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Attn: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, D.C. 20460-0001

Dear Mr. [redacted] an

Thank you for your letter of August 2 relative to Document ID 8EHQ-0494-13008.

Full copies of reports:

1. Acute Toxicity of Sodium Chlorite to the Sheepshead Minnow
2. Acute Toxicity of Sodium Chlorite to MYSID SHRIMP
3. Effect of Sodium Chlorite on New Shell Growth in Eastern Oyster

are enclosed for your information per your request.

Very truly yours,

John J. McCallion

John J. McCallion
Senior Vice President

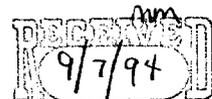


8EHQ-94-13008
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Date Submitted:

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Volume:

ACUTE TOXICITY OF SODIUM CHLORITE TO THE SHEEPSHEAD MINNOW
(CYPRINODON VARIEGATUS)

U.S.-EPA-FIFRA 40 CFR SERIES 72-3

AUTHORS:

Study Director: J. J. Yurk
Principal Investigator: M. A. Overman
Environmental Science & Engineering, Inc.

FINAL REPORT DATE:

May 18, 1994

SUBMITTED TO:

Chemical Manufacturers Association (CMA)
1501 M Street NW
Washington, D.C. 20037

PERFORMING LABORATORY:

Environmental Science & Engineering, Inc., (ESE)
Department of Aquatic Toxicology
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LABORATORY PROJECT ID:

ESE Project No. 3943014-0100-3140
CMA Reference No. CD-7.0-3AT-ESE

Contains No CBI

STATEMENT OF NO DATA CONFIDENTIALITY

Acute Toxicity of Sodium Chlorite
to the Sheepshead Minnow
(Cyprinodon variegatus)

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No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA para. 10(d) (1) (A), (B), or (C). Nor is any supplemental claim of confidentiality made for any information in this study on the basis of FIFRA para. 10(A) or (B). This document, however, is propriety to Chemical Manufacturers Association and is considered confidential and trade-secret information in all other countries and for all purposes other than those enunciated in FIFRA para. 3 & 10. In addition, this data is compensative under FIFRA and must be handled accordingly by all parties receiving this data. Information contained in this study should not be reproduced, stored in a retrieval system or transmitted in any form or by any means by persons other than EPA without the express written consent of Chemical Manufacturers Association except as required to carry out the requirements of FIFRA.

Company: CHLORINE DIOXIDE PANEL OF THE CHEMICAL MANUFACTURERS ASSOCIATION

Company Agent: ROBERT R. ROMANO (NAME)

Date: MAY 23, 1994

Title: ASSOCIATE DIRECTOR, CHEMSTAR

Signature: Robert R. Romano

STATEMENT OF GLP COMPLIANCE

Compound: Sodium Chlorite

Title: Acute toxicity of sodium chlorite to the sheepshead minnow (Cyprinodon variegatus)

The study described in this report was conducted in accordance with the following Good Laboratory Practice Standards:

United States Environmental Protection Agency,
Title 40 Code of Federal Regulations Part 160,
1 July 1993 Edition

Japan Ministry of Agriculture, Forestry and Fisheries,
59 NohSan, Notification No. 3850.
Agricultural Products Bureau
10 August 1984

Organization for Economic Co-Operation and Development
ISBN 92-64-12367-9, Paris 1982

Study Director: Jeffrey J. Turk 5-18-94
Jeffrey J. Turk Date
ESE

Principal Investigator: Max Overman 5-18-94
Max A. Overman Date
ESE

Study Sponsor: Robert R. Romano 5/19/94
Robert Romano Date
CMA

Submitter: _____ Date

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CMA #: CD-7.0-3AT-ESE

STATEMENT OF QUALITY ASSURANCE

The study data were reviewed by the ESE Quality Assurance Unit to assure that standard operating procedures and guidelines used to conduct this study were followed and that this report is an accurate reflection of the raw data.

Audit Type	Date	Date Reported To Management and Study Director
In-phase including: filling out chain of custody and sampling for chemical analysis	03/25/94	04/05/94
Draft Report	04/15/94	04/15/94
Final Report	05/18/94	05/18/94

Joe Owusu-Yaw

Dr. Joe Owusu-Yaw, Quality Assurance Unit

5/18/94

Date

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SCIENTIFIC PERSONNEL SIGNATURE PAGE

Study Director: Jeffrey J. Yurk 5/18/94
Jeffrey J. Yurk Date

Principal Investigator: Max Overman 5/18/94
Max A. Overman Date

Chemistry Coordinator: Patrick Wilber 5-18-94
Patrick Wilber Date

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STUDY SUMMARY

Study Title: Acute Toxicity of Sodium Chlorite to the Sheepshead Minnow (Cyprinodon variegatus)

Data Requirement: Section 72-3 of the EPA Pesticide Assessment Guidelines, Subdivision E

Sponsor: Chemical Manufacturers Association (CMA),
2501 M Street NW,
Washington D.C. 20037

Location of Study: Environmental Science & Engineering, Inc. (ESE)
P.O. Box 1703
Gainesville, FL 32602-1703

Study Director: Jeffrey J. Yurk; Tel No. (904) 333-2626

Study Initiation Date: February 11, 1994

Experimental Start Date: March 21, 1994

Experimental Termination Date: March 25, 1994

Test Substance: Sodium Chlorite, Batch No. 5064, CAS No. 7758-19-2, purity 79.0%

Test Organism and Source: Sheepshead Minnow (Cyprinodon variegatus), juveniles, obtained from Aquatox Inc., Hot Springs, Arkansas

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Test Conditions:

Dilution water: filtered sea water adjusted to approximately 20‰ salinity with well water from a well located at test site; photoperiod: 16-h light and 8-h dark with 30-minute dawn/dusk transition; temperature: 22 ± 2 °C

Test Results:

96 hour LC50: 75.0 mg/L Sodium Chlorite; 95% confidence limits: 62.6 and 89.8 mg/L Sodium Chlorite; NOEC: 13.9 mg/L

Location of Raw Data and Final Report:

Environmental Science & Engineering, Inc. (ESE)
P.O. Box 1703
Gainesville, FL 32602-1703

1.0 INTRODUCTION

A flow-through toxicity test was conducted at Environmental Science & Engineering, Inc., in Gainesville, Florida to determine the acute toxicity of sodium chlorite to the sheepshead minnow (Cyprinodon variegatus). The criterion for effect was mortality and results of the test are expressed as a 96-hour median lethal concentration (LC50), the concentration of sodium chlorite estimated to be lethal to 50 percent of the test population of sheepshead minnows under specified conditions of exposure. All raw data related to this study are maintained by Environmental Science & Engineering, Inc., Gainesville, Florida.

2.0 MATERIALS AND METHODS

2.1 Test substance

The test substance, sodium chlorite (Batch No. 5064, CAS No. 7758-19-2) was received by ESE in two 1000 mL plastic bottles on December 27, 1993. The test substance was a white crystalline material and was stored in the dark at ambient temperatures. The water solubility of the test substance was reported by Vulcan Chemicals to be 39% at 25°C. The purity of the test substance was reported to be 79.0%. All sodium chlorite stock solutions were

made by dissolving the test substance in filtered seawater. Measured test concentrations are reported as milligrams (mg) of sodium chlorite per liter (L) of filtered seawater.

2.2 Test organisms

Juvenile sheepshead minnows (Cyprinodon variegatus) that were used in the definitive test, were obtained from Aquatox Inc., Hot Springs, Arkansas on February 15, 1994. During the 48-hours prior to test start, test organisms were maintained in filtered seawater with a salinity of 20 ± 3 ‰, a pH of 7.5 ± 1.0 and a temperature of 22 ± 1 °C. During holding, the sheepshead minnows were fed a daily diet of brine shrimp, although they were not fed during the 48-hour pretest period. Chemical analyses of fish feed is performed when fresh feed is purchased; the results of the most recently reported chemical analyses of fish feed are provided in Appendix A. No mortality of the test population was observed over the 48-hour pretest period. Before test start, a representative sample of test organisms were weighed and measured. The average weight and length were 0.19 g (ranging from 0.11 to 0.31 g) and 21.8 mm (ranging from 20 to 25 mm), respectively.

2.3 Dilution water

Sand filtered Atlantic Ocean water was obtained from Whitney Laboratory, Marineland, FL and was diluted to a salinity of approximately 20‰ with well water from a well located at the test site. Chemical analyses of 20‰ filtered sea water are performed semi-annually and the results of the most recently reported analyses are presented in Appendix B.

2.4 Test Methods

Methods for the 96-hour flow-through toxicity test are described in the ESE test protocol entitled: "Acute Toxicity of sodium chlorite to the Sheepshead Minnow (Cyprinodon variegatus)".

2.4.1 Range Finding Test

A static range-finding test (see Appendix C) was conducted at nominal test concentrations of 0 (control), 10, 100, 500, and 1000 mg sodium chlorite/L in 10 L chambers. Five organisms were exposed per concentration for 96 hours, during which time they were not fed. After 96 hours of exposure, there was 80% survival in the control (0 mg/L), 100% survival in the lowest concentration (10 mg/L), 60% survival at 100 mg/L, and 0% survival in the two highest concentrations (500 and 1000 mg/L). Based upon these results, nominal concentrations selected for the definitive test were 0

(dilution water control), 12.5, 25, 50, 100, and 200 mg sodium chlorite/L of dilution water.

2.4.2 Definitive Test

The definitive test was conducted under flow-through conditions in a proportional diluter system (see Appendix C). The stock solution, with a nominal concentration of 3.23 g sodium chlorite/L, was prepared in dilution water. Stock was prepared daily and was continuously mixed throughout the test to insure homogeneity. The mean measured stock concentration was 3.344 g/L (104% of nominal). Approximately 64 mL of stock solution were pumped to the chemical mixing chamber (total volume 0.97 L) each diluter cycle providing a high nominal concentration of 221 mg/L. This test solution was proportionally diluted in the diluter system to provide the four lower test concentrations (i.e., 111, 55.3, 27.6, and 13.8 mg/L). A dilution water control was maintained concurrently with the five test solutions.

The toxicity test was conducted using 10.5 L, rectangular glass chambers (28 cm length X 25 cm width X 15 cm height), filled to approximately 5 L with dilution water or test solution. All test chambers were kept covered during the test, except when daily observations of test organisms were made and water quality parameters measured. A test solution volume of approximately 250

mL was delivered to each test chamber during every cycle. The diluter cycled at a rate of 5.1 cycles per hour providing approximately 6.1 volume additions every 24 hours. Diluter function including stock delivery, stock usage, and cycle time were checked and recorded daily throughout the test.

Test vessels were labeled using the compound name, nominal concentrations, and a letter (A or B) designating concentration replicates. Test vessels were randomly positioned in a water bath under fluorescent lighting regulated to provide a photoperiod of 16 hours light and 8 hours dark with a 15 to 30 minute transition period of lower light intensity to simulate dawn and dusk. Light intensity averaged 616 Lux over the test system, as measured by a Lux meter. The 96-hour test was started on March 21, 1994 by indiscriminately distributing ten organisms to each and replicate test vessel. Organisms were indiscriminately netted and added to test vessels in lots of five, until each vessel held ten organisms.

Survival of test organisms was monitored daily and any dead organisms were recorded and removed. Any abnormalities in behavior or physical appearance of the test organisms were also noted. Test organisms were not fed during the test. Test solutions were not aerated during the test.

Water quality was monitored daily during the test in exposure chambers with live test organisms present. Water temperature in one control exposure was continuously monitored with a Supco Temperature Recorder and spot checked daily with a NIST-calibrated thermocouple. Dissolved oxygen, salinity and pH were measured daily in all test chambers containing surviving organisms. Dissolved oxygen concentrations were determined using a YSI Model 58 meter, salinity was measured using an American Optical refractometer, and pH was measured with an Orion Model SA 250 pH meter.

2.5 Statistical Analyses

Statistical analyses were conducted using recommended procedures (EPA Pesticide Assessment Guidelines). Mortality data for the test organisms and the mean measured concentrations of the test material were used to compute the 24-, 48-, 72- and 96-hour LC50 values and their 95 percent confidence limits, using a statistical computer program (Stephan, 1982). The method of statistical analysis selected by the program was determined by characteristics of the data, i.e., the presence or absence of 0-percent and 100-percent mortality and the number of concentrations in which mortalities between 0 and 100 percent occurred (1). A 96-hour no-observed-effect concentration (NOEC) of the test substance was defined as

the highest test concentration where no mortality or other toxic signs occurred.

2.6 Chemical Analysis

Samples were collected from the B-replicate of the control and each test concentration on day 0 and from the A-replicate of each on day 4 for chemical analysis. Test solutions were sampled from the approximate center of the test vessel using a serological pipet, and transported in scintillation vials to analytical services for analysis. Samples were analyzed by ion chromatography with visible detection following the methodology provided in the Method Validation report (Appendix D).

3.0 RESULTS AND DISCUSSION

Nominal and measured concentrations of sodium chlorite, in the definitive test, are presented in Table 1. The mean measured concentrations ranged from 13.9 mg/L sodium chlorite in the lowest concentration to 229 mg/L sodium chlorite in the highest concentration. Mean measured concentrations ranged from 101 to 109% of nominal. The concentration in the control exposure was determined to be below the limit of validation for this test of 0.27 mg/L. Analytical quality control data are presented in Table 2. Individual standard matrix spike recoveries ranged from 101 to

102 percent for all quality control data. Data for exposure water quality parameters, and their corresponding ranges and median values are presented in Tables 3, 4 and 5. Dilution water salinity remained at 21 ‰ throughout the test. All ranges are within the acceptable limits (2).

The mean measured sodium chlorite concentrations and corresponding mortalities of test organisms, presented in Table 6, were used to calculate the LC50 and 95% confidence limits for various lengths of exposures (Table 7). The 96-hour LC50 was calculated to be 75.0 mg/L sodium chlorite using the Probit method. Behavioral changes in organisms such as lethargic behavior were noted in daily observations of organisms in exposures of 112 mg/L and above (Appendix C). The 96-hour NOEC of the test substance was 13.9 mg/L sodium chlorite in this test.

4.0 CONCLUSION

The 96-hour LC50 for sodium chlorite in the sheepshead minnow (Cyprinodon variegatus) was determined to be 75.0 mg/L with 95% confidence limits of 62.6 and 89.8 mg/L. The 96-hour NOEC of the test substance was estimated to be 13.9 mg/L.

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REFERENCES

(1) Stephan, C.E. 1977. Methods for Calculating an LC50. IN: American Society for Testing and Materials (ASTM) Aquatic Toxicology and Hazard Evaluation, pp. 65-84, F.L. Mayer and J.L. Hamelink (Editors). ASTM STP 524, Philadelphia, PA.

(2) American Society for Testing and Materials (ASTM). 1980. Standard Practice for Conducting Acute Toxicity Tests With Fishes, Macroinvertebrates, and Amphibians. ASTM, Philadelphia, PA. Publication E 729-80.

Table 1. Nominal and measured concentrations of sodium chlorite in exposure water during a 96-h flow-through, acute toxicity test with the sheepshead minnow (*C. variegatus*).

Nominal concentration (mg/L) of sodium chlorite	Measured concentration (mg/L) of sodium chlorite at test start and test termination		Mean measured concentration (mg/L)	Percent of nominal ^a
	0 h	96 h		
0 (control)	ND ^b	ND	ND	NC ^c
13.8	13.6	14.1	13.9	101
27.6	26.1	33.8	30.0	109
55.3	58.5	60.4	59.5	108
111	109	115	112	101
221	224	234	229	104

^a Mean measured concentration x 100/nominal concentration.
^b ND = not detected below a limit of validation for this test of 0.27 mg/L.
^c Cannot be calculated.

Source: ESE 1994

Table 2. Analytical quality control data from a 96-h flow-through, acute toxicity test of sodium chlorite to the sheepshead minnow (*C. variegatus*).

Sample	Target chlorite concentration (mg/L)	Measured chlorite concentration (mg/L)	Percent recovery ^a
<u>DAY-0</u>			
Std Matrix Spike	10.0	10.19	102
Std Matrix Spike	10.0	10.23	102
<u>DAY-4</u>			
Std Matrix Spike	10.0	10.13	101
Std Matrix Spike	10.0	10.20	102
Sample Matrix Spike	100.0	103.3	103
Sample Matrix Spike	100.0	102.0	102
Sample Matrix Spike	5.00	5.160	103
Sample Matrix Spike	5.00	5.054	101

^a Measured concentration X 100/target concentration.

Source: ESE 1994

Table 3. Daily temperature of exposure water during a 96-h flow-through, acute toxicity test of sodium chlorite to the sheepshead minnow (*C. variegatus*).

Mean measured concentration (mg/L) of sodium chlorite	Replicate exposure	Temperature (°C)					n ^a	Range	Median
		0 h	24 h	48 h	72 h	96 h			
0 (control)	A	22.2	22.2	22.6	21.8	22.1	10	21.8-22.6	22.2
	B	21.8	22.3	22.6	21.8	22.1			
13.9	A	22.2	22.3	22.6	21.8	22.1	10	21.8-22.6	22.2
	B	22.2	22.3	22.6	21.8	22.1			
30.0	A	22.1	22.3	22.7	21.9	22.1	10	21.8-22.7	22.2
	B	22.2	22.3	22.6	21.8	22.2			
59.5	A	22.2	22.3	22.6	21.9	22.1	10	21.8-22.6	22.2
	B	22.2	22.2	22.6	21.8	22.1			
112	A	22.2	22.3	22.7	22.0	22.2	10	21.9-22.7	22.2
	B	22.2	22.3	22.6	21.9	22.1			
229	A	22.2	22.3	- ^b	- ^b	- ^b	4	22.2-22.3	22.3
	B	22.2	22.3	- ^b	- ^b	- ^b			

^a n = total number of measurements.

^b - = value not determined after 100% mortality.

Note: temperatures as measured with a continuous temperature monitor were within the range of 21 to 23°C.

Source: ESE 1994

Table 4. Exposure water pH during a 96-h flow-through, acute toxicity test of sodium chlorite to the sheepshead minnow (*C. variegatus*).

Mean measured concentration (mg/L) of sodium chlorite	Replicate exposure	pH					n ^a	Range	Median
		0 h	24 h	48 h	72 h	96 h			
0 (control)	A	8.1	8.3	8.3	8.3	8.3	10	8.1-8.3	8.3
	B	8.1	8.3	8.3	8.3	8.3			
13.9	A	8.1	8.3	8.3	8.3	8.3	10	8.1-8.3	8.3
	B	8.2	8.3	8.3	8.3	8.3			
27.6	A	8.2	8.3	8.3	8.3	8.3	10	8.2-8.4	8.3
	B	8.2	8.4	8.3	8.3	8.3			
55.3	A	8.2	8.4	8.3	8.3	8.3	10	8.2-8.4	8.3
	B	8.2	8.4	8.3	8.3	8.3			
111	A	8.2	8.4	8.4	8.4	8.4	10	8.2-8.4	8.4
	B	8.2	8.4	8.4	8.4	8.4			
221	A	8.3	8.4	- ^b	- ^b	- ^b	4	8.3-8.4	8.4
	B	8.3	8.4	- ^b	- ^b	- ^b			

^a n : total number of measurements.

^b - : value not determined after 100% mortality.

Source: ESE 1994

Table 5. Dissolved oxygen concentrations of exposure water during a 96-h flow-through, acute toxicity test of sodium chlorite to the sheepshead minnow (*C. variegatus*).

Mean measured concentration (mg/L) of sodium chlorite	Replicate exposure	Dissolved Oxygen Concentration (mg/L) (percent saturation)					n ^a	Range	Median
		0 h	24 h	48 h	72 h	96 h			
0 (control)	A	8.2(112)	7.6(104)	7.4(99)	7.8(106)	7.6(104)	10	7.4-8.2	7.7
	B	8.2(112)	7.7(105)	7.4(99)	7.8(106)	7.6(104)			
13.9	A	7.9(108)	7.5(102)	7.4(99)	7.8(106)	7.6(104)	10	7.4-8.2	7.6
	B	8.2(112)	7.6(104)	7.5(101)	7.9(108)	7.6(104)			
30.0	A	8.1(110)	7.7(105)	7.3(98)	7.8(106)	7.6(104)	10	7.3-8.1	7.7
	B	8.1(110)	7.6(104)	7.5(101)	7.9(108)	7.7(105)			
59.5	A	8.1(110)	7.5(102)	7.4(99)	7.8(106)	7.6(104)	10	7.4-8.1	7.6
	B	8.1(110)	7.5(102)	7.4(99)	7.9(108)	7.6(104)			
112	A	8.2(112)	7.5(102)	7.4(99)	8.0(109)	7.7(105)	10	7.4-8.2	7.7
	B	8.2(112)	7.4(101)	7.5(101)	8.1(110)	7.6(104)			
229	A	8.1(110)	7.6(104)	- ^b	- ^b	- ^b	4	7.6-8.1	7.9
	B	8.1(110)	7.6(104)	- ^b	- ^b	- ^b			

^a n : total number of measurements.

^b - : value not determined after 100% mortality.

Source: ESE 1994

Table 6. Mortalities of sheepshead minnows (*C. variegatus*) exposed to sodium chlorite in a 96-h flow-through, acute toxicity test.

Mean measured concentration (mg/L) of sodium chlorite	Cumulative number of dead organisms and (percent mortality of total organisms exposed)			
	24 h	48 h	72 h	96 h
0 (control)	0(0)	0(0)	0(0)	0(0)
13.9	0(0)	0(0)	0(0)	0(0)
30.0	1(5)	1(5)	1(5)	1(5)
59.5	1(5)	2(10)	3(15)	3(15)
112	12(60)	16(80)	18(90)	18(90)
229	20(100)	20(100)	20(100)	20(100)

Source: ESE 1994

Table 7. LC50 values of sodium chlorite for the sheephead minnow (C. variegatus) for different lengths of exposure.

Exposure period	LC50 (mg/L) ^a	95% Confidence limits for LC50	Statistical method used for LC50 calculation
24 h	101	59.5-112	Binomial
48 h	82.9	68.8-100	Probit
72 h	75.0	62.6-89.8	Probit
96 h	75.0	62.6-89.8	Probit

^a Calculated based on mean measured concentrations of sodium chlorite.

Source: ESE 1994

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Appendix A- Sheepshead Food Characterizations

BRINE SHRIMP CHEMICAL CHARACTERIZATION
February 1993

Component	Analytical Method	Brine Shrimp ^a
Organochlorine pesticides ($\mu\text{g/g}$ wet)	EPA 8080	<20
Hydrocarbon, Petroleum ($\mu\text{g/g}$ tissue)	EPA 418.1	<29

^a Brine shrimp (lot number SFO 65034) were purchased from Florida Aqua Farms, Inc., Dade City, FL and hatched using filtered seawater diluted to a salinity of approximately 5 ppt.

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Appendix B- Filtered Seawater Characterization

FILTERED SEAWATER CHARACTERIZATION*

<u>PARAMETER</u>	<u>CONCENTRATION</u>	<u>METHOD</u>
Arsenic	2.9 µg/L	EPA 206.2
Aluminum	<40 µg/L	EPA 200.7
Cadmium	<0.5 µg/L	EPA 213.3
Calcium	215 mg/L	EPA 200.7
Chromium	<10.0 µg/L	EPA 200.7
Cobalt	<20 µg/L	EPA 200.7
Copper	11.9 µg/L	EPA 200.7
Iron	<45.0 µg/L	EPA 200.7
Lead	<2.0 µg/L	EPA 239.2
Mercury	<0.20 µg/L	EPA 245.1
Magnesium	590 mg/L	EPA 200.7
Nickel	<15.0 µg/L	EPA 200.7
Selenium	<2.5 µg/L	EPA 270.2
Silver	<5.0 µg/L	EPA 200.7
Sodium	5120 mg/L	EPA 200.7
Zinc	<20.0 µg/L	EPA 200.7
Nitrogen, NH ₄ +NH ₃	<0.020 mg/L as N	EPA 350.1
Nitrogen, NH ₃ Unionized	<0.001 mg/L	Calculated
Nitrogen, NO ₂ +NO ₃	0.034 mg/L as N	EPA 353.2
pH	8.3	Meter
Alkalinity	171 mg/L as CaCO ₃	EPA 310.1
Sulfide	<0.05 mg/L as S	EPA 376.2
Hydrogen Sulfide	<0.03 mg/L	Std Method (428E)
Turbidity	<0.40 NTU	EPA 180.1
Dissolved Solids	20100 mg/L	EPA 160.1
Suspended Solids	27 mg/L	EPA 160.2
Fluoride	0.65 mg/L	EPA 340.2
Cyanide	<0.002 mg/L	EPA 335.3
Carbon, TOC	2.3 mg/L	EPA 415.1
COD	83 mg/L	EPA 410.1
Pesticides-Organochlorine and PCB's	<0.005 µg/L	EPA 8080
Pesticides-Organophosphate	<2.52 µg/L	EPA 8140

* The characterized seawater is natural seawater collected from the Atlantic Ocean near Marineland, Florida and diluted to a salinity of 20 parts per thousand using ESE's well water. The diluted seawater sample was collected from ESE's seawater delivery system on September 9, 1993.

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SODIUM CHLORITE CVARIEGATUS
ESE PROJECT #: 3943014-0100-3140
CMA #: CD-7.0-3AT-ESE

Appendix C- Pertinent Raw Data

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Page: _____
 ESE QA Form No.: 015A
 Effective: APR 1993

ACUTE TOXICITY DATA SHEET-BIOLOGICAL DATA								
SPONSOR: <u>CMA</u>		PROJECT NUMBER: <u>3943014-0100-3140</u>						
TEST SUBSTANCE: <u>Sodium Chlorite</u>		TEST SPECIES: <u>C. variegatus</u>						
ANIMAL HISTORY								
Sample Information and Description Found on Page <u>50</u> of Test Substance Log								
Lot No./Batch No.: <u>44-16</u> (Circle One)		Date Maintenance/Acclimation Began: <u>2/15/94</u>						
See Page No.: <u>58</u> of Animal Collection/Receipt Log		Condition of Animals: <u>Normal</u>						
See Page No.: <u>93</u> of <u>Fish Holding Log</u> For Raw Data on Animal Holding		Life Stage/Age: <u>Juvenile</u>						
		Mortality in Tank 48 Hours Prior To Test Initiation: <u>0</u> (%)						
TEST CONDITIONS								
<input checked="" type="checkbox"/> Range-Finding <input type="checkbox"/> Definitive	<input checked="" type="checkbox"/> Static <input type="checkbox"/> Flow-Through	Time Added Substance/Animals <u>1120 / 1145</u>		Test Location <u>Water bath 2</u>				
Test System: <input checked="" type="checkbox"/> Covered <input type="checkbox"/> Open	Test Container Dimensions (cm) <u>28 x 15 x 25.5</u>	Test Solution Height: <u>4.6cm</u>		Test Container Composition: <u>Glass</u>				
Test Chamber Volume (L) 0.34, 1.6, 3.8, 30 - <u>10L</u>		Test Solution Volume (L) 0.20, 1.0, <u>3.0</u> 10 -						
Protocol: Acute toxicity of sodium chlorite to the sheepshead minnow (<u>Cyprinodon variegatus</u>)								
Dilution Water: <u>Filtered Seawater</u> . Type of Lighting: <u>Fluorescent</u> Photoperiod: <u>16L/8D/8DK</u>								
Cicle One: Test Substance as Active Ingredient/Whole Material								
Test Concentration % <u>mg/L</u> ug/L	Control	Solv Cont	10.0	100.0	500.0	1000.0	—	Solvent/Carrier: <u>NA</u>
Amount of Substance/Stock Added (mL)	NA	NA	3.0	30.0	150.0	300.0	—	
Stock Solution Used (e.g., 1', 2')	NA	NA	1'	1'	1'	1'	—	Solvent/Carrier Concentration: <u>NA</u>
Amount of Dilution Water Added (mL)	3000.0	NA	2997.0	2970.0	2850.0	2700.0	—	
Amount of Solvent Added (—)	NA	NA	NA	NA	NA	NA	NA	<u>NA</u>

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CMA #: CD-7.0-3AT-ESE

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 ESE QA Form No.: 015B
 Effective: MAR 1993

Ranges Under 3943014-0100-3140

SUBJECT: ACUTE TOXICITY DATA SHEET - SURVIVAL											
		0-HOUR		24-HOUR		48-HOUR		72-HOUR		96-HOUR	
DATE		3/8/94		3/9/94		3/10/94		3-11-94		3-12-94	
ANIMALS FED		No		No		No		no		no	
TIME		1145		1215		1345		1340		1300	
DATA BY		HJT		HJT		HJT		B		B	
NOMINAL CONCENTRATION % mg/L ug/L	R E P	NO.		NO.		NO.		NO.		NO.	
		OBS.	ALIVE	OBS.	ALIVE	OBS.	ALIVE	OBS.	ALIVE	OBS.	ALIVE
CONTROL	A	None	5	None	5	None	5	none	5	1 dead	4
10 mg/h	A	None	5	None	5	None	5	none	5	none	5
100 mg/h	A	None	5	1 Dead	4	1 both	^{100 mg/h} 43	none	3	none	3
500 mg/h	A	None	5	5 Dead	0	-	-	-	-	-	-
1000 mg/h	A	None	5	5 Dead	0	-	-	-	-	-	-

OBSERVATION KEY

OBS - OBSERVATIONS	DRK - DARKER THAN CONTROLS	NF - NOT FOUND
NONE - NORMAL CONDITIONS	LT - LIGHTER THAN CONTROLS	BS - BRINE SHRIMP
AS - AT SURFACE	RAR - RAPID RESPIRATION	
LE - LOSS OF EQUILIBRIUM	PRE - PRECIPITATE	
LETH - LETHARGIC	FOS - FILM ON SURFACE	
HYP - HYPERACTIVE	UC - UNDISSOLVED CHEMICAL	

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PAGE:
 ESE QA FORM: 016
 EFFECTIVE: JUNE 1990

SUBJECT: ACUTE TOXICITY DATA SHEET--WATER QUALITY DATA																									
SPONSOR: <u>CMA</u>										PROJECT NUMBER: <u>3943014-0100-3140</u>															
TEST SUBSTANCE: <u>Sodium Chlorite</u>										TEST SPECIES: <u>C. variegatus</u>															
DATE	w/PHN survey					3/9/94					3/10/94					3-11-94					3/12/94				
TIME	1120					1210					1410					1330					1300				
DATA BY	NAT					NAT					NAT					B					B				
NOMINAL CONCENTRATION mg/L ug/L	REP	TEMP	SL	HD	PH	DO	ALK	COND	TEMP	SL	HD	PH	DO	TEMP	SL	HD	PH	DO	TEMP	SL	HD	PH	DO		
																								P	M
CONTROL	A	21.7	20	8.3	7.9	-	-	-	21.9	20	8.1	7.9	21.0	-	-	-	22.1	22	8.2	8.2	22.1	20	8.1	7.5	
	B																								
10mg/l	A	21.7	20	8.4	7.8	-	-	-	21.8	21	8.2	7.2	21.1	-	-	-	22.1	22	8.2	8.3	22.0	21	8.1	7.6	
	B																								
100mg/l	A	21.6	21	8.4	7.8	-	-	-	21.7	21	8.2	7.0	21.1	-	-	-	22.1	22	8.2	8.3	21.8	21	8.2	7.6	
	B																								
500mg/l	A	21.6	21	8.5	7.8	-	-	-	21.7	21	8.2	7.1	21.0	-	-	-	-	-	-	-	-	-	-	-	
	B																								
1000mg/l	A	21.6	21	8.7	7.8	-	-	-	21.7	22	8.5	7.3	21.0	-	-	-	-	-	-	-	-	-	-		
	B																								
Instrument/Method:	FS-4	REF-6	SH-25F	DO-1	-	-	-	-	FS-4	REF-5	SH-25F	DO-1	FS-2	REF-5	SH-25F	DO-1	FS-2	REF-5	SH-25F	DO-1	FS-2	REF-5	SH-25F	DO-1	

KEY: TEMP - Temperature DO - Dissolved Oxygen
 SL - Salinity ALK - Alkalinity
 HD - Hardness COND - Conductivity

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 Effective: APR 1993

DILUTER CALIBRATION	
Sponsor: <u>CMA</u>	Test Substance: <u>Sodium Chlorite</u>
Project Number: <u>3943014-0100-3140</u>	Test Species: <u>M. bahia + C. variegatus</u>
Date Calibrated: <u>3/11/94</u>	Calibrated By: <u>NZT</u>
Diluter ID Number: <u>5</u>	Location: <u>In-house</u>
Calibrated for a <u>50</u> % diluter.	

Cell ID	Desired Cell Volumes (mL)		Measured Cell Volumes (mL)	
	Water	Chemical	Water	Chemical
Control	500	0	500.0, 496.0, 498.0	0
6.25%	468.75	31.25	465.0, 470.0, 470.0	31.5, 32.0, 32.0
12.5%	437.5	62.5	435.0, 438.0, 437.0	62.0, 61.5, 62.5
25%	375	125	370.0, 375.0, 375.0	124.0, 125.0, 125.0
50%	250	250	250.0, 248.0, 250.0	253.0, 250.0, 252.0
100%	0	500	0	500.0, 505.0, 502.0

SPLITTER BOX DELIVERY CALIBRATION							
Splitter Box ID	Control	0.125m/h	0.25m/h	0.5m/h	1.0m/h	2.0m/h	
Replicate A Volume (mL)	250.0	248.0	250.0	252.0	248.0	260.0	
	252.0	250.0	253.0	265.0	252.0	255.0	
	255.0	252.0	255.0	255.0	245.0	250.0	
Replicate B Volume (mL)	245.0	255.0	235.0	250.0	260.0	265.0	
	250.0	253.0	252.0	250.0	255.0	250.0	
	250.0	250.0	250.0	252.0	255.0	260.0	

Comments: _____

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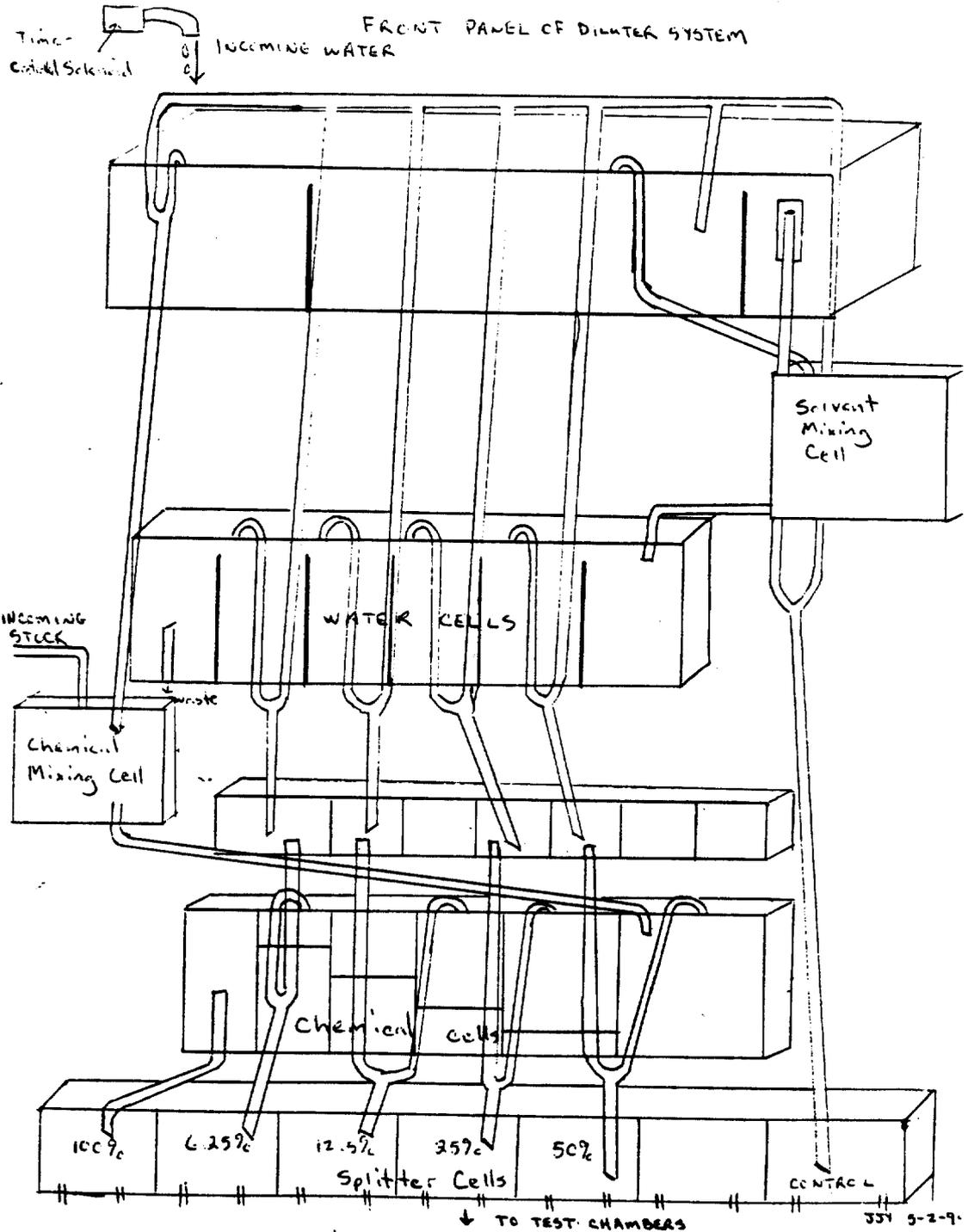
DILUTER CALIBRATION	
Sponsor: <u>CMA</u>	Test Substance: <u>Sodium Chlorite</u>
Project Number: <u>3943014-0100-3140</u>	Test Species: <u>C. variegatus & m. bahia</u>
Date Calibrated: <u>4/01/94</u>	Calibrated By: <u>JK</u>
Diluter ID Number: <u>5</u>	Location: <u>In-house</u>
Calibrated for a <u>50</u> % diluter.	

