Survival				
	0 hour	24 hour	48 hour	72 hour	96 hour
100	20	9	0	0	0
75	20	0	0	0	0

56	20	11	8	7	5
42	20	13	11	11	11
32	20	18	17	15	15
24	20	20	20	20	20

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: "Estimation was made of the TL_{50} for 96 hours only. The 96-hour TL_{50} for Bluegill Sunfish, Lepomis macrochirus, was calculated to be 43.0 mg/L (upper limit 49.5 mg/L -- lower limit 37.4 mg/L)." A Quality Assurance statement was included in the report, "at the time of this inspection, all work had been and was being performed in manner consistent with the GLP's."

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

A. Test Procedure: The overall procedures appear to be in accordance with the Guidelines. The following items were not included in the report and are required in the Guidelines:

- o The composition and percent active ingredient of the test chemical
- o The age, sex, size, life stage, and/or weight of the test organisms
- o The source of the test organisms
- o The culture and/or holding history of test fish
- o The survival of fish during the 48 hours prior to testing
- o The acclimation and feeding regimes
- o The method of assigning test organisms to test and control groups
- o The data provided does not mention a control, the Guidelines require that a control be included and that the survival in the control chamber(s) be reported

The following items deviated from the Guidelines:

- o Test temperature was 20 $\pm$ 2 ^{o}C, Guidelines specify 17 or 22 $\pm$ 1 oC
- o Photoperiod was 14 hours light : 10 hours darkness, the Guidelines require 16 hours light : 8 hours darkness
- o Tap water was used, the Guidelines specify that "dechlorinated water should not be used because removal of chlorine is rarely complete and residual chlorine can be quite toxic to aquatic organisms"
- o Loading was reported as 1 fish per liter of water, and it should be reported in grams of fish tissue per liter of water, the weight of the fish was not provided

B. Statistical Analysis: The reviewer recalculated the 96-hour LC_{50} using the Binomial Test (96-hour LC_{50} of 44.0 mg/L), the Moving Average Method and the Probit Method (both provided

96-hour LC_{50}s of 42.7 mg/L, with upper and lower confidence limits of 47 and 38 mg/L). These results confirm the reported results of 43 mg/L.

C. Discussion/Results: The study results appear scientifically valid. But, the missing data critically affect the validation of this study. The most critical discrepancies are the lack of:

- o controls
- o background information on the test species
- o acclimation and feeding regimes
- o survival of test organisms in the 48 hours prior to testing
- o size of the test fish (age or weight), and
- o the percent active ingredient of the test material.

Other issues critical to the validity are the use of chlorinated tap water and the lack of documentation of the acceptability of this dilution water to the test species (ie. no controls).

D. Adequacy of the Study:

(1) Classification: Invalid.

(2) Rationale: This study is considered invalid because sufficient information is missing to appropriately evaluate the appropriateness of the protocols. The use of dechlorinated water, the lack of information on the test species, the lack of controls, and the unknown loading rate, all make this study unacceptable according to the Guidelines.

(3) Repairability: Yes. If all the information missing under 14 A is provided, and considered scientifically acceptable, this study can be upgraded to supplemental. This study can not be upgraded to core because chlorinated tap water was used as dilution water, and this is unacceptable according to the Guidelines.

15. COMPLETION OF ONE-LINER: Yes, January 10, 1988.

UCF

ISABEL C. JOHNSON BIONOX BLUEGILL SUNFISH 01-10-88

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
100	20	20	100	9.536742E-05
75	20	20	100	9.536742E-05
56	20	15	75	2.069473
42	20	9	45	41.19014
32	20	5	25	2.069473
24	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 32 AND 56 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 43.99281

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	S	LC50	95 PERCENT CONFIDENCE LIMITS	
8	5.135016E-02	42.732	38.2156	47.10104

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	S	=	GOODNESS OF FIT PROBABILITY
4	9.221782E-02	1	.5652403

SLOPE = 3.782037

95 PERCENT CONFIDENCE LIMITS = 5.430092 AND 10.16668

LC50 = 42.7251

95 PERCENT CONFIDENCE LIMITS = 38.66063 AND 47.17651

LC10 = 24.365

95 PERCENT CONFIDENCE LIMITS = 24.04772 AND 33.19569

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F

DATA EVALUATION RECORD
Accession Number 403842-02

1. **CHEMICAL:** BIONOX A

2. **TEST MATERIAL:** BIONOX A, Lot Number 342861, strength 4251 parts per million (ppm). Percent Active Ingredient not provided.