Cell ID	Desired Cell Volumes (mL)		Measured Cell Volumes (mL)	
	Water	Chemical	Water	Chemical
<u>Control</u>	<u>500.0</u>	<u>-</u>	<u>498.0</u>	<u>-</u>
<u>0.125mg/L</u>	<u>468.75</u>	<u>31.25</u>	<u>474.0</u>	<u>31.0</u>
<u>0.250</u>	<u>437.50</u>	<u>62.5</u>	<u>443.0</u>	<u>66.0</u>
<u>0.500</u>	<u>375.0</u>	<u>125.0</u>	<u>380.0</u>	<u>130.0</u>
<u>1.00</u>	<u>250.0</u>	<u>250.0</u>	<u>251.0</u>	<u>250.0</u>
<u>2.00</u>	<u>-</u>	<u>500.0</u>	<u>-</u>	<u>510.0</u>

SPLITTER BOX DELIVERY CALIBRATION						
Splitter Box ID						
Replicate A Volume ()						
Replicate B Volume ()						

Comments: _____
* Post-calibration check of diluter

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 Effective: APR 1993

ACUTE TOXICITY DATA SHEET-BIOLOGICAL DATA	
SPONSOR: <u>CMA</u>	PROJECT NUMBER: <u>3943014-0100-3140</u>
TEST SUBSTANCE: <u>Sodium chlorite</u>	TEST SPECIES: <u>C. variegatus</u>

ANIMAL HISTORY	
Sample Information and Description Found on Page <u>532</u> of Test Substance Log	
Lot No. / Batch No.: <u>94-16</u> (Circle One)	Date Maintenance/Acclimation Began: <u>2/15/94</u>
See Page No.: <u>58</u> of Animal Collection/ Receipt Log	Condition of Animals: <u>Normal</u>
See Page No.: <u>86</u> of <u>Fish Holding Log</u> For Raw Data on Animal Holding	Life Stage/Age: <u>Juvenile (78 days)</u>
	Mortality in Tank 48 Hours Prior To Test Initiation: <u>0</u> (%)

TEST CONDITIONS			
<input type="checkbox"/> Range-Finding	<input type="checkbox"/> Static	Time Added Substance/Animals <i>Flow through</i> / 1130	Test Location Diluter S
<input checked="" type="checkbox"/> Definitive	<input checked="" type="checkbox"/> Flow-Through		
Test System: <input checked="" type="checkbox"/> Covered <input type="checkbox"/> Open	Test Container Dimensions (cm) L 28 W 25 H 15	Test Solution Height: 7.5 cm	Test Container Composition: Glass
Test Chamber Volume (L) 0.34, 1.6, 3.8, 30 - <u>10.5</u>		Test Solution Volume (L) 0.20, 1.0, 3.0, 10 - <u>5</u>	

Protocol: Acute toxicity of sodium chlorite to the sheepshead minnow (*Cyprinodon variegatus*)
 Dilution Water: Natural Filtered Seawater Type of Lighting: Fluorescent Photoperiod: (6 hr. L : 8 hr. D)

Cycle One: Test Substance as Active Ingredient/Whole Material									
Test Concentration % (mg/L ug/L)	Con- trol	Solv Cont	12.5	25	50	100	200	Solvent/ Carrier: <u>Natural filtered Seawater</u>	
Amount of Substance/ Stock Added ()	NA	NA			<u>see</u>	<u>diluter</u>			Solvent/ Carrier Concen- traion: <u>NA</u>
Stock Solution Used (e.g., 1', 2')	NA	NA			<u>calibration sheet</u>				
Amount of Dilution Water Added ()		NA							
Amount of Solvent Added ()	NA	NA	NA	NA	NA	NA	NA		

Analyst: ms Date: 3/21/94 Comments in Daily Log

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 ESE QA Form No.: 015B
 Effective: MAR 1993

Proj. # 3943014-0100-3140

C. variegatus

SUBJECT: ACUTE TOXICITY DATA SHEET - SURVIVAL											
		0-HOUR		24-HOUR		48-HOUR		72-HOUR		96-HOUR	
DATE		3/21/94		3/22/94		3/23/94		3/24/94		3/25/94	
ANIMALS FED		No		No		No		No		No	
TIME		1130		1115		1030		1130		1030	
DATA BY		MO		MO		MO		MO		MO	
NOMINAL CONCENTRATION % (mg/L) ug/L	R E P	NO.	NO.	NO.	NO.	NO.	NO.	NO.	NO.	NO.	NO.
		OBS.	ALIVE	OBS.	ALIVE	OBS.	ALIVE	OBS.	ALIVE	OBS.	ALIVE
CONTROL	A	None	10	None	10	None	10	None	10	None	10
	B	↓	10	↓	10	↓	10	↓	10	↓	10
12.5	A	None	10	None	10	None	10	None	10	None	10
	B	↓	10	↓	10	↓	10	↓	10	↓	10
25	A	None	10	None	10	None	10	None	10	None	10
	B	↓	10	1 Dead	9	↓	9	↓	9	↓	9
50	A	None	10	1 Dead	9	None	9	None	9	None	9
	B	↓	10	None	10	1 Dead 1 leth	9	1 dead	8	↓	8
100	A	None	10	4 DEAD	6	3 Dead 3 leth	3	1 Dead	2	2 leth	2
	B	↓	10	8 DEAD	2	1 Dead 1 leth	1	1 Dead	0	-	-
200	A	None	10	10 DEAD	0	-	-	-	-	-	-
	B	↓	10	10 DEAD	0	-	-	-	-	-	-

OBSERVATION KEY

OBS - OBSERVATIONS	DRK - DARKER THAN CONTROLS	NF - NOT FOUND
NONE - NORMAL CONDITIONS	LT - LIGHTER THAN CONTROLS	
AS - AT SURFACE	RAR - RAPID RESPIRATION	
LE - LOSS OF EQUILIBRIUM	PRE - PRECIPITATE	BS - BRINE SHRIMP
LETH - LETHARGIC	FOS - FILM ON SURFACE	
HYP - HYPERACTIVE	UC - UNDISSOLVED CHEMICAL	

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 ESE QA FORM: 010
 EFFECTIVE: JUNE 1990

SUBJECT: ACUTE TOXICITY DATA SHEET--WATER QUALITY DATA																							
SPONSOR: <u>CMA</u>					PROJECT NUMBER: <u>3943014-0100-3140</u>																		
TEST SUBSTANCE: <u>Sodium chlorite</u>										TEST SPECIES: <u>C. variegatus</u>													
DATE	3/21/94				3/22/94				3/23/94				3/24/94				3/25/94						
TIME	1045				1045				1000				1115				1000						
DATA BY	CN				MO				MO				MO				MO						
NOMINAL CONCENTRATION mg/L ug/L	REP	TEMP		DO	ALK	COND	TEMP		DO	ALK	COND	TEMP		DO	ALK	COND	TEMP		DO	ALK	COND		
		SL	HD				SL	HD				SL	HD				SL	HD					
CONTROL	A	22.2	21	8.1	8.2	-	-	22.2	21	8.3	7.6	22.6	21	8.3	7.4	21.6	21	8.3	7.8	22.1	21	8.3	7.6
	B	21.8	21	8.1	8.2	-	-	22.3	21	8.3	7.7	22.6	21	8.3	7.4	21.8	21	8.3	7.8	22.1	21	8.3	7.6
12.5	A	22.2	21	8.1	7.9	-	-	22.3	21	8.3	7.5	22.6	21	8.3	7.4	21.6	21	8.3	7.8	22.1	21	8.3	7.6
	B	22.2	21	8.2	8.2	-	-	22.3	21	8.3	7.6	22.6	21	8.3	7.5	21.8	21	8.3	7.9	22.1	21	8.3	7.6
25	A	22.2	21	8.2	8.1	-	-	22.3	21	8.3	7.7	22.7	21	8.3	7.3	21.9	21	8.3	7.8	22.1	21	8.3	7.6
	B	22.2	21	8.2	8.1	-	-	22.3	21	8.4	7.6	22.6	21	8.3	7.5	21.8	21	8.3	7.9	22.2	21	8.3	7.7
50	A	22.2	21	8.2	8.1	-	-	22.3	21	8.4	7.5	22.6	21	8.3	7.4	21.9	21	8.3	7.8	22.1	21	8.3	7.6
	B	22.2	21	8.2	8.1	-	-	22.3	21	8.4	7.5	22.6	21	8.3	7.4	21.8	21	8.3	7.9	22.1	21	8.3	7.6
100	A	22.2	21	8.2	8.2	-	-	22.3	21	8.4	7.5	22.7	21	8.4	7.4	22.0	21	8.4	8.0	22.2	21	8.4	7.7
	B	22.2	21	8.2	8.2	-	-	22.3	21	8.4	7.4	22.6	21	8.4	7.5	21.9	21	8.4	8.1	22.1	21	8.4	7.6
200	A	22.2	21	8.3	8.1	-	-	22.3	21	8.4	7.6	-	-	-	-	-	-	-	-	-	-	-	-
	B	22.2	21	8.3	8.1	-	-	22.3	21	8.4	7.6	-	-	-	-	-	-	-	-	-	-	-	-

Instrument/Method: FS 2 5 SA 250 1 DO 1 FS 2 5 SA 250 1 DO 1

KEY: TEMP - Temperature DO - Dissolved Oxygen
 SL - Salinity ALK - Alkalinity
 HD - Hardness COND - Conductivity

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Page _____

72h 96-h LC50 Calculations

Results calculated using the Probit Method.

Client : CMA Species : C. variegatus

Dates of test : 3/21/94 to 3/25/94

Test Material : Sodium Chlorite Project # 3943014-0100-3141

Report run by : JJY Date : 03-29-1994

Concentration units (Circle One) : ‡ PPTH PPM PPB Other _____

Conc.	Number Exposed	Number Dead	Percent Dead
229.0	20	20	100.0
112.0	20	18	90.0
59.5	20	3	15.0
30.0	20	1	5.0
13.9	20	0	0.0
Control	20	0	0.0

Iterations	G	H	Chi-Square	Probability
5	0.158	1.00	5.3245	0.15

95% confidence limits

Slope =	5.8320	3.5161	8.1479
LC50 =	75.0258	62.6039	89.8320
LC1 =	29.9451	15.7494	40.3323

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VULCAN CHEMICALS R&D
ANALYTICAL REPORT

DATE: 03/18/94
PAGE NO: 1

SAMPLE NO.	PROJ. NO.	COMPONENT	RESULTS	ANALYST	COMMENTS

os#5064	60	Vulcan Chemical NaClO2 (Same drum as Submitted By: BCC			

		NaClO2	81.41%	SLG	Titration w/ Na2S2O3
		NaCl	10.621%	SLG	Titration w/ AgNO3
		Na2CO3	0.504%	SLG	Titration w/ H2SO4
		Na2SO4	0.364%	DMM	IC, 89.3% spike recovery
		H2O2	0.003%	SLG	Titration w/ NaOBr
		water by di	6.156%	DMM	By difference
		Pb	< 0.5 ppm	DMM	GF, 88.4% spike recovery
		NaOH	0.028%	SLG	Titration w/ H2SO4 w/ BaCl2
		NaClO3	0.914%	DMM	IC, 101.3% spike recovery
		Hg	< 65 ppb	SLG	MHS, 89.0% spike recovery
		As	< 1.3 ppm	DMM	MHS-20, 89.6% spike recovery

END OF REPORT

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CMA #: CD-7.0-3AT-ESE

Appendix D- Analytical Method Validation

CONFIDENTIAL
SODIUM CHLORITE CVARIEGATUS
ESE PROJECT #: 3943014-0100-3140
CMA #: CD-7.0-3AT-ESE

FINAL REPORT

STUDY TITLE

Method Validation for the Determination
of Sodium Chlorite in Seawater

AUTHOR

David H. Greer, Jr.

STUDY COMPLETED

May 18, 1994

REPORT DATE

May 18, 1994

SPONSOR

Chemical Manufacturers Association
2501 M Street N.W.
Washington, D.C. 20037

PERFORMING LABORATORY

Environmental Science & Engineering, Inc.
14220 Newberry Road
Gainesville, Florida 32607
(904) 332-3318

LABORATORY STUDY NUMBER

1944015V-L202

LABORATORY PROTOCOL NUMBER

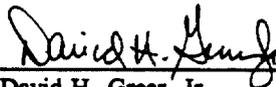
ASG-94-02

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SODIUM CHLORITE CVARIEGATUS
ESE PROJECT #: 3943014-0100-3140
CMA #: CD-7.0-3AT-ESE

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COMPLIANCE STATEMENT

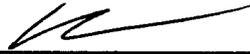
The data presented in this report was an accurate representation of the investigations conducted. This study was conducted in compliance with current Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Good Laboratory Practice Regulations (40 CFR Part 160). All records pertaining to this study were maintained at Chemical Manufacturers Association GLP archives located at 2501 M Street, N.W., Washington, D.C. 20037. A copy of the original raw data will be archived in the ESE Gainesville Analytical Services GLP archives located at 14220 West Newberry Road, Gainesville, Florida 32607.



David H. Greer, Jr.,
Program Manager, Study Director
ESE, Inc.

5/18/94

Date



Kathleen K. Allen
Water Quality Department Manager
ESE, Inc.

5/18/94

Date



Robert Romano, Ph.D.
Director of Special Programs
Chemical Manufacturers Association

5/19/94

Date

QUALITY ASSURANCE STATEMENT

This study was monitored by Environmental Science & Engineering, Inc. (ESE) Quality Assurance Unit to assure that the guidelines and Standard Operating Procedures (SOPs) used to conduct this study were followed.

The final report for this study was reviewed by the Quality Assurance Unit and, accurately reflects the results of the study as conducted, including all areas of compliance or noncompliance with Good Laboratory Practice Standards, the study protocol and ESE's SOPs.

Joe Owusu-Yaw, Ph.D.
Joe Owusu-Yaw
QAU GLP Coordinator

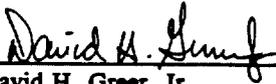
5-18-94
Date

QUALITY ASSURANCE AUDITS

<u>Audit Date</u>	<u>Type of Report</u>	<u>Date Reported to Management</u>	<u>Date Reported Study Director</u>
2/25/94	Protocol	5/18/94	2/25/94
5/16/94	Data Review	5/18/94	5/16/94
5/16/94	Draft Report	5/18/94	5/16/94
5/17/94	Revised Report	5/18/94	5/17/94
5/18/94	Final Report	5/18/94	5/18/94

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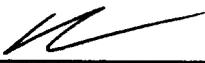
REPORT SIGNATURES



David H. Greer, Jr.,
Program Manager, Study Director
ESE, Inc.

5/18/94

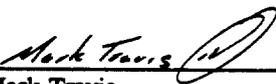
Date



Kathleen K. Allen
Water Quality Department Manager
ESE, Inc.

5/18/94

Date



Mark Travis
Water Quality Analyst
ESE, Inc.

5/18/94

Date

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CMA #: CD-7.0-3AT-ESE

STUDY SUMMARY

SPONSOR: Chemical Manufacturers Association
2501 M Street, N.W.
Washington, D.C. 20037
Sponsor Representative: Robert Romero, Ph.D.
Director of Special Programs
(202) 887-1198

SPONSOR STUDY NUMBER: ASG-94-02 .

STUDY DIRECTOR: David H. Greer, Jr.
Environmental Science & Engineering, Inc.
14220 West Newberry Road
Gainesville, Florida 32607
(904) 333-1608

STUDY TITLE: Method Validation for the Determination of
Sodium Chlorite in Seawater

LOCATION OF STUDY: Environmental Science & Engineering, Inc.
14220 West Newberry Road
Gainesville, Florida 32602-1703

LABORATORY STUDY IDENTIFICATION: 1944015V-L202

TEST MATERIAL: Sodium Chlorite - CAS No.: 7758-19-2

STUDY INITIATION DATE: March 2, 1994

EXPERIMENTAL INITIATION DATE: March 2, 1994

EXPERIMENTAL COMPLETION DATE: March 18, 1994

STUDY COMPLETION DATE: May 18, 1994

DETERMINATION OF SODIUM CHLORITE IN SEAWATER

1.0 SCOPE AND APPLICATION

This method describes the analysis of Sodium Chlorite at the milligram per liter (mg/L) level in seawater.

2.0 SUMMARY OF METHOD

The reference analytical method used for this validation study was "The Measurement of Chlorite Ion in Seawater by IC Using the Chlorpromazine Post Column Reaction" [NOVATEK Application Note for the Chemical Manufacturers Association (Contract CD-6.0-NOV, Appendix D)]. This method is based on an ion chromatography post-column procedure using USEPA Method 300.0 with a borate buffer eluent. The post-column procedure uses photometric detection at 530 nanometers (nm). Following separation, chlorite (ClO_2^-) reacts with chlorpromazine in acidic media. This detection chemistry shows no interference from most common anions.

3.0 APPARATUS AND MATERIALS

- 3.1 Ion Chromatography System: Dionex IC Model DX-300.
- 3.2 Analytical Column: Dionex AS11.
- 3.3 Post Column Derivatization: Technicon II reagent manifold and colorimeter.
- 3.4 Data system: Dionex AI 450.
- 3.5 Volumetric Flasks: Various.
- 3.6 Analytical Balance: Mettler AE160.
- 3.7 Test Materials: Sodium Chlorite (Aldrich Lot # 01615KY and #05801JZ, 80% purity and NOVATEK, 99.75% purity).
- 3.8 Boric Acid: Aldrich lot# 05625MY, ACS reagent grade.
- 3.9 Sodium Hydroxide: Fisher lot# 9338003-24, 50/50 (w/w) solution.
- 3.10 Eluent: Borate eluent, 25 millimolar (mM) NaOH/50 mM H_3BO_3 .

- 3.11 Regenerant: Supressor Regenerant, 12.5 mM H₂SO₄.
- 3.12 Water: Deionized (D.I.) water.
- 3.13 Seawater Test Solution: Ethylenediamine, 50 mg/L in full strength natural seawater.
- 3.14 Derivatization Reagent: Chlorpromazine hydrochloride (Sigma Lot# 112H0125), 0.56 mM in D.I. water.
- 3.15 Hydrochloric Acid Solution: 350 mL concentrated hydrochloric acid in 2 L D.I. water.

4.0 ELUENT PREPARATION

The eluent was prepared by dissolving 12.4 grams (g) of boric acid into 1 liter of D.I. water in a 2 liter volumetric flask. To the boric acid solution, 2.6 milliliters (mL) of 50/50 (w/w) sodium hydroxide solution was added, and the flask brought to volume with D.I. water. The contents of the flask were mixed thoroughly by inverting several times.

5.0 DERIVATIZATION REAGENT PREPARATION

The derivatization reagent was prepared by dissolving 0.2 g of chlorpromazine hydrochloride in 1 L of D.I. water. This solution was degassed with nitrogen prior to use.

6.0 STANDARD PREPARATION

6.1 PREPARATION OF CALIBRATION STOCK STANDARD

A calibration stock (spiking stock) was prepared by accurately weighing 1.676 g of sodium chlorite (80 %purity, Aldrich lot # 05801JZ) into a 1,000 mL volumetric flask and bringing to volume with deionized water. The solution was mixed thoroughly by inverting the stoppered flask several times. The concentration of this stock solution was 1000 mg-chlorite/L. This solution was used to prepare the instrument calibration standards.

6.2 PREPARATION OF CONTROL STOCK STANDARD

A control stock standard was prepared by accurately weighing 1.676 g of sodium chlorite (80 % percent purity, Aldrich lot # 01615KY) into a 1000 mL volumetric flask. The flask was brought to volume with D.I. water and mixed thoroughly by inverting the stoppered flask several times. The concentration of this stock was 1000 mg-chlorite/L. This solution was used to prepare the fortified samples for the method validation.

6.3 PREPARATION OF CALIBRATION VERIFICATION STOCK

An independent calibration verification stock (ICV) standard was prepared by accurately weighing 0.1676 g of sodium chlorite (99.75 % purity, NOVATEK) into a 100 mL volumetric flask. The flask was brought to volume with D.I. water and mixed thoroughly by inverting the stoppered flask several times. The concentration of this stock was 1250 mg-chlorite/L. This standard was used to verify the preparation of the calibration and control stocks.

6.4 STANDARDIZATION OF CHLORITE STOCKS

All Chlorite stock solutions were standardized using the iodometric procedure (Standard Methods, 15th Edition, Method 410A). Twenty five milliliter (mL) of each stock solution was volumetrically transferred to separate 125 mL erlenmeyer flask and 0.5 g of potassium iodine (KI) was added to the flask and dissolved. After the KI was dissolved, 0.5 mL of concentrated sulfuric acid was added and the flask was stoppered and placed in the dark for 5 minutes. Each flask was titrated with 0.1 N thiosulfate, adding 1 mL of starch indicator solution when the brown color became light yellow. Titration was continued until the solution became clear.

6.5 PREPARATION OF WORKING CALIBRATION STANDARDS

One mL of the 1000 mg/L calibration stock was diluted with D.I. water to 10 mL in a 10 mL volumetric flask. The concentration of this intermediate calibration stock was 100 mg/L. A series of 7 dilutions of the calibration stock were prepared for use in instrument calibration. The following dilution schedule was used to prepare the calibration standards.

Intermediate Chlorite Calibration Stock (mL) (100 mg/L)	Final Volume (mL D.I. water)	Calibration Standard Concentration (mg/L)
0.02	100	0.02
0.05	100	0.05
0.10	100	0.10
0.20	100	0.20
0.40	100	0.40
1.00	100	1.00
2.00	100	2.00

7.0 PREPARATION OF FORTIFICATION SOLUTIONS

7.1 SECONDARY FORTIFICATION SOLUTION

A secondary fortification solution was prepared by transferring 1 mL of the control stock into a 100 mL volumetric flask. The flask was brought to volume with D.I. water and inverted several times to mix thoroughly. The concentration of this solution was 10 mg-chlorite/L.

7.2 PREPARATION OF FORTIFICATION SAMPLES

Low level fortification samples, 0.1 mg-chlorite/L, were prepared by transferring 100 microliters of the secondary fortification solution (10 mg-chlorite/L) to a 10 mL volumetric flask. The flask was brought to volume with filtered natural seawater fortified with 50 mg/L of ethylenediamine. The fortification samples were prepared in triplicate and mixed thoroughly by inverting the stoppered flask several times.

High level fortification samples, 250 mg-chlorite/L, were prepared by diluting 1 mL of the control stock (1000 mg-chlorite/L) with 3 mL of filtered natural seawater fortified with 50 mg/L ethylenediamine. The fortification samples were prepared in triplicate and mixed thoroughly by inverting the stoppered flask several times.

8.0 SAMPLE ANALYSIS

8.1 INSTRUMENT OPERATING CONDITIONS

The following instrumental conditions were used during analysis of test samples.

System I.D.	IC Model DX-300, #1
Separation Column	AS11 #1700
Suppression	AMMSII #8909
Injection Loop	50 μ L
Flow Rate	1.0 mL/min
Pressure	680 psig
Offset	5.1 micro-Siemens (μ S)
Eluent	12.5 mM NaOH/25 mM H ₃ BO ₃
Regenerant	25 mN H ₂ SO ₄

The configuration of the post column derivatization and visible detection system is presented as Figure 8-1.

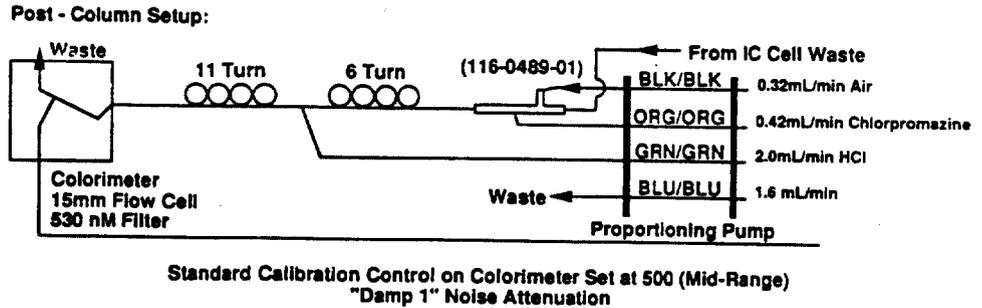


Figure 8-1 Post Column Derivatization/Visible Detection System Configuration

8.2 INSTRUMENT CALIBRATION AND SAMPLE ANALYSIS

- 8.2.1 After the instrument had warmed up and stabilized, the column was 'primed' with injection of the 2.0 mg/L calibration standard followed by an injection of D.I water.
- 8.2.2 Each calibration standard, beginning with a D.I. water blank was injected in order of increasing chlorite concentration.
- 8.2.3 A 1.25 mg/L instrument calibration verification standard (ICV) was analyzed to confirm that instrument calibration standard preparation was correct.
- 8.2.4 After calibration was complete, each set of fortified test samples were injected beginning with a seawater blank. Seawater samples were diluted by a factor of 4 to prevent overload of the column and subsequent retention time shifts. Dilution factors of 4 were used for the low level fortification samples and 250 was used for the high level fortification samples.
- 8.2.5 After all sample analyses were complete, a 1.0 mg/L continuing calibration verification standard (CCV) was analyzed to verify instrument stability throughout the analytical run.

9.0 QUANTIFICATION

- 9.1 A quadratic regression curve of Chlorite concentration versus instrument response, expressed as peak area, was generated from the data obtained during instrument calibration. The regression was used to calculate concentrations of Chlorite in samples and quality control fortifications by substitution of peak areas found in samples and quality control fortifications into the regression equation generated from the calibration data.
- 9.2 Recoveries of Chlorite from the fortification test samples were calculated by comparing the calculated found concentrations in the fortified samples with the nominal fortification levels. Simple arithmetic means and standard deviations for the method validation fortification levels were calculated and used to generate the quality control limits for the toxicity test sample determinations.

10.0 DATA ACCEPTABILITY

- 10.1 Calibrations were considered acceptable if the correlation coefficient of the curve was greater than or equal to 0.995.
- 10.2 Continuing calibration standard response (post-run) agreed within plus or minus 10 percent of the original analysis response for that standard.

- 10.3 Quality control limits were established using results obtained during the method validation study. Warning limits were set at plus or minus 2 standard deviations from the mean of all recovery values. Control limits were set at plus or minus 3 standard deviations from the mean.

11.0 SAFETY

All reagents and test materials should be handled with care to avoid unnecessary exposure of any personnel to these potentially toxic materials. Material Safety Data Sheets should be read by all personnel involved with the determination of Chlorite. Avoid skin contact or ingestion of Chlorite.

12.0 METHOD VALIDATION RESULTS

- Recoveries for fortified samples ranged from 99.8 to 109 percent. The calculated mean recovery was 103.5 percent with a standard deviation of 3.7. Result for individual fortification samples are presented in Table 12-1. The reporting limit for chlorite using this method was 0.08 mg/L in seawater.

Low level fortification samples were analyzed undiluted. Due to column overload, there was no recovery of chlorite from the undiluted samples. There were no significant interferences noted during the analysis of chlorite when utilizing the post column derivatization and visible detection system proceeded by separation of chlorite by ion chromatography.

Example chromatograms for standards and fortification samples depicting both the ion chromatographic conductivity and post column derivatization visible detection systems are presented as Figures 12-1 through 12-4.

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Table 12-1 Fortification Results of Chlorite Analytical Method Validation

Fortification Level	Target Conc. (mg/L)	Found Conc. (mg/L)	Percent Recovery
Low SPK #1	0.10	0.109	109
Low SPK #2	0.10	0.107	107
Low SPK #3	0.10	0.103	103
High SPK #1	250.00	249.4	99.8
High SPK #2	250.00	251.1	100.4
High SPK #3	250.00	254.9	102
		Mean	103.5
		Std Dev	3.7

Std Dev = Standard Deviation

Source: ESE, 1994

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Area Report

Data Reprocessed On 03/09/1994 10:08:17

```

-----
| Sample Name: AUTOCAL7R - A10           Date: 03/08/1994 16:38:39
| Data File : C:\DX\DATA4\ch030801.d10
| Method    : C:\DX\METHOD\CLO2.met
| ACI Address: 1 System: 1 Inject#: 10 Vial:A10   Detector:CDM-2
| Analyst   :                               Column:
-----
  
```

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area	Reject
External	50	1	2280	2Hz	0.00	18.99	500	

***** Peak Report: All Peaks *****

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
1	1.25			0.000	5484475	0.000	1
2	3.00	0.00	CLO2	1.000	11585140	0.000	1
3	5.20			0.000	4040705	0.000	1
Totals				1.000	21110320		

File: ch030801.d10 Sample: AUTOCAL7R - A10

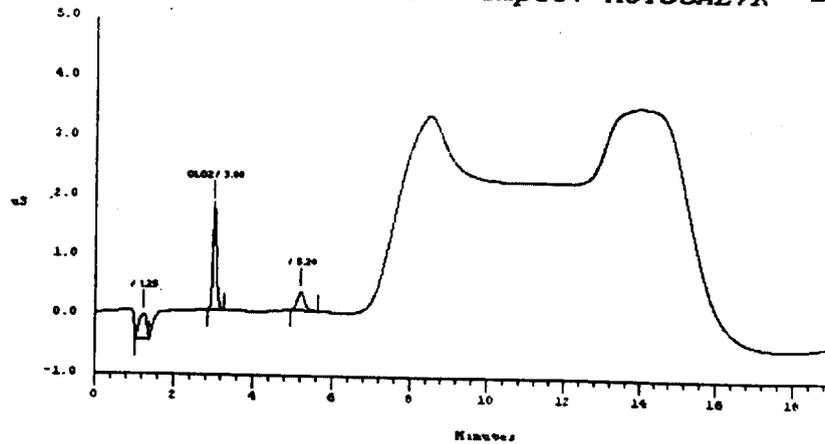


Figure 12-1 Example Chromatogram of Chlorite Standard (1.0 mg/L) - Conductivity Detection.

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Area Report

Data Reprocessed On 03/09/1994 10:09:25

```

-----
Sample Name: SP1*QC*3 - A23           Date: 03/08/1994 21:06:34
Data File  : C:\DX\DATA4\ch030801.d23
Method     : C:\DX\METHOD\CLO2.met
ACI Address: 1 System: 1 Inject#: 23 Vial:A23   Detector:CDM-2
Analyst    :                          Column:
-----

```

```

-----
Calibration Volume Dilution Points Rate Start Stop Area Reject
-----
External           50           250  2280  2Hz  0.00 16.99      500
-----

```

***** Peak Report: All Peaks *****

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
1	1.25			0.000	5052670	0.000	1
2	3.03	1.11	CLO2	255.740	11863418	0.000	1
3	4.98			0.000	1656239100	0.000	1
Totals				255.740	1673155188		

File: ch030801.d23 Sample: SP1*QC*3 - A23

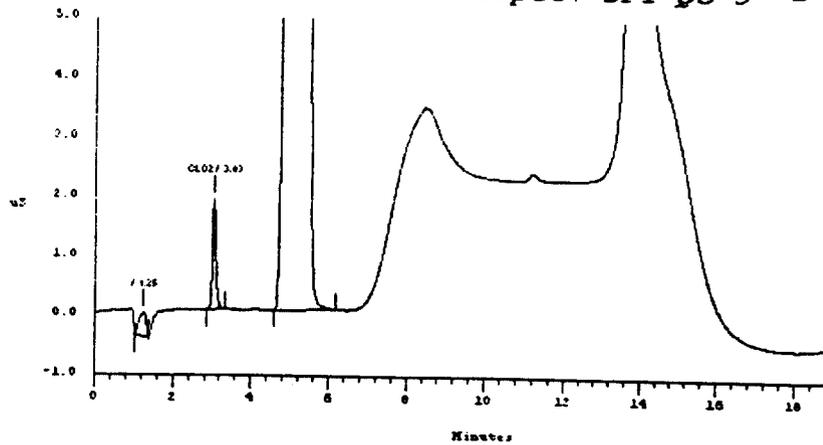


Figure 12-2 Example Chromatogram of Chlorite Seawater Fortification (250 mg/L) - Conductivity Detection.

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Area Report

Data Reprocessed On 03/09/1994 10:39:43

```

-----
: Sample Name: AUTOCAL7R - A10           Date: 03/08/1994 16:38:39
: Data File  : C:\DX\DATA4\ch030802.d10
: Method     : C:\DX\METHOD\CLO2.me2
: ACI Address: 1 System: 1 Inject#: 10 Vial:A10   Detector:OTHER
: Analyst    :                               Column:
-----
  
```

```

-----
Calibration Volume Dilution Points Rate Start Stop Area Reject
External          50           1 2280 2Hz 0.00 18.99      100
-----
  
```

***** Component Report: All Components *****

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
1	4.52	0.00	50074*IC-6 CLO2	1.000	34796	0.000	1
Totals				1.000	34796		

***** Peak Report: Unknown Peaks *****

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
Totals				0.000	0		

File: ch030802.d10 Sample: AUTOCAL7R - A10

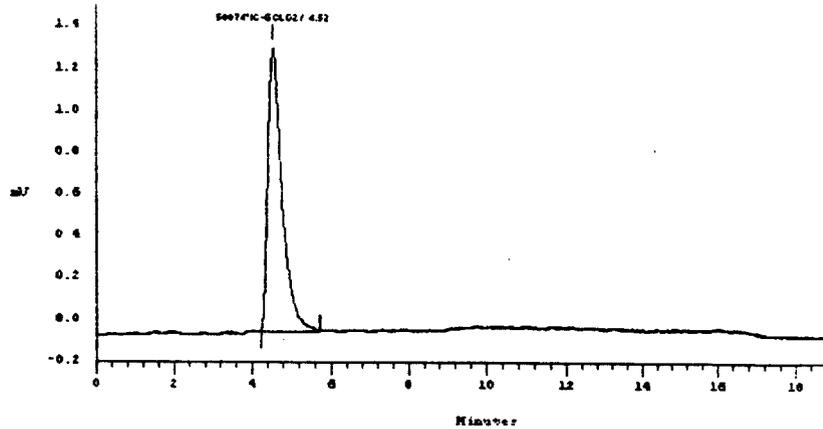


Figure 12-3 Example Chromatogram of Chlorite Seawater Fortification (250 mg/L) - Visible Detection.

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CMA #: CD-7.0-3AT-ESE

Area Report

Data Reprocessed On 03/09/1994 10:41:09

```

-----
: Sample Name: SP1*QC*3 - A23                               Date: 03/08/1994 21:08:34
: Data File  : C:\DX\DATA4\ch030802.d23
: Method     : C:\DX\METHOD\CLO2.me2
: ACI Address: 1 System: 1 Inject#: 23 Vial:A23             Detector:OTHER
: Analyst    :                                             Column:
-----
  
```

```

-----
Calibration Volume Dilution Points Rate Start Stop Area Reject
-----
External          50          250    2280 2Hz  0.00 18.99          100
-----
  
```

***** Component Report: All Components *****

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
2	4.55	1.11	50074*IC-G CLO2	249.858	34547	0.000	1
Totals				249.858	34547		

***** Peak Report: Unknown Peaks *****

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
1	1.87			0.000	776	0.000	1
Totals				0.000	776		

File: ch030802.d23 Sample: SP1*QC*3 - A23

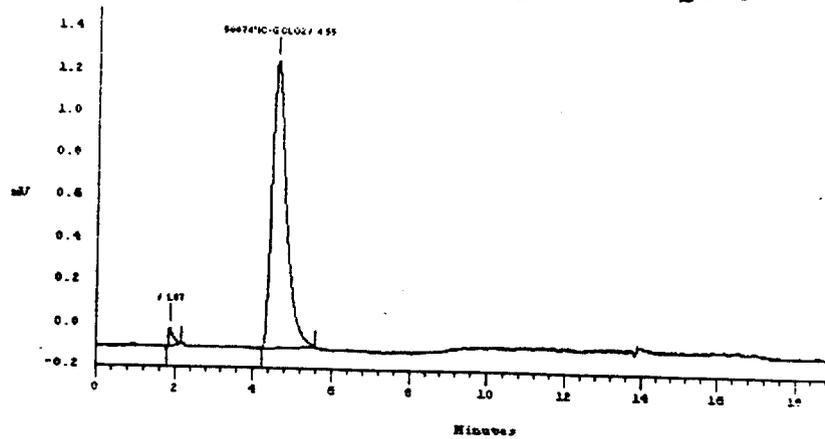


Figure 12-4 Example Chromatogram of Chlorite Standard (1.0 mg/L) - Visible Detection.

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APPENDIX I
PROTOCOL

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ESE PROTOCOL NO.: ASG-94-02

ENVIRONMENTAL SCIENCE & ENGINEERING, INC.

ANALYTICAL SERVICES

METHOD VALIDATION PROTOCOL

STUDY TITLE

Method Validation for the Determination of Sodium Chlorite in Seawater

TEST SUBSTANCE IDENTIFICATION

Sodium Chlorite

CAS No.: 7758-19-2

SPONSOR

Chemical Manufacturers Association

Sponsor Representative: Mr. Robert Romano, Ph.D.
Director of Special Programs
Chemical Manufacturers Association
2501 M. Street N.W.
Washington, D.C. 20037
(202) 887-1198

Study Number: Not Assigned

TEST FACILITY

Environmental Science & Engineering, Inc. (ESE)
Analytical Services
14220 Newberry Road
Gainesville, Florida 32607

ESE Study Director: David H. Greer, Jr.
GLP Program Manager
(904) 333-1608
(904) 333-6622 FAX

ESE PROTOCOL NO.: ASG-94-02

PROPOSED EXPERIMENTAL START DATE

Upon acceptance of the protocol by the study sponsor. The proposed experimental start date for this method validation study is February 16, 1994.

PROPOSED EXPERIMENTAL TERMINATION DATE

March 10, 1994.

OBJECTIVE

To validate an analytical method for the determination of sodium chlorite in seawater utilizing ion chromatography with visible (530 nanometers) light detection in support of aquatic toxicity studies.

REFERENCE MATERIALS

1. Sodium Chlorite, 80% purity (Aldrich Chemical Co. Lot # 0161SKY)

METHODS

The analytical method used for this validation study will be "The Measurement of Chlorite Ion in Seawater by IC Using the Chlorpromazine Post Column Reaction" (Novatek Application Note for the Chemical Manufacturers Association (Contract CD-6.0-NOV, Appendix I). Modifications to the method will be made only after consultation with the Study Sponsor Representative.

Analytical Method Validation

The analytical method validation will consist of the preparation of a blank seawater matrix, triplicate fortifications of seawater at the anticipated low test concentration (0.1 milligrams per liter (mg/L)), and triplicate fortifications of seawater at the anticipated high test concentration (250 mg/L). The samples will be analyzed according to the referenced method and the resulting data will be analyzed to determine the precision and accuracy of the method.

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Statistical Analysis

Recoveries of Chlorite from the analytical method validation fortification spike samples will be calculated by comparing the calculated found concentrations in the fortified samples with the nominal fortification levels. Simple means and standard deviations for the two fortification levels will be calculated and used to generate the quality control limits for field sample determinations.

Quality Assurance/Quality Control

Prior to each analytical sequence, a series of analytical calibration standards will be prepared, analyzed and used to determine the concentration of the target compounds in the test solutions.

Acceptance of the instrument calibration will depend upon the correlation coefficients for the standard curves. Calibration will be considered acceptable if the correlation coefficient of the calibration curve is greater than or equal to 0.995 using either linear or quadratic regression analysis.

At the end of each analytical sequence, a continuing calibration standard (CCS) will be analyzed to determine the instrument stability throughout the analytical sequence. The CCS must be within plus or minus 15 percent of the same concentration standard run at the beginning of the analytical sequence for the run to be considered valid.

The upper and lower warning and control limits for the analysis field samples will be set by mutual agreement between the Study Sponsor Representative and the ESE Study Director upon completion of the method validation study and review of the recoveries obtained during the method validation study.

REPORT

A method validation report will be prepared in U.S. Environmental Protection Agency PR Notice 86-5 format documenting all activities associated with the determination of Chlorite ion in seawater matrices. The report will contain a detailed write-up of the method for analysis of Chlorite, a data summary of the results of the method validation, raw data, a quality assurance statement and a Good Laboratory Practices compliance statement.

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SAFETY

Material Safety Data Sheets (MSDS) will be provided to all personnel involved in the study. All reagents and test materials will be handled in manners described in the ESE Laboratory Safety Manual. All chemical reagents and test materials will be handled with respect to their respective MSDS. It will be the responsibility of the various ESE Department Managers to impart the hazards associated with each reagent or test material to the personnel participating in this study.

GOOD LABORATORY PRACTICES

The study will be conducted using Good Laboratory Practices. All raw data generated in the study will be sent to and archived at the Chemical Manufacturers Association GLP archives located at 2501 M. Street, N.W., Washington, D.C. 20037. A copy of the original raw data will be archived in the ESE Analytical Services GLP archives located at 14220 Newberry Road, Gainesville, Florida. The Study Director will be responsible for developing the protocol and writing the report. The ESE Principal Investigator is responsible for the conduct of the study, and for providing the Study Director with a final report and all data generated. Additionally the ESE Principal Investigator will review the protocol, Good Laboratory Practices, materials and methods, and safety guidelines with all support personnel prior to their participation in the study. Any deviations from the protocol will be recorded and changes in plans will be discussed with the Study Director and the Study Sponsor Representative in advance, as well as being written and attached to this protocol as amendments.

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CMA #: CD-7.0-3AT-ESE

ESE PROTOCOL NO.: ASG-94-02

APPROVAL SIGNATURES

Sponsor Representative: Robert R. Romano Date Feb. 23, 1994
Robert Romano, Ph.D.
Director of Special Programs
Chemical Manufacturers Association

Study Director: David H. Greer, Jr. Date 3/2/94
David H. Greer, Jr.
GLP Program Manager
ESE

Principal Investigator: [Signature] Date 2/25/94
Kathleen K. Allen
Water Quality Department Manger
ESE

Quality Assurance: Portia O. Pisigan Date 3/2/94
Portia O. Pisigan
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ESE PROTOCOL NO.: ASG-94-02

APPENDIX I
ANALYTICAL METHOD

6

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June 1993

NOVATEK APPLICATION NOTE
FOR THE
CHEMICAL MANUFACTURERS ASSOCIATION
(Contract CD-6.0-NOV)

The Measurement of Chlorite Ion in Seawater by IC
Using the Chlorpromazine Post Column Reaction

SCOPE AND APPLICATION

This method incorporates a chlorpromazine reaction chemistry to measure chlorite ion (ClO_2^-) following the separation of anions by USEPA Method 300.0. This method can be used to measure chlorite ion from 0.05 to 1.0 mg/L in a seawater matrix. The method detection limit (MDL) was determined to be 0.04 mg/L chlorite ion.

SUMMARY OF THE METHOD

This method is an ion chromatography post-column procedure using USEPA Method 300.0 with a borate eluent. The post-column procedure uses photometric detection at 530 nm. Following separation, ClO_2^- reacts with chlorpromazine in acidic media by a charge transfer mechanism. This method overcomes common problems encountered in the ion chromatographic measurement of seawater samples from Cl^- and SO_4^{2-} . This detection chemistry shows no interference from all common anions with the exception of bromate ion and nitrite ion.

The sample (25 - 200 μL) is injected into the eluent at a flow rate of 1.0 mL/min. The sample passes through a metal free column (MFC) and a guard column before separation on the analytical column. An anion micromembrane suppressor (AMMS II) is used with a weak sulfuric acid regenerant solution flowing at 10 mL/min. The suppressed eluent from the ion chromatograph is directed to a post column manifold in which the eluent is first merged with the chlorpromazine reagent. The combined eluent-chlorpromazine reagent stream is then merged with an acid stream to form the photometric product in the presence of ClO_2^- (Figure 1).

SAMPLE HANDLING

All samples should be analyzed as soon as possible. Check that the sample pH is between 6.5 and 7.5. Adjust the sample with dilute hydrochloric acid or sodium

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Chlorite Ion
Chlorpromazine Post Column

hydroxide solutions if necessary. Limit sample exposure to hv by using opaque sample bottles and storing the samples in the dark at 4°C.

Sample bottles should not be cleaned with soap or surfactants. All sample bottles should be rinsed a minimum of five times with distilled water and an additional five times with deionized water displaying a minimum resistance of 17.8 megohms-cm. The bottles are then allowed to air dry for 48 hours.

Sample treatment - Treat the sample with ethylenediamine (50 ppm/100 mL) and let it react for a minimum of 15 minutes to mask any chlorine that may be present in the sample. Store the sample at 4°C and filter any precipitates that may be present before analysis.

If you plan to ship or transport a sample, ship at least 100 mL of treated sample making sure that the sample bottle is completely filled thereby minimizing headspace. Ship the sample under blue ice in a suitable container.

Holding Times - Holding times are as listed for finished water samples in the Federal Register 40 CFR and in the text of USEPA Method 300.0 for the anions separated by this procedure. Laboratory tests have shown that chlorite ion samples in a seawater matrix are not stable.

GLASSWARE

It is important to keep all glassware clean and free of interferences. During storage all glassware openings should be covered with cellophane wrap or ground glass stoppers and stored in the dark to prevent contamination. This includes beakers, volumetric flasks and pipettes.

The following cleaning procedure is recommended:

1. Clean all glassware with a detergent and rinse well with tap water.
2. Soak the glassware in 1:1 nitric acid overnight.
3. Rinse 5X with tap water.
4. Rinse 5X with doubly distilled and deionized water.
5. Store glassware as described above.

APPARATUS

Ion Chromatography System to include:
Ion Chromatograph (Dionex DX-100)

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Metal-Free Column (Dionex, MFC)
Guard Column (Dionex, AG9)
Analytical Column (Dionex, AS9-SC)
Integrator

Post-Column System to include:

Two Reagent Manifold (Tecator Chemifold SR, Type III)
Two Channel Peristaltic Pump
UV/Visible Detector (Perstorp Analytical 510 or equivalent)
Integrator

REAGENTS

All water used to prepare reagent working solutions is best prepared by the passage of distilled water through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Regeneration of the column should be carried out according to the manufacturers specifications. The water should then be distilled and collected. Commercial water purifications systems that deionize and doubly distill the water are preferred. Water with a minimum resistance of 17.8 megohm-cm should be used to prepare all solutions.

Distilled Water: Deionized and doubly distilled as described above.

Ethylenediamine: EDA should be purchased in as pure a form as possible. A concentrated solution is prepared by diluting the pure EDA 1:100 with DI water. This yields an EDA stock solution concentration of 8990 mg/L. To 100 mL sample add 0.5 mL of EDA stock to give an EDA final concentration of ca. 45 mg/L.

Borate Eluent: 12.5 mM NaOH/50 mM H₃BO₃. The eluent is prepared by dissolving 0.5 g sodium hydroxide (NaOH) and 6.2 g boric acid (H₃BO₃) into 2 liters of deionized water.

Suppressor Regenerant: 12.5 mM H₂SO₄. The regenerant is prepared by diluting 2.75 mL sulfuric acid (H₂SO₄) in 4 liters of deionized water.

Chlorpromazine Solution: 0.56 mM Chlorpromazine. The indicator is prepared fresh daily by dissolving 0.2 g chlorpromazine (Sigma) into 1 liter of deionized water. This solution must be degassed with either nitrogen or helium for at least 10 minutes and be protected from light.

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HCl Solution: 2.1 M HCl. The acid solution is prepared by diluting 350 mL hydrochloric acid (HCl) in 2 liters of deionized water. This solution must be degassed with either nitrogen or helium for at least 10 minutes.

STANDARDS

A series of standards should be prepared for the chlorite ion measurement.

NOTE

Most commercial sodium chlorite contains impurities. Highly purified sodium chlorite is essential for the establishment of valid calibration curves (i.e. correlation coefficients ≥ 0.999). It is recommended that the purity of the sodium chlorite reagent be determined prior to its use.

A series of standards should be prepared for the chlorite ion and chlorate ion measurement. Chlorite ion standards are stable for a maximum of 2 weeks if kept cold (4°C) and shielded from light.

Stock Chlorite Ion Standard Solution (100 mg/L): To a 100 mL volumetric flask containing 50 mL of doubly distilled water, place 13.41 mg of sodium chlorite. Mix to dissolve and dilute to 100 mL with doubly distilled water.

Working Standard Solutions: To six 100.0 mL volumetric flasks, add the following volumes of stock standard solution and dilute to volume with distilled, deionized water.

ClO₂ Working Stock (mL)	Standard Solution (mg/L)
0.1	0.1
0.2	0.2
0.4	0.4
0.8	0.8
1.0	1.0
check 0.4	0.4

Chlorite Ion
Chlorpromazine Post Column

PROCEDURE

Instrument Set-up

1. Turn the power on for the IC, UV/Visible detector and integrators.
2. Fill the eluent and regenerant bottles with the appropriate reagents.
3. Fill the chlorpromazine and acid bottles with the appropriate reagents.
4. Check to make sure that the following parameters are set on the IC:

Columns	MFC/AG9/AS9-SC
Sensitivity	1 μ S full-scale
Eluent	12.5 mM NaOH/50 mM H ₃ BO ₃
Eluent Flow	1.0 mL/min
Detection	Suppressed conductivity
System Backpressure	950-1150 psi
Background Conductivity	7-12 μ S
Suppressor	Anion Micro Membrane (AMMS II)
Suppressor Regenerant	12.5 mM H ₂ SO ₄
Regenerant Flow	10 mL/min
Sample Injection Volume	25 - 200 μ L

5. Check to make sure that the following parameters are set on the post-column detection system:

Indicator	0.56 mM Chlorpromazine
Indicator Flow	0.4 mL/min
Acid	2.1 M HCl
Acid Flow	2.0 mL/min
Mixing Coils	M1=60 cm, M2=120 cm
Pathlength	10 mm flow cell
Wavelength	530 nm
Detector Settings	0.002 AUFS/10 sec rise time

6. Equilibrate the system by allowing the IC and post column reagents to flow for a minimum of 30 minutes. Set the auto offset on IC to zero. Check the system backpressure and background conductivity.

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NOTE

We have noticed that fluorescent lights seem to effect the post column chemistry sensitivity. Precautions should be taken to shield reagents from fluorescent lights.

7. Check the post-column system for leaks and air bubbles.
8. Set the integrators according to the instructions provided by the manufacturer.
9. Place the injection valve on the IC in the load position. Fill the sample loop with the most concentrated sample.
10. Inject the sample. A normal anion profile will be recorded by the IC integrator. The ClO_2^- typically is detected ca. 2.5 minutes. The post-column tracing can be expected in an additional 30 - 45 seconds (Figure 2).
11. The high standard should be injected until the calculated peak area values are within $\pm 5\%$.
12. Prepare a calibration curve by injecting standards in random order.
13. Analyze the check standard. The calculated value for the standard from the calibration curve should be within ± 0.02 mg/L of the known concentration. If the value is outside this range, the instrument is not properly calibrated.

NOTE

Calibration curves should exhibit a correlation coefficient better than 0.999. If this level cannot be attained, check your reagents, standards and the instrument for potential causes that prevent adequate calibration. Make adjustments as necessary. Do not analyze samples until a suitable calibration curve is recorded.

STANDARDIZATION AND QUANTITATION

The quantitation of bromate ion separated by this method is performed by injecting standard solutions and integrating the detector response at a defined retention time to calculate a peak area. A calibration curve is developed from a series of standard injections (concentration vs. peak area) and used to quantitate injected samples.

1. Establish the IC and post-column operating parameters.

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2. Prepare calibration standards at a minimum of four concentration levels and a blank by adding accurately measured volumes of the dilute chlorite ion standard solution to a volumetric flask and diluting to volume.
3. Prepare a calibration curve. This curve should be developed each day and checked every 10 samples. If the response or retention time for any analyte varies from the expected value by more than 10%, the test should be repeated using fresh calibration standards.
4. Calculate the sample concentration using a statistics program. Enter the data into the program to generate a worksheet.

NOTE

Data quality should be evaluated on multiple levels:

1. The correlation coefficient should be ≥ 0.999 .
2. Calculated standard peak area values should be within 5% of the measured area.
3. Calculated standard concentration values should be within 0.01 mg/L of the analyte being calibrated. This test indicates the error associated with the calculated regression line.
4. The concentration of the check sample should be within 0.02 mg/L of the theoretical concentration. This test indicates the accuracy of the calibration.

SAFETY

The chlorite ion measurement requires the careful handling of solutions. Refer to the appropriate Material Safety Data Sheet for reagent handling instructions. For added protection, the laboratory should be well ventilated and safety goggles should be worn at all times.

REFERENCES

1. USEPA Method 300.0
2. Hautman/Bolyard personal communication.

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4. Gordon, G.; Cooper, W.J.; Rice, R.G.; Pacey, G.E. A Survey of the Current Status of Residual Disinfectant Measurement Methods for all Chlorine Species and Ozone, J. Am. Water Works, (ISBN 0-89867-409-3) Distributed by AWWA-RF to Water Treatment Plants in North America, (1987), 29 pp.
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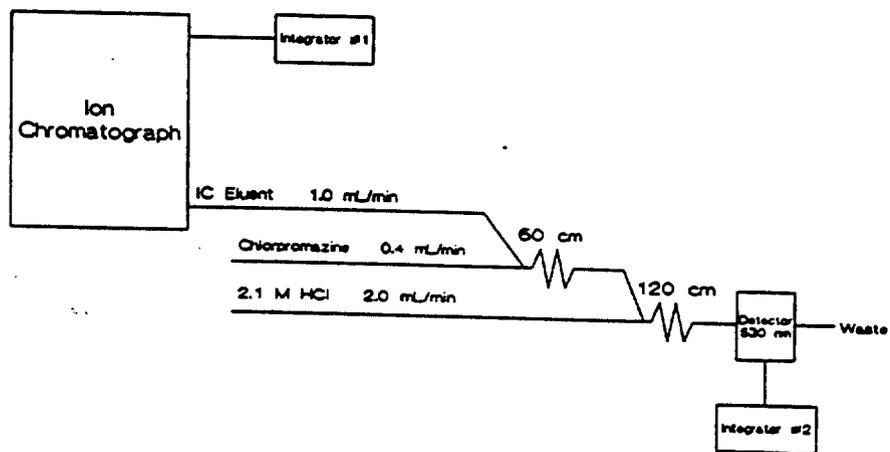


Figure 1. IC post-column diagram.

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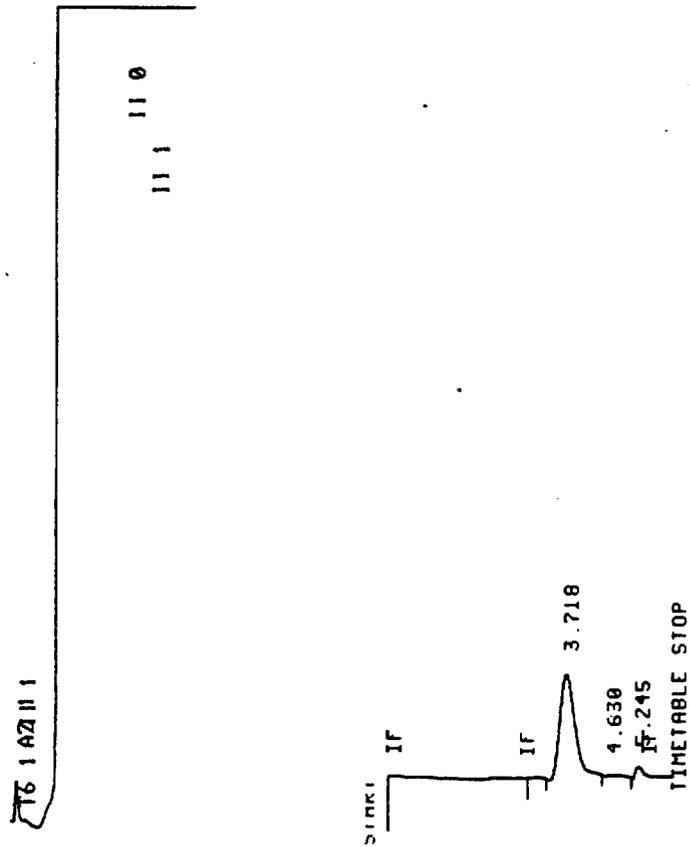


Figure 2. US EPA Method 300.0 conductivity and post-column detection of ClO_2^- .

Date Submitted: _____ MIRAD No: _____ Volume: _____

**ACUTE TOXICITY OF SODIUM CHLORITE TO MYSID SHRIMP
(MYSIDOPSIS BAHIA)**

U.S.-EPA-FIFRA 40 CFR SERIES 72-3

AUTHORS:

Study Director: J. J. Yurk
Principal Investigator: M. A. Overman
Environmental Science & Engineering, Inc.

Contains No CBI

FINAL REPORT DATE:

May 18, 1984

SUBMITTED TO:

Chemical Manufacturers Association (CMA)
2501 M Street NW
Washington, D.C. 20037

PERFORMING LABORATORY:

Environmental Science & Engineering, Inc., (ESE)
Department of Aquatic Toxicology
PO Box 1703
Gainesville, FL 32602-1703
Tel No. (904) 332-3318

LABORATORY PROJECT ID:

ESE Project No. 3943014-0200-3140
CMA No. CD-7.0-3AT-ESE

STATEMENT OF NO DATA CONFIDENTIALITY

Acute Toxicity of Sodium Chlorite
to Mysid Shrimp
(Mysidopsis bahia)

Contains No CBI

This data submission contains a statement of "no data confidentiality" pursuant to PR notice 86-5. This claim is valid. Please disregard the statement "confidential" located in the header on pages 3 - 78. This statement was erroneously generated by a computer.

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA para. 10(d) (1) (A), (B), or (C). Nor is any supplemental claim of confidentiality made for any information in this study on the basis of FIFRA para. 10(A) or (B). This document, however, is propriety to Chemical Manufacturers Association and is considered confidential and trade-secret information in all other countries and for all purposes other than those enunciated in FIFRA para. 3 & 10. In addition, this data is compensative under FIFRA and must be handled accordingly by all parties receiving this data. Information contained in this study should not be reproduced, stored in a retrieval system or transmitted in any form or by any means by persons other than EPA without the express written consent of Chemical Manufacturers Association except as required to carry out the requirements of FIFRA.

Company: CHLORINE DIOXIDE PANEL OF THE CHEMICAL MANUFACTURERS ASSOCIATION

Company Agent: ROBERT R. ROMANO (NAME)

Date: MAY 23, 1994

Title: ASSOCIATE DIRECTOR, CHEMSTAR

Signature: *Robert R. Romano*

STATEMENT OF GLP COMPLIANCE

Compound: Sodium Chlorite

Title: Acute toxicity of sodium chlorite to the mysid shrimp
(Mysidopsis bahia)

The study described in this report was conducted in accordance with the following Good Laboratory Practice Standards:

United States Environmental Protection Agency,
Title 40 Code of Federal Regulations Part 160,
1 July 1993 Edition

Japan Ministry of Agriculture, Forestry and Fisheries
59 NohSan, Notification No. 3850,
Agricultural Products Bureau
10 August 1984

Organization for Economic Co-Operation and Development
ISBN 92-64-12367-9, Paris 1982

Study Director: Jeffrey J. Yurk 5-18-94
Jeffrey J. Yurk Date
ESE

Principal Investigator: Max Overman 5/18/94
Max A. Overman Date
ESE

Study Sponsor: Robert R. Romano 5/19/94
Robert Romano Date
CMA

Submitter: _____ Date

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The study data were reviewed by the ESE Quality Assurance Unit to assure that standard operating procedures and guidelines used to conduct this study were followed and that this report is an accurate reflection of the raw data.

Audit Type	Date	Date Reported To Management and the Study Director
In-phase including organism counts and general test conditions	03/31/94	04/05/94
Draft Report	04/15/94	04/15/94
Final Report	05/18/94	05/18/94

Joe Owusu-Yaw

Dr. Joe Owusu-Yaw, Quality Assurance Unit

5/18/94

Date

SCIENTIFIC PERSONNEL SIGNATURE PAGE

Study Director: Jeffrey J. Jurk 5-18-94
Jeffrey J. Jurk Date

Principal Investigator: Max Overman 5-18-94
Max A. Overman Date

Chemistry Coordinator: Patrick Wilber 5-18-94
Patrick Wilber Date

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STUDY SUMMARY

Study Title: Acute Toxicity of Sodium Chlorite to the Mysid Shrimp (Mysidopsis bahia)

Data Requirement: Section 72-3 of the EPA Pesticide Assessment Guidelines, Subdivision E

Sponsor: Chemical Manufacturers Association (CMA), 2501 M Street NW, Washington, D.C. 20037

Location of Study: Environmental Science & Engineering, Inc.
P.O. Box 1703
Gainesville, FL 32602-1703

Study Director: Jeffrey J. Yurk; Tel No. (904) 333-2626

Study Initiation Date: February 11, 1994

Experimental Start Date: March 28, 1994

Experimental Termination Date: April 1, 1994

Test Substance: Sodium Chlorite, Batch No. 5064, CAS No. 7758-19-2, purity 79%

Test Organism and Source: Mysid shrimp (Mysidopsis bahia), less than 24 h old, obtained from Aquatic Indicators, Inc., St. Augustine, FL

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Test Conditions:

Dilution water: filtered sea water adjusted to approximately 20‰ salinity with well water from a well located at test site, photoperiod: 16-h light and 8-h dark with 30-minute dawn/dusk transition, temperature: 21 to 23 °C

Test Results:

96-hour LC50: 0.576 mg sodium chlorite/L; 95% confidence limits: 0.441 and 0.754 mg/L

Location of Raw Data and Final Report:

Environmental Science & Engineering, Inc. (ESE), P.O. Box 1703, Gainesville, FL 32602-1703

1.0 INTRODUCTION

A flow-through toxicity test was conducted at Environmental Science & Engineering, Inc., in Gainesville, Florida to determine the acute toxicity of sodium chlorite to mysid shrimp (Mysidopsis bahia). The criterion for effect was mortality and results of the test are expressed as a 96-hour median lethal concentration (LC50), the concentration of sodium chlorite estimated to be lethal to 50 percent of the test population of mysid shrimp under specified conditions of exposure. All raw data related to this study are maintained by Environmental Science & Engineering, Inc., Gainesville, Florida.

2.0 MATERIALS AND METHODS

2.1 Test substance

The test substance, sodium chlorite (Batch no. 5064; CAS no. 7758-19-2) was received at ESE in two 1000 mL plastic bottles on December 27, 1993. The test substance was a white crystalline material and was stored in the dark at ambient temperatures. The water solubility of the test substance was reported by Vulcan Chemicals to be 39% at 25 °C and the purity of the test substance was reported to be 79.0%. All sodium chlorite solutions were made by dissolving the test substance in filtered seawater. Measured

test concentrations are reported as milligrams (mg) of sodium chlorite per liter (L) of filtered seawater.

2.2 Test organisms

Less than 24-hour old mysid shrimp (Mysidopsis bahia), were obtained from brood stock cultures maintained at Aquatic Indicators, Inc., St. Augustine, Florida on March 28, 1994. During the 48-hours prior to test start, brood cultures were maintained in filtered seawater with a salinity of 23 ‰, a pH of 8.2 ± 0.03 and a temperature of 25 ± 1 °C. Brood mysids were fed daily with live brine shrimp nauplii. Chemical analyses of the brine shrimp nauplii are performed when fresh feed is purchased, and the results of the most recently reported chemical analyses of feed are provided in Appendix A. No mortality of the mysid brood population was observed over the 48-hour period before the start of the test.

2.3 Dilution water

Sand filtered Atlantic Ocean water was obtained near Whitney Laboratory, Marineland, FL and was diluted to a salinity of approximately 20‰ with well water from a well located at the test site. Chemical analyses of 20‰ filtered seawater are performed

semi-annually and the most recently reported analyses are presented in Appendix B.

2.4 Test Methods

Methods for the 96-hour flow-through toxicity test are described in the ESE test protocol entitled: "Acute Toxicity of sodium chlorite to mysid shrimp (Mysidopsis bahia)".

2.4.1 Range Finding Test

A static range-finding test (Appendix C) was conducted at nominal test concentrations of 0, 0.1, 0.5, 1.0 and 10 mg sodium chlorite/L, in 1.6 L glass bowls. Five mysids were exposed per concentration for 96 hours, during which the test organisms were fed live brine shrimp nauplii daily to reduce cannibalism. After 96 hours of exposure, there was 100% survival in the control and low concentration (0.1 mg/L), and 60%, 40%, and 0% survival in the 0.5 mg/L, 1.0 mg/L and 10 mg/L exposures, respectively. Based upon these results, nominal concentrations selected for the definitive test were 0 (dilution water control), 0.125, 0.25, 0.50, 1.0 and 2.0 mg sodium chlorite/L of dilution water.

2.4.2 Definitive Test

The definitive test was conducted under flow-through conditions in a proportional diluter system (see Appendix C). The stock

solution, with a nominal concentration of 9.69 g sodium chlorite/L, was prepared in filtered seawater. Stock was prepared daily and was continuously mixed throughout the test to insure homogeneity. The mean measured stock concentration was 9.77 mg/L (101 percent of nominal). Approximately 200 μ L of stock solution were pumped to the chemical mixing chamber (total volume of 0.97 L) each diluter cycle providing a high nominal concentration of 2.0 mg/L. This test solution was proportionally diluted in the diluter system to provide the four lower test concentrations (i.e., 1.0, 0.5, 0.25, and 0.125 mg/L). A dilution water control was maintained concurrently with the five test solutions.

The toxicity test was conducted using 10.5 L, rectangular glass chambers (28 cm length X 25 cm width X 15 cm height). Inside the test chambers, test organisms were contained in two cylindrical Nytex® retention cups (each with 9 cm diameter and 16 cm height). The chambers were filled to approximately 5 L with dilution water or test solution. A test solution volume of approximately 250 mL was delivered to each test chamber during every cycle. The diluter cycled at a rate of 5.4 cycles per hour providing approximately 6.5 volume additions every 24 hours. All test chambers were kept covered during the test, except when daily observations of test organisms were made and water quality parameters measured. Diluter

function including stock delivery, stock usage, and cycle time were checked and recorded daily throughout the test.

Test vessels were labeled using the compound name, nominal concentrations, and a letter (A or B) designating concentration replicates. Test vessels were indiscriminately positioned in a water bath under fluorescent lighting regulated to provide a photoperiod of 16 hours light and 8 hours dark with a 15 to 30 minute transition period of lower light intensity to simulate dawn and dusk. Light intensity averaged 643 Lux over the test system, as measured by a Lux meter. The 96-hour test was started on March 28, 1994 with the indiscriminate distribution of five organisms into individual Nytex® retention cups (two per test vessel; four per concentration).

Survival of test organisms was monitored daily and any dead organisms were removed. Any abnormalities in behavior or physical appearance of the test organisms were also noted. Test organisms were fed two drops of concentrated brine shrimp nauplii daily during the test. Test solutions were not aerated during the test.

Water quality was monitored daily during the test. Water temperature in one control vessel was continuously monitored with a Supco Temperature Recorder and spot checked daily with a NIST-

calibrated thermocouple. Dissolved oxygen, salinity, and pH were measured daily in all test solutions containing surviving mysids. Dissolved oxygen concentrations were determined using a YSI Model 58 meter, salinity was measured using an American Optical refractometer, and pH was measured with an Orion Model SA 250 pH meter.

2.5 Statistical Analyses

Statistical analyses were conducted using recommended procedures (EPA Pesticide Assessment Guidelines). Mortality data for the test organisms and the mean measured concentrations of the test substance were used to compute the 24-, 48-, 72- and 96-hour LC50 values and their 95 percent confidence limits, using a statistical computer program (Stephan, 1982). The method of statistical analysis selected by the program was determined by characteristics of the data, i.e., the presence or absence of 0-percent and 100-percent mortality and the number of concentrations in which mortalities between 0 and 100 percent occurred (1). A 96-hour no-observed-effect concentration (NOEC) of the test substance was defined as the highest test concentration where no mortality or other toxic signs occurred.

2.6 Chemical Analysis

Twelve days prior to test start, a sample of test stock solution and test samples from the low, middle and high test solutions were collected and analyzed to verify stock concentration and diluter function, respectively. During the test, samples were collected in amber glass bottles from the A-replicate of the control and each test concentration on day 0 and from the B-replicate of each on day 4. Test solutions were sampled from the approximate center of the test vessel using a serological pipet, and transported in scintillation vials to analytical services for analysis. Samples were analyzed by ion chromatography with visible detection following the methodology provided in the Method Validation report (Appendix D).

3.0 RESULTS AND DISCUSSION

Nominal and measured concentrations of sodium chlorite, in the definitive study, are presented in Table 1. The measured concentrations ranged from a mean of 0.148 mg/L in the lowest concentration to 2.25 mg/L in the highest concentration. Mean measured concentrations ranged from 111 to 118% of nominal concentrations. The control exposure was determined to be below the limit of validation for this test of 0.107 mg/L. Analytical quality control data are presented in Table 2. Spike recoveries

ranged from 95.9 to 104 percent for all quality control data. Data for exposure water quality parameters, and their corresponding ranges and median values are presented in Tables 3, 4 and 5. Dilution water salinity remained at 21 ‰ throughout the test. All ranges are within the acceptable limits (2).

The mean measured sodium chlorite concentrations and corresponding mortalities of test organisms, presented in Table 6, were used to calculate the LC50 values and their 95% confidence limits for various lengths of exposure (Table 7). The 96-hour LC50 was calculated to be 0.576 mg of sodium chlorite/L using the moving average method. Behavioral changes in mysids such as erratic motion and lethargic behavior were noted in daily observations of organisms in exposures of 0.574 mg/L and above (Appendix C). A 96 hour no-observed-effect concentration (NOEC) of the test substance was not determined in this test since there was 10 percent mortality in the lowest tested concentration, 0.148 mg of sodium chlorite/L.

4.0 CONCLUSION

The 96-hour LC50 for sodium chlorite in mysid shrimp (Mysidopsis bahia) was determined to be 0.576 mg/L with 95% confidence limits of 0.441 and 0.754 mg/L. The NOEC was < 0.148 mg/L.

REFERENCES

- (1) Stephan, C.E. 1977. Methods for Calculating an LC50. IN: American Society for Testing and Materials (ASTM) Aquatic Toxicology and Hazard Evaluation, pp. 65-84, F.L. Mayer and J.L. Hamelink (Editors). ASTM STP 524, Philadelphia, PA.
- (2) American Society for Testing and Materials (ASTM). 1980. Standard Practice for Conducting Acute Toxicity Tests With Fishes, Macroinvertebrates, and Amphibians. ASTM, Philadelphia, PA. Publication E 729-80.

Table 1. Nominal and measured concentrations of sodium chlorite in exposure water during a 96-h flow-through, acute toxicity test with mysid shrimp (S. bahia).

Nominal concentration (mg/L) of sodium chlorite	Measured concentration (mg/L) of sodium chlorite at test start and test termination		Mean measured concentration (mg/L)	Percent of nominal ^a
	0 h	96 h		
0 (control)	ND ^b	ND	ND	NC ^c
0.125	0.146	0.149	0.148	118
0.250	0.319	0.272	0.296	118
0.500	0.581	0.567	0.574	115
1.00	1.12	1.09	1.11	111
2.00	2.24	2.25	2.25	113

^a Mean measured concentration x 100/nominal concentration.
^b ND = not detected below a limit of validation for this test of 0.107 mg/L.
^c Cannot be calculated.

Source: ESE 1994

Table 2. Analytical quality control data from a 96-h flow-through, acute toxicity test of sodium chlorite to the mysid shrimp (*M. bahia*).