3. **STUDY TYPE:** Static Acute Freshwater Fish.
Species Tested: Rainbow Trout (Salmo gairdneri).

4. **CITATION:** Terrell, Y. and B. Till. 1987. Acute Toxicity Bioassay of BIONOX A (342861) on Rainbow Trout. American Standards Biosciences Corp. Project Number 87-368. Prepared by American Standards Biosciences Corp., Reading, Pennsylvania. Submitted by Metrex Research Corporation, Parker, Colorado. Accession Number 403842-02.

5. **REVIEWED BY:**

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6. **APPROVED BY:**

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Principal Scientist
KBN Engineering and
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Signature:
Date:

James W. Akerman
Chief, EEB/HED
USEPA

Signature:
Date:

7. **CONCLUSIONS:** This study does not fulfill the Guideline requirements for a cold water static acute fish study due to numerous deviations from the Guidelines (lack of controls, inapprociate temperature range, etc.). Bionox is considered practically nontoxic to rainbow trout with a 96-hour LC_{50} greater than 100 mg/L under the conditions tested.

8. RECOMMENDATIONS: The registrant should conduct another study following 72-1 Guidelines which will include items listed under 14A below.

9. BACKGROUND: This study was submitted in response to Bionox registration standard.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Rainbow trout (Salmo gairdneri) were used for testing. No other information was provided on the test fish.

B. Test System: Glass aquaria of 50 liters capacity were used. The test solution was 20 liters (approximately 10.5-centimeter depth). Tap water from the City of Reading was used. Tap water was left standing for 24 hours to dechlorinate naturally. No other treatments were reported. Water characteristics included: pH of 7.8; alkalinity of 94 milligrams per liter (mg/L) as calcium carbonate ($CaCO_3$); and hardness of 132 mg/L as $CaCO_3$. Lighting was 14 hours light: 10 hours darkness. Water temperature was 15 $\pm$ 2 degrees Celsius (^{O}C).

C. Dosage: Static acute test.

D. Design: A range-finding and a definitive test were conducted. Both tests were conducted for 96 hours. The range-finding test concentrations were 0.1, 1.0, 10.0, and 100.0 mg/L of the test material. The range-finding test was not replicated, and 5 fish were tested per concentration. The definitive test was conducted using only one test concentration, 100 mg/L. This test was conducted in triplicate and 30 fish were tested per concentration. The reported loading was 1 fish per 2 liters of water, but the individual fish weight or age was not provided. The tests were examined at 6, 24, 48, 72, and 96 hours.

E. Statistics: The estimation of Median Tolerance Limits for each day and the 95 percent confidence limits was calculated using the J.T. Litchfield, Jr. and F.W. Wilcoxon Method.

12. REPORTED RESULTS: The range-finding results indicated that the 96-hour LC_{50} was greater than 100 mg/L. During the definitive test no mortalities were observed after 96 hours of exposure.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 96-hour LC_{50} for rainbow trout, Salmo gairdneri, was calculated to be greater than 100 mg/L. A Quality Assurance statement was included in the report, "at the time of this inspection, all work had been and was being performed in manner consistent with the GLP's."

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

A. Test Procedure: The overall procedures appear to be in accordance with the Guidelines. The following items were not included in the report and are required in the Guidelines:

- The composition and percent active ingredient of the test chemical
- The age, sex, size, life stage, and/or weight of the test organisms
- The source of the test organisms
- The culture and/or holding history of test fish
- The survival of fish during the 48 hours prior to testing
- The acclimation and feeding regimes
- The method of assigning test organisms to test and control groups
- The data provided does not mention a control, the Guidelines require that a control be included and that the survival in the control chamber(s) be reported

The following items deviated from the Guidelines:

- Test temperature was 15 $\pm$ 2 ^{o}C, Guidelines specify 12 $\pm$ 1 oC
- Photoperiod was 14 hours light : 10 hours darkness, the Guidelines require 16 hours light : 8 hours darkness
- Tap water was used, the Guidelines specify that "dechlorinated water should not be used because removal of chlorine is rarely complete and residual chlorine can be quite toxic to aquatic organisms"
- Loading was reported as 1 fish per 2 liters of water, and it should be reported in grams of fish tissue per liter of water, the weight of the fish was not provided

B. Statistical Analysis: No statistical analysis are required when only one test concentration is used.

C. Discussion/Results: The study results appear scientifically valid. But, the missing data and protocol deviations critically affect the validation of this study. The most critical discrepancies are the lack of:

No controls

- o background information on the test species
- o acclimation and feeding regimes
- o survival of test organisms in the 48 hours prior to testing
- o size of the test fish (age or weight), and
- o the percent active ingredient of the test material.

Other issues critical to the validity are the use of chlorinated tap water, test temperature range of 15 to 17 ^{o}C (compared to the 11 to 13 ^{o}C range allowed), and the lack of documentation of the acceptability of the dilution water to the test species (ie. no controls).

D. Adequacy of the Study:

(1) Classification: Invalid.

(2) Rationale: This study is considered invalid because sufficient information is missing to appropriately evaluate the appropriateness of the protocols. The use of dechlorinated water, the lack of information on the test species, the lack of controls, the inappropriate temperature range for a cold water test, and the unknown loading rate, all make this study unacceptable according to the Guidelines.

(3) Repairability: No. The test was conducted at an unacceptable test temperature, therefore even if all the information missing was provided, it can not be upgraded to core.

15. COMPLETION OF ONE-LINER: Yes, January 10, 1988.

DATA EVALUATION RECORD
Accession Number 403842-03

1. **CHEMICAL:** BIONOX A

2. **TEST MATERIAL:** BIONOX A, Lot Number 342861, strength 4251 parts per million (ppm). Percent Active Ingredient not provided.

3. **STUDY TYPE:** Static Acute Freshwater Invertebrate. Species Tested: Cladoceran. Daphnia magna.

4. **CITATION:** Terrell, Y. and B. Till. 1987. Acute Toxicity Bioassay of BIONOX A (342861) on Daphnia magna. American Standards Biosciences Corp. Project Number 87-370. Prepared by American Standards Biosciences Corp., Reading, Pennsylvania. Submitted by Metrex Research Corporation, Parker, Colorado. Accession Number 403842-03.

5. **REVIEWED BY:**

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6. **APPROVED BY:**

James R. Newman, Ph.D.
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KBN Engineering and
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Date:

WCW
C.E.Z.

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Signature:
Date:

7. **CONCLUSIONS:** This study does not fulfill the Guideline requirements for an acute static toxicity determination for an invertebrate species due to the lack of critical data needed for the evaluation of this study. Bionox is considered slightly toxic to Daphnia magna with a 48-hour LC_{50} of 49 mg/L of test material.

8. **RECOMMENDATIONS:** The registrant should conduct another invertebrate study following the 72-2 Guidelines which will include items listed under 14A below, providing the data under 14A cannot be generated.

9. BACKGROUND: This study was submitted in response to Bionox Registration Standard.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Daphnia magna less than 24 hours old were used for the tests. No other information was provided on the test organisms.

B. Test System: Glass beakers of 500 milliliters capacity were used. The test solution was 400 milliliters (approximately 10.5-centimeter depth). Stream water was used. No chemical characterization or other information was provided on the dilution water. Lighting was 14 hours light: 10 hours darkness. Water temperature was 19 ± 2 degrees Celsius (^{o}C).

C. Dosage: Static acute test.

D. Design: A range-finding and a definitive test were conducted. Both tests were conducted for 48 hours. The range-finding test concentrations were 0.1, 1.0, 10.0, and 100.0 mg/L of the test material. The range-finding test was not replicated, and 5 cladocerans were tested per concentration. The definitive test concentrations were: 24.0, 32.0, 42.0, 56.0, 75.0, and 100 mg/L. This test was replicated and 20 cladocerans were tested per concentration. The reported loading was 10 cladocerans per 400 milliliters of water. The tests were examined at 6, 24, and 48 hours.

E. Statistics: The estimation of Median Tolerance Limits and the 95 percent confidence limits was calculated using the J.T. Litchfield, Jr. and F.W. Wilcoxon Method.

12. REPORTED RESULTS: The range-finding results indicated that the 96-hour LC_{50} was between 10 an 100 mg/L. The definitive data are shown below:

Concentration (mg/L)	Survival 0 hour	24 hour	48 hour
100	20	1	0
75	20	5	3
56	20	8	5
42	20	18	12
32	20	20	19
24	20	20	20

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: "Estimation was made of the TL_{50} for 48 hours only, and was found to be 49.0 mg/L (upper limit 55.4 mg/L -- lower limit 43.4 mg/L)." A Quality Assurance statement was included in the report, "at the time of this inspection, all work had been and was being performed in manner consistent with the GLP's."