Sample	Target chlorite concentration (mg/L)	Measured chlorite concentration (mg/L)	Percent recovery ^a
<u>DAY-0</u>			
Std Matrix Spike	10.0	10.00	100
Std Matrix Spike	10.0	9.862	98.6
Sample Matrix Spike	5.00	4.824	96.5
Sample Matrix Spike	5.00	4.794	95.9
<u>DAY-4</u>			
Std Matrix Spike	10.0	9.992	99.9
Std Matrix Spike	10.0	10.02	100
Sample Matrix Spike	50.0	51.25	103
Sample Matrix Spike	50.0	51.85	104

^a Measured concentration X 100/target concentration.

Source: ESE 1994

Table 3. Daily temperature of exposure water during a 96-h flow-through, acute toxicity test of sodium chlorite to the mysid shrimp (*M. bahig*).

Mean measured concentration (mg/L) of sodium chlorite	Replicate exposure	Temperature (°C)					n ^a	Range	Median
		0 h	24 h	48 h	72 h	96 h			
0 (control)	A	21.7	21.7	21.9	21.5	21.5	10	21.3-21.9	21.7
	B	21.9	21.7	21.9	21.4	21.3			
0.148	A	21.7	21.7	21.9	21.4	21.1	10	21.1-21.9	21.7
	B	21.7	21.6	21.9	21.4	21.1			
0.296	A	21.8	21.7	22.0	21.4	21.2	10	21.2-22.0	21.7
	B	21.8	21.7	21.9	21.4	21.2			
0.574	A	21.8	21.7	21.9	21.4	21.2	10	21.2-21.9	21.7
	B	21.7	21.8	21.9	21.4	21.2			
1.11	A	22.0	21.9	21.8	21.3	21.2	10	21.2-22.0	21.8
	B	21.8	21.7	22.0	21.3	21.3			
2.25	A	21.8	21.8	21.7	21.3	- ^b	7	21.3-21.8	21.8
	B	21.8	21.8	21.8	- ^b	- ^b			

^a n = total number of measurements.

^b - = value not determined after 100% mortality.

Note: temperatures as measured with a continuous temperature monitor were within the range of 21 to 23°C.

Source: ESE 1994

Table 4. Exposure water pH during a 96-h flow-through, acute toxicity test of sodium chlorite to the mysid shrimp (*M. bahig*).

Mean measured concentration (mg/L) of sodium chlorite	Replicate exposure	pH					n ^a	Range	Median
		0 h	24 h	48 h	72 h	96 h			
0 (control)	A	7.9	8.1	8.2	8.2	8.3	10	7.9-8.4	8.2
	B	7.9	8.1	8.2	8.2	8.4			
0.148	A	8.0	8.1	8.3	8.3	8.4	10	8.0-8.4	8.3
	B	8.0	8.2	8.3	8.3	8.4			
0.296	A	8.0	8.2	8.3	8.3	8.4	10	8.0-8.4	8.3
	B	8.0	8.2	8.3	8.3	8.4			
0.574	A	8.0	8.2	8.3	8.3	8.4	10	8.0-8.4	8.3
	B	8.0	8.2	8.3	8.3	8.4			
1.11	A	8.0	8.2	8.3	8.3	8.4	10	8.0-8.4	8.3
	B	8.0	8.2	8.3	8.3	8.4			
2.25	A	8.0	8.2	8.3	- ^b	- ^b	6	8.0-8.3	8.2
	B	8.0	8.2	8.3	- ^b	- ^b			

^a n : total number of measurements.

^b - : value not determined after 100% mortality.

Source: ESE 1994

Table 5. Dissolved oxygen concentrations of exposure water during a 96-h flow-through, acute toxicity test of sodium chlorite to the mysid shrimp (*M. bahia*)

Mean measured concentration (mg/L) of sodium chlorite	Replicate exposure	Dissolved Oxygen Concentration (mg/L) (percent saturation)					n ^a	Range	Median
		0 h	24 h	48 h	72 h	96 h			
0 (control)	A	7.8(106)	7.9(108)	7.7(105)	7.7(105)	7.9(108)	10	7.6-7.9	7.8
	B	7.6(104)	7.8(106)	7.7(105)	7.8(101)	7.9(103)			
0.148	A	8.0(109)	7.5(102)	7.6(104)	7.8(101)	7.9(103)	10	7.5-8.0	7.8
	B	7.9(108)	7.7(105)	7.7(105)	7.8(101)	7.9(103)			
0.296	A	7.9(108)	7.8(106)	7.6(104)	7.9(103)	8.0(104)	10	7.6-8.0	7.9
	B	8.0(109)	7.7(105)	7.6(104)	7.8(101)	8.0(104)			
0.574	A	7.8(106)	7.8(106)	7.7(105)	7.8(101)	7.9(103)	10	7.7-8.0	7.8
	B	8.0(109)	7.7(105)	7.7(105)	7.8(101)	8.0(104)			
1.11	A	8.0(109)	8.0(109)	7.7(105)	7.9(103)	7.9(103)	10	7.7-8.0	7.9
	B	7.9(108)	7.7(105)	7.7(105)	7.8(101)	7.9(103)			
2.25	A	7.8(106)	7.3(99)	7.7(105)	- ^b	- ^b	6	7.3-7.9	7.7
	B	7.9(108)	7.5(102)	7.7(105)	- ^b	- ^b			

^a n : total number of measurements.

^b - : value not determined after 100% mortality.

Source: ESE 1994

Table 6. Mortalities of mysid shrimp (*M. bahia*) exposed to sodium chlorite in a 96-h flow-through, acute toxicity test.

Mean measured concentration (mg/L) of sodium chlorite	Cumulative number of dead organisms and (percent mortality of total organisms exposed)			
	24 h	48 h	72 h	96 h
0 (control)	0(0)	0(0)	0(0)	0(0)
0.148	0(0)	2(10)	2(10)	2(10)
0.296	0(0)	1(5)	2(10)	2(10)
0.574	0(0)	2(10)	5(25)	6(30)
1.11	3(15)	10(50)	18(90)	19(95)
2.25	6(30)	20(100)	20(100)	20(100)

Source: ESE 1994

Table 7. LC50 values of sodium chlorite for the mysid shrimp (M. bahia) for different lengths of exposure.

Exposure period	LC50 (mg/L) ^a	95% Confidence limits for LC50	Statistical method used for LC50 calculation
24 h	2.81	0.925-4.71	Probit
48 h	1.11	0.574-2.25	Binomial
72 h	0.628	0.484-0.830	Moving Average
96 h	0.576	0.441-0.754	Moving Average

^a Calculated based on mean measured concentrations of sodium chlorite.

Source: ESE 1994

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Appendix A- Mysid Food Characterization

BRINE SHRIMP CHEMICAL CHARACTERIZATION
February 1993

Component	Analytical Method	Brine Shrimp ^a
Organochlorine pesticides ($\mu\text{g/g}$ wet)	EPA 8080	<20
Hydrocarbon, Petroleum ($\mu\text{g/g}$ tissue)	EPA 418.1	<29

^a Brine shrimp (lot number SFO 65034) were purchased from Florida Aqua Farms, Inc., Dade City, FL and hatched using filtered seawater diluted to a salinity of approximately 5 ppt.

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Appendix B- Filtered Sea Water Characterization

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FILTERED SEAWATER CHARACTERIZATION*

<u>PARAMETER</u>	<u>CONCENTRATION</u>	<u>METHOD</u>
Arsenic	2.9 µg/L	EPA 206.2
Aluminum	<40 µg/L	EPA 200.7
Cadmium	<0.5 µg/L	EPA 213.3
Calcium	215 mg/L	EPA 200.7
Chromium	<10.0 µg/L	EPA 200.7
Cobalt	<20 µg/L	EPA 200.7
Copper	11.9 µg/L	EPA 200.7
Iron	<45.0 µg/L	EPA 200.7
Lead	<2.0 µg/L	EPA 239.2
Mercury	<0.20 µg/L	EPA 245.1
Magnesium	590 mg/L	EPA 200.7
Nickel	<15.0 µg/L	EPA 200.7
Selenium	<2.5 µg/L	EPA 270.2
Silver	<5.0 µg/L	EPA 200.7
Sodium	5120 mg/L	EPA 200.7
Zinc	<20.0 µg/L	EPA 200.7
Nitrogen, NH ₄ +NH ₃	<0.020 mg/L as N	EPA 350.1
Nitrogen, NH ₃ Unionized	<0.001 mg/L	Calculated
Nitrogen, NO ₂ +NO ₃	0.034 mg/L as N	EPA 353.2
pH	8.3	Meter
Alkalinity	171 mg/L as CaCO ₃	EPA 310.1
Sulfide	<0.05 mg/L as S	EPA 376.2
Hydrogen Sulfide	<0.03 mg/L	Std Method (428E)
Turbidity	<0.40 NTU	EPA 180.1
Dissolved Solids	20100 mg/L	EPA 160.1
Suspended Solids	27 mg/L	EPA 160.2
Fluoride	0.65 mg/L	EPA 340.2
Cyanide	<0.002 mg/L	EPA 335.3
Carbon, TOC	2.3 mg/L	EPA 415.1
COD	83 mg/L	EPA 410.1
Pesticides-Organochlorine and PCB's	<0.005 µg/L	EPA 8080
Pesticides-Organophosphate	<2.52 µg/L	EPA 8140

* The characterized seawater is natural seawater collected from the Atlantic Ocean near Marineland, Florida and diluted to a salinity of 20 parts per thousand using ESE's well water. The diluted seawater sample was collected from ESE's seawater delivery system on September 9, 1993.

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Appendix C- Pertinent Raw Data

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 Gainesville, Florida

Page: _____
 ESE QA Form No.: 015A
 Effective: APR 1993

ACUTE TOXICITY DATA SHEET-BIOLOGICAL DATA								
SPONSOR: <u>CMA</u>			PROJECT NUMBER: <u>3943014-0200-3140</u>					
TEST SUBSTANCE: <u>Sodium Chlorite</u>			TEST SPECIES: <u>M. bahia</u>					
ANIMAL HISTORY								
Sample Information and Description Found on Page <u>532</u> of Test Substance Log								
Lot No./Batch No.: <u>94-22</u> (Circle One)			Date Maintenance/Acclimation Began: <u>3/8/94</u>					
See Page No.: <u>58</u> of Animal Collection/Receipt Log			Condition of Animals: <u>Normal</u>					
See Page No.: <u>61</u> of <u>Invertebrate Holding Log</u> For Raw Data on Animal Holding			Life Stage/Age: <u>24 Hrs.</u>					
			Mortality in Tank 48 Hours Prior To Test Initiation: <u>0</u> (%)					
TEST CONDITIONS								
<input checked="" type="checkbox"/> Range-Finding <input type="checkbox"/> Definitive		<input checked="" type="checkbox"/> Static <input type="checkbox"/> Flow-Through		Time Added Substance/Animals <u>1400 / 1410</u>		Test Location <u>Lab. bath 2</u>		
<input checked="" type="checkbox"/> Covered <input type="checkbox"/> Open		Test Container Dimensions (cm) (dia) (height) <u>9.5 x 6.5</u>		Test Solution Height: <u>3.5 cm</u>		Test Container Composition: <u>Glass</u>		
Test Chamber Volume (L) 0.34, <u>0.6</u> , 3.8, 30 - <u>NA</u> ^{WARRANT} _{3/8/94}				Test Solution Volume (L) 0.20, <u>0.6</u> , 3.0, 10 -				
Protocol: Acute toxicity of sodium chlorite to the mysid shrimp (<u>Mysidopsis bahia</u>)								
Dilution Water: <u>Filtered Seawater</u> Type of Lighting: <u>Fluorescent</u> Photoperiod: <u>16 light / 8 dark</u>								
Cycle One: Test Substance as Active Ingredient/Whole Material								
Test Concentration & <u>mg/L</u> ug/L	Control	Solv Cont	0.1	0.5	1.0	10.0	-	Solvent/Carrier:
Amount of Substance/Stock Added (ml)	NA	NA	.1	.5	1.0	10.0	-	NA
Stock Solution Used (e.g., 1', 2')	NA	NA	i	i	i	i	-	Solvent/Carrier Concentration:
Amount of Dilution Water Added (ml)	1000.0	NA	999.9	999.5	999.0	999.0	-	NA
Amount of Solvent Added (<u>NA</u>)	NA	NA	NA	NA	NA	NA	NA	
Analyst: <u>NZT</u>			Date: <u>3/8/94</u>			Comments in Daily Log		

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PAGE:
 ESE QA FORM: 016
 EFFECTIVE: JUNE 1990

SUBJECT: ACUTE TOXICITY DATA SHEET--WATER QUALITY DATA																									
SPONSOR: <u>CMA</u>										PROJECT NUMBER: <u>3943014-0200-3140</u>															
TEST SUBSTANCE: <u>Sodium Chlorite</u>										TEST SPECIES: <u>M. bahia</u>															
DATE	3/8/94					3/9/94					3/10/94					3/11/94					3/12/94				
TIME	1400					1345					1335					1330					1300				
DATA BY	HNT					HNT					HNT					(S)					(S)				
NOMINAL CONCENTRATION mg/L ug/L	R E P	T E M (SL) OR P HD			A L K	C O N D	T E M (SL) OR P HD			D O	T E M (SL) OR P HD			D O	T E M (SL) OR P HD			D O	T E M (SL) OR P HD			D O			
		P	HD	pH			P	HD	pH		P	HD	pH		P	HD	pH		P	HD	pH		P	HD	pH
CONTROL	A	22.0	20	8.3	7.8	-	-	21.9	20	8.1	7.5	21.1	-	-	22.3	22	8.4	8.1	22.1	20	8.3	7.4			
	B																								
0.1mg/l	A	21.8	20	8.4	7.8	-	-	21.9	21	8.3	7.5	21.7	-	-	22.4	20	8.4	8.2	22.1	21	8.3	7.3			
	B																								
0.5mg/l	A	21.7	21	8.4	7.8	-	-	21.7	21	8.3	7.4	21.2	-	-	22.3	22	8.4	8.3	22.0	21	8.3	7.1			
	B																								
1.0mg/l	A	21.2	21	8.4	7.6	-	-	21.7	21	8.3	7.5	21.3	-	-	22.3	22	8.4	8.3	22.4	22	8.3	7.1			
	B																								
10mg/l	A	21.7	21	8.4	7.6	-	-	21.7	21	8.3	7.5	21.2	-	-	22.3	22	8.4	8.3	22.4	22	8.3	7.1			
	B																								
Instrument/Method:	TS-4	AL-6	SA-200	DO-1	-	-	TS-4	AL-5	SA-200	DO-1	TS-4	-	-	TS-2	REF-5	SA-200	DO-1	TS-2	REF-5	SA-200	DO-1				

KEY: TEMP - Temperature DO - Dissolved Oxygen
 SL - Salinity ALK - Alkalinity
 HD - Hardness COND - Conductivity

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Page: _____
 QA Form Number: 031
 Effective: APR 1993

DILUTER CALIBRATION	
Sponsor: <u>CMA</u>	Test Substance: <u>Sodium Chlorite</u>
Project Number: <u>3943014-0200-3140</u>	Test Species: <u>M. bahia + C. variegatus</u>
Date Calibrated: <u>3/14/94</u>	Calibrated By: <u>NZT</u>
Diluter ID Number: <u>5</u>	Location: <u>In-house</u>
Calibrated for a <u>50</u> % diluter.	

Cell ID	Desired Cell Volumes (mL)		Measured Cell Volumes (mL)	
	Water	Chemical	Water	Chemical
Control	500	0	500, 496, 498	0
6.25% 0.125mg/L	468.75	31.25	465.0, 470.0, 470.0	31.5, 32.0, 32.0
12.5% 0.25mg/L	437.5	62.5	435.0, 438.0, 437.0	62.0, 61.5, 62.5
25% 0.5mg/L	375	125	370.0, 375.0, 375.0	124.0, 125.0, 125.0
50% 1.0mg/L	250	250	250.0, 248.0, 250.0	253.0, 250.0, 252.0
100% 2.0mg/L	0	500	0	500.0, 505.0, 502.0

SPLITTER BOX DELIVERY CALIBRATION							
Splitter Box ID	Control	0.125mg/L	0.25mg/L	0.5mg/L	1.0mg/L	2.0mg/L	
Replicate A	250.0	248.0	250.0	257.0	248.0	260.0	
Volume (mL)	252.0	260.0	253.0	265.0	252.0	255.0	
	253.0	252.0	263.0	255.0	245.0	250.0	
Replicate B	245.0	253.0	235.0	260.0	260.0	265.0	
Volume (mL)	250.0	253.0	252.0	260.0	265.0	250.0	
	250.0	250.0	250.0	252.0	255.0	260.0	

Comments: _____

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Gainesville, Florida

Page:
QA Form Number: 102
Effective: July 1990

SUBJECT: METERING PUMP CALIBRATION

Sponsor: CMA Project Number: 3943014-0200-3140
Test Substance: Sodium Chlorite Test Species: M. bahia

^{#1} Calibration Substance: Milli-Quartz Pump Setting: 2ml
Desired Volume: 2.0ml Metering Pump: Digital
Tare Weight: 1.3855g Total Weight: 3.3763g
Difference: 1.9908g Number of Cycles: 10
Volume Per Cycle: 0.19908ul
Date: 3/11/94 Analyst: HZT

Comments: _____

^{#2} Tare Weight: 1.3730g Total weight: 3.3670g
Difference: 1.9940g Number of Cycles: 10
Volume per cycle: 0.19940ul

^{#3} Tare Weight: 1.3423g Total weight: 3.3358g
Difference: 1.9935g Number of Cycles: 10
Volume per cycle: 0.19935ul

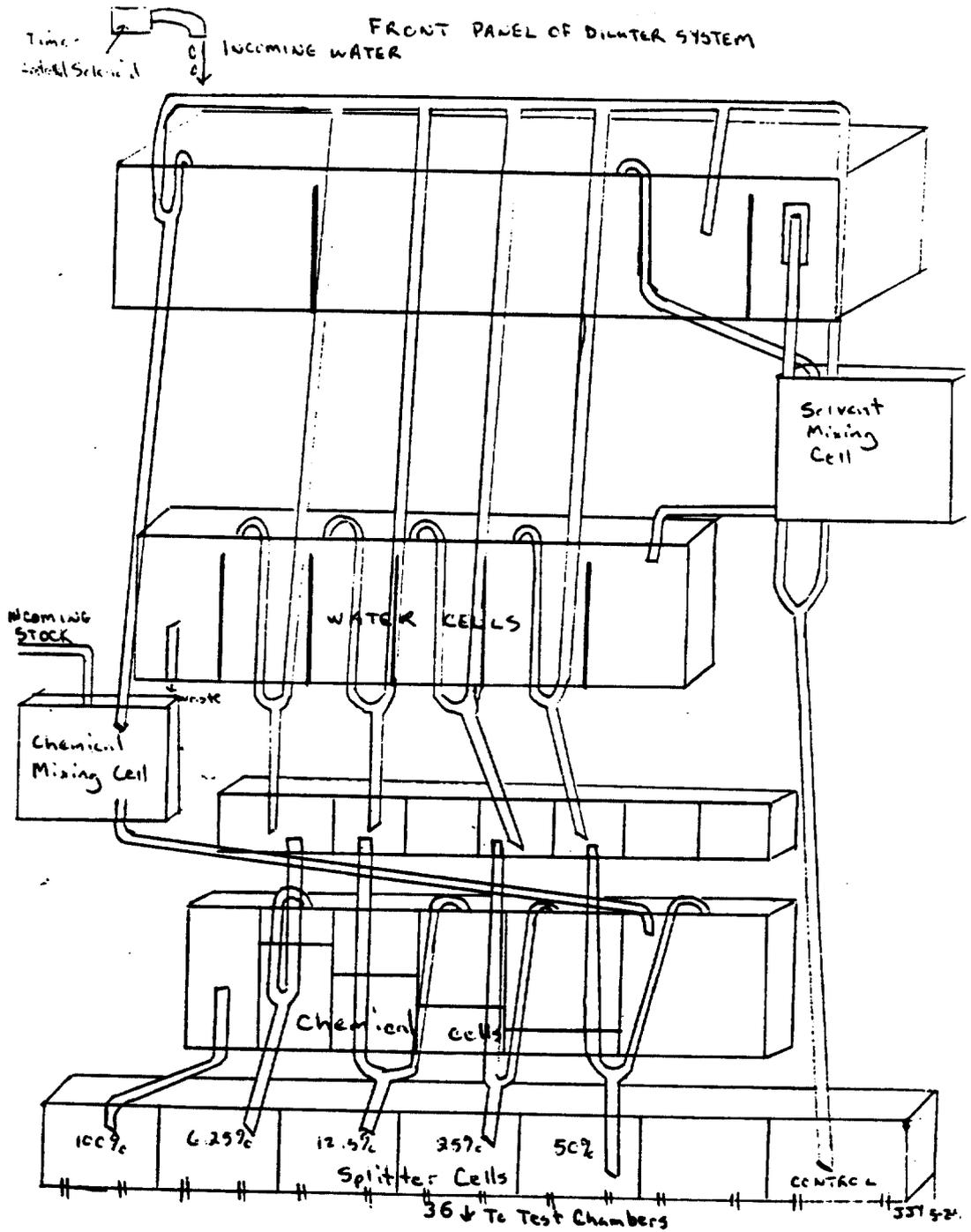
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 QA Form Number: 031
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DILUTER CALIBRATION				
Sponsor: <u>CMA</u>		Test Substance: <u>Sodium Chlorite</u>		
Project Number: <u>3943014-0200-3140</u>		Test Species: <u>C. variegatus & m. bahia</u>		
Date Calibrated: <u>4/21/94</u>		Calibrated By: <u>JK</u>		
Diluter ID Number: <u>5</u>		Location: <u>In-house</u>		
Calibrated for a <u>50</u> % diluter.				
Cell ID	Desired Cell Volumes (mL)		Measured Cell Volumes (mL)	
	Water	Chemical	Water	Chemical
<u>Control</u>	<u>500.0</u>	<u>-</u>	<u>498.0</u>	<u>-</u>
<u>0.125mg/L</u>	<u>468.75</u>	<u>31.25</u>	<u>474.0</u>	<u>31.0</u>
<u>0.250</u>	<u>437.50</u>	<u>62.5</u>	<u>443.0</u>	<u>66.0</u>
<u>0.500</u>	<u>375.0</u>	<u>125.0</u>	<u>380.0</u>	<u>130.0</u>
<u>1.00</u>	<u>250.0</u>	<u>250.0</u>	<u>251.0</u>	<u>250.0</u>
<u>2.00</u>	<u>-</u>	<u>500.0</u>	<u>-</u>	<u>510.0</u>
SPLITTER BOX DELIVERY CALIBRATION				
Splitter Box ID				
Replicate A Volume ()				
Replicate B Volume ()				
Comments:	<u>* Post-calibration check of diluter</u> _____ _____ _____			

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SODIUM CHLORITE-MBAHIA
ESE PROJECT #: 3943014-0200-3140
CMA #: CD-7.0-3AT-ESE



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Environmental Science & Engineering, Inc.
 Aquatic Toxicology Laboratory
 Gainesville, Florida

Page: _____
 ESE QA Form No.: 015A
 Effective: APR 1993

ACUTE TOXICITY DATA SHEET-BIOLOGICAL DATA								
SPONSOR: <u>CMA</u>		PROJECT NUMBER: <u>3943014-0200-3140</u>						
TEST SUBSTANCE: <u>Sodium Chlorite</u>		TEST SPECIES: <u>Musidopsis bulia</u>						
ANIMAL HISTORY								
Sample Information and Description Found on Page <u>532</u> of Test Substance Log								
Lot No./Batch No.: <u>94-26</u> (Circle One)		Date Maintenance/Acclimation Began: <u>N/A</u>						
See Page No.: <u>58</u> of Animal Collection/Receipt Log		Condition of Animals: <u>Normal</u>						
See Page No.: <u>65</u> of <u>Inventory holding log</u> For Raw Data on Animal Holding		Life Stage/Age: <u>2-24 hours</u>						
		Mortality in Tank 48 Hours Prior To Test Initiation: <u>0</u> (%)						
TEST CONDITIONS								
Range-Finding		Static		Time Added Substance/Animals		Test Location		
<input checked="" type="checkbox"/> Definitive		<input checked="" type="checkbox"/> Flow-Through		N/A / 1500		Diluter		
Test System: <input checked="" type="checkbox"/> Covered <input type="checkbox"/> Open		Test Container Dimensions (cm) L W H 25 25 15		Test Solution Height: 7.5 cm		Test Container Composition: Glass		
Test Chamber Volume (L) 0.34, 1.6, 3.8, 30 - <u>(10.5)</u>				Test Solution Volume (L) 0.20, 1.0, 3.0, 10 - <u>(5)</u>				
Protocol: <u>acute toxicity of sodium chlorite to the marine shrimp (Musidopsis bulia)</u>								
Dilution Water: <u>Natural Filtered Seawater</u>		Type of Lighting: <u>Fluorescent</u>		Photoperiod: <u>12L:12D</u>				
Circle One: Test Substance as Active Ingredient/Whole Material								
Test Concentration & (ug/L ug/L)	Control	Solv Cont	0.125	0.250	0.500	1.00	2.00	Solvent/Carrier:
Amount of Substance/Stock Added ()	NA	NA	see	diluter				NA
Stock Solution Used (e.g., 1', 2')	NA	NA	calibration sheet		sheet			Solvent/Carrier Concentration:
Amount of Dilution Water Added (-)								
Amount of Solvent Added (-)	NA		NA	NA	NA	NA	NA	

Analyst: mc Date: 3/24/93 Comments in Daily Log

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Environmental Science and Engineering, Inc.
 Gainesville, Florida
 DEFINITIVE TEST

PAGE:
 ESE QA FORM: 016
 EFFECTIVE: JUNE 1990

SUBJECT: ACUTE TOXICITY DATA SHEET--WATER QUALITY DATA																																	
SPONSOR: <u>CMA</u>										PROJECT NUMBER: <u>3943014-0200-3140</u>																							
TEST SUBSTANCE: <u>Sodium chlorite</u>										TEST SPECIES: <u>m. bahia</u>																							
DATE		3-28-94					3/29/94					3/30/94					3/31/94					4/1/94											
TIME		1330					1345					1145					1145					1415											
DATA BY		CM					CM					CM					CM					CM											
NOMINAL CONCENTRATION mg/L ug/L	R P	T P	SL		DO	ALK	COND	T P	SL		DO	ALK	COND	T P	SL		DO	ALK	COND	T P	SL		DO	ALK	COND	T P	SL		DO	ALK	COND		
			HD	pH					HD	pH					HD	pH					HD	pH					HD	pH				HD	pH
CONTROL	A	27	21	79	7.8	-	-	217	21	8.1	79	219	21	8.2	77	245	21	8.2	77	215	21	8.3	79										
	B	219	21	79	7.6	-	-	217	21	8.1	78	219	21	8.2	77	214	21	8.2	78	213	21	8.4	79										
0.125	A	27	21	80	8.0	-	-	217	21	8.1	75	219	21	8.3	76	214	21	8.3	77	211	21	8.4	79										
	B	217	21	80	7.9	-	-	214	21	8.2	77	219	21	8.3	77	214	21	8.3	78	211	21	8.4	79										
0.25	A	219	21	80	7.9	-	-	217	21	8.2	78	219	21	8.3	76	214	21	8.3	79	212	21	8.4	8.0										
	B	219	21	80	8.0	-	-	217	21	8.2	77	219	21	8.3	76	214	21	8.3	78	212	21	8.4	8.0										
0.50	A	27	21	80	7.8	-	-	217	21	8.2	78	219	21	8.3	77	214	21	8.3	78	212	21	8.4	79										
	B	27	21	80	8.0	-	-	218	21	8.2	77	219	21	8.3	77	214	21	8.3	78	212	21	8.4	8.0										
1.00	A	220	21	80	8.0	-	-	219	21	8.2	8.0	218	21	8.3	77	213	21	8.3	78	212	21	8.4	79										
	B	218	21	80	7.9	-	-	217	21	8.2	77	220	21	8.3	77	213	21	8.3	78	213	21	8.4	79										
2.00	A	218	21	80	7.8	-	-	218	21	8.2	7.5	217	21	8.3	77	213	-	-	-	-	-	-	-										
	B	218	21	80	7.9	-	-	218	21	8.2	7.5	218	21	8.3	77	-	-	-	-	-	-	-	-										
Instrument/Method:		FS	REF	SL	DO	-	-	FS	REF	SL	DO	-	-	FS	REF	SL	DO	FS	REF	SL	DO	FS	REF	SL	DO	FS	REF	SL	DO	FS	REF	SL	DO
		2	5	2	1	-	-	2	5	2	1	-	-	2	5	2	1	4	5	2	1	4	5	2	1	4	5	2	1	4	5	2	

KEY: TEMP - Temperature DO - Dissolved Oxygen
 SL - Salinity ALK - Alkalinity
 HD - Hardness COND - Conductivity

96-h LC50

Results calculated using the Moving Average Method.

Client : CMA Species : M. bahia

Dates of test : 3/28/94 to 4/1/94

Test Material : Sodium Chlorite proj # 3943014-0200-3140

Report run by : JJY Date : 04-06-1994

Concentration units (Circle One) : ‡ PPTH **PPM** PPB Other _____

Conc.	Number Exposed	Number Dead	Percent Dead
2.3	20	20	100.0
1.1	20	19	95.0
0.6	20	6	30.0
0.3	20	2	10.0
0.1	20	2	10.0
Control	20	0	0.0

Span	G	LC50	95 Percent Confidence Limits	
	0.085	0.576	0.441	0.754
3	0.078	0.614	0.476	0.758
2	0.103	0.538	0.530	0.787
1	0.184	0.688	0.558	0.792

Note -- An LC50 calculated using the Moving Average Method may not be a very good estimate for Spans less than 4 .

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VULCAN CHEMICALS R&D
ANALYTICAL REPORT

DATE: 03/18/94
 PAGE NO: 1

SAMPLE NO.	PROJ. NO.	COMPONENT	RESULTS	ANALYST	COMMENTS

osf5064	60	Vulcan Chemical NaClO2 (Same drum a Submitted By: BCC			
		NaClO2	81.41%	SLG	Titration w/ Na2S2O3
		NaCl	10.621%	SLG	Titration w/ AgNO3
		Na2CO3	0.504%	SLG	Titration w/ H2SO4
		Na2SO4	0.364%	DMM	IC, 89.3% spike recovery
		H2O2	0.003%	SLG	Titration w/ NaOH
		water by di	6.156%	DMM	By difference
		Pb	< 0.5 ppm	DMM	GF, 88.4% spike recovery
		NaOH	0.028%	SLG	Titration w/ H2SO4 w/ BaCl2
		NaClO3	0.914%	DMM	IC, 101.3% spike recovery
		Hg	< 65 ppb	SLG	MHS, 89.0% spike recovery
		As	< 1.3 ppm	DMM	MHS-20, 89.6% spike recovery

 END OF REPORT

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Appendix D- Analytical Method Validation

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FINAL REPORT

STUDY TITLE

**Method Validation for the Determination
of Sodium Chlorite in Seawater**

AUTHOR

David H. Greer, Jr.

STUDY COMPLETED

May 18, 1994

REPORT DATE

May 18, 1994

SPONSOR

**Chemical Manufacturers Association
2501 M Street N.W.
Washington, D.C. 20037**

PERFORMING LABORATORY

**Environmental Science & Engineering, Inc.
14220 Newberry Road
Gainesville, Florida 32607
(904) 332-3318**

LABORATORY STUDY NUMBER

1944015V-L202

LABORATORY PROTOCOL NUMBER

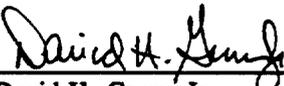
ASG-94-02

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COMPLIANCE STATEMENT

The data presented in this report was an accurate representation of the investigations conducted. This study was conducted in compliance with current Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Good Laboratory Practice Regulations (40 CFR Part 160). All records pertaining to this study were maintained at Chemical Manufacturers Association GLP archives located at 2501 M Street, N.W., Washington, D.C. 20037. A copy of the original raw data will be archived in the ESE Gainesville Analytical Services GLP archives located at 14220 West Newberry Road, Gainesville, Florida 32607.



David H. Greer, Jr.,
Program Manager, Study Director
ESE, Inc.

5/18/94

Date



Kathleen K. Allen
Water Quality Department Manager
ESE, Inc.

5/18/94

Date



Robert Romano, Ph.D.
Director of Special Programs
Chemical Manufacturers Association

5/19/94

Date

QUALITY ASSURANCE STATEMENT

This study was monitored by Environmental Science & Engineering, Inc. (ESE) Quality Assurance Unit to assure that the guidelines and Standard Operating Procedures (SOPs) used to conduct this study were followed.

The final report for this study was reviewed by the Quality Assurance Unit and, accurately reflects the results of the study as conducted, including all areas of compliance or noncompliance with Good Laboratory Practice Standards, the study protocol and ESE's SOPs.

Joe Owusu-Yaw, Ph.D.
Joe Owusu-Yaw
QAU GLP Coordinator

5-18-94
Date

QUALITY ASSURANCE AUDITS

<u>Audit Date</u>	<u>Type of Report</u>	<u>Date Reported to Management</u>	<u>Date Reported Study Director</u>
2/25/94	Protocol	5/18/94	2/25/94
5/16/94	Data Review	5/18/94	5/16/94
5/16/94	Draft Report	5/18/94	5/16/94
5/17/94	Revised Report	5/18/94	5/17/94
5/18/94	Final Report	5/18/94	5/18/94

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Appendix B- Filtered Sea Water Characterization

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FILTERED SEAWATER CHARACTERIZATION*

<u>PARAMETER</u>	<u>CONCENTRATION</u>	<u>METHOD</u>
Arsenic	2.9 µg/L	EPA 206.2
Aluminum	<40 µg/L	EPA 200.7
Cadmium	<0.5 µg/L	EPA 213.3
Calcium	215 mg/L	EPA 200.7
Chromium	<10.0 µg/L	EPA 200.7
Cobalt	<20 µg/L	EPA 200.7
Copper	11.9 µg/L	EPA 200.7
Iron	<45.0 µg/L	EPA 200.7
Lead	<2.0 µg/L	EPA 239.2
Mercury	<0.20 µg/L	EPA 245.1
Magnesium	590 mg/L	EPA 200.7
Nickel	<15.0 µg/L	EPA 200.7
Selenium	<2.5 µg/L	EPA 270.2
Silver	<5.0 µg/L	EPA 200.7
Sodium	5120 mg/L	EPA 200.7
Zinc	<20.0 µg/L	EPA 200.7
Nitrogen, NH ₄ +NH ₃	<0.020 mg/L as N	EPA 350.1
Nitrogen, NH ₃ Unionized	<0.001 mg/L	Calculated
Nitrogen, NO ₂ +NO ₃	0.034 mg/L as N	EPA 353.2
pH	8.3	Meter
Alkalinity	171 mg/L as CaCO ₃	EPA 310.1
Sulfide	<0.05 mg/L as S	EPA 376.2
Hydrogen Sulfide	<0.03 mg/L	Std Method (428E)
Turbidity	<0.40 NTU	EPA 180.1
Dissolved Solids	20100 mg/L	EPA 160.1
Suspended Solids	27 mg/L	EPA 160.2
Fluoride	0.65 mg/L	EPA 340.2
Cyanide	<0.002 mg/L	EPA 335.3
Carbon, TOC	2.3 mg/L	EPA 415.1
COD	83 mg/L	EPA 410.1
Pesticides-Organochlorine and PCB's	<0.005 µg/L	EPA 8080
Pesticides-Organophosphate	<2.52 µg/L	EPA 8140

* The characterized seawater is natural seawater collected from the Atlantic Ocean near Marineland, Florida and diluted to a salinity of 20 parts per thousand using ESE's well water. The diluted seawater sample was collected from ESE's seawater delivery system on September 9, 1993.