14. REVIEWER'S DISCUSSION AND INTERPRETATION O F STUDY RESULTS:

A. Test Procedure: The overall procedures appear to be in accordance with the Guidelines. The following items were not included in the report and are required in the Guidelines:

- o The composition and percent active ingredient of the test chemical
- o The source of the test organisms
- o The culture and/or holding history of test organisms
- o The survival of adult cladocerans during the 48 hours prior to testing
- o The acclimation and feeding regimes
- o The method of assigning test organisms to test and control groups
- o The data provided does not mention a control, the Guidelines require that a control be included and that the survival in the control chamber(s) be reported
- o The chemical characterization of the dilution water or its acceptability of the dilution water for rearing the test organisms
- o The dissolved oxygen concentration must be reported at the beginning and the end of the test. Measurements should be taken from the control and the high, medium, and low test concentration.
- o Test temperature should be reported and should be measured hourly in at least one test vessel, unless water temperature is controlled by a constant water temperature bath, then recordings can be taken every 6 hours
- o The pH should be measured and reported at the beginning and end of the test in the control and the high, medium, and low toxicant concentrations.

The following item deviated from the Guidelines:

- o Photoperiod was 14 hours light : 10 hours darkness, the Guidelines require 16 hours light : 8 hours darkness.

B. Statistical Analysis: The reviewer recalculated the 96-hour LC_{50} using the Binomial Test (48-hour LC_{50} of 45.5 mg/L), the Moving Average Method calculated an LC_{50} of 49 mg/L (54 and 44 mg/L were the confidence limits), and the Probit Method provided a 48-hour LC_{50} of 48 mg/L, with upper and lower confidence limits of 53 and 44 mg/L. These results confirm the reported results of 49 mg/L.

C. Discussion/Results: The study results appear scientifically valid. But, the missing data critically affect the validation of this study. The most critical discrepancies are the lack of:

- o controls
- o background information on the test species
- o acclimation and feeding regimes
- o survival of the adult brood organisms in the 48 hours prior to testing
- o chemical characterization of test solutions
- o information of the quality of the dilution water, and
- o the percent active ingredient of the test material.

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: This study is considered supplemental because sufficient information is missing to appropriately evaluate the appropriateness of the protocol, including the lack of information on the test species, the lack of controls, and the unknown quality of the stream water used as dilution water, all make this study unacceptable according to the Guidelines.

(3) Repairability: Yes. If all the information missing under 14 A is provided, and considered scientifically acceptable, this study can be upgraded to core.

15. COMPLETION OF ONE-LINER: Yes, January 10, 1988.

Laird Bionox Daphnia magna 03-21-88

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
100	20	20	100	9.536742E-05
75	20	17	85	.1288414
56	20	15	75	2.069473
42	20	8	40	25.17223
32	20	1	5	2.002716E-03
24	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 32 AND 56 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 45.50333

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	5.135019E-02	48.79366	44.16767	53.90435

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	8.701264E-02	1	.5073517

SLOPE = 7.539153
95 PERCENT CONFIDENCE LIMITS = 5.315261 AND 9.763044

LC50 = 48.34194
95 PERCENT CONFIDENCE LIMITS = 43.71434 AND 53.50283

LC10 = 32.79972
95 PERCENT CONFIDENCE LIMITS = 26.94938 AND 37.1052

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DATA EVALUATION RECORD
Accession Number 403842-04

1. **CHEMICAL:** BIONOX A and Bionox B

2. **TEST MATERIAL:** BIONOX A, Lot Number 342861, strength 4251 parts per million (ppm) and Bionox B, Lot 152861; used in equal parts. Percent Active Ingredient not provided.

3. **STUDY TYPE:** Static Acute Freshwater Fish.
Species Tested: Bluegill Sunfish, Lepomis macrochirus.

4. **CITATION:** Terrell, Y. and B. Till. 1987. The Acute Toxicity Bioassay of BIONOX A 342861 and BIONOX B 152861 on Bluegill Sunfish. American Standards Biosciences Corp. Project Number 87-367. Prepared by American Standards Biosciences Corp., Reading, Pennsylvania. Submitted by Metrex Research Corporation, Parker, Colorado. Accession Number 403842-04.

5. **REVIEWED BY:**

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6. **APPROVED BY:**

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Signature:
Date:

James W. Akerman
Chief, EEB/HED
USEPA

Signature:
Date:

7. **CONCLUSIONS:** This study does not fulfill the requirements for an acute static toxicity determination for a warmwater fish species due to the lack of critical data needed for the evaluation of this study and the use of an unacceptable dilution

water. The combination of Bionox A and Bionox B is considered slightly toxic to Bluegill Sunfish with a 96-hour LC50 between 75.0 and 100 mg/L of test material.

8. RECOMMENDATIONS: The registrant should conduct another study following 72-1 Guidelines which will include items under 14A below.

9. BACKGROUND: This study was submitted in response to Bionox registration standard.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Bluegill Sunfish, Lepomis macrochirus. No other information was provided on the test fish.

B. Test System: Glass aquaria of 20 liters capacity were used. The test solution was 10 liters (approximately 10.5-centimeter depth). Tap water from the City of Reading was used. Tap water was left standing for 24 hours to dechlorinate naturally. No other treatments were reported. Water characteristics included: pH of 7.8; alkalinity of 94 milligrams per liter (mg/L) as calcium carbonate ($CaCO_3$); and hardness of 132 mg/L as $CaCO_3$. Lighting was 14 hours light: 10 hours darkness. Water temperature was 20 ± 2 degrees Celsius (^oC).

C. Dosage: Static acute test.

D. Design: A range-finding and a definitive test were conducted. Both were conducted for 96 hours. The range-finding test concentrations were 0.1, 1.0, 10.0, and 100.0 mg/L of the test material. The range-finding test was not replicated, and 5 fish were tested per concentration. The definitive test concentrations were: 32.0, 42.0, 56.0, 75.0, and 100 mg/L. This test was replicated and 20 fish were tested per concentration. The reported loading was 1 fish per liter of water, but the individual fish weight or age was not provided. The tests were examined at 6, 24, 48, 72, and 96 hours.

E. Statistics: The estimation of Median Tolerance Limits for each day and the 95 percent confidence limits was calculated using the J.T. Litchfield, Jr. and F.W. Wilcoxon Method.

12. REPORTED RESULTS: The range-finding results indicated that the 96-hour LC_{50} was between 10 an 100 mg/L. During the definitive test "no mortality was observed at 32.0, 42.0, 56.0, or 75.0 mg/L, while 80 percent mortality was exhibited at 100 mg/L.

Concentration (mg/L) hour	Survival 0 hour	24 hour	48 hour	72 hour	96 hour
100	4	4	4	4	4
75	20	20	20	20	20
56	20	20	20	20	20
42	20	20	20	20	20
32	20	20	20	20	20

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: "Estimation was made of the TL_{50} for 96 hours only. The 96-hour TL_{50} for Bluegill Sunfish, Lepomis macrochirus, was calculated to be greater than 75.0 mg/L but less than 100.0 mg/L. A Quality Assurance statement was included in the report, "at the time of this inspection, all work had been and was being performed in manner consistent with the GLP's."

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

A. Test Procedure: The overall procedures appear to be in accordance with the Guidelines. The following items were not included in the report and are required in the Guidelines:

- The composition and percent active ingredient of the test chemicals
- The age, sex, size, life stage, and/or weight of the test organisms
- The source of the test organisms
- The culture and/or holding history of test fish
- The survival of fish during the 48 hours prior to testing
- The acclimation and feeding regimes
- The method of assigning test organisms to test and control groups
- The data provided does not mention a control, the Guidelines require that a control be included and that the survival in the control chamber(s) be reported

The following items deviated from the Guidelines:

- Photoperiod was 14 hours light : 10 hours darkness, the Guidelines require 16 hours light : 8 hours darkness
- Tap water was used, the Guidelines specify that "dechlorinated water should not be used because removal of chlorine is rarely complete and residual

chlorine can be quite toxic to aquatic organisms"

- Loading was reported as 1 fish per liter of water, and it should be reported in grams of fish tissue per liter of water, the weight of the fish was not provided

B. Statistical Analysis: Due to the mortality pattern an LC_{50} value can not be calculated. The test concentrations did not allow for partial mortalities.