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Appendix C- Pertinent Raw Data

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SODIUM CHLORITE-MBAHIA

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 Aquatic Toxicology Laboratory
 Gainesville, Florida

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 Effective: APR 1993

ACUTE TOXICITY DATA SHEET-BIOLOGICAL DATA								
SPONSOR: <u>CMA</u>			PROJECT NUMBER: <u>3943014-0200-3140</u>					
TEST SUBSTANCE: <u>Sodium Chlorite</u>			TEST SPECIES: <u>M. bahia</u>					
ANIMAL HISTORY								
Sample Information and Description Found on Page <u>532</u> of Test Substance Log								
Cof. No./Batch No.: <u>94-22</u> (Circle One)			Date Maintenance/Acclimation Began: <u>5/8/94</u>					
See Page No.: <u>58</u> of Animal Collection/Receipt Log			Condition of Animals: <u>Normal</u>					
See Page No.: <u>61</u> of <u>Invertebrate Holding Log</u> For Raw Data on Animal Holding			Life Stage/Age: <u><24 Hrs.</u>					
			Mortality in Tank 48 Hours Prior To Test Initiation: <u>0</u> (%)					
TEST CONDITIONS								
<input checked="" type="checkbox"/> Range-Finding	<input checked="" type="checkbox"/> Static		Time Added Substance/Animals		Test Location			
<input type="checkbox"/> Definitive	<input type="checkbox"/> Flow-Through		<u>140c / 1410</u>		<u>Lab tank 2</u>			
Test System: <input checked="" type="checkbox"/> Covered <input type="checkbox"/> Open	Test Container Dimensions (cm) (Dia) (Height) <u>19.5 x 6.5</u>		Test Solution Height: <u>3.5 cm</u>		Test Container Composition: <u>Glass</u>			
Test Chamber Volume (L) 0.34, <u>1.0</u> , 3.8, 30 - <u>NA</u> ^{WENT} _{1/18/94}			Test Solution Volume (L) 0.20, <u>1.0</u> , 3.0, 10 -					
Protocol: Acute toxicity of sodium chlorite to the mysid shrimp (<u>Mysidopsis bahia</u>)								
Dilution Water: <u>Filtered Seawater</u> Type of Lighting: <u>Fluorescent</u> Photoperiod: <u>12 light / 8 dark</u>								
Circle One: Test Substance as Active Ingredient/Whole Material								
Test Concentration & <u>29/L</u> ug/L	Control	Solv Cont	0.1	0.5	1.0	10.0	-	Solvent/Carrier:
Amount of Substance/Stock Added (ml)	NA	NA	.1	.5	1.0	10.0	-	NA
Stock Solution Used (e.g., 1', 2')	NA	NA	1	1	1	1	-	Solvent/Carrier Concentration:
Amount of Dilution Water Added (ml)	1000.0	NA	999.9	999.5	<u>999.0</u> <u>998.5</u>	<u>998.0</u>	-	NA
Amount of Solvent Added (NA)	NA	NA	NA	NA	NA	NA	NA	
Analyst: <u>NT</u>			Date: <u>3/1/94</u>			Comments in Daily Log		

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 Aquatic Toxicology Laboratory
 Gainesville, Florida

Page: _____
 QA Form Number: 031
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DILUTER CALIBRATION						
Sponsor: <u>CMA</u>		Test Substance: <u>Sodium Chlorite</u>				
Project Number: <u>3943014-0200-3140</u> <small>- 0100 -</small>		Test Species: <u>M. bahia & C. variegatus</u>				
Date Calibrated: <u>3/11/94</u>		Calibrated By: <u>NJF</u>				
Diluter ID Number: <u>5</u>		Location: <u>In-house</u>				
Calibrated for a <u>50</u> % diluter.						
Cell ID	Desired Cell Volumes (mL)		Measured Cell Volumes (mL)			
	Water	Chemical	Water	Chemical		
Control	500	0	500.0, 496.0, 498.0	0		
6.25% 0.125 mg/L	468.75	31.25	465.0, 470.0, 470.0	31.5, 32.0, 32.0		
12.5% 0.25 mg/L	437.5	62.5	435.0, 438.0, 432.0	62.0, 61.5, 62.5		
25% 0.5 mg/L	375	125	370.0, 375.0, 375.0	124.0, 125.0, 125.0		
50% 1.0 mg/L	250	250	250.0, 248.0, 250.0	253.0, 250.0, 252.0		
100% 2.0 mg/L	0	500	0	500.0, 505.0, 502.0		
SPLITTER BOX DELIVERY CALIBRATION						
Splitter Box ID	Control	0.125 mg/L	0.25 mg/L	0.5 mg/L	1.0 mg/L	2.0 mg/L
Replicate A Volume (mL)	250.0 252.0 255.0	240.0 250.0 252.0	250.0 253.0 255.0	257.0 265.0 255.0	248.0 252.0 245.0	260.0 255.0 250.0
Replicate B Volume (mL)	245.0 250.0 250.0	255.0 253.0 250.0	235.0 252.0 250.0	250.0 250.0 252.0	260.0 265.0 255.0	265.0 250.0 260.0
Comments: _____ _____ _____ _____						

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Aquatic Toxicology Department
Gainesville, Florida

Page: _____
QA Form Number: 102
Effective: July 1990

SUBJECT: METERING PUMP CALIBRATION

Sponsor: CMA Project Number: 3943014-0200-3140
Test Substance: Sodium Chlorite Test Species: M. bahia

^{#1} Calibration Substance: Milli-Quater Pump Setting: 2ml
Desired Volume: 2.0ml Metering Pump: Digiflex
Tare Weight: 1.3855g Total Weight: 3.3763g
Difference: 1.9908g Number of Cycles: 10
Volume Per Cycle: 0.19908ul
Date: 3/11/94 Analyst: jt

Comments: _____

^{#2} Tare Weight: 1.3730g Total weight: 3.3670g
Difference: 1.9940g Number of Cycles: 10
Volume per cycle: 0.19940ul

^{#3} Tare weight: 1.3423g Total weight: 3.3358g
Difference: 1.9935g Number of Cycles: 10
Volume per cycle: 0.19935ul

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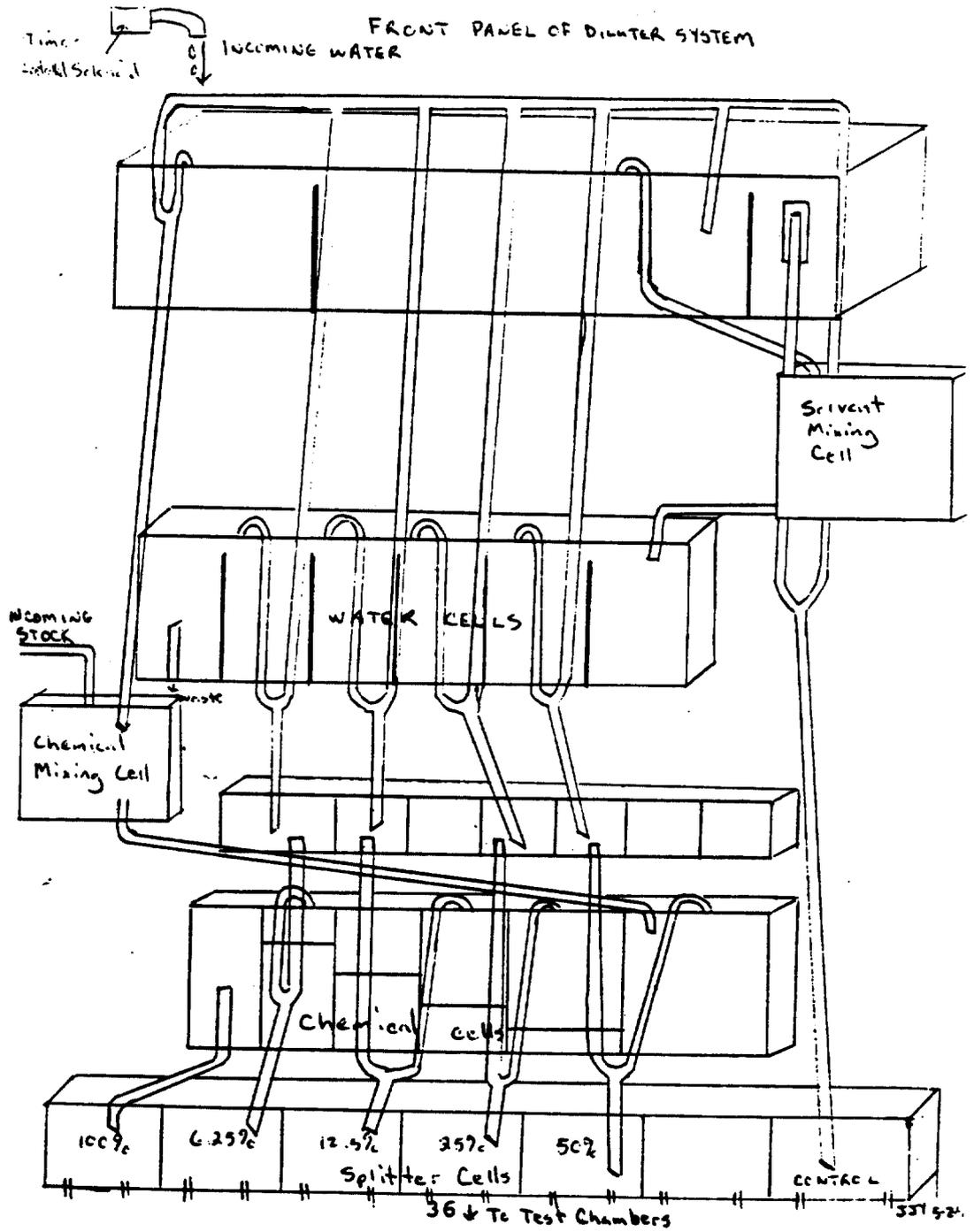
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DILUTER CALIBRATION				
Sponsor: <u>CMA</u>		Test Substance: <u>Sodium Chlorite</u>		
Project Number: <u>3943014-0200-3140</u>		Test Species: <u>C. virginicus & M. bahia</u>		
Date Calibrated: <u>4/6/94</u>		Calibrated By: <u>JK</u>		
Diluter ID Number: <u>5</u>		Location: <u>In-house</u>		
Calibrated for a <u>50</u> % diluter.				
Cell ID	Desired Cell Volumes (mL)		Measured Cell Volumes (mL)	
	Water	Chemical	Water	Chemical
<u>Control</u>	<u>500.0</u>	<u>-</u>	<u>498.0</u>	<u>-</u>
<u>0.125-ml</u>	<u>468.75</u>	<u>31.25</u>	<u>474.0</u>	<u>31.0</u>
<u>0.250</u>	<u>437.50</u>	<u>62.5</u>	<u>443.0</u>	<u>66.0</u>
<u>0.500</u>	<u>375.0</u>	<u>125.0</u>	<u>380.0</u>	<u>130.0</u>
<u>1.00</u>	<u>250.0</u>	<u>250.0</u>	<u>251.0</u>	<u>250.0</u>
<u>2.00</u>	<u>-</u>	<u>500.0</u>	<u>-</u>	<u>510.0</u>
SPLITTER BOX DELIVERY CALIBRATION				
Splitter Box ID				
Replicate A Volume ()				
Replicate B Volume ()				
Comments: <u>* Past-calibration check of diluter</u>				

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 Gainesville, Florida

Page: _____
 ESE QA Form No.: 015A
 Effective: APR 1993

ACUTE TOXICITY DATA SHEET-BIOLOGICAL DATA								
SPONSOR: <u>CMA</u>		PROJECT NUMBER: <u>3943014-0200-3140</u>						
TEST SUBSTANCE: <u>Sodium Chlorite</u>		TEST SPECIES: <u>Musidopsis halia</u>						
ANIMAL HISTORY								
Sample Information and Description Found on Page <u>532</u> of Test Substance Log								
Lot No./Batch No.: <u>94-36</u> (Circle One)				Date Maintenance/Acclimation Began: <u>N/A</u>				
See Page No.: <u>58</u> of Animal Collection/Receipt Log				Condition of Animals: <u>Normal</u>				
See Page No.: <u>65</u> of <u>Inventory Worksheet</u> For Raw Data on Animal Holding				Life Stage/Age: <u>4-24 hours</u>				
				Mortality in Tank 48 Hours Prior To Test Initiation: <u>0</u> (%)				
TEST CONDITIONS								
<input type="checkbox"/> Range-Finding		<input type="checkbox"/> Static		Time Added Substance/Animals		Test Location		
<input checked="" type="checkbox"/> Definitive		<input checked="" type="checkbox"/> Flow-Through		<u>N/A / 15cc</u>		<u>Diluter</u>		
Test System: <input checked="" type="checkbox"/> Covered <input type="checkbox"/> Open		Test Container Dimensions (cm) L: <u>35</u> W: <u>25</u> H: <u>15</u>		Test Solution Height: <u>7.5 cm</u>		Test Container Composition: <u>Glass</u>		
Test Chamber Volume (L) 0.34, 1.6, 3.8, 30 - <u>(10.5)</u>				Test Solution Volume (L) 0.20, 1.0, 3.0, 10 - <u>(5)</u>				
Protocol: <u>acute toxicity of sodium chlorite to the marine shrimp (Musidopsis halia)</u>								
Dilution Water: <u>Natural Filtered Seawater</u>		Type of Lighting: <u>Fluorescent</u>			Photoperiod: <u>12 L: 12 D</u>			
Circle One: Test Substance as Active Ingredient/Whole Material								
Test Concentration & (ug/L ug/L)	Control	Solv Cont	0.100	0.350	0.500	1.00	2.00	Solvent/Carrier:
Amount of Substance/Stock Added ()	NA	NA	<u>see</u>	<u>diluter</u>				NA
Stock Solution Used (e.g., 1', 2')	NA	NA	<u>calibration sheet</u>					Solvent/Carrier Concentration:
Amount of Dilution Water Added (-)								
Amount of Solvent Added (-)	NA		NA	NA	NA	NA	NA	

Analyst: MA Date: 11/26/93 Comments in Daily Log

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 ESE PROJECT #: 3943014-0200-3140
 CMA #: CD-7.0-3AT-ESE

Environmental Science & Engineering, Inc.
 Aquatic Toxicology Laboratory
 Gainesville, Florida

Page: _____
 ESE QA Form No.: 015B
 Effective: MAR 1993

DEFINITIVE TEST

Proj: 3943014-0200-3140

M. L. White

SUBJECT: ACUTE TOXICITY DATA SHEET - SURVIVAL											
		0-HOUR		24-HOUR		48-HOUR		72-HOUR		96-HOUR	
DATE		3/28/94		3/29/94		3/30/94		3/31/94		4/1/94	
ANIMALS FED		Yes, brine shrimp		Yes, brine shrimp		Yes, brine shrimp		Yes, brine shrimp		Yes, brine shrimp	
TIME		1500		1430		1400		1200		1400	
DATA BY		MO		CN		CN		CN		CN	
NOMINAL CONCENTRATION & $\mu\text{g}/\text{L}$	REP	OBS.	NO. ALIVE								
	CONTROL	A	None	10	None	10	None	10	None	10	None
	B	↓	10	↓	10	↓	10	↓	10	↓	10
0.125	A	None	10	None	10	1 dead	9	1 NF	8	None	8
	B	↓	10	↓	10	1 dead	9	1 NF	8	↓	8
0.250	A	None	10	None	10	1 dead	9	1 NF	8	None	8
	B	↓	10	↓	10	1 LEH	10	None	10	↓	10
0.500	A	None	10	None	10	1 dead	9	2 NF	7	None	7
	B	↓	10	↓	10	1 dead 1 LEH	9	1 dead	8	1 NF	7
1.00	A	None	10	1 LEH	10	3 dead	7	5 dead 1 NF	1	None	1
	B	↓	10	3 dead	7	4 dead	3	2 dead 1 LEH	1	1 dead	0
2.00	A	None	10	2 dead	8	8 dead	0	None	0	None	0
	B	↓	10	4 dead	6	6 dead	0	↓	0	↓	0

OBSERVATION KEY

OBS - OBSERVATIONS	DRK - DARKER THAN CONTROLS	NF - NOT FOUND
NONE - NORMAL CONDITIONS	LT - LIGHTER THAN CONTROLS	
AS - AT SURFACE	RAR - RAPID RESPIRATION	BS - BRINE SHRIMP
LE - LOSS OF EQUILIBRIUM	PRE - PRECIPITATE	
LETH - LETHARGIC	POS - FILM ON SURFACE	
HYP - HYPERACTIVE	UC - UNDISSOLVED CHEMICAL	
SPM - SPERMATIC MOVEMENT	CLDY - CLOUDY SOLUTION	

96-h LC50

Results calculated using the Moving Average Method.

Client : CMA Species : M. bahia

Dates of test : 3/28/94 to 4/1/94

Test Material : Sodium Chlorite proj # 3943014-0200-3140

Report run by : JJY Date : 04-06-1994

Concentration units (Circle One) : % PPTH **PPM** PPB Other _____

Conc.	Number Exposed	Number Dead	Percent Dead
2.3	20	20	100.0
1.1	20	19	95.0
0.6	20	6	30.0
0.3	20	2	10.0
0.1	20	2	10.0
Control	20	0	0.0

Span	G	LC50	95 Percent Confidence Limits	
	0.085	0.576	0.441	0.754
3	0.078	0.614	0.476	0.758
2	0.103	0.638	0.530	0.787
1	0.184	0.688	0.558	0.792

Note -- An LC50 calculated using the Moving Average Method may not be a very good estimate for Spans less than 4 .

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VULCAN CHEMICALS RAD
ANALYTICAL REPORT

DATE: 03/18/94
 PAGE NO: 1

SAMPLE NO.	PROJ. NO.	COMPONENT	RESULTS	ANALYST	COMMENTS

os95064	60	Vulcan Chemical NaClO2 (Same drum a Submitted By: BCC			

		NaClO2	81.41%	SLG	Titration w/ Na2S2O3
		NaCl	10.821%	SLG	Titration w/ AgNO3
		Na2CO3	0.504%	SLG	Titration w/ H2SO4
		Na2SO4	0.364%	DMM	IC, 89.3% spike recovery
		H2O2	0.003%	SLG	Titration w/ NaOH
		water by di	6.156%	DMM	By difference
		Pb	< 0.5 ppm	DMM	GF, 88.4% spike recovery
		NaOH	0.028%	SLG	Titration w/ H2SO4 w/ BaCl2
		NaClO3	0.914%	DMM	IC, 101.3% spike recovery
		Hg	< 65 ppb	SLG	MHS, 89.0% spike recovery
		As	< 1.3 ppm	DMM	MHS-20, 89.6% spike recovery

 END OF REPORT

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Appendix D- Analytical Method Validation

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FINAL REPORT

STUDY TITLE

**Method Validation for the Determination
of Sodium Chlorite in Seawater**

AUTHOR

David H. Greer, Jr.

STUDY COMPLETED

May 18, 1994

REPORT DATE

May 18, 1994

SPONSOR

**Chemical Manufacturers Association
2501 M Street N.W.
Washington, D.C. 20037**

PERFORMING LABORATORY

**Environmental Science & Engineering, Inc.
14220 Newberry Road
Gainesville, Florida 32607
(904) 332-3318**

LABORATORY STUDY NUMBER

1944015V-L202

LABORATORY PROTOCOL NUMBER

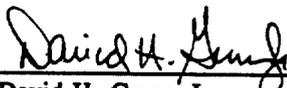
ASG-94-02

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ESE PROJECT #: 3943014-0200-3140
CMA #: CD-7.0-3AT-ESE

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COMPLIANCE STATEMENT

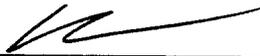
The data presented in this report was an accurate representation of the investigations conducted. This study was conducted in compliance with current Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Good Laboratory Practice Regulations (40 CFR Part 160). All records pertaining to this study were maintained at Chemical Manufacturers Association GLP archives located at 2501 M Street, N.W., Washington, D.C. 20037. A copy of the original raw data will be archived in the ESE Gainesville Analytical Services GLP archives located at 14220 West Newberry Road, Gainesville, Florida 32607.



David H. Greer, Jr.,
Program Manager, Study Director
ESE, Inc.

5/18/94

Date



Kathleen K. Allen
Water Quality Department Manager
ESE, Inc.

5/18/94

Date



Robert Romano, Ph.D.
Director of Special Programs
Chemical Manufacturers Association

5/19/94

Date

QUALITY ASSURANCE STATEMENT

This study was monitored by Environmental Science & Engineering, Inc. (ESE) Quality Assurance Unit to assure that the guidelines and Standard Operating Procedures (SOPs) used to conduct this study were followed.

The final report for this study was reviewed by the Quality Assurance Unit and, accurately reflects the results of the study as conducted, including all areas of compliance or noncompliance with Good Laboratory Practice Standards, the study protocol and ESE's SOPs.

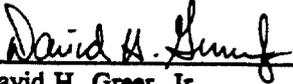
Joe Owusu-Yaw, Ph.D.
Joe Owusu-Yaw
QAU GLP Coordinator

5-18-94
Date

QUALITY ASSURANCE AUDITS

<u>Audit Date</u>	<u>Type of Report</u>	<u>Date Reported to Management</u>	<u>Date Reported Study Director</u>
2/25/94	Protocol	5/18/94	2/25/94
5/16/94	Data Review	5/18/94	5/16/94
5/16/94	Draft Report	5/18/94	5/16/94
5/17/94	Revised Report	5/18/94	5/17/94
5/18/94	Final Report	5/18/94	5/18/94

CONFIDENTIAL
SODIUM CHLORITE-MBAHIA
ESE PROJECT #: 3943014-0200-3140
CMA #: CD-7.0-3AT-ESE
REPORT SIGNATURES



David H. Greer, Jr.,
Program Manager, Study Director
ESE, Inc.

5/18/94

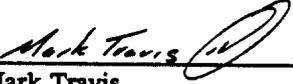
Date



Kathleen K. Allen
Water Quality Department Manager
ESE, Inc.

5/18/94

Date



Mark Travis
Water Quality Analyst
ESE, Inc.

5/18/94

Date

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SODIUM CHLORITE-MBAHIA
ESE PROJECT #: 3943014-0200-3140
CMA #: CD-7.0-3AT-ESE

STUDY SUMMARY

SPONSOR: Chemical Manufacturers Association
2501 M Street, N.W.
Washington, D.C. 20037
Sponsor Representative: Robert Romero, Ph.D.
Director of Special Programs
(202) 887-1198

SPONSOR STUDY NUMBER: ASG-94-02 .

STUDY DIRECTOR: David H. Greer, Jr.
Environmental Science & Engineering, Inc.
14220 West Newberry Road
Gainesville, Florida 32607
(904) 333-1608

STUDY TITLE: Method Validation for the Determination of
Sodium Chlorite in Seawater

LOCATION OF STUDY: Environmental Science & Engineering, Inc.
14220 West Newberry Road
Gainesville, Florida 32602-1703

LABORATORY STUDY IDENTIFICATION: 1944015V-L202

TEST MATERIAL: Sodium Chlorite - CAS No.: 7758-19-2

STUDY INITIATION DATE: March 2, 1994

EXPERIMENTAL INITIATION DATE: March 2, 1994

EXPERIMENTAL COMPLETION DATE: March 18, 1994

STUDY COMPLETION DATE: May 18, 1994

DETERMINATION OF SODIUM CHLORITE IN SEAWATER

1.0 SCOPE AND APPLICATION

This method describes the analysis of Sodium Chlorite at the milligram per liter (mg/L) level in seawater.

2.0 SUMMARY OF METHOD

The reference analytical method used for this validation study was "The Measurement of Chlorite Ion in Seawater by IC Using the Chlorpromazine Post Column Reaction" [NOVATEK Application Note for the Chemical Manufacturers Association (Contract CD-6.0-NOV, Appendix I)]. This method is based on an ion chromatography post-column procedure using USEPA Method 300.0 with a borate buffer eluent. The post-column procedure uses photometric detection at 530 nanometers (nm). Following separation, chlorite (ClO_2^-) reacts with chlorpromazine in acidic media. This detection chemistry shows no interference from most common anions.

3.0 APPARATUS AND MATERIALS

- 3.1 Ion Chromatography System: Dionex IC Model DX-300.
- 3.2 Analytical Column: Dionex AS11.
- 3.3 Post Column Derivatization: Technicon II reagent manifold and colorimeter.
- 3.4 Data system: Dionex AI 450.
- 3.5 Volumetric Flasks: Various.
- 3.6 Analytical Balance: Mettler AE160.
- 3.7 Test Materials: Sodium Chlorite (Aldrich Lot # 01615KY and #05801JZ, 80% purity and NOVATEK, 99.75% purity).
- 3.8 Boric Acid: Aldrich lot# 05625MY, ACS reagent grade.
- 3.9 Sodium Hydroxide: Fisher lot# 9338003-24, 50/50 (w/w) solution.
- 3.10 Eluent: Borate eluent, 25 millimolar (mM) NaOH/50 mM H_3BO_3 .

- 3.11 Regenerant: Suppressor Regenerant, 12.5 mM H₂SO₄.
- 3.12 Water: Deionized (D.I.) water.
- 3.13 Seawater Test Solution: Ethylenediamine, 50 mg/L in full strength natural seawater.
- 3.14 Derivatization Reagent: Chlorpromazine hydrochloride (Sigma Lot# 112H0125), 0.56 mM in D.I. water.
- 3.15 Hydrochloric Acid Solution: 350 mL concentrated hydrochloric acid in 2 L D.I. water.

4.0 ELUENT PREPARATION

The eluent was prepared by dissolving 12.4 grams (g) of boric acid into 1 liter of D.I. water in a 2 liter volumetric flask. To the boric acid solution, 2.6 milliliters (mL) of 50/50 (w/w) sodium hydroxide solution was added, and the flask brought to volume with D.I. water. The contents of the flask were mixed thoroughly by inverting several times.

5.0 DERIVATIZATION REAGENT PREPARATION

The derivatization reagent was prepared by dissolving 0.2 g of chlorpromazine hydrochloride in 1 L of D.I. water. This solution was degassed with nitrogen prior to use.

6.0 STANDARD PREPARATION

6.1 PREPARATION OF CALIBRATION STOCK STANDARD

A calibration stock (spiking stock) was prepared by accurately weighing 1.676 g of sodium chlorite (80 %purity, Aldrich lot # 05801JZ) into a 1,000 mL volumetric flask and bringing to volume with deionized water. The solution was mixed thoroughly by inverting the stoppered flask several times. The concentration of this stock solution was 1000 mg-chlorite/L. This solution was used to prepare the instrument calibration standards.

6.2 PREPARATION OF CONTROL STOCK STANDARD

A control stock standard was prepared by accurately weighing 1.676 g of sodium chlorite (80 % percent purity, Aldrich lot # 01615KY) into a 1000 mL volumetric flask. The flask was brought to volume with D.I. water and mixed thoroughly by inverting the stoppered flask several times. The concentration of this stock was 1000 mg-chlorite/L. This solution was used to prepare the fortified samples for the method validation.

6.3 PREPARATION OF CALIBRATION VERIFICATION STOCK

An independent calibration verification stock (ICV) standard was prepared by accurately weighing 0.1676 g of sodium chlorite (99.75 % purity, NOVATEK) into a 100 mL volumetric flask. The flask was brought to volume with D.I. water and mixed thoroughly by inverting the stoppered flask several times. The concentration of this stock was 1250 mg-chlorite/L. This standard was used to verify the preparation of the calibration and control stocks.

6.4 STANDARDIZATION OF CHLORITE STOCKS

All Chlorite stock solutions were standardized using the iodometric procedure (Standard Methods, 15th Edition, Method 410A). Twenty five milliliter (mL) of each stock solution was volumetrically transferred to separate 125 mL erlenmeyer flask and 0.5 g of potassium iodine (KI) was added to the flask and dissolved. After the KI was dissolved, 0.5 mL of concentrated sulfuric acid was added and the flask was stoppered and placed in the dark for 5 minutes. Each flask was titrated with 0.1 N thiosulfate, adding 1 mL of starch indicator solution when the brown color became light yellow. Titration was continued until the solution became clear.

6.5 PREPARATION OF WORKING CALIBRATION STANDARDS

One mL of the 1000 mg/L calibration stock was diluted with D.I. water to 10 mL in a 10 mL volumetric flask. The concentration of this intermediate calibration stock was 100 mg/L. A series of 7 dilutions of the calibration stock were prepared for use in instrument calibration. The following dilution schedule was used to prepare the calibration standards.

Intermediate Chlorite Calibration Stock (mL) (100 mg/L)	Final Volume (mL D.I. water)	Calibration Standard Concentration (mg/L)
0.02	100	0.02
0.05	100	0.05
0.10	100	0.10
0.20	100	0.20
0.40	100	0.40
1.00	100	1.00
2.00	100	2.00

7.0 PREPARATION OF FORTIFICATION SOLUTIONS

7.1 SECONDARY FORTIFICATION SOLUTION

A secondary fortification solution was prepared by transferring 1 mL of the control stock into a 100 mL volumetric flask. The flask was brought to volume with D.I. water and inverted several times to mix thoroughly. The concentration of this solution was 10 mg-chlorite/L.

7.2 PREPARATION OF FORTIFICATION SAMPLES

Low level fortification samples, 0.1 mg-chlorite/L, were prepared by transferring 100 microliters of the secondary fortification solution (10 mg-chlorite/L) to a 10 mL volumetric flask. The flask was brought to volume with filtered natural seawater fortified with 50 mg/L of ethylenediamine. The fortification samples were prepared in triplicate and mixed thoroughly by inverting the stoppered flask several times.

High level fortification samples, 250 mg-chlorite/L, were prepared by diluting 1 mL of the control stock (1000 mg-chlorite/L) with 3 mL of filtered natural seawater fortified with 50 mg/L ethylenediamine. The fortification samples were prepared in triplicate and mixed thoroughly by inverting the stoppered flask several times.

8.0 SAMPLE ANALYSIS

8.1 INSTRUMENT OPERATING CONDITIONS

The following instrumental conditions were used during analysis of test samples.

System I.D.	IC Model DX-300, #1
Separation Column	AS11 #1700
Suppression	AMMSII #8909
Injection Loop	50 μ L
Flow Rate	1.0 mL/min
Pressure	680 psig
Offset	5.1 micro-Siemens (μ S)
Eluent	12.5 mM NaOH/25 mM H ₃ BO ₃
Regenerant	25 mN H ₂ SO ₄

The configuration of the post column derivatization and visible detection system is presented as Figure 8-1.

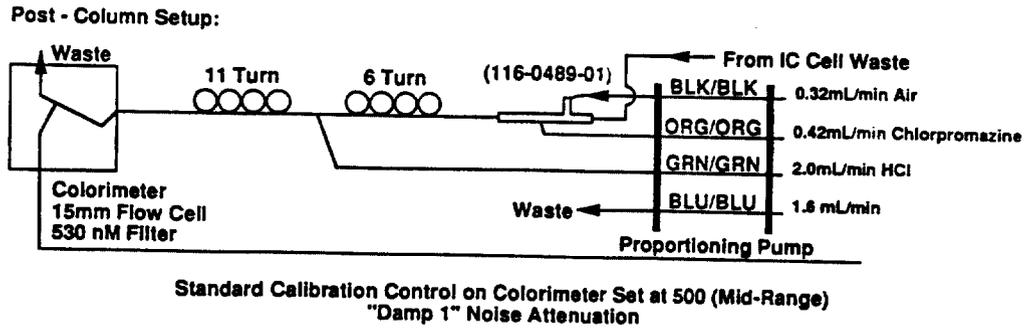


Figure 8-1 Post Column Derivatization/Visible Detection System Configuration

8.2 INSTRUMENT CALIBRATION AND SAMPLE ANALYSIS

- 8.2.1 After the instrument had warmed up and stabilized, the column was 'primed' with injection of the 2.0 mg/L calibration standard followed by an injection of D.I water.
- 8.2.2 Each calibration standard, beginning with a D.I. water blank was injected in order of increasing chlorite concentration.
- 8.2.3 A 1.25 mg/L instrument calibration verification standard (ICV) was analyzed to confirm that instrument calibration standard preparation was correct.
- 8.2.4 After calibration was complete, each set of fortified test samples were injected beginning with a seawater blank. Seawater samples were diluted by a factor of 4 to prevent overload of the column and subsequent retention time shifts. Dilution factors of 4 were used for the low level fortification samples and 250 was used for the high level fortification samples.
- 8.2.5 After all sample analyses were complete, a 1.0 mg/L continuing calibration verification standard (CCV) was analyzed to verify instrument stability throughout the analytical run.

9.0 QUANTITATION

- 9.1 A quadratic regression curve of Chlorite concentration versus instrument response, expressed as peak area, was generated from the data obtained during instrument calibration. The regression was used to calculate concentrations of Chlorite in samples and quality control fortifications by substitution of peak areas found in samples and quality control fortifications into the regression equation generated from the calibration data.
- 9.2 Recoveries of Chlorite from the fortification test samples were calculated by comparing the calculated found concentrations in the fortified samples with the nominal fortification levels. Simple arithmetic means and standard deviations for the method validation fortification levels were calculated and used to generate the quality control limits for the toxicity test sample determinations.

10.0 DATA ACCEPTABILITY

- 10.1 Calibrations were considered acceptable if the correlation coefficient of the curve was greater than or equal to 0.995.
- 10.2 Continuing calibration standard response (post-run) agreed within plus or minus 10 percent of the original analysis response for that standard.

10.3 Quality control limits were established using results obtained during the method validation study. Warning limits were set at plus or minus 2 standard deviations from the mean of all recovery values. Control limits were set at plus or minus 3 standard deviations from the mean.

11.0 SAFETY

All reagents and test materials should be handled with care to avoid unnecessary exposure of any personnel to these potentially toxic materials. Material Safety Data Sheets should be read by all personnel involved with the determination of Chlorite. Avoid skin contact or ingestion of Chlorite.

12.0 METHOD VALIDATION RESULTS

Recoveries for fortified samples ranged from 99.8 to 109 percent. The calculated mean recovery was 103.5 percent with a standard deviation of 3.7. Results for individual fortification samples are presented in Table 12-1. The reporting limit for chlorite using this method was 0.08 mg/L in seawater.

Low level fortification samples were analyzed undiluted. Due to column overload, there was no recovery of chlorite from the undiluted samples. There were no significant interferences noted during the analysis of chlorite when utilizing the post column derivatization and visible detection system proceeded by separation of chlorite by ion chromatography.

Example chromatograms for standards and fortification samples depicting both the ion chromatographic conductivity and post column derivatization visible detection systems are presented as Figures 12-1 through 12-4.

Table 12-1 Fortification Results of Chlorite Analytical Method Validation

Fortification Level	Target Conc. (mg/L)	Found Conc. (mg/L)	Percent Recovery
Low SPK #1	0.10	0.109	109
Low SPK #2	0.10	0.107	107
Low SPK #3	0.10	0.103	103
High SPK #1	250.00	249.4	99.8
High SPK #2	250.00	251.1	100.4
High SPK #3	250.00	254.9	102
		Mean	103.5
		Std Dev	3.7

Std Dev = Standard Deviation

Source: ESE, 1994

Area Report

Data Reprocessed On 03/09/1994 10:08:17

```

-----
| Sample Name: AUTOCAL7R - A10           Date: 03/08/1994 16:38:39
| Data File : C:\DX\DATA4\ch030801.d10
| Method    : C:\DX\METHOD\CLO2.met
| ACI Address: 1 System: 1 Inject#: 10 Vial:A10   Detector:CDM-2
| Analyst   :                               Column:
-----

```

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area	Reject
External	50	1	2280	2Hz	0.00	18.99		500

***** Peak Report: All Peaks *****

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
1	1.25			0.000	5484475	0.000	1
2	3.00	0.00	CLO2	1.000	11585140	0.000	1
3	5.20			0.000	4040705	0.000	1
Totals				1.000	21110320		

File: ch030801.d10 Sample: AUTOCAL7R - A10

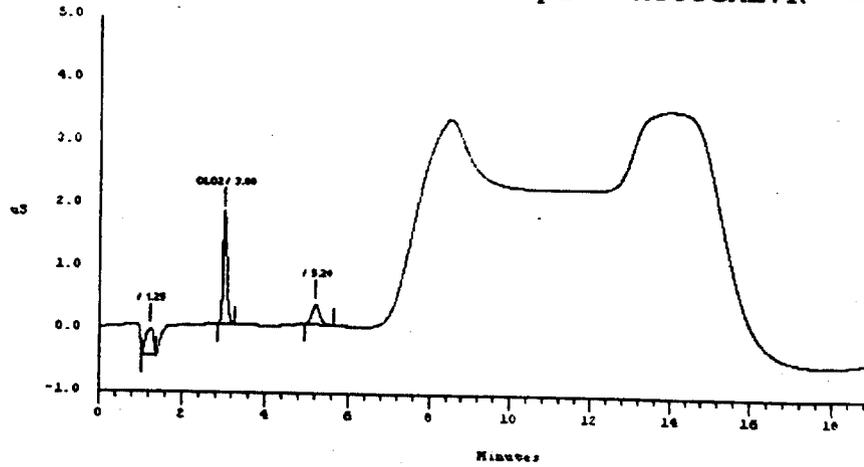


Figure 12-1 Example Chromatogram of Chlorite Standard (1.0 mg/L) - Conductivity Detection.