C. Discussion/Results: The study results appear scientifically valid. But, the missing data critically affect the validation of this study. The most critical discrepancies are the lack of:

- controls
- background information on the test species
- acclimation and feeding regimes
- survival of test organisms in the 48 hours prior to testing
- size of the test fish (age or weight), and
- the percent active ingredient of the test materials.

Other issues critical to the validity are the use of chlorinated tap water and the lack of documentation of the acceptability of this dilution water to the test species (ie. no controls).

D. Adequacy of the Study:

(1) Classification: Invalid.

(2) Rationale: This study is considered invalid because sufficient information is missing to appropriately evaluate the appropriateness of the protocols. The use of dechlorinated water, the lack of information on the test species, the lack of controls, and the unknown loading rate, all make this study unacceptable according to the Guidelines.

(3) Repairability: Yes. If all the information missing under 14 A is provided, and considered scientifically acceptable, this study can be upgraded to supplemental. This study can not be upgraded to core because chlorinated tap water was used as dilution water, and this is unacceptable according to the Guidelines.

15. COMPLETION OF ONE-LINER: Yes, January 10, 1988.

Laird Bionox Bluegill sunfish 03-18-88

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
100	20	16	80	.5908966
75	20	0	0	9.536742E-05
56	20	0	0	9.536742E-05
42	20	0	0	9.536742E-05
32	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 75 AND 100 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 91.44447

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.

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DATA EVALUATION RECORD
Accession Number 403842-05

1. **CHEMICAL:** BIONOX A and Bionox B

2. **TEST MATERIAL:** BIONOX A, Lot Number 342861, strength 4251 parts per million (ppm) and Bionox B, Lot Number 152861; used in equal parts. Percent Active Ingredient not provided.

3. **STUDY TYPE:** Static Acute Freshwater Fish.
Species Tested: Rainbow Trout (Salmo gairdneri).

4. **CITATION:** Terrell, Y. and B. Till. 1987. Acute Toxicity Bioassay of BIONOX A (342861) and BIONOX B on Rainbow Trout. American Standards Biosciences Corp. Project Number 87-369. Prepared by American Standards Biosciences Corp., Reading, Pennsylvania. Submitted by Metrex Research Corporation, Parker, Colorado. Accession Number 403842-05.

5. **REVIEWED BY:**

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature:
Date:

6. **APPROVED BY:**

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

Signature:
Date:

MCW
C.E.Z.

James W. Akerman
Chief, EEB/HED
USEPA

Signature:
Date:

7. **CONCLUSIONS:** This study does not fulfill the Guideline requiremnets for a coldwater static acute fish study due to the numerous deviations from the Guidelines (lack of controls, inappropriate temperature range, etc.). This compound is considered practically nontoxic under the conditions tested with the reported 96-hour LC_{50} greater than 100 mg/L.

8. RECOMMENDATIONS: The registrant should conduct another study following 72-1 Guidelines which will include items listed under 14A below.

9. BACKGROUND: This study was submitted in response to Bionox registration standard.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Rainbow trout (Salmo gairdneri) were used for testing. No other information was provided on the test fish.

B. Test System: Glass aquaria of 50 liters capacity were used. The test solution was 20 liters (approximately 10.5-centimeter depth). Tap water from the City of Reading was used. Tap water was left standing for 24 hours to dechlorinate naturally. No other treatments were reported. Water characteristics included: pH of 7.8; alkalinity of 94 milligrams per liter (mg/L) as calcium carbonate ($CaCO_3$); and hardness of 132 mg/L as $CaCO_3$. Lighting was 14 hours light: 10 hours darkness. Water temperature was 15 $\pm$ 2 degrees Celsius (^{o}C).

C. Dosage: Static acute test.

D. Design: A range-finding and a definitive test were conducted. Both tests were conducted for 96 hours. The range-finding test concentrations were 0.1, 1.0, 10.0, and 100.0 mg/L of the test material. The range-finding test was not replicated, and 5 fish were tested per concentration. The definitive test was conducted using only one test concentration, 100 mg/L. This test was conducted in triplicate and 30 fish were tested per concentration. The reported loading was 1 fish per 2 liters of water, but the individual fish weight or age was not provided. The tests were examined at 6, 24, 48, 72, and 96 hours.

E. Statistics: The estimation of Median Tolerance Limits for each day and the 95 percent confidence limits was calculated using the J.T. Litchfield, Jr. and F.W. Wilcoxon Method.

12. REPORTED RESULTS: The range-finding results indicated that the 96-hour LC_{50} was greater than 100 mg/L. During the definitive test no mortalities were observed after 96 hours of exposure.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 96-hour LC_{50} for rainbow trout, Salmo gairdneri, was calculated to be greater than 100 mg/L. A Quality Assurance statement was included in the report, "at the time of this inspection, all work had been and was being performed in manner consistent with the GLP's."

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

A. Test Procedure: The overall procedures appear to be in accordance with the Guidelines. The following items were not included in the report and are required in the Guidelines:

- o The composition and percent active ingredient of the test chemicals
- o The age, sex, size, life stage, and/or weight of the test organisms
- o The source of the test organisms
- o The culture and/or holding history of test fish
- o The survival of fish during the 48 hours prior to testing
- o The acclimation and feeding regimes
- o The method of assigning test organisms to test and control groups
- o The data provided does not mention a control, the Guidelines require that a control be included and that the survival in the control chamber(s) be reported

The following items deviated from the Guidelines:

- o Test temperature was 15 ± 2 ^{o}C, Guidelines specify 12 ± 1 oC
- o Photoperiod was 14 hours light : 10 hours darkness, the Guidelines require 16 hours light : 8 hours darkness
- o Tap water was used, the Guidelines specify that "dechlorinated water should not be used because removal of chlorine is rarely complete and residual chlorine can be quite toxic to aquatic organisms"

- o Loading was reported as 1 fish per 2 liters of water, and it should be reported in grams of fish tissue per liter of water, the weight of the fish was not provided

B. Statistical Analysis: No statistical analysis are required when only one test concentration is used.

C. Discussion/Results: The study results appear scientifically valid. But, the missing data and protocol

deviations critically affect the validation of this study. The most critical discrepancies are the lack of:

- o controls
- o background information on the test species
- o acclimation and feeding regimes
- o survival of test organisms in the 48 hours prior to testing
- o size of the test fish (age or weight), and
- o the percent active ingredient of the test materials.

Other issues critical to the validity are the use of chlorinated tap water, test temperature range of 15 to 17 ^{o}C (compared to the 11 to 13 ^{o}C range allowed), and the lack of documentation of the acceptability of the dilution water to the test species (ie. no controls).

D. Adequacy of the Study:

(1) Classification: Invalid.

(2) Rationale: This study is considered invalid because sufficient information is missing to appropriately evaluate the appropriateness of the protocols. The use of dechlorinated water, the lack of information on the test species, the lack of controls, the inappropriate temperature range for a cold water test, and the unknown loading rate, all make this study unacceptable according to the Guidelines.

(3) Repairability: No. The test was conducted at an unacceptable test temperature using unacceptable dilution water, therefore even if all the information missing was provided, it can not be upgraded to core.

15. COMPLETION OF ONE-LINER: Yes, January 10, 1988.

DATA EVALUATION RECORD
Accession Number 403842-06

1. **CHEMICAL:** BIONOX A and BIONOX B

2. **TEST MATERIAL:** BIONOX A, Lot Number 342861, strength 4251 parts per million (ppm) and BIONOX B, Lot Number 152861; mixed in equal parts. Percent Active Ingredient not provided.

3. **STUDY TYPE:** Static Acute Freshwater Invertebrate.
Species Tested: Cladoceran. Daphnia magna.

4. **CITATION:** Terrell, Y. and B. Till. 1987. Acute Toxicity Bioassay of BIONOX A (342861) and BIONOX B on Daphnia magna. American Standards Biosciences Corp. Project Number 87-371. Prepared by American Standards Biosciences Corp., Reading, Pennsylvania. Submitted by Metrex Research Corporation, Parker, Colorado. Accession Number 403842-06.

5. **REVIEWED BY:**

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Signature:
Date:

6. **APPROVED BY:**

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Project Manager/
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KBN Engineering and
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Signature:
Date:

Hawk C.E.L.

James W. Akerman
Chief, EEB/HED
USEPA

Signature:
Date:

7. **CONCLUSIONS:** This study does not fulfill the Guideline requirements for an acute static toxicity determination for an invertebrate species due to the lack of critical data needed for the evaluation of this study. Bionox A and Bionox B combined in equal parts, is considered slightly toxic to Daphnia magna with a 48-hour LC_{50} between 75.0 and 100.0 mg/L.