Area Report

Data Reprocessed On 03/09/1994 10:09:25

```

-----
| Sample Name: SP1*QC*3 - A23           Date: 03/08/1994 21:08:34
| Data File : C:\DX\DATA4\ch030801.d23
| Method    : C:\DX\METHOD\CLO2.met
| ACI Address: 1 System: 1 Inject#: 23 Vial:A23   Detector:CDM-2
| Analyst   :                               Column:
-----
  
```

```

-----
Calibration Volume Dilution Points Rate Start Stop Area Reject
-----
External          50          250  2280  2Hz  0.00 18.99      500
-----
  
```

***** Peak Report: All Peaks *****

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
1	1.25			0.000	5052670	0.000	1
2	3.03	1.11	CLO2	255.740	11863418	0.000	1
3	4.98			0.000	1656239100	0.000	1
Totals				255.740	1673155188		

File: ch030801.d23 Sample: SP1*QC*3 - A23

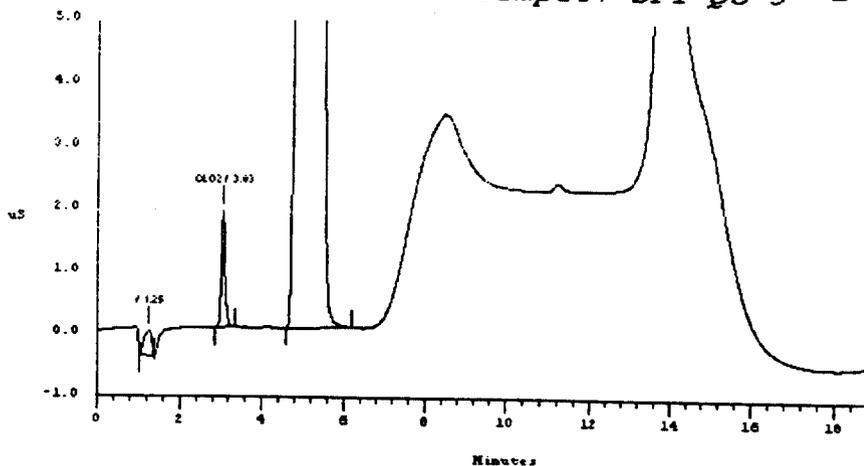


Figure 12-2 Example Chromatogram of Chlorite Seawater Fortification (250 mg/L) - Conductivity Detection.

CONFIDENTIAL
SODIUM CHLORITE-MBAHIA
ESE PROJECT #: 3943014-0200-3140
 Area Report
CMA #: CD-7.0-3AT-ESE

Data Reprocessed On 03/09/1994 10:39:43

```

-----
| Sample Name: AUTOCAL7R - A10           Date: 03/08/1994 16:38:39
| Data File : C:\DX\DATA4\ch030802.d10
| Method    : C:\DX\METHOD\CLO2.me2
| ACI Address: 1 System: 1 Inject#: 10 Vial:A10      Detector:OTHER
| Analyst   :                               Column:
-----
  
```

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area	Reject
External	50	1	2280	2Hz	0.00	18.99		100

***** Component Report: All Components *****

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
1	4.52	0.00	50074*IC-G CLO2	1.000	34796	0.000	1
Totals				1.000	34796		

***** Peak Report: Unknown Peaks *****

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
Totals				0.000	0		

File: ch030802.d10 Sample: AUTOCAL7R - A10

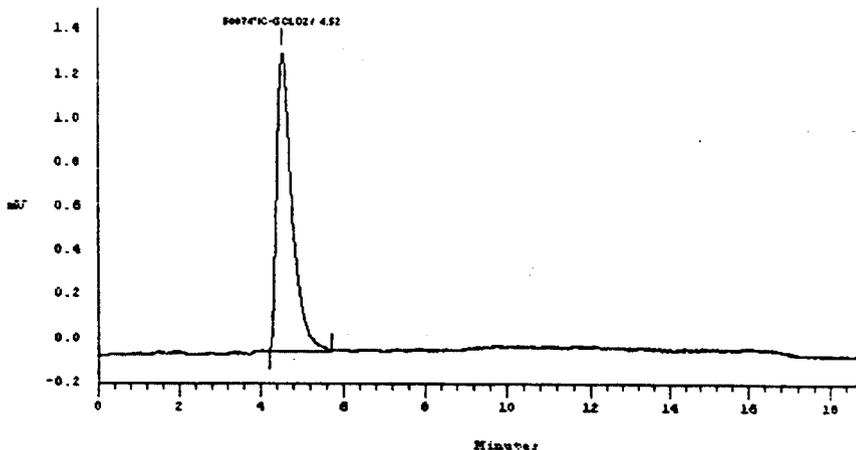


Figure 12-3 Example Chromatogram of Chlorite Seawater Fortification (250 mg/L) - Visible Detection.

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Data Reprocessed On 03/09/1994 10:41:09

```

-----
: Sample Name: SP1*QC*3 - A23                      Date: 03/08/1994 21:08:34
: Data File : C:\DX\DATA4\ch030802.d23
: Method    : C:\DX\METHOD\CLO2.me2
: ACI Address: 1 System: 1 Inject#: 23 Vial:A23      Detector:OTHER
: Analyst   :                                     Column:
-----
  
```

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area	Reject
External	50	250	2280	2Hz	0.00	18.99		100

***** Component Report: All Components *****

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
2	4.55	1.11	50074*IC-G CLO2	249.858	34547	0.000	1
Totals				249.858	34547		

***** Peak Report: Unknown Peaks *****

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
1	1.87			0.000	776	0.000	1
Totals				0.000	776		

File: ch030802.d23 Sample: SP1*QC*3 - A23

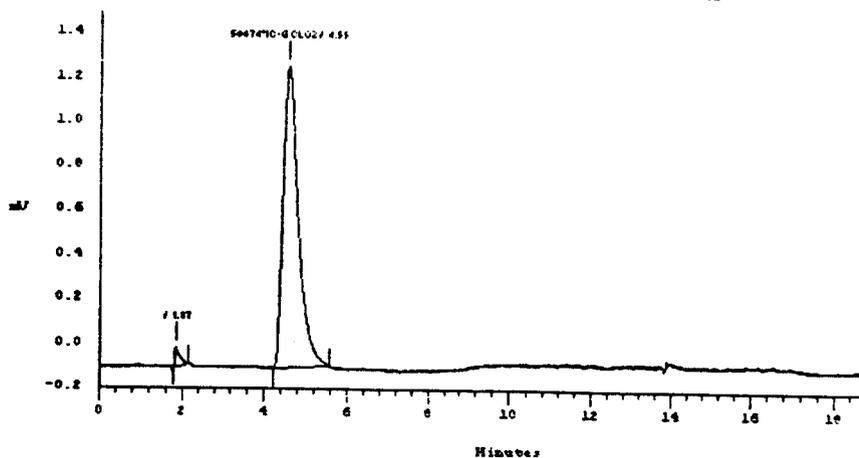


Figure 12-4 Example Chromatogram of Chlorite Standard (1.0 mg/L) - Visible Detection.

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APPENDIX I
PROTOCOL

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CMA #: CD-7.0-3AT-ESE
ESE PROTOCOL NO.: ASG-94-02

ENVIRONMENTAL SCIENCE & ENGINEERING, INC.

ANALYTICAL SERVICES

METHOD VALIDATION PROTOCOL

STUDY TITLE

Method Validation for the Determination of Sodium Chlorite in Seawater

TEST SUBSTANCE IDENTIFICATION

Sodium Chlorite

CAS No.: 7758-19-2

SPONSOR

Chemical Manufacturers Association

Sponsor Representative: Mr. Robert Romano, Ph.D.
Director of Special Programs
Chemical Manufacturers Association
2501 M. Street N.W.
Washington, D.C. 20037
(202) 887-1198

Study Number: Not Assigned

TEST FACILITY

Environmental Science & Engineering, Inc. (ESE)
Analytical Services
14220 Newberry Road
Gainesville, Florida 32607
ESE Study Director: David H. Greer, Jr.
GLP Program Manager
(904) 333-1608
(904) 333-6622 FAX

PROPOSED EXPERIMENTAL START DATE

Upon acceptance of the protocol by the study sponsor. The proposed experimental start date for this method validation study is February 16, 1994.

PROPOSED EXPERIMENTAL TERMINATION DATE

March 10, 1994.

OBJECTIVE

To validate an analytical method for the determination of sodium chlorite in seawater utilizing ion chromatography with visible (530 nanometers) light detection in support of aquatic toxicity studies.

REFERENCE MATERIALS

1. Sodium Chlorite, 80% purity (Aldrich Chemical Co. Lot # 0161SKY)

METHODS

The analytical method used for this validation study will be "The Measurement of Chlorite Ion in Seawater by IC Using the Chlorpromazine Post Column Reaction" (Novatek Application Note for the Chemical Manufacturers Association (Contract CD-6.0-NOV, Appendix I). Modifications to the method will be made only after consultation with the Study Sponsor Representative.

Analytical Method Validation

The analytical method validation will consist of the preparation of a blank seawater matrix, triplicate fortifications of seawater at the anticipated low test concentration (0.1 milligrams per liter (mg/L)), and triplicate fortifications of seawater at the anticipated high test concentration (250 mg/L). The samples will be analyzed according to the referenced method and the resulting data will be analyzed to determine the precision and accuracy of the method.

Statistical Analysis

Recoveries of Chlorite from the analytical method validation fortification spike samples will be calculated by comparing the calculated found concentrations in the fortified samples with the nominal fortification levels. Simple means and standard deviations for the two fortification levels will be calculated and used to generate the quality control limits for field sample determinations.

Quality Assurance/Quality Control

Prior to each analytical sequence, a series of analytical calibration standards will be prepared, analyzed and used to determine the concentration of the target compounds in the test solutions.

Acceptance of the instrument calibration will depend upon the correlation coefficients for the standard curves. Calibration will be considered acceptable if the correlation coefficient of the calibration curve is greater than or equal to 0.995 using either linear or quadratic regression analysis.

At the end of each analytical sequence, a continuing calibration standard (CCS) will be analyzed to determine the instrument stability throughout the analytical sequence. The CCS must be within plus or minus 15 percent of the same concentration standard run at the beginning of the analytical sequence for the run to be considered valid.

The upper and lower warning and control limits for the analysis field samples will be set by mutual agreement between the Study Sponsor Representative and the ESE Study Director upon completion of the method validation study and review of the recoveries obtained during the method validation study.

REPORT

A method validation report will be prepared in U.S. Environmental Protection Agency PR Notice 86-5 format documenting all activities associated with the determination of Chlorite ion in seawater matrices. The report will contain a detailed write-up of the method for analysis of Chlorite, a data summary of the results of the method validation, raw data, a quality assurance statement and a Good Laboratory Practices compliance statement.

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SAFETY

Material Safety Data Sheets (MSDS) will be provided to all personnel involved in the study. All reagents and test materials will be handled in manners described in the ESE Laboratory Safety Manual. All chemical reagents and test materials will be handled with respect to their respective MSDS. It will be the responsibility of the various ESE Department Managers to impart the hazards associated with each reagent or test material to the personnel participating in this study.

GOOD LABORATORY PRACTICES

The study will be conducted using Good Laboratory Practices. All raw data generated in the study will be sent to and archived at the Chemical Manufacturers Association GLP archives located at 2501 M. Street, N.W., Washington, D.C. 20037. A copy of the original raw data will be archived in the ESE Analytical Services GLP archives located at 14220 Newberry Road, Gainesville, Florida. The Study Director will be responsible for developing the protocol and writing the report. The ESE Principal Investigator is responsible for the conduct of the study, and for providing the Study Director with a final report and all data generated. Additionally the ESE Principal Investigator will review the protocol, Good Laboratory Practices, materials and methods, and safety guidelines with all support personnel prior to their participation in the study. Any deviations from the protocol will be recorded and changes in plans will be discussed with the Study Director and the Study Sponsor Representative in advance, as well as being written and attached to this protocol as amendments.

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APPROVAL SIGNATURES

Sponsor Representative: Robert R. Romano Date Feb. 23, 1994
Robert Romano, Ph.D.
Director of Special Programs
Chemical Manufacturers Association

Study Director: David H. Greer, Jr. Date 3/2/94
David H. Greer, Jr.
GLP Program Manager
ESE

Principal Investigator: [Signature] Date 2/25/94
Kathleen K. Allen
Water Quality Department Manger
ESE

Quality Assurance: Portia O. Pisigan Date 3/2/94
Portia O. Pisigan
Quality Assurance Unit Supervisor
ESE

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APPENDIX I
ANALYTICAL METHOD

**NOVATEK APPLICATION NOTE
FOR THE
CHEMICAL MANUFACTURERS ASSOCIATION
(Contract CD-6.0-NOV)**

**The Measurement of Chlorite Ion in Seawater by IC
Using the Chlorpromazine Post Column Reaction**

SCOPE AND APPLICATION

This method incorporates a chlorpromazine reaction chemistry to measure chlorite ion (ClO_2^-) following the separation of anions by USEPA Method 300.0. This method can be used to measure chlorite ion from 0.05 to 1.0 mg/L in a seawater matrix. The method detection limit (MDL) was determined to be 0.04 mg/L chlorite ion.

SUMMARY OF THE METHOD

This method is an ion chromatography post-column procedure using USEPA Method 300.0 with a borate eluent. The post-column procedure uses photometric detection at 530 nm. Following separation, ClO_2^- reacts with chlorpromazine in acidic media by a charge transfer mechanism. This method overcomes common problems encountered in the ion chromatographic measurement of seawater samples from Cl^- and SO_4^{2-} . This detection chemistry shows no interference from all common anions with the exception of bromate ion and nitrite ion.

The sample (25 - 200 μL) is injected into the eluent at a flow rate of 1.0 mL/min. The sample passes through a metal free column (MFC) and a guard column before separation on the analytical column. An anion micromembrane suppressor (AMMS II) is used with a weak sulfuric acid regenerant solution flowing at 10 mL/min. The suppressed eluent from the ion chromatograph is directed to a post column manifold in which the eluent is first merged with the chlorpromazine reagent. The combined eluent-chlorpromazine reagent stream is then merged with an acid stream to form the photometric product in the presence of ClO_2^- (Figure 1).

SAMPLE HANDLING

All samples should be analyzed as soon as possible. Check that the sample pH is between 6.5 and 7.5. Adjust the sample with dilute hydrochloric acid or sodium

**Chlorite Ion
Chlorpromazine Post Column**

hydroxide solutions if necessary. Limit sample exposure to hv by using opaque sample bottles and storing the samples in the dark at 4°C.

Sample bottles should not be cleaned with soap or surfactants. All sample bottles should be rinsed a minimum of five times with distilled water and an additional five times with deionized water displaying a minimum resistance of 17.8 megohms-cm. The bottles are then allowed to air dry for 48 hours.

Sample treatment - Treat the sample with ethylenediamine (50 ppm/100 mL) and let it react for a minimum of 15 minutes to mask any chlorine that may be present in the sample. Store the sample at 4°C and filter any precipitates that may be present before analysis.

If you plan to ship or transport a sample, ship at least 100 mL of treated sample making sure that the sample bottle is completely filled thereby minimizing headspace. Ship the sample under blue ice in a suitable container.

Holding Times - Holding times are as listed for finished water samples in the Federal Register 40 CFR and in the text of USEPA Method 300.0 for the anions separated by this procedure. Laboratory tests have shown that chlorite ion samples in a seawater matrix are not stable.

GLASSWARE

It is important to keep all glassware clean and free of interferences. During storage all glassware openings should be covered with cellophane wrap or ground glass stoppers and stored in the dark to prevent contamination. This includes beakers, volumetric flasks and pipettes.

The following cleaning procedure is recommended:

1. Clean all glassware with a detergent and rinse well with tap water.
2. Soak the glassware in 1:1 nitric acid overnight.
3. Rinse 5X with tap water.
4. Rinse 5X with doubly distilled and deionized water.
5. Store glassware as described above.

APPARATUS

Ion Chromatography System to include:
Ion Chromatograph (Dionex DX-100)

Metal-Free Column (Dionex, MFC)
Guard Column (Dionex, AG9)
Analytical Column (Dionex, AS9-SC)
Integrator

Post-Column System to include:
Two Reagent Manifold (Tecator Chemifold SR, Type III)
Two Channel Peristaltic Pump
UV/Visible Detector (Perstorp Analytical 510 or equivalent)
Integrator

REAGENTS

All water used to prepare reagent working solutions is best prepared by the passage of distilled water through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Regeneration of the column should be carried out according to the manufacturers specifications. The water should then be distilled and collected. Commercial water purifications systems that deionize and doubly distill the water are preferred. Water with a minimum resistance of 17.8 megohm-cm should be used to prepare all solutions.

Distilled Water: Deionized and doubly distilled as described above.

Ethylenediamine: EDA should be purchased in as pure a form as possible. A concentrated solution is prepared by diluting the pure EDA 1:100 with DI water. This yields an EDA stock solution concentration of 8990 mg/L. To 100 mL sample add 0.5 mL of EDA stock to give an EDA final concentration of ca. 45 mg/L.

Borate Eluent: 12.5 mM NaOH/50 mM H₃BO₃. The eluent is prepared by dissolving 0.8 g sodium hydroxide (NaOH) and 6.2 g boric acid (H₃BO₃) into 2 liters of deionized water.

Suppressor Regenerant: 12.5 mM H₂SO₄. The regenerant is prepared by diluting 2.75 mL sulfuric acid (H₂SO₄) in 4 liters of deionized water.

Chlorpromazine Solution: 0.56 mM Chlorpromazine. The indicator is prepared fresh daily by dissolving 0.2 g chlorpromazine (Sigma) into 1 liter of deionized water. This solution must be degassed with either nitrogen or helium for at least 10 minutes and be protected from light.

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HCl Solution: 2.1 M HCl. The acid solution is prepared by diluting 350 mL hydrochloric acid (HCl) in 2 liters of deionized water. This solution must be degassed with either nitrogen or helium for at least 10 minutes.

STANDARDS

A series of standards should be prepared for the chlorite ion measurement.

NOTE

Most commercial sodium chlorite contains impurities. Highly purified sodium chlorite is essential for the establishment of valid calibration curves (i.e. correlation coefficients ≥ 0.999). It is recommended that the purity of the sodium chlorite reagent be determined prior to its use.

A series of standards should be prepared for the chlorite ion and chlorate ion measurement. Chlorite ion standards are stable for a maximum of 2 weeks if kept cold (4°C) and shielded from light.

Stock Chlorite Ion Standard Solution (100 mg/L): To a 100 mL volumetric flask containing 50 mL of doubly distilled water, place 13.41 mg of sodium chlorite. Mix to dissolve and dilute to 100 mL with doubly distilled water.

Working Standard Solutions: To six 100.0 mL volumetric flasks, add the following volumes of stock standard solution and dilute to volume with distilled, deionized water.

ClO₂⁻ Working Stock (mL)	Standard Solution (mg/L)
0.1	0.1
0.2	0.2
0.4	0.4
0.8	0.8
1.0	1.0
check 0.4	0.4

PROCEDURE

Instrument Set-up

1. Turn the power on for the IC, UV/Visible detector and integrators.
2. Fill the eluent and regenerant bottles with the appropriate reagents.
3. Fill the chlorpromazine and acid bottles with the appropriate reagents.
4. Check to make sure that the following parameters are set on the IC:

Columns	MFC/AG9/AS9-SC
Sensitivity	1 μ S full-scale
Eluent	12.5 mM NaOH/50 mM H ₃ BO ₃
Eluent Flow	1.0 mL/min
Detection	Suppressed conductivity
System Backpressure	950-1150 psi
Background Conductivity	7-12 μ S
Suppressor	Anion Micro Membrane (AMMS II)
Suppressor Regenerant	12.5 mM H ₂ SO ₄
Regenerant Flow	10 mL/min
Sample Injection Volume	25 - 200 μ L
5. Check to make sure that the following parameters are set on the post-column detection system:

Indicator	0.56 mM Chlorpromazine
Indicator Flow	0.4 mL/min
Acid	2.1 M HCl
Acid Flow	2.0 mL/min
Mixing Coils	M1=60 cm, M2=120 cm
Pathlength	10 mm flow cell
Wavelength	530 nm
Detector Settings	0.002 AUFS/10 sec rise time
6. Equilibrate the system by allowing the IC and post column reagents to flow for a minimum of 30 minutes. Set the auto offset on IC to zero. Check the system backpressure and background conductivity.

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NOTE

We have noticed that fluorescent lights seem to effect the post column chemistry sensitivity. Precautions should be taken to shield reagents from flouorescent lights.

7. Check the post-column system for leaks and air bubbles.
8. Set the integrators according to the instructions provided by the manufacturer.
9. Place the injection valve on the IC in the load position. Fill the sample loop with the most concentrated sample.
10. Inject the sample. A normal anion profile will be recorded by the IC integrator. The ClO_2^- typically is detected ca. 2.5 minutes. The post-column tracing can be expected in an additional 30 - 45 seconds (Figure 2).
11. The high standard should be injected until the calculated peak area values are within $\pm 5\%$.
12. Prepare a calibration curve by injecting standards in random order.
13. Analyze the check standard. The calculated value for the standard from the calibration curve should be within ± 0.02 mg/L of the known concentration. If the value is outside this range, the instrument is not properly calibrated.

NOTE

Calibration curves should exhibit a correlation coefficient better than 0.999. If this level cannot be attained, check your reagents, standards and the instrument for potential causes that prevent adequate calibration. Make adjustments as necessary. Do not analyze samples until a suitable calibration curve is recorded.

STANDARDIZATION AND QUANTITATION

The quantitation of bromate ion separated by this method is performed by injecting standard solutions and integrating the detector response at a defined retention time to calculate a peak area. A calibration curve is developed from a series of standard injections (concentration vs. peak area) and used to quantitate injected samples.

1. Establish the IC and post-column operating parameters.

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2. Prepare calibration standards at a minimum of four concentration levels and a blank by adding accurately measured volumes of the dilute chlorite ion standard solution to a volumetric flask and diluting to volume.
3. Prepare a calibration curve. This curve should be developed each day and checked every 10 samples. If the response or retention time for any analyte varies from the expected value by more than 10%, the test should be repeated using fresh calibration standards.
4. Calculate the sample concentration using a statistics program. Enter the data into the program to generate a worksheet.

NOTE

Data quality should be evaluated on multiple levels:

1. The correlation coefficient should be ≥ 0.999 .
2. Calculated standard peak area values should be within 5% of the measured area.
3. Calculated standard concentration values should be within 0.01 mg/L of the analyte being calibrated. This test indicates the error associated with the calculated regression line.
4. The concentration of the check sample should be within 0.02 mg/L of the theoretical concentration. This test indicates the accuracy of the calibration.

SAFETY

The chlorite ion measurement requires the careful handling of solutions. Refer to the appropriate Material Safety Data Sheet for reagent handling instructions. For added protection, the laboratory should be well ventilated and safety goggles should be worn at all times.

REFERENCES

1. USEPA Method 300.0
2. Hautman/Bolyard personal communication.

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3. Gordon, G.; Cooper, W.J.; Rice, R.G.; Pacey, G.E. Disinfectant Residual Measurement Methods, American Water Works Association - Research Foundation (AWWA-RFISBN0 -89867-408-5) Denver Colorado, 1987, 815pp
4. Gordon, G.; Cooper, W.J.; Rice, R.G.; Pacey, G.E. A Survey of the Current Status of Residual Disinfectant Measurement Methods for all Chlorine Species and Ozone, J. Am. Water Works, (ISBN 0-89867-409-3) Distributed by AWWA-RF to Water Treatment Plants in North America, (1987), 29 pp.
5. Gordon, G. personal communication.
6. Kojlo, A.; Puzanowska-Tarasiewicz, H.; Calatayud, J.M. "Immobilization of Hexacyanoferrate (III) for a Flow Injection-Spectrophotometric Determination of Promazine", Analytical Letters, 1993, 26(3), 593-604.

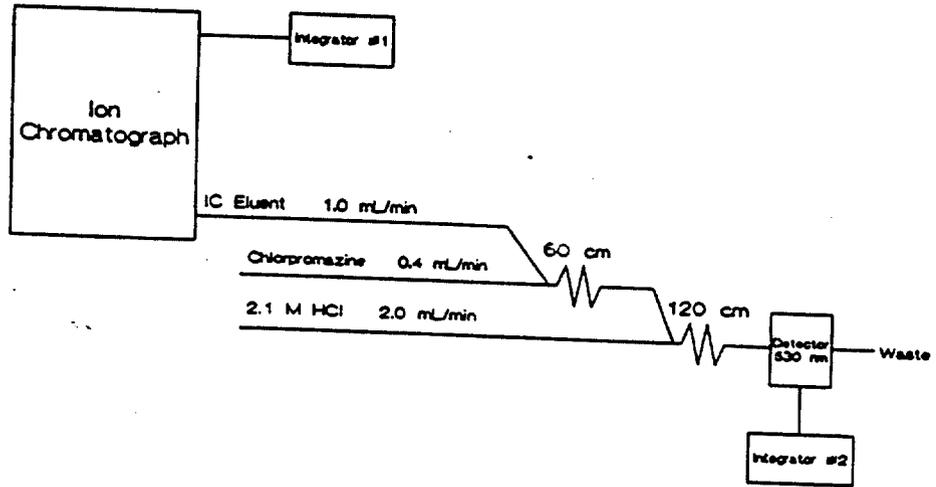


Figure 1. IC post-column diagram.

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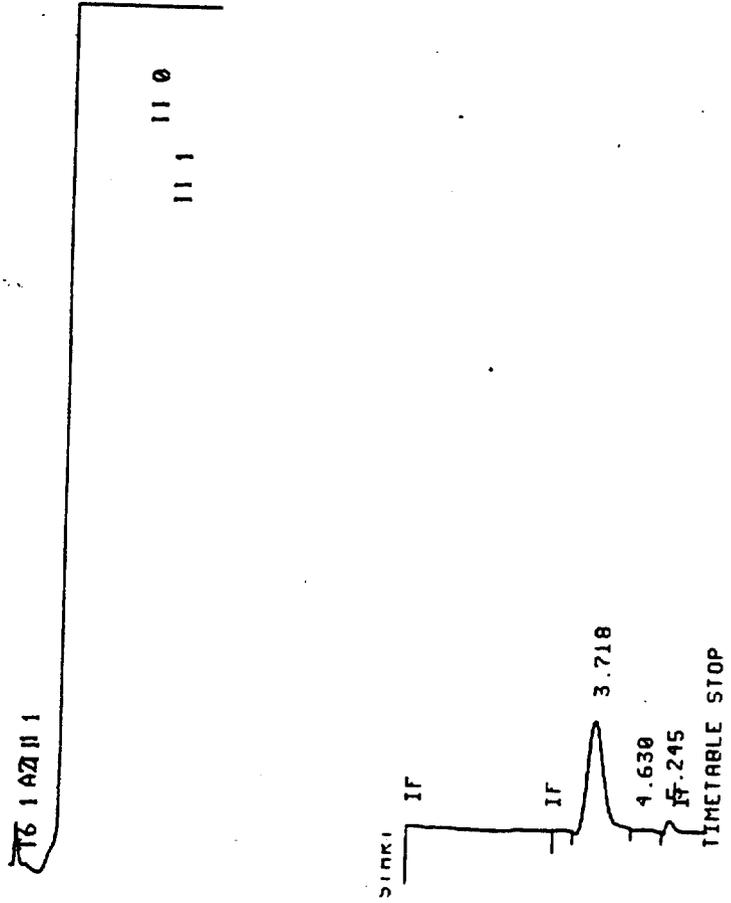


Figure 2. US EPA Method 300.0 conductivity and post-column detection of ClO_2^- .

Date Submitted:

MRID No.:

Volume:

STUDY TITLE

EFFECT OF SODIUM CHLORITE ON NEW SHELL GROWTH IN
EASTERN OYSTER (Crassostrea virginica)

U.S.-EPA-FIFRA CFR 40 - SERIES 72-3

AUTHORS

Study Director: J. J. Yurk
Principal Investigator: M. A. Overman
Environmental Science & Engineering, Inc.

FINAL REPORT DATE

May 18, 1994

SUBMITTED TO:

Chemical Manufacturers Association (CMA)
2501 M Street NW
Washington, D.C. 20037

PERFORMING LABORATORY

Environmental Science & Engineering, Inc. (ESE)
Department of Aquatic Toxicology
PO Box 1703, Gainesville, FL 32602-1703
Tel No. (904) 332-3318

LABORATORY PROJECT ID

ESE Project No. 3943014-0300-3140
CMA Reference No. CD-7.0-3AT-ESE

Contains No CBI

Contains No CBI

STATEMENT OF NO DATA CONFIDENTIALITY

Effect of Sodium Chlorite of New Shell Growth
in Eastern Oyster
(Crassotrea virginica)

This data submission contains a statement of "no data confidentiality" pursuant to PR notice 86-5. This claim is valid. Please disregard the statement "confidential" located in the header on pages 3 - 75. This statement was erroneously generated by a computer.

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA para. 10(d) (1) (A), (B), or (C). Nor is any supplemental claim of confidentiality made for any information in this study on the basis of FIFRA para. 10(A) or (B). This document, however, is propriety to Chemical Manufacturers Association and is considered confidential and trade-secret information in all other countries and for all purposes other than those enunciated in FIFRA para. 3 & 10. In addition, this data is compensative under FIFRA and must be handled accordingly by all parties receiving this data. Information contained in this study should not be reproduced, stored in a retrieval system or transmitted in any form or by any means by persons other than EPA without the express written consent of Chemical Manufacturers Association except as required to carry out the requirements of FIFRA.

Company: CHLORINE DIOXIDE PANEL OF THE CHEMICAL MANUFACTURERS ASSOCIATION

Company Agent: ROBERT R. ROMANO (NAME)

Date: MAY 23, 1994

Title: ASSOCIATE DIRECTOR, CHEMSTAR

Signature: Robert R. Romano

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STATEMENT OF GLP COMPLIANCE

Compound: Sodium Chlorite

Title: Effect of sodium chlorite on new shell growth in Eastern Oyster (Crassostrea virginica)

The study described in this report was conducted in accordance with the following Good Laboratory Practice Standards:

United States Environmental Protection Agency,
Title 40 Code of Federal Regulations Part 160,
1 July 1993 Edition

Japan Ministry of Agriculture, Forestry and Fisheries,
59 NohSan, Notification No. 3850.
Agricultural Products Bureau
10 August 1984

Organization for Economic Co-Operation and Development
ISBN 92-64-12367-9, Paris 1982

Study Director: Jeffrey J. Burk 5/18/94
Jeffrey J. Burk Date
ESE

Principal Investigator: Max Overman 5/18/94
Max A. Overman Date
ESE

Study Sponsor: Robert R. Romano 5/19/94
Robert Romano Date
CMA

Submitter: _____ Date

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SCIENTIFIC PERSONNEL SIGNATURE PAGE

Study Director: Jeffrey J. Gurky 5-18-94
Jeffrey J. Gurky Date

Principal Investigator: Max Overman 5/18/94
Max A. Overman Date

Chemistry Coordinator: Patrick Wilber 5-18-94
Patrick Wilber Date

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STUDY SUMMARY

Study Title: Effect of Sodium Chlorite on New Shell Growth in Eastern Oyster (Crassostrea virginica)

Data Requirement: Section 72-3 of the EPA Pesticide Assessment Guidelines, Subdivision E

Sponsor: Chemical Manufacturers Association (CMA),
2501 M Street NW,
Washington, D.C. 20037

Location of Study: Environmental Science & Engineering, Inc. (ESE)
P.O. Box 1703
Gainesville, FL 32602-1703

Study Director: Jeffrey J. Yurk; Tel No. (904) 333-2626

Study Initiation Date: February 11, 1994

Experimental Start Date: March 28, 1994

Experimental Termination Date: April 1, 1994

Test Substance: Sodium Chlorite, Batch No. 5064, CAS No. 7758-19-2, purity 79.0%

Test Organism and Source: Eastern Oyster (Crassostrea virginica), 25 to 50 mm valve height, obtained from P. Cummings Oyster Company, Pasadena, MD

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Test Conditions:

Dilution water: filtered sea water adjusted to approximately 28‰ salinity with well water from a well located at test site; photoperiod--16-h light and 8-h dark with 30-minute dawn/dusk transition; temperature--23 ± 2 °C

Test Results:

96 hour EC50: 21.4 mg/L Sodium Chlorite; 95% confidence limits: 14.3 and 27.1 mg/L Sodium Chlorite; NOEC: 14.3 mg/L

Location of Raw Data and Final Report:

Environmental Science & Engineering, Inc. (ESE)
P.O. Box 1703
Gainesville, FL 32602-1703

1.0 INTRODUCTION

A flow-through toxicity test was conducted at Environmental Science & Engineering, Inc., in Gainesville, Florida to determine the effect of sodium chlorite on new shell growth in eastern oyster (Crassostrea virginica). The criterion for effect was new shell growth and results of the test are expressed as a 96-hour median effect concentration (EC50), the concentration of sodium chlorite estimated to reduce shell growth by 50 percent of the test population of eastern oyster under specified conditions of exposure. All raw data related to this study are maintained by Environmental Science & Engineering, Inc., Gainesville, Florida.

2.0 MATERIALS AND METHODS

2.1 Test substance

The test substance, sodium chlorite (Batch No. 5064, CAS No. 7758-19-2) was received by ESE in two 1000 mL plastic bottles on December 27, 1993. The test substance was a white crystalline material and was stored in the dark at ambient temperatures. The water solubility of the test substance was reported by Vulcan Chemicals to be 39% at 25°C. The purity of the test substance was reported to be 79.0%. All sodium chlorite stock solutions were

made by dissolving the test substance in seawater. Measured test concentrations are reported as milligrams (mg) of sodium chlorite per liter (L) of seawater.

2.2 Test organisms

Eastern oysters (Crassostrea virginica) that were used in the definitive test, were obtained from P. Cummins Oyster Company, Pasadena, CA on March 18, 1994. During the 48-hours prior to test start, test organisms were maintained in seawater with a salinity of 28 ± 1 ‰, a pH of 7.5 ± 1.0 and a temperature of 23 ± 1 °C. The oyster were fed a natural algal diet supplement (Diet A; Coast Oyster Co., Seattle, WA) at least once daily during holding and throughout the test. No mortality of the test population was observed over the 48-hour pretest period.

2.3 Dilution water

Atlantic Ocean water was obtained near the Whitney Laboratory, Marineland, FL and was diluted to a salinity of approximately 28‰ with well water from a well located at the test site. Chemical analyses of unfiltered sea water are performed semi-annually by ESE and the results of the most recently reported analyses are presented in Appendix A.

2.4 Test Methods

Methods for the 96-hour flow-through toxicity test are described in the ESE test protocol entitled: "Effect of sodium chlorite on New Shell Growth in Eastern Oyster (Crassostrea virginica)."

2.4.1 Range Finding Test

Results of a preliminary 96-hour flow-through oyster shell deposition test of sodium chlorite, conducted by a different laboratory, was provided by the Sponsor (Appendix B). Based upon these results, nominal concentrations selected for the definitive test were 0 (dilution water control), 12.5, 25, 50, 100, and 200 mg sodium chlorite/L of dilution water.

2.4.2 Definitive Test

Six days prior to test start, the periphery of the shell margin of each oyster was ground (approximately 1-2 mm) with a fine grit grinder in order to establish a baseline for new shell growth. To begin the test, any new shell growth was removed to the baseline and 20 oysters were indiscriminately distributed to each of the test and control vessels. There was one test vessel for each test concentration, loaded so that 0.5 liters of test solution was delivered per hour per oyster.

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The definitive test was conducted under flow-through conditions in a continuous flow diluter system (Appendix B). A stock solution, with a nominal concentration of 3.23 g sodium chlorite/L, was prepared in seawater. Stock was prepared daily and continuously mixed by a high speed mixing pump throughout the study to insure homogeneity. The mean measured stock concentration was 3.28 g/L (102% of nominal). Approximately 20 mL/minute of stock solution and 304 mL/minute of dilution water were pumped to the chemical mixing chamber per minute providing a high nominal concentration of 200 mg/L. This test solution was proportionally diluted in the diluter system to provide the four lower test concentrations (i.e., 100, 50, 25, and 12.5 mg/L). A dilution water control was maintained concurrently with the five test solutions.