8. **RECOMMENDATIONS:** The registrant should conduct another aquatic invertebrate study following the 72-2 Guidelines which will include items listed under 14A below, providing the registrant cannot generate the data listed under 14A.

9. BACKGROUND: This study was submitted in response to Bionox Registration Standard.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Daphnia magna less than 24 hours old were used for the tests. No other information was provided on the test organisms.

B. Test System: Glass beakers of 500 milliliters capacity were used. the test solution was 400 milliliters (approximately 10.5-centimeter depth). Stream water was used. No chemical characterization or other information was provided on the dilution water. Lighting was 14 hours light: 10 hours darkness. Water temperature was 19 ± 2 degrees Celsius (^{o}C).

C. Dosage: Static acute test.

D. Design: A range-finding and a definitive test were conducted. Both tests were conducted for 48 hours. The range-finding test concentrations were 0.1, 1.0, 10.0, and 100.0 mg/L of the test material. The range-finding test was not replicated, and 5 cladocerans were tested per concentration. The definitive test concentrations were: 24.0, 32.0, 42.0, 56.0, 75.0, and 100 mg/L. This test was replicated and 20 cladocerans were tested per concentration. The reported loading was 10 cladocerans per 400 milliliters of water. The tests were examined at 6, 24, and 48 hours.

E. Statistics: The estimation of Median Tolerance Limits and the 95 percent confidence limits was calculated using the J.T. Litchfield, Jr. and F.W. Wilcoxon Method.

12. REPORTED RESULTS: The range-finding results indicated that the 96-hour LC_{50} was between 10 an 100 mg/L. The definitive data are shown below:

Concentration (mg/L)	Survival 0 hour	24 hour	48 hour
100	20	20	7
75	20	20	20
56	20	20	20
42	20	20	20
32	20	20	20
24	20	20	20

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: "Estimation was made of the TL_{50} for 48 hours only, and was found to be greater than 75.0 mg/L, but less than 100.0 mg/L. A Quality Assurance statement was included in the report, "at the time of this inspection, all work had been and was being performed in manner consistent with the GLP's."

14. REVIEWER'S DISCUSSION AND INTERPRETATION O F STUDY RESULTS:

A. Test Procedure: The overall procedures appear to be in accordance with the Guidelines. The following items were not included in the report and are required in the Guidelines:

- The composition and percent active ingredient of the test chemicals
- The source test organisms
- The culture and/or holding history of test organisms
- The survival of adult cladocerans during the 48 hours prior to testing
- The acclimation and feeding regimes
- The method of assigning test organisms to test and control groups
- The data provided does not mention a control, the Guidelines require that a control be included and that the survival in the control chamber(s) be reported
- The chemical characterization of the dilution water or its acceptability of the dilution water for rearing the test organisms
- The dissolved oxygen concentration must be reported at the beginning and the end of the test. Measurements should be taken from the control and the high, medium, and low test concentration.
- Test temperature should be reported and should be measured hourly in at least one test vessel, unless water temperature is controlled by a constant water temperature bath, then recordings can be taken every 6 hours
- The pH should be measured and reported at the beginning and end of the test in the control and the high, medium, and low toxicant concentrations.

The following item deviated from the Guidelines:

- Photoperiod was 14 hours light : 10 hours darkness, the Guidelines require 16 hours light : 8 hours darkness.

B. **Statistical Analysis:** Due to the lack of partial mortalities,statistical analysis were not conducted.

C. **Discussion/Results:** The study results appear scientifically valid. But, the missing data critically affect the validation of this study. The most critical discrepancies are the lack of:

- o controls
- o background information on the test species
- o acclimation and feeding regimes
- o survival of the adult brood organisms in the 48 hours prior to testing
- o chemical characterization of test solutions
- o information of the quality of the dilution water, and
- o the percent active ingredient of the test material.

D. **Adequacy of the Study:**

(1) Classification: Supplemental.

(2) Rationale: This study is considered supplemental because sufficient information is missing to appropriately evaluate the appropriateness of the protocol, including the lack of information on the test species, the lack of controls, and the unknown quality of the stream water used as dilution water, all make this study unacceptable according to the Guidelines.

(3) Repairability: Yes. If all the information missing under 14 A is provided, and considered scientifically acceptable, this study can be upgraded to core.

15. **COMPLETION OF ONE-LINER:** Yes, January 10, 1988.