The toxicity test was conducted using 16.3 L, rectangular glass chambers (65 cm length X 16.5 cm width X 15.4 cm height), filled to approximately 8.2 L with dilution water or test solution. One test chamber containing 20 oysters was used for each test concentration or control. Test vessels were labeled using the compound name and nominal concentrations. All test chambers were kept covered during the test, except when daily observations of test organisms were made and water quality parameters measured. Test temperature was maintained at $23 \pm 1^{\circ}\text{C}$ throughout the test. A 16-h light and 8-h

dark daily photoperiod with a 30 minute dawn/dusk transition period was maintained using cool white fluorescent lights controlled by a timer. Light intensity averaged 200 Lux over the test system, as measured by a Lux meter.

Test organisms were observed daily for mortality and any physical or behavioral changes. Mortality was defined as the inability to close the shell on gentle prodding. A definitive determination of mortality was made at test termination, when oysters were removed from the test system. New shell growth, the effect criterion, was measured with vernier calipers at test termination. New shell growth was defined as the length of the longest finger of growth on the peripheral shell margin.

Water quality was monitored daily during the test in exposure chambers with live test organisms present. Water temperature in the control exposure was continuously monitored with a Supco Temperature Recorder and spot checked daily with a NIST-calibrated thermocouple. Dissolved oxygen, salinity and pH were measured daily in all test solutions containing surviving organisms. Dissolved oxygen concentrations were determined using a YSI Model 58 meter, salinity was measured using an American Optical refractometer, and pH was measured with an Orion Model SA 250 pH meter.

2.5 Statistical Analyses

Statistical analyses of the new shell growth data were performed using the mean measured concentrations of test substance. A 96-hour EC50 and 95% confidence limits were determined by computer program developed by Stephen (1), using the following statistical methods: moving average angle, Probit, and non-linear interpolation. Prior to statistical analysis, new shell growth data for each treatment were converted to a percentage reduction of new shell growth as compared control new shell growth as follows:

$$\frac{\text{Control mean new growth} - \text{Treatment mean new growth}}{\text{Control mean new growth}} \times 100$$

To determine a no-observed-effect concentration (NOEC), statistical significance ($p \leq 0.05$) between the control and treatment new shell growth data was evaluated using Steel's Many-One Rank test (2).

2.6 Chemical Analysis

Samples were collected from the control and each test concentration on day 0 and day 4. Test solutions were sampled from the approximate center of the test vessel using a serological pipet, and transported in scintillation vials to analytical services for analysis. Samples were analyzed by ion chromatography with visible detection following the methodology provided in the Method Validation report (Appendix C).

3.0 RESULTS AND DISCUSSION

Nominal and measured concentrations of sodium chlorite are presented in Table 1. The mean measured concentrations ranged from 14.3 mg/L sodium chlorite in the lowest concentration to 211 mg/L sodium chlorite in the highest concentration. The mean measured concentrations ranged from 101 to 114% of nominal. The concentration in the control exposure was determined to be below the limit of validation for this test of 0.27 mg/L. Analytical quality control data are presented in Table 2. Individual standard matrix spike recoveries ranged from 96.5 to 104 percent for all quality control data. Data for exposure water quality parameters, and their corresponding ranges and median values are presented in Tables 3, 4 and 5. Dilution water salinity remained between 27 and 28 ‰ throughout the test. All ranges are within the acceptable limits (3).

The mean measured sodium chlorite concentrations and corresponding new shell growth of test organisms are presented in Table 6. The mean measured sodium chlorite concentrations and corresponding percent reduction in new shell growth (Table 7) were used to calculate the 96-hour EC50 and 95% confidence limits. The 96-hour EC50 was calculated to be 21.4 mg/L sodium chlorite using nonlinear

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interpolation with 95% confidence limits of 14.3 and 27.1 mg/L. The 96-hour no-observed-effect concentration (NOEC) of the test substance was 14.3 mg/L sodium chlorite in this test since shell growth at this concentration is not statistically different from the control value.

4.0 CONCLUSION

The 96-hour EC50 for sodium chlorite for the eastern oyster (Crassostrea virginica), based on new shell growth was determined to be 21.4 mg/L with 95% confidence limits of 14.3 and 27.1 mg/L. The NOEC of sodium chlorite was determined to be 14.3 mg/L.

REFERENCES

- (1) Stephan, C.E. 1977. Methods for Calculating an LC50. IN: American Society for Testing and Materials (ASTM) Aquatic Toxicology and Hazard Evaluation, pp. 65-84, F.L. Mayer and J.L. Hamelink (Editors). ASTM STP 524, Philadelphia, PA.
- (2) Gully, D.D., A.M. Boelter, and H.L. Bergman. 1991. Toxstat 3.3. Department of Zoology and Physiology, University of Wyoming. April, 1991.
- (3) American Society for Testing and Materials (ASTM). 1980. Standard Practice for Conducting Acute Toxicity Tests With Fishes, Macroinvertebrates, and Amphibians. ASTM, Philadelphia, PA. Publication E 729-80.

Table 1. Nominal and measured concentrations of sodium chlorite in exposure water during a 96-h flow-through, acute toxicity test with eastern oyster (*C. virginica*)

Nominal concentration (mg/L) of sodium chlorite	Measured concentration (mg/L) of sodium chlorite at test start and test termination		Mean measured concentration (mg/L)	Percent of nominal ^a
	0 h	96 h		
0 (control)	ND ^b	ND	ND	NC ^c
12.5	14.09	14.48	14.29	114
25.0	26.92	27.33	27.13	109
50.0	53.76	54.46	54.12	108
100	102.8	99.39	101.1	101
200	209.7	211.2	210.5	105

^a Mean measured concentration X 100/nominal concentration.

^b ND = not detected below a limit of validation for this test of 0.27 mg/L.

^c Cannot be calculated.

Source: ESE 1994

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Table 2. Analytical quality control data from a 96-h flow-through, acute toxicity test of sodium chlorite to the eastern oyster (*C. virginica*).

Sample	Target chlorite concentration (mg/L)	Measured chlorite concentration (mg/L)	Percent recovery ^a
<u>DAY-0</u>			
Std Matrix Spike	10.0	10.00	100
Std Matrix Spike	10.0	9.862	98.6
Sample Matrix Spike	5.00	4.824	96.5
Sample Matrix Spike	5.00	4.794	95.9
<u>DAY-4</u>			
Std Matrix Spike	10.0	9.992	99.9
Std Matrix Spike	10.0	10.02	100
Sample Matrix Spike	50.0	51.25	103
Sample Matrix Spike	50.0	51.85	104

^a Measured concentration X 100/target concentration.

Source: ESE 1994

Table 3. Daily temperature of exposure water during a 96-h flow-through, acute toxicity test of sodium chlorite to the eastern oyster (*C. virginica*)

Mean measured concentration (mg/L) of sodium chlorite	Temperature (°C)					n ^a	Range	Median
	0 h	24 h	48 h	72 h	96 h			
0 (control)	23.6	23.0	22.5	22.4	22.5	5	22.4-23.6	22.5
14.3	23.9	23.1	22.4	22.4	22.6	5	22.4-23.9	22.6
27.1	23.7	23.0	22.4	22.4	22.6	5	22.4-23.7	22.6
54.1	23.8	23.1	22.5	22.5	22.8	5	22.5-23.8	22.8
101	23.8	23.3	22.7	22.7	23.0	5	22.7-23.8	23.0
211	24.0	23.7	22.9	22.9	23.7	5	22.9-24.0	23.7

^a n = total number of measurements.

Note: temperatures as measured with a continuous temperature monitor were within the range of 22 to 24°C.

Source: ESE 1994

Table 4. Exposure water pH during a 96-h flow-through, acute toxicity test of sodium chlorite to the eastern oyster (*C. virginica*).

Mean measured concentration (mg/L) of sodium chlorite	Replicate exposure	pH					n ^a	Range	Median
		0 h	24 h	48 h	72 h	96 h			
0 (control)	A	7.9	7.7	7.9	7.8	8.0	5	7.7-8.0	7.9
14.3	A	7.9	7.8	7.9	7.8	8.1	5	7.8-8.1	7.9
27.1	A	8.0	7.8	7.9	7.9	8.1	5	7.8-8.1	7.9
54.1	A	8.0	7.9	8.0	7.9	8.0	5	7.9-8.0	8.0
101	A	8.0	7.9	8.0	7.9	8.1	5	7.9-8.1	8.0
211	A	8.0	7.9	8.0	7.9	8.1	5	7.9-8.1	8.0

^a n : total number of measurements.

Source: ESE 1994

Table 5. Dissolved oxygen concentrations of exposure water during a 96-h flow-through, acute toxicity test of sodium chlorite to the eastern oyster (*C. virginica*)

Mean measured concentration (mg/L) of sodium chlorite	Replicate exposure	Dissolved Oxygen Concentration (mg/L) (percent saturation)					n ^a	Range	Median
		0 h	24 h	48 h	72 h	96 h			
0 (control)	A	7.6(108)	7.1(99)	7.0(98)	7.2(103)	7.3(102)	5	7.0-7.6	7.2
14.3	A	7.6(108)	7.1(99)	7.1(101)	7.1(101)	7.3(102)	5	7.1-7.6	7.1
27.1	A	7.4(105)	7.1(99)	7.1(101)	7.2(103)	7.4(103)	5	7.1-7.4	7.2
54.1	A	7.4(105)	7.2(100)	7.1(99)	7.2(100)	7.4(103)	5	7.1-7.4	7.2
101	A	7.4(105)	7.2(100)	7.2(100)	7.2(100)	7.4(103)	5	7.2-7.4	7.2
211	A	7.3(104)	7.1(101)	7.2(100)	7.2(100)	7.4(103)	5	7.1-7.4	7.2

^a n : total number of measurements.

Source: ESE 1994

Table 6. New shell growth in eastern oyster (*C. virginica*) exposed to sodium chlorite in a 96-h flow-through, shell deposition test.

Mean measured concentration (mg/L) of sodium chlorite	Individual new shell growth (mm) ^a				
0 - control	2.4	3.0	1.8	2.6	3.2
	2.4	2.8	1.7	3.0	2.1
	1.5	0.5	0.9	1.7	1.3
	3.4	2.3	2.9	5.3	2.4
	Treatment mean and standard deviation				
	2.36 ± 1.04				
14.3	2.9	2.0	2.0	2.2	4.2
	2.3	1.6	1.0	1.1	1.2
	2.0	2.5	1.9	0.7	1.8
	1.9	1.0	2.0	1.8	2.8
	Treatment mean and standard deviation				
	1.95 ± 0.80				
27.1	0.7	0.7	0.6	1.5	1.0
	1.0	0.5	1.4	1.7	1.0
	0.6	1.1	0.7	0.9	0.7
	Treatment mean and standard deviation				
	0.71 ± 0.52				
54.1	0.5	0.5	0.8	0.7	1.1
	0.7	1.0	0.5	0.8	
	Treatment mean and standard deviation				
	0.33 ± 0.40				

Table 6 continued.

Mean measured concentration (mg/L) of sodium chlorite	Individual new shell growth (mm) ^a			
101	0.4	0.8	0.7	0.7
	Treatment mean and standard deviation			
	0.13 ± 0.28			
211	0.5	1.0	0.7	
	Treatment mean and standard deviation			
	0.11 ± 0.28			

^a Each treatment had 20 organisms exposed at test start. Only those organisms with new shell growth (≥ 0.1 mm) are listed. All 20 values (less than 0.1 mm being considered 0) were used to determine mean and standard deviation.
Source: ESE 1994

Table 7. Percent reduction in new shell growth in eastern oyster (*C. virginica*) exposed to sodium chlorite in a 96-h flow-through shell deposition test

Mean measured concentration (mg/L) of sodium chlorite	Percent reduction in new shell growth ^a
0 - control	0%
14.3	17.6%
27.1	70.1% ^b
54.1	86.0% ^b
101	94.5% ^b
211	95.3% ^b

^a $\frac{\text{mean control growth} - \text{mean treatment growth}}{\text{mean control growth}} \times 100$

^b Significant reduction based on computer program for Steel's Many-One Rank Test.

Source: ESE 1994

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Appendix A- Copy of Unfiltered Seawater Characterization

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UNFILTERED SEAWATER CHARACTERIZATION*

<u>PARAMETER</u>	<u>CONCENTRATION</u>	<u>METHOD</u>
Arsenic	5.8 µg/L	EPA 206.2
Aluminum	251 µg/L	EPA 200.7
Cadmium	<0.5 µg/L	EPA 213.3
Calcium	303 mg/L	EPA 200.7
Chromium	<10 µg/L	EPA 200.7
Cobalt	<20 µg/L	EPA 200.7
Copper	<5.0 µg/L	EPA 200.7
Iron	316 µg/L	EPA 200.7
Lead	<2.0 µg/L	EPA 239.2
Mercury	<0.20 µg/L	EPA 245.1
Magnesium	902 mg/L	EPA 200.7
Nickel	<15.0 µg/L	EPA 200.7
Selenium	<2.5 µg/L	EPA 270.2
Silver	<5.0 µg/L	EPA 200.7
Sodium	8210 mg/L	EPA 200.7
Zinc	<20 µg/L	EPA 200.7
Nitrogen, NH ₄ + NH ₃	<0.02 mg/L as N	EPA 350.1
Nitrogen, NH ₃ Unionized	<0.001 mg/L	Calculated
Nitrogen, NO ₂ + NO ₃	<0.034 mg/L as N	EPA 353.2
pH	8.3	Meter
Alkalinity	180 mg/L as CaCO ₃	EPA 310.1
Sulfide	<0.05 mg/L as S	EPA 376.2
Hydrogen Sulfide	<0.03 mg/L	Std Method(428E)
Turbidity	<0.40 NTU	EPA 180.1
Dissolved Solids	32600 mg/L	EPA 160.1
Suspended Solids	40 mg/L	EPA 160.2
Fluoride	0.87 mg/L	EPA 340.2
Cyanide	<0.002 mg/L	EPA 335.3
Carbon, TOC	11.5 mg/L	EPA 415.1
COD	232 mg/L	EPA 410.1
Pesticides-Organochlorine and PCB's	<0.005 µg/L	EPA 8080
Pesticides-Organophosphate	<2.52 µg/L	EPA 8140

* The characterized seawater is natural seawater collected from the Atlantic Ocean near Marineland, Florida. The seawater sample was collected from ESE's seawater delivery system on September 9, 1993.

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Appendix B- Pertinent Raw Data

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Table 3. Shell growth data from toxicity test with sodium chlorite

Rangefinding Study

Oyster Number	Shell Growth at Longest Finger (mm)					
	Nominal Concentration of Sodium Chlorite (mg/L)					
	(con) 0.0	70.6	121	252	504	1,008
1	2.2	1.9	1.3	0.0	0.0	0.0
2	2.1	1.7	1.0	0.0	0.0	0.0
3	1.9	2.2	1.2	0.0	0.0	-
4	2.4	2.4	1.6	0.2	0.0	-
5	2.1	2.3	1.4	0.0	0.0	-
6	2.1	2.0	1.3	0.0	0.0	-
7	2.3	2.1	1.0	0.1	0.0	-
8	1.9	2.2	0.9	0.0	0.0	-
9	2.3	1.9	1.4	0.0	0.0	-
10	2.2	2.2	1.5	0.0	0.0	-
11	2.0	2.4	1.3	0.0	0.0	-
12	2.4	2.0	1.6	0.0	0.0	-
13	2.0	2.1	1.0	0.1	-	-
14	2.1	2.1	0.7	0.1	-	-
15	2.1	1.7	0.8	0.0	-	-
16	1.8	2.3	1.1	0.0	-	-
17	1.7	2.4	0.9	0.0	-	-
18	2.2	1.8	1.3	0.0	-	-
19	2.4	2.1	1.2	0.0	-	-
20	2.1	2.0	1.0	-	-	-
Mean	2.1	2.1	1.2	<0.1	0.0	0.0
Std. dev.	0.2	0.2	0.3	<0.1	-	-
Percent of control	100.0	100.0	57.1	1.4	0.0	0.0

Note: con = control.

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 Aquatic Toxicology Department
 Gainesville, Florida

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 ESE QA Form: 069
 Effective: February 1990

SUBJECT: TEST CALCULATIONS FOR DILUTER STUDIES	
SPONSOR: <u>CMA</u>	PROJECT NUMBER: <u>3943114-0300-3140</u>
TEST SUBSTANCE: <u>Sodium chlorite</u>	TEST SPECIES: <u>C. virginica</u>
Stock Delivery (define units): Mixing Chamber Volume (define units): Highest Test Concentration (define units): Percent Active Ingredient: Solve for x, where x = stock concentration as active ingredient (Mixing Chamber Volume) (Highest Test Concentration) $x = \frac{\text{Stock Delivery}}{\text{Stock Delivery}}$	
Show Calculations:	
$\text{Stock delivery} = \frac{(0.3235L) (200 \text{ ng/L})}{3229.2 \text{ ng/L}} = 0.02L \Rightarrow 20.0 \text{ mL/min.}$	
STOCK PREPARATION	
(Stock Concentration) (Volume of Stock Prepared) = Test Substance as Active Ingredient (Test Substance as Active Ingredient / Percent Active Ingredient) = Test Substance to be Weighed Out	
Show Calculations:	
$(3.2292 \text{ g/L}) (10L) = 32.292 \text{ g} / 0.79 = 40.8759 \text{ g} / 10L \text{ stock}$ $\Rightarrow 163.50 \text{ g} / 40L \text{ stock}$	
SOLVENT CONTROL DATA AND CALCULATIONS	
Solvent: <u>NA</u>	Solvent Mixing Chamber Volume: <u>NA</u>
Solvent Delivery Volume: <u>NA</u>	Solvent Concentration: <u>NA</u>
Calculate the solvent concentration by the following equation: $x = \text{stock solution delivery volume} / \text{mixing chamber delivery volume}$	
Show Calculations:	

Calculate the solvent delivery volume by the following equation: $x = (\text{solvent concentration}) (\text{volume of solvent mixing chamber})$	
Show Calculations:	

FORM: DILCALC

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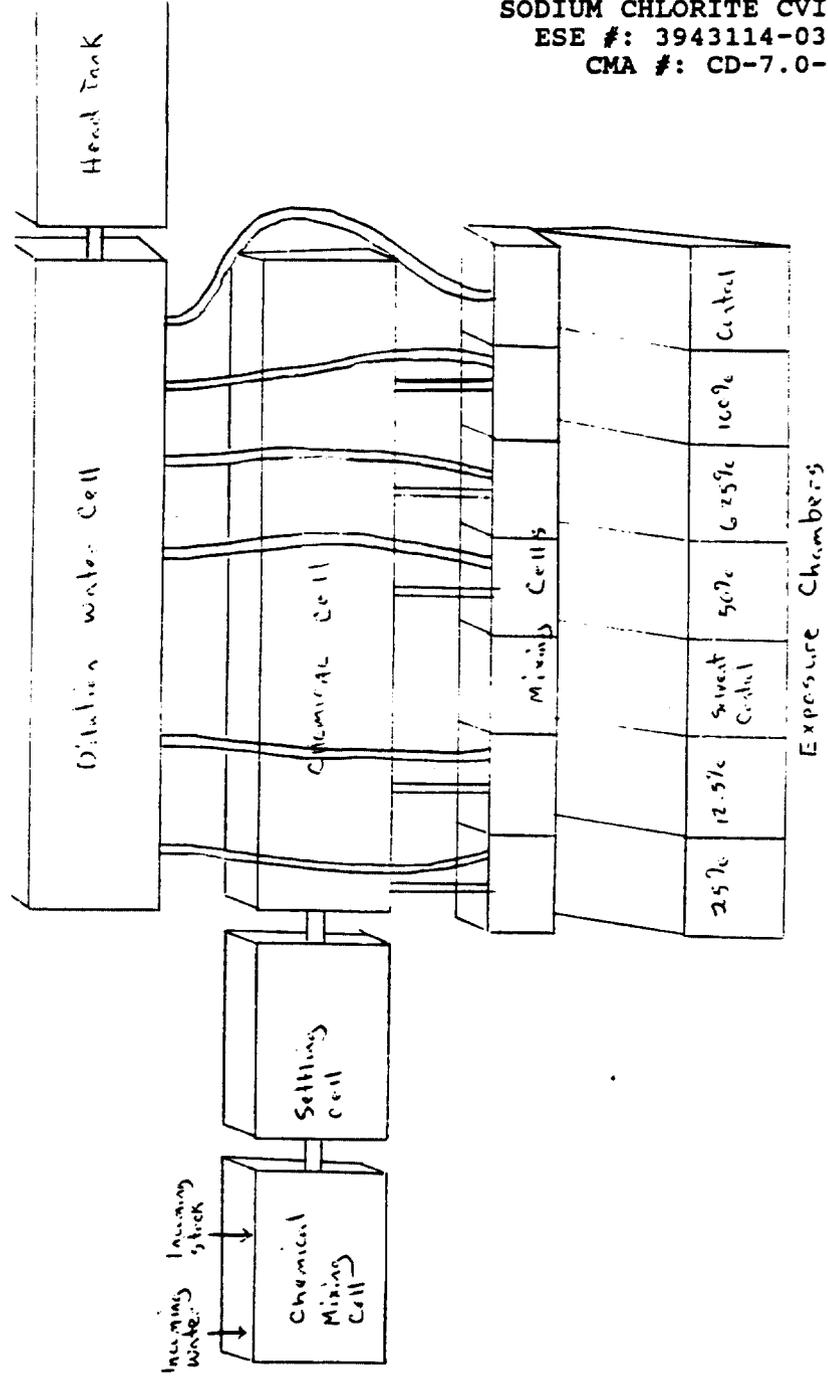
Environmental Science & Engineering, Inc.
 Aquatic Toxicology Laboratory
 Gainesville, Florida

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 QA Form Number: 031
 Effective: APR 1993

DILUTER CALIBRATION				
Sponsor: <u>CMA</u>		Test Substance: <u>Sodium Chlorite</u>		
Project Number: <u>3943114-0300-3140</u>		Test Species: <u>C virginica</u>		
Date Calibrated: <u>3-16-94</u>		Calibrated By: <u>AD</u>		
Diluter ID Number: <u>3</u>		Location: <u>Oyster diluter ROOM</u>		
Calibrated for a <u>50</u> % diluter.				
Cell ID	Desired Cell Volumes (mL)		Measured Cell Volumes (mL)	
	Water	Chemical	Water	Chemical
CONTROL	167.0	0	169, 168, 168	-
6.25%	156.6	10.4	154, 155, 155.5	10.5, 10.5, 10.2
12.5%	146.1	20.9	146.0, 145.8, 146.0	21.0, 21.2, 21.2
25%	125.25	41.75	125.0, 126.1, 126.1	42.0, 42.2, 41.9
50%	83.5	83.5	83.5, 84.0, 84.0	82.0, 82.0, 83.0
100%	0	167	-	168, 168, 167
SPLITTER BOX DELIVERY CALIBRATION				
Splitter Box ID				
Replicate A Volume ()				
Replicate B Volume ()				
Comments:				

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Continuous-Flow Oyster Diluter

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 Effective: APR 1993

ACUTE TOXICITY DATA SHEET-BIOLOGICAL DATA									
SPONSOR: <u>CMA</u>			PROJECT NUMBER: <u>3rd 3000-300 300</u>						
TEST SUBSTANCE: <u>Sodium Chlorite</u>			TEST SPECIES: <u>C. virginica</u>						
ANIMAL HISTORY									
Sample Information and Description Found on Page <u>532</u> of Test Substance Log									
Lot No./Batch No.: <u>94-25</u> (Circle One)			Date Maintenance/Acclimation Began: <u>3/18/94</u>						
See Page No.: <u>58</u> of Animal Collection/ Receipt Log			Condition of Animals: <u>Normal</u>						
See Page No.: <u>64</u> of Immediate Holding Log For Raw Data on Animal Holding			Life Stage/Age: <u>N/A</u>						
Mortality in Tank 48 Hours Prior To Test Initiation: <u>0</u> (%)									
TEST CONDITIONS									
<input type="checkbox"/> Range-Finding		<input type="checkbox"/> Static		Time Added Substance/Animals		Test Location			
<input checked="" type="checkbox"/> Definitive		<input checked="" type="checkbox"/> Flow-Through		/ 1430		Cyster column			
Test System: <input checked="" type="checkbox"/> Covered <input type="checkbox"/> Open		Test Container Dimensions (cm) <small>length width height</small> 65 x 16.5 x 15.4		Test Solution Height: 7.6 cm		Test Container Composition: Glass			
Test Chamber Volume (L) 0.34, 1.6, 3.8, 30 - <u>16.3</u>				Test Solution Volume (L) 0.20, 1.0, 3.0, 10 - <u>8.2</u>					
Protocol: <u>Effect of sodium chlorite on New Shell Growth in Eastern Cyster (C. virginica)</u>									
Dilution Water: <u>Natural or filtered seawater</u>			Type of Lighting: <u>Fluorescent</u>			Photoperiod: <u>16L: 8D</u>			
Cycle One: Test Substance as Active Ingredient/Whole Material									
Test Concentration % <u>(mg/L)</u> ug/L		Con- trol	Solv Cont	12.5	25	50	100	200	Solvent/ Carrier:
Amount of Substance/ Stock Added ()		NA	NA	see dilution calibration					NSF
Stock Solution Used (e.g., 1', 2')		NA	NA	sheet					Solvent/ Carrier Concen- traion:
Amount of Dilution Water Added ()									NSF
Amount of Solvent Added ()		NA		NA	NA	NA	NA	NA	

Analyst: NO Date: 3/29/94 Comments in Daily Log

CONFIDENTIAL
SODIUM CHLORITE CVIRGINICA
ESE #: 3943114-0300-3140
CMA #: CD-7.0-3AT-ESE

ENVIRONMENTAL PROTECTION AND ENGINEERING DEPT., JUNE
 GAINESVILLE, Florida

PAGE:
 ESE QA FORM: 016
 EFFECTIVE: JUNE 1990

SUBJECT: ACUTE TOXICITY DATA SHEET--WATER QUALITY DATA																								
SPONSOR: <u>CMA</u>										PROJECT NUMBER: <u>3943114-0300-3140</u>														
TEST SUBSTANCE: <u>Sodium chlorite</u>										TEST SPECIES: <u>C. virginica</u>														
DATE	3-28-94				3-29-94				3-30-94				3-31-94				4/01/94							
TIME	1330				1330				1130				1130				1400							
DATA BY	cm				cm				cm				cm				JK							
NOMINAL CONCENTRATION mg/L ug/L	R P	T P	E P	SL or HD	pH	DO	ALK	COND	T P	E P	SL or HD	pH	DO	T P	E P	SL or HD	pH	DO	T P	E P	SL or HD	pH	DO	
																								TEMP
CONTROL	A	23.4	28	7.9	7.6	-	-	-	23.0	27	7.7	7.4	25	27	7.9	7.0	22.4	27	7.8	7.2	22.5	27	8.0	7.3
	B	23.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
12.5	A	23.9	28	7.9	7.6	-	-	-	23.1	27	7.8	7.1	22.4	27	7.9	7.1	22.4	27	7.8	7.1	22.6	27	8.1	7.3
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
25	A	23.7	28	8.0	7.4	-	-	-	23.0	27	7.8	7.1	22.4	27	7.9	7.1	22.4	27	7.9	7.2	22.6	27	8.1	7.4
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
50	A	23.8	28	8.0	7.4	-	-	-	23.1	27	7.9	7.2	22.5	27	8.0	7.1	22.5	27	7.9	7.2	22.8	27	8.0	7.4
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
100	A	23.8	28	8.0	7.4	-	-	-	23.3	27	7.9	7.2	22.7	27	8.0	7.2	22.7	27	7.9	7.2	23.0	27	8.1	7.4
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
200	A	24.0	28	8.0	7.3	-	-	-	23.7	27	7.9	7.1	22.9	27	8.0	7.2	22.9	27	7.9	7.2	23.1	27	8.1	7.4
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Instrument/Method :		FS	REF	SA	OC				FS	REF	SA	OC	FS	REF	SA	OC	FS	REF	SA	OC	FS	REF	SA	OC
		2	5	2	1				2	5	2	1	2	5	2	1	2	5	2	1	2	5	2	1

KEY: TEMP - Temperature DO - Dissolved Oxygen
 SL - Salinity ALK - Alkalinity
 HD - Hardness COND - Conductivity

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Environmental Science & Engineering, Inc.
 Aquatic Toxicology Laboratory
 Gainesville, Florida

Page: _____
 ESE QA Form No.: 015B
 Effective: MAR 1993

SUBJECT: ACUTE TOXICITY DATA SHEET - SURVIVAL

		0-HOUR		24-HOUR		48-HOUR		72-HOUR		96-HOUR	
DATE		3/28/94		3/29/94		3/30/94		3/31/94		4/1/94	
ANIMALS FED		Yes		Yes		Yes		Yes		NO	
TIME		1030		1330		1000		1100		1530	
DATA BY		MO		MO		MO		MO		CY	
NOMINAL CONCENTRATION % (mg/L) ug/L	REP	OBS.	NO. ALIVE								
CONTROL	A	NONE	20								
	-	-	-	-	-	-	-	-	-	-	-
100	A	NONE	20								
	-	-	-	-	-	-	-	-	-	-	-
25	A	NONE	20								
	-	-	-	-	-	-	-	-	-	-	-
50	A	NONE	20								
	-	-	-	-	-	-	-	-	-	-	-
100	A	NONE	20								
	-	-	-	-	-	-	-	-	-	-	-
300	A	NONE	20	NONE	20	1 dead	19	4 dead	15	7 DEAD	8
	-	-	-	-	-	-	-	-	-	-	-

OBS - OBSERVATIONS	DRK - DARKER THAN CONTROLS	NF - NOT FOUND
NONE - NORMAL CONDITIONS	LT - LIGHTER THAN CONTROLS	BS - BRINE SHRIMP
AS - AT SURFACE	RAR - RAPID RESPIRATION	
LE - LOSS OF EQUILIBRIUM	PRE - PRECIPITATE	
LETH - LETHARGIC	FOS - FILM ON SURFACE	
HYP - HYPERACTIVE	UC - UNDISSOLVED CHEMICAL	
ERR - ERRATIC MOVEMENT	CLDY - CLOUDY SOLUTION	

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SODIUM CHLORITE CVIRGINICA
 ESE #: 3943114-0300-3140
 CMA #: CD-7.0-3AT-ESE

ESE Bioassay Department
 Gainesville, Florida

Page: _____
 QA Form: 042
 Effective: September 1985

SUBJECT: OYSTER SHELL GROWTH DATA								
Sponsor: <u>CMA</u>		Project No: <u>3943114-0300-3140</u>						
Test Substance: <u>Sodium Chlorite</u>		Test Species: <u>Eastern Oyster</u>						
Oyster No.	New Shell Growth (millimeters)							
	CTL	12.5mg/L	25 mg/L	50 mg/L	100 mg/L	200 mg/L		
1	2.4	2.9	0.7	0.0	0.0	0.0		
2	3.0	2.0	0.7	0.0	0.0	0.0		
3	1.8	2.0	0.6	0.0	0.0	0.0		
4	2.6	2.7	0.0	0.5	0.0	0.5		
5	3.2	4.2	0.0	0.0	0.4	1.0		
6	2.4	2.3	1.5	0.5	0.0	0.0		
7	2.8	1.6	0.0	0.0	0.0	0.0		
8	1.7	1.0	0.0	0.8	0.0	0.0		
9	3.0	1.1	0.0	0.0	0.0	0.0		
10	2.1	0.4	1.0	0.7	0.0	0.0		
11	1.5	2.0	1.0	0.0	0.8	0.0		
12	0.5	2.5	0.5	1.1	0.7	0.0		
13	0.9	1.9	1.4	0.7	0.0	0.0		
14	1.7	0.7	1.7	1.0	0.7	0.0		
15	1.3	1.8	1.0	0.0	0.0	0.7		
16	3.4	1.9	0.6	0.0	0.0			
17	2.3	1.0	1.1	0.0	0.0			
18	2.9	2.0	0.7	0.5	0.0			
19	5.3	1.8	0.9	0.0	0.0			
20	2.4	2.8	0.7	0.8	0.0			
Mean	2.36	1.945	0.705	0.33	0.13	0.11		
S.D.	1.04	0.795	0.519	0.400	0.275	0.231		
Difference	-	0.415	1.655	3.03	2.23	2.25		
2 Change	-	17.6	70.1	86.0	94.5	95.3		
DATE: <u>4-1-94</u>		TIME: <u>1500</u>		DATA BY: <u>GM</u>				

96-h EC50

 Results calculated using the Binomial Method. 3943014-0300-3140

Client : CMA Species : Eastern Oyster
 Dates of test : 3/28/94 to 4/1/94
 Test Material : Sodium Chlorite
 Report run by : JJY Date : 04-08-1994
 Concentration units (Circle One) : % PPTH PPM PPB Other _____

Conc. (mg/L)	Number Exposed	% Reduction in Growth Compared to Controls		Binomial Prob. (%)
		Number Dead	Percent Dead	
210.5	100	95	95.0	< 0.001
101.1	100	95	95.0	< 0.001
54.1	100	86	86.0	< 0.001
27.1	100	70	70.0	0.004
14.3	100	18	18.0	< 0.001
Control	100	0	0.0	N/A

Note -- The Binomial test shows that 14.29 and 27.13 can be used as statistically sound conservative 95 % confidence limits because the actual confidence level associated with these limits is greater than 95 %.

Note -- An approximate LC50 of 21.37 is obtained by nonlinear interpolation between 14.29 and 27.13

Calculations based on mean
 reduction in new shell growth

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 3943114 - 0300 - 3140

Sodium Chlorite/Eastern Oyster NOEC
 File: c:qqq.jjy Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	8.040	29.040	45.840	29.040	8.040
OBSERVED	2	26	62	19	11

Calculated Chi-Square goodness of fit test statistic = 15.1135
 Table Chi-Square value (alpha = 0.01) = 13.277

Data FAIL normality test. Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normal data and should not be performed.

Sodium Chlorite/Eastern Oyster NOEC
 File: c:qqq.jjy Transform: NO TRANSFORMATION

Hartley's test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 14.22
 Closest, conservative, Table H statistic = 6.4 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 15
 Actual values ==> R (# groups) = 6, df (# avg reps-1) = 19.00

Data FAIL homogeneity test. Try another transformation.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, Hartley's test may still be used as an approximate test (average df are used).

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 SODIUM CHLORITE CVIRGINICA
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3943014-0300-3140

Sodium Chlorite/Eastern Oyster NOEC
 File: c:qqq.jyy Transform: NO TRANSFORMATION

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	Control	2.360				
2	14.3 mg/L	1.945	353.50	327.00	20.00	
3	27.1 mg/L	0.705	237.50	327.00	20.00	*
4	54.1 mg/L	0.330	219.50	327.00	20.00	*
5	101 mg/L	0.130	213.00	327.00	20.00	*
6	211 mg/L	0.110	213.50	327.00	20.00	*

Critical values use k = 5, are 1 tailed, and alpha = 0.05

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Appendix C- Analytical Method Validation

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ESE #: 3943114-0300-3140
CMA #: CD-7.0-3AT-ESE

FINAL REPORT

STUDY TITLE

Method Validation for the Determination
of Sodium Chlorite in Seawater

AUTHOR

David H. Greer, Jr.

STUDY COMPLETED

May 18, 1994

REPORT DATE

May 18, 1994

SPONSOR

Chemical Manufacturers Association
2501 M Street N.W.
Washington, D.C. 20037

PERFORMING LABORATORY

Environmental Science & Engineering, Inc.
14220 Newberry Road
Gainesville, Florida 32607
(904) 332-3318

LABORATORY STUDY NUMBER

1944015V-L202

LABORATORY PROTOCOL NUMBER

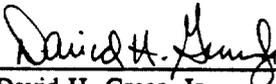
ASG-94-02

CONFIDENTIAL
SODIUM CHLORITE CVIRGINICA
ESE #: 3943114-0300-3140
CMA #: CD-7.0-3AT-ESE

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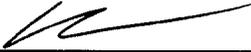
COMPLIANCE STATEMENT

The data presented in this report was an accurate representation of the investigations conducted. This study was conducted in compliance with current Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Good Laboratory Practice Regulations (40 CFR Part 160). All records pertaining to this study were maintained at Chemical Manufacturers Association GLP archives located at 2501 M Street, N.W., Washington, D.C. 20037. A copy of the original raw data will be archived in the ESE Gainesville Analytical Services GLP archives located at 14220 West Newberry Road, Gainesville, Florida 32607.



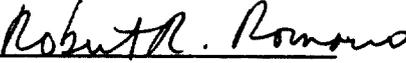
David H. Greer, Jr.,
Program Manager, Study Director
ESE, Inc.

5/18/94
Date



Kathleen K. Allen
Water Quality Department Manager
ESE, Inc.

5/18/94
Date



Robert Romano, Ph.D.
Director of Special Programs
Chemical Manufacturers Association

5/19/94
Date

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SODIUM CHLORITE CVIRGINICA
ESE #: 3943114-0300-3140
CMA #: CD-7.0-3AT-ESE

QUALITY ASSURANCE STATEMENT

This study was monitored by Environmental Science & Engineering, Inc. (ESE) Quality Assurance Unit to assure that the guidelines and Standard Operating Procedures (SOPs) used to conduct this study were followed.

The final report for this study was reviewed by the Quality Assurance Unit and, accurately reflects the results of the study as conducted, including all areas of compliance or noncompliance with Good Laboratory Practice Standards, the study protocol and ESE's SOPs.

Joe Owusu-Yaw, Ph.D.
Joe Owusu-Yaw
QAU GLP Coordinator

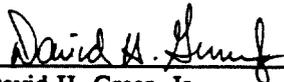
5-18-94
Date

QUALITY ASSURANCE AUDITS

<u>Audit Date</u>	<u>Type of Report</u>	<u>Date Reported to Management</u>	<u>Date Reported Study Director</u>
2/25/94	Protocol	5/18/94	2/25/94
5/16/94	Data Review	5/18/94	5/16/94
5/16/94	Draft Report	5/18/94	5/16/94
5/17/94	Revised Report	5/18/94	5/17/94
5/18/94	Final Report	5/18/94	5/18/94

CONFIDENTIAL
SODIUM CHLORITE CVIRGINICA
ESE #: 3943114-0300-3140
CMA #: CD-7.0-3AT-ESE

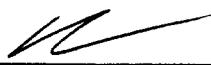
REPORT SIGNATURES



David H. Greer, Jr.,
Program Manager, Study Director
ESE, Inc.

5/18/94

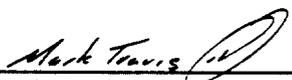
Date



Kathleen K. Allen
Water Quality Department Manager
ESE, Inc.

5/18/94

Date



Mark Travis
Water Quality Analyst
ESE, Inc.

5/18/94

Date

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CONFIDENTIAL
SODIUM CHLORITE CVIRGINICA
ESE #: 3943114-0300-3140
CMA #: CD-7.0-3AT-ESE

STUDY SUMMARY

SPONSOR: Chemical Manufacturers Association
2501 M Street, N.W.
Washington, D.C. 20037
Sponsor Representative: Robert Romero, Ph.D.
Director of Special Programs
(202) 887-1198

SPONSOR STUDY NUMBER: ASG-94-02 .

STUDY DIRECTOR: David H. Greer, Jr.
Environmental Science & Engineering, Inc.
14220 West Newberry Road
Gainesville, Florida 32607
(904) 333-1608

STUDY TITLE: Method Validation for the Determination of
Sodium Chlorite in Seawater

LOCATION OF STUDY: Environmental Science & Engineering, Inc.
14220 West Newberry Road
Gainesville, Florida 32602-1703

LABORATORY STUDY IDENTIFICATION: 1944015V-L202

TEST MATERIAL: Sodium Chlorite - CAS No.: 7758-19-2

STUDY INITIATION DATE: March 2, 1994

EXPERIMENTAL INITIATION DATE: March 2, 1994

EXPERIMENTAL COMPLETION DATE: March 18, 1994

STUDY COMPLETION DATE: May 18, 1994

DETERMINATION OF SODIUM CHLORITE IN SEAWATER

1.0 SCOPE AND APPLICATION

This method describes the analysis of Sodium Chlorite at the milligram per liter (mg/L) level in seawater.

2.0 SUMMARY OF METHOD

The reference analytical method used for this validation study was "The Measurement of Chlorite Ion in Seawater by IC Using the Chlorpromazine Post Column Reaction" [NOVATEK Application Note for the Chemical Manufacturers Association (Contract CD-6.0-NOV, Appendix I)]. This method is based on an ion chromatography post-column procedure using USEPA Method 300.0 with a borate buffer eluent. The post-column procedure uses photometric detection at 530 nanometers (nm). Following separation, chlorite (ClO_2^-) reacts with chlorpromazine in acidic media. This detection chemistry shows no interference from most common anions.

3.0 APPARATUS AND MATERIALS

- 3.1 Ion Chromatography System: Dionex IC Model DX-300.
- 3.2 Analytical Column: Dionex AS11.
- 3.3 Post Column Derivatization: Technicon II reagent manifold and colorimeter.
- 3.4 Data system: Dionex AI 450.
- 3.5 Volumetric Flasks: Various.
- 3.6 Analytical Balance: Mettler AE160.
- 3.7 Test Materials: Sodium Chlorite (Aldrich Lot # 01615KY and #05801JZ, 80% purity and NOVATEK, 99.75% purity).
- 3.8 Boric Acid: Aldrich lot# 05625MY, ACS reagent grade.
- 3.9 Sodium Hydroxide: Fisher lot# 9338003-24, 50/50 (w/w) solution.
- 3.10 Eluent: Borate eluent, 25 millimolar(mM) NaOH/50 mM H_3BO_3 .

- 3.11 Regenerant: Suppressor Regenerant, 12.5 mM H₂SO₄.
- 3.12 Water: Deionized (D.I.) water.
- 3.13 Seawater Test Solution: Ethylenediamine, 50 mg/L in full strength natural seawater.
- 3.14 Derivatization Reagent: Chlorpromazine hydrochloride (Sigma Lot# 112H0125), 0.56 mM in D.I. water.
- 3.15 Hydrochloric Acid Solution: 350 mL concentrated hydrochloric acid in 2 L D.I. water.

4.0 ELUENT PREPARATION

The eluent was prepared by dissolving 12.4 grams (g) of boric acid into 1 liter of D.I. water in a 2 liter volumetric flask. To the boric acid solution, 2.6 milliliters (mL) of 50/50 (w/w) sodium hydroxide solution was added, and the flask brought to volume with D.I. water. The contents of the flask were mixed thoroughly by inverting several times.

5.0 DERIVATIZATION REAGENT PREPARATION

The derivatization reagent was prepared by dissolving 0.2 g of chlorpromazine hydrochloride in 1 L of D.I. water. This solution was degassed with nitrogen prior to use.

6.0 STANDARD PREPARATION

6.1 PREPARATION OF CALIBRATION STOCK STANDARD

A calibration stock (spiking stock) was prepared by accurately weighing 1.676 g of sodium chlorite (80 %purity, Aldrich lot # 05801JZ) into a 1,000 mL volumetric flask and bringing to volume with deionized water. The solution was mixed thoroughly by inverting the stoppered flask several times. The concentration of this stock solution was 1000 mg-chlorite/L. This solution was used to prepare the instrument calibration standards.

6.2 PREPARATION OF CONTROL STOCK STANDARD

A control stock standard was prepared by accurately weighing 1.676 g of sodium chlorite (80 % percent purity, Aldrich lot # 01615KY) into a 1000 mL volumetric flask. The flask was brought to volume with D.I. water and mixed thoroughly by inverting the stoppered flask several times. The concentration of this stock was 1000 mg-chlorite/L. This solution was used to prepare the fortified samples for the method validation.

6.3 PREPARATION OF CALIBRATION VERIFICATION STOCK

An independent calibration verification stock (ICV) standard was prepared by accurately weighing 0.1676 g of sodium chlorite (99.75 % purity, NOVATEK) into a 100 mL volumetric flask. The flask was brought to volume with D.I. water and mixed thoroughly by inverting the stoppered flask several times. The concentration of this stock was 1250 mg-chlorite/L. This standard was used to verify the preparation of the calibration and control stocks.

6.4 STANDARDIZATION OF CHLORITE STOCKS

All Chlorite stock solutions were standardized using the iodometric procedure (Standard Methods, 15th Edition, Method 410A). Twenty five milliliter (mL) of each stock solution was volumetrically transferred to separate 125 mL erlenmeyer flask and 0.5 g of potassium iodine (KI) was added to the flask and dissolved. After the KI was dissolved, 0.5 mL of concentrated sulfuric acid was added and the flask was stoppered and placed in the dark for 5 minutes. Each flask was titrated with 0.1 N thiosulfate, adding 1 mL of starch indicator solution when the brown color became light yellow. Titration was continued until the solution became clear.

6.5 PREPARATION OF WORKING CALIBRATION STANDARDS

One mL of the 1000 mg/L calibration stock was diluted with D.I. water 10 mL in a 10 mL volumetric flask. The concentration of this intermediate calibration stock was 100 mg/L. A series of 7 dilutions of the calibration stock were prepared for use in instrument calibration. The following dilution schedule was used to prepare the calibration standards.

Intermediate Chlorite Calibration Stock (mL) (100 mg/L)	Final Volume (mL D.I. water)	Calibration Standard Concentration (mg/L)
0.02	100	0.02
0.05	100	0.05
0.10	100	0.10
0.20	100	0.20
0.40	100	0.40
1.00	100	1.00
2.00	100	2.00

7.0 PREPARATION OF FORTIFICATION SOLUTIONS

7.1 SECONDARY FORTIFICATION SOLUTION

A secondary fortification solution was prepared by transferring 1 mL of the control stock into a 100 mL volumetric flask. The flask was brought to volume with D.I. water and inverted several times to mix thoroughly. The concentration of this solution was 10 mg-chlorite/L.

7.2 PREPARATION OF FORTIFICATION SAMPLES

Low level fortification samples, 0.1 mg-chlorite/L, were prepared by transferring 100 microliters of the secondary fortification solution (10 mg-chlorite/L) to a 10 mL volumetric flask. The flask was brought to volume with filtered natural seawater fortified with 50 mg/L of ethylenediamine. The fortification samples were prepared in triplicate and mixed thoroughly by inverting the stoppered flask several times.

High level fortification samples, 250 mg-chlorite/L, were prepared by diluting 1 mL of the control stock (1000 mg-chlorite/L) with 3 mL of filtered natural seawater fortified with 50 mg/L ethylenediamine. The fortification samples were prepared in triplicate and mixed thoroughly by inverting the stoppered flask several times.

8.0 SAMPLE ANALYSIS

8.1 INSTRUMENT OPERATING CONDITIONS

The following instrumental conditions were used during analysis of test samples.

System I.D.	IC Model DX-300, #1
Separation Column	AS11 #1700
Suppression	AMMSII #8909
Injection Loop	50 μ L
Flow Rate	1.0 mL/min
Pressure	680 psig
Offset	5.1 micro-Siemens (μ S)
Eluent	12.5 mM NaOH/25 mM H ₃ BO ₃
Regenerant	25 mN H ₂ SO ₄

The configuration of the post column derivatization and visible detection system is presented as Figure 8-1.

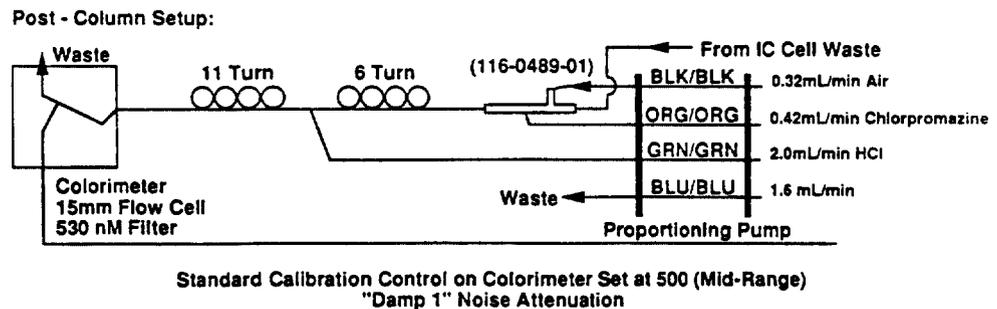


Figure 8-1 Post Column Derivatization/Visible Detection System Configuration

8.2 INSTRUMENT CALIBRATION AND SAMPLE ANALYSIS

- 8.2.1 After the instrument had warmed up and stabilized, the column was 'primed' with injection of the 2.0 mg/L calibration standard followed by an injection of D.I water.
- 8.2.2 Each calibration standard, beginning with a D.I. water blank was injected in order of increasing chlorite concentration.
- 8.2.3 A 1.25 mg/L instrument calibration verification standard (ICV) was analyzed to confirm that instrument calibration standard preparation was correct.
- 8.2.4 After calibration was complete, each set of fortified test samples were injected beginning with a seawater blank. Seawater samples were diluted by a factor of 4 to prevent overload of the column and subsequent retention time shifts. Dilution factors of 4 were used for the low level fortification samples and 250 was used for the high level fortification samples.
- 8.2.5 After all sample analyses were complete, a 1.0 mg/L continuing calibration verification standard (CCV) was analyzed to verify instrument stability throughout the analytical run.

9.0 QUANTITATION

- 9.1 A quadratic regression curve of Chlorite concentration versus instrument response, expressed as peak area, was generated from the data obtained during instrument calibration. The regression was used to calculate concentrations of Chlorite in samples and quality control fortifications by substitution of peak areas found in samples and quality control fortifications into the regression equation generated from the calibration data.
- 9.2 Recoveries of Chlorite from the fortification test samples were calculated by comparing the calculated found concentrations in the fortified samples with the nominal fortification levels. Simple arithmetic means and standard deviations for the method validation fortification levels were calculated and used to generate the quality control limits for the toxicity test sample determinations.

10.0 DATA ACCEPTABILITY

- 10.1 Calibrations were considered acceptable if the correlation coefficient of the curve was greater than or equal to 0.995.
- 10.2 Continuing calibration standard response (post-run) agreed within plus or minus 10 percent of the original analysis response for that standard.

- 10.3 Quality control limits were established using results obtained during the method validation study. Warning limits were set at plus or minus 2 standard deviations from the mean of all recovery values. Control limits were set at plus or minus 3 standard deviations from the mean.

11.0 SAFETY

All reagents and test materials should be handled with care to avoid unnecessary exposure of any personnel to these potentially toxic materials. Material Safety Data Sheets should be read by all personnel involved with the determination of Chlorite. Avoid skin contact or ingestion of Chlorite.

12.0 METHOD VALIDATION RESULTS

Recoveries for fortified samples ranged from 99.8 to 109 percent. The calculated mean recovery was 103.5 percent with a standard deviation of 3.7. Result for individual fortification samples are presented in Table 12-1. The reporting limit for chlorite using this method was 0.08 mg/L in seawater.

Low level fortification samples were analyzed undiluted. Due to column overload, there was no recovery of chlorite from the undiluted samples. There were no significant interferences noted during the analysis of chlorite when utilizing the post column derivatization and visible detection system proceeded by separation of chlorite by ion chromatography.

Example chromatograms for standards and fortification samples depicting both the ion chromatographic conductivity and post column derivatization visible detection systems are presented as Figures 12-1 through 12-4.

Table 12-1 Fortification Results of Chlorite Analytical Method Validation

Fortification Level	Target Conc. (mg/L)	Found Conc. (mg/L)	Percent Recovery
Low SPK #1	0.10	0.109	109
Low SPK #2	0.10	0.107	107
Low SPK #3	0.10	0.103	103
High SPK #1	250.00	249.4	99.8
High SPK #2	250.00	251.1	100.4
High SPK #3	250.00	254.9	102
		Mean	103.5
		Std Dev	3.7

Std Dev = Standard Deviation

Source: ESE, 1994

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CMA #: CD-7.0-3AT-ESE

Area Report

Data Reprocessed On 03/09/1994 10:08:17

```

-----
| Sample Name: AUTOCAL7R - A10           Date: 03/08/1994 16:38:39
| Data File : C:\DX\DATA4\ch030801.d10
| Method    : C:\DX\METHOD\CLO2.met
| ACI Address: 1 System: 1 Inject#: 10 Vial:A10   Detector:CDM-2
| Analyst   :                               Column:
-----

```

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area	Reject
External	50	1	2280	2Hz	0.00	18.99	500	

***** Peak Report: All Peaks *****

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
1	1.25			0.000	5484475	0.000	1
2	3.00	0.00	CLO2	1.000	11585140	0.000	1
3	5.20			0.000	4040705	0.000	1
Totals				1.000	21110320		

File: ch030801.d10 Sample: AUTOCAL7R - A10

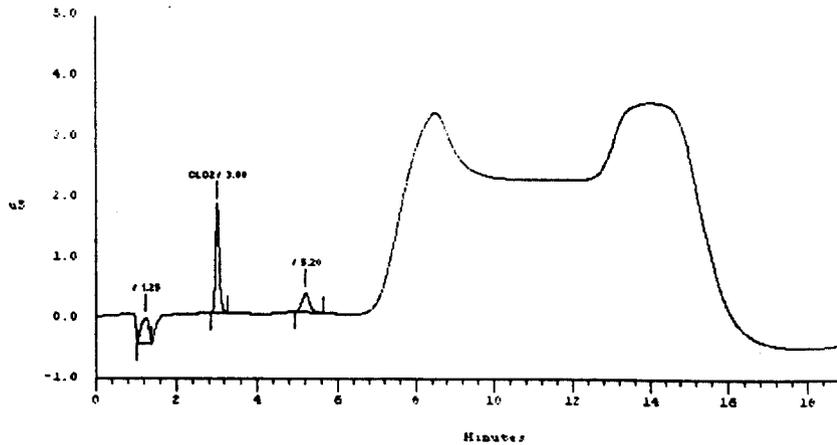


Figure 12-1 Example Chromatogram of Chlorite Standard (1.0 mg/L) - Conductivity Detection.

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Area Report

Data Reprocessed On 03/09/1994 10:09:25

```

-----
: Sample Name: SP1*QC*3 - A23           Date: 03/08/1994 21:06:34
: Data File  : C:\DX\DATA4\ch030801.d23
: Method     : C:\DX\METHOD\CLO2.met
: ACI Address: 1 System: 1 Inject#: 23  Vial: A23   Detector: CDM-2
: Analyst    :                               Column:
-----

```

```

-----
Calibration Volume Dilution Points Rate Start Stop Area Reject
-----
External          50          250  2280  2Hz  0.00 16.99      500
-----

```

***** Peak Report: All Peaks *****

PK. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
1	1.25			0.000	5052670	0.000	1
2	3.03	1.11	CLO2	255.740	11863418	0.000	1
3	4.98			0.000	1656239100	0.000	1
Totals				255.740	1673155186		

File: ch030801.d23 Sample: SP1*QC*3 - A23

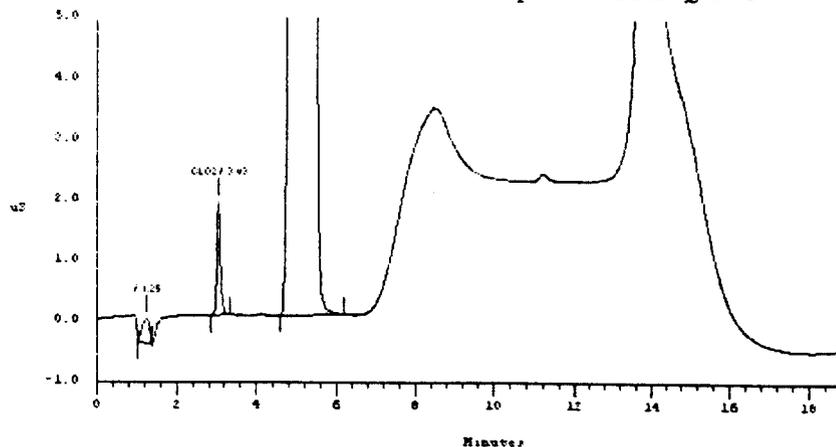


Figure 12-2 Example Chromatogram of Chlorite Seawater Fortification (250 mg/L) - Conductivity Detection.

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Area Report

Data Reprocessed On 09/1994 10:39:43

```

-----
: Sample Name: AUTOCAL7R - A10           Date: 03/08/1994 16:38:39
: Data File  : C:\DX\DATA4\ch030802.d10
: Method     : C:\DX\METHOD\CLO2.me2
: ACI Address: 1 System: 1 Inject#: 10  Vial:A10   Detector:OTHER
: Analyst    :                               Column:
-----

```

Calibration	Volume	Dilution	Point	Start	Stop	Area	Reject
External	50	1	2250	2Hz	0.00	16.99	100

***** Component Report: All Components *****

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
1	4.52	0.00	50074*IC-G CLO2	1.000	34796	0.000	1
Totals				1.000	34796		

***** Peak Report: Unknown Peaks *****

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
Totals				0.000	0		

File: ch030802.d10 Sample: AUTOCAL7R - A10

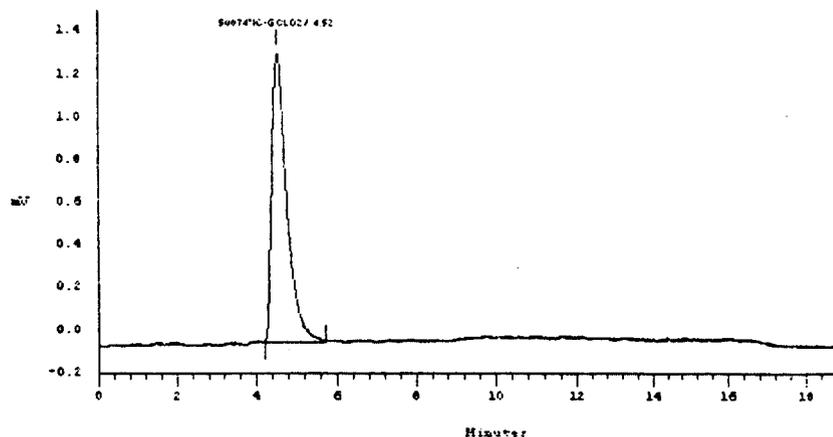


Figure 12-3 Example Chromatogram of Chlorite Seawater Fortification (250 mg/L) - Visible Detection.

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Area Report

Data Reprocessed On 03/09/1994 10:41:09

```

-----
Sample Name: SP1*QC*3 - A23                               Date: 03/08/1994 21:08:34
Data File  : C:\DX\DATA4\ch030802.d23
Method     : C:\DX\METHOD\CL02.me2
PCI Address: 1 System: 1 Inject#: 23 Vial:A23             Detector:OTHER
Analyst    :                                             Column:
-----
  
```

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area	Reject
External	50	250	2260	2Hz	0.00	18.99		100

..... Component Report: All Components

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
2	4.55	1.11	50074*IC-G CL02	249.858	34547	0.000	1
Totals				249.858	34547		

..... Peak Report: Unknown Peaks

Pk. Num	Ret% Time	Delta	Component Name	Concentration MG/L	Area	Response Factor	Bl. Code
	1.87			0.000	776	0.000	1
Totals				0.000	776		

File: ch030802.d23 Sample: SP1*QC*3 - A23

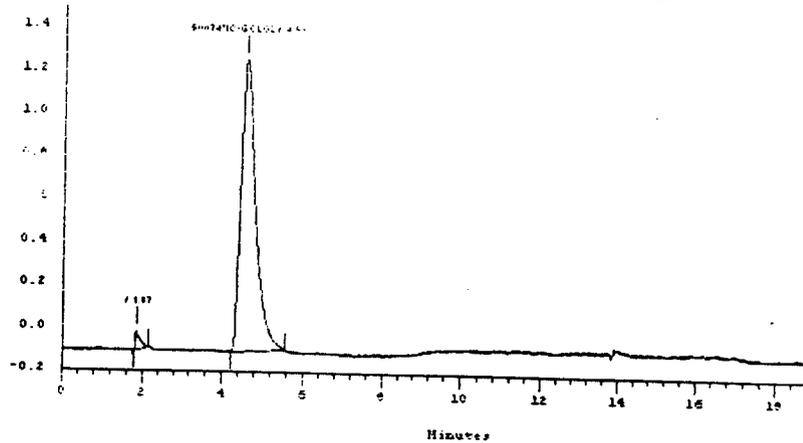


Figure 12-4 Example Chromatogram of Chlorite Standard (1.0 mg/L) - Visible Detection.

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APPENDIX I
PROTOCOL

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ESE PROTOCOL NO.: ASG-94-02

ENVIRONMENTAL SCIENCE & ENGINEERING, INC.

ANALYTICAL SERVICES

METHOD VALIDATION PROTOCOL

STUDY TITLE

Method Validation for the Determination of Sodium Chlorite in Seawater

TEST SUBSTANCE IDENTIFICATION

Sodium Chlorite

CAS No.: 7758-19-2

SPONSOR

Chemical Manufacturers Association

Sponsor Representative: Mr. Robert Romano, Ph.D.
Director of Special Programs
Chemical Manufacturers Association
2501 M. Street N.W.
Washington, D.C. 20037
(202) 887-1198

Study Number: Not Assigned

TEST FACILITY

Environmental Science & Engineering, Inc. (ESE)
Analytical Services
14220 Newberry Road
Gainesville, Florida 32607
ESE Study Director: David H. Greer, Jr.
GLP Program Manager
(904) 333-1608
(904) 333-6622 FAX

PROPOSED EXPERIMENTAL START DATE

Upon acceptance of the protocol by the study sponsor. The proposed experimental start date for this method validation study is February 16, 1994.

PROPOSED EXPERIMENTAL TERMINATION DATE

March 10, 1994.

OBJECTIVE

To validate an analytical method for the determination of sodium chlorite in seawater utilizing ion chromatography with visible (530 nanometers) light detection in support of aquatic toxicity studies.

REFERENCE MATERIALS

1. Sodium Chlorite, 80% purity (Aldrich Chemical Co. Lot # 0161SKY)

METHODS

The analytical method used for this validation study will be "The Measurement of Chlorite Ion in Seawater by IC Using the Chlorpromazine Post Column Reaction" (Novatek Application Note for the Chemical Manufacturers Association (Contract CD-6.0-NOV, Appendix I). Modifications to the method will be made only after consultation with the Study Sponsor Representative.

Analytical Method Validation

The analytical method validation will consist of the preparation of a blank seawater matrix, triplicate fortifications of seawater at the anticipated low test concentration (0.1 milligrams per liter (mg/L)), and triplicate fortifications of seawater at the anticipated high test concentration (250 mg/L). The samples will be analyzed according to the referenced method and the resulting data will be analyzed to determine the precision and accuracy of the method.

Statistical Analysis

Recoveries of Chlorite from the analytical method validation fortification spike samples will be calculated by comparing the calculated found concentrations in the fortified samples with the nominal fortification levels. Simple means and standard deviations for the two fortification levels will be calculated and used to generate the quality control limits for field sample determinations.

Quality Assurance/Quality Control

Prior to each analytical sequence, a series of analytical calibration standards will be prepared, analyzed and used to determine the concentration of the target compounds in the test solutions.

Acceptance of the instrument calibration will depend upon the correlation coefficients for the standard curves. Calibration will be considered acceptable if the correlation coefficient of the calibration curve is greater than or equal to 0.995 using either linear or quadratic regression analysis.

At the end of each analytical sequence, a continuing calibration standard (CCS) will be analyzed to determine the instrument stability throughout the analytical sequence. The CCS must be within plus or minus 15 percent of the same concentration standard run at the beginning of the analytical sequence for the run to be considered valid.

The upper and lower warning and control limits for the analysis field samples will be set by mutual agreement between the Study Sponsor Representative and the ESE Study Director upon completion of the method validation study and review of the recoveries obtained during the method validation study.

REPORT

A method validation report will be prepared in U.S. Environmental Protection Agency PR Notice 86-5 format documenting all activities associated with the determination of Chlorite ion in seawater matrices. The report will contain a detailed write-up of the method for analysis of Chlorite, a data summary of the results of the method validation, raw data, a quality assurance statement and a Good Laboratory Practices compliance statement.

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SAFETY

Material Safety Data Sheets (MSDS) will be provided to all personnel involved in the study. All reagents and test materials will be handled in manners described in the ESE Laboratory Safety Manual. All chemical reagents and test materials will be handled with respect to their respective MSDS. It will be the responsibility of the various ESE Department Managers to impart the hazards associated with each reagent or test material to the personnel participating in this study.

GOOD LABORATORY PRACTICES

The study will be conducted using Good Laboratory Practices. All raw data generated in the study will be sent to and archived at the Chemical Manufacturers Association GLP archives located at 2501 M. Street, N.W., Washington, D.C. 20037. A copy of the original raw data will be archived in the ESE Analytical Services GLP archives located at 14220 Newberry Road, Gainesville, Florida. The Study Director will be responsible for developing the protocol and writing the report. The ESE Principal Investigator is responsible for the conduct of the study, and for providing the Study Director with a final report and all data generated. Additionally the ESE Principal Investigator will review the protocol, Good Laboratory Practices, materials and methods, and safety guidelines with all support personnel prior to their participation in the study. Any deviations from the protocol will be recorded and changes in plans will be discussed with the Study Director and the Study Sponsor Representative in advance, as well as being written and attached to this protocol as amendments.

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APPROVAL SIGNATURES

Sponsor Representative: Robert R. Romano Date Feb. 23, 1994
Robert Romano, Ph.D.
Director of Special Programs
Chemical Manufacturers Association

Study Director: David H. Greer, Jr. Date 3/2/94
David H. Greer, Jr.
GLP Program Manager
ESE

Principal Investigator: [Signature] Date 2/25/94
Kathleen K. Allen
Water Quality Department Manger
ESE

Quality Assurance: Portia O. Pisigan Date 3/2/94
Portia O. Pisigan
Quality Assurance Unit Supervisor
ESE

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APPENDIX I
ANALYTICAL METHOD

6

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June 1993

**NOVATEK APPLICATION NOTE
FOR THE
CHEMICAL MANUFACTURERS ASSOCIATION
(Contract CD-6.0-NOV)**

**The Measurement of Chlorite Ion in Seawater by IC
Using the Chlorpromazine Post Column Reaction**

SCOPE AND APPLICATION

This method incorporates a chlorpromazine reaction chemistry to measure chlorite ion (ClO_2^-) following the separation of anions by USEPA Method 300.0. This method can be used to measure chlorite ion from 0.05 to 1.0 mg/L in a seawater matrix. The method detection limit (MDL) was determined to be 0.04 mg/L chlorite ion.

SUMMARY OF THE METHOD

This method is an ion chromatography post-column procedure using USEPA Method 300.0 with a borate eluent. The post-column procedure uses photometric detection at 530 nm. Following separation, ClO_2^- reacts with chlorpromazine in acidic media by a charge transfer mechanism. This method overcomes common problems encountered in the ion chromatographic measurement of seawater samples from Cl^- and SO_4^{2-} . This detection chemistry shows no interference from all common anions with the exception of bromate ion and nitrite ion.

The sample (25 - 200 μL) is injected into the eluent at a flow rate of 1.0 mL/min. The sample passes through a metal free column (MFC) and a guard column before separation on the analytical column. An anion micromembrane suppressor (AMMS II) is used with a weak sulfuric acid regenerant solution flowing at 10 mL/min. The suppressed eluent from the ion chromatograph is directed to a post column manifold in which the eluent is first merged with the chlorpromazine reagent. The combined eluent-chlorpromazine reagent stream is then merged with an acid stream to form the photometric product in the presence of ClO_2^- (Figure 1).

SAMPLE HANDLING

All samples should be analyzed as soon as possible. Check that the sample pH is between 6.5 and 7.5. Adjust the sample with dilute hydrochloric acid or sodium

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Chlorite Ion
Chlorpromazine Post Column

hydroxide solutions if necessary. Limit sample exposure to hv by using opaque sample bottles and storing the samples in the dark at 4°C.

Sample bottles should not be cleaned with soap or surfactants. All sample bottles should be rinsed a minimum of five times with distilled water and an additional five times with deionized water displaying a minimum resistance of 17.8 megohms-cm. The bottles are then allowed to air dry for 48 hours.

Sample treatment - Treat the sample with ethylenediamine (50 ppm/100 mL) and let it react for a minimum of 15 minutes to mask any chlorine that may be present in the sample. Store the sample at 4°C and filter any precipitates that may be present before analysis.

If you plan to ship or transport a sample, ship at least 100 mL of treated sample making sure that the sample bottle is completely filled thereby minimizing headspace. Ship the sample under blue ice in a suitable container.

Holding Times - Holding times are as listed for finished water samples in the Federal Register 40 CFR and in the text of USEPA Method 300.0 for the anions separated by this procedure. Laboratory tests have shown that chlorite ion samples in a seawater matrix are not stable.

GLASSWARE

It is important to keep all glassware clean and free of interferences. During storage all glassware openings should be covered with cellophane wrap or ground glass stoppers and stored in the dark to prevent contamination. This includes beakers, volumetric flasks and pipettes.

The following cleaning procedure is recommended:

1. Clean all glassware with a detergent and rinse well with tap water.
2. Soak the glassware in 1:1 nitric acid overnight.
3. Rinse 5X with tap water.
4. Rinse 5X with doubly distilled and deionized water.
5. Store glassware as described above.

APPARATUS

Ion Chromatography System to include:
Ion Chromatograph (Dionex DX-100)

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Metal-Free Column (Dionex, MFC)
Guard Column (Dionex, AG9)
Analytical Column (Dionex, AS9-SC)
Integrator

Post-Column System to include:

Two Reagent Manifold (Tecator Chemifold SR, Type III)
Two Channel Peristaltic Pump
UV/Visible Detector (Perstorp Analytical 510 or equivalent)
Integrator

REAGENTS

All water used to prepare reagent working solutions is best prepared by the passage of distilled water through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Regeneration of the column should be carried out according to the manufacturers specifications. The water should then be distilled and collected. Commercial water purifications systems that deionize and doubly distill the water are preferred. Water with a minimum resistance of 17.8 megohm-cm should be used to prepare all solutions.

Distilled Water: Deionized and doubly distilled as described above.

Ethylenediamine: EDA should be purchased in as pure a form as possible. A concentrated solution is prepared by diluting the pure EDA 1:100 with DI water. This yields an EDA stock solution concentration of 8990 mg/L. To 100 mL sample add 0.5 mL of EDA stock to give an EDA final concentration of ca. 45 mg/L.

Borate Eluent: 12.5 mM NaOH/50 mM H₃BO₃. The eluent is prepared by dissolving 0.8 g sodium hydroxide (NaOH) and 6.2 g boric acid (H₃BO₃) into 2 liters of deionized water.

Suppressor Regenerant: 12.5 mM H₂SO₄. The regenerant is prepared by diluting 2.75 mL sulfuric acid (H₂SO₄) in 4 liters of deionized water.

Chlorpromazine Solution: 0.56 mM Chlorpromazine. The indicator is prepared fresh daily by dissolving 0.2 g chlorpromazine (Sigma) into 1 liter of deionized water. This solution must be degassed with either nitrogen or helium for at least 10 minutes and be protected from light.

Chlorite Ion
Chlorpromazine Post Column

HCl Solution: 2.1 M HCl. The acid solution is prepared by diluting 350 mL hydrochloric acid (HCl) in 2 liters of deionized water. This solution must be degassed with either nitrogen or helium for at least 10 minutes.

STANDARDS

A series of standards should be prepared for the chlorite ion measurement.

NOTE

Most commercial sodium chlorite contains impurities. Highly purified sodium chlorite is essential for the establishment of valid calibration curves (i.e. correlation coefficients ≥ 0.999). It is recommended that the purity of the sodium chlorite reagent be determined prior to its use.

A series of standards should be prepared for the chlorite ion and chlorate ion measurement. Chlorite ion standards are stable for a maximum of 2 weeks if kept cold (4°C) and shielded from light.

Stock Chlorite Ion Standard Solution (100 mg/L): To a 100 mL volumetric flask containing 50 mL of doubly distilled water, place 13.41 mg of sodium chlorite. Mix to dissolve and dilute to 100 mL with doubly distilled water.

Working Standard Solutions: To six 100.0 mL volumetric flasks, add the following volumes of stock standard solution and dilute to volume with distilled, deionized water.

ClO₂⁻ Working Stock (mL)	Standard Solution (mg/L)
0.1	0.1
0.2	0.2
0.4	0.4
0.8	0.8
1.0	1.0
check 0.4	0.4

PROCEDURE

Instrument Set-up

1. Turn the power on for the IC, UV/Visible detector and integrators.
2. Fill the eluent and regenerant bottles with the appropriate reagents.
3. Fill the chlorpromazine and acid bottles with the appropriate reagents.
4. Check to make sure that the following parameters are set on the IC:

Columns	MFC/AG9/AS9-SC
Sensitivity	1 μ S full-scale
Eluent	12.5 mM NaOH/50 mM H ₃ BO ₃
Eluent Flow	1.0 mL/min
Detection	Suppressed conductivity
System Backpressure	950-1150 psi
Background Conductivity	7-12 μ S
Suppressor	Anion Micro Membrane (AMMS II)
Suppressor Regenerant	12.5 mM H ₂ SO ₄
Regenerant Flow	10 mL/min
Sample Injection Volume	25 - 200 μ L
5. Check to make sure that the following parameters are set on the post-column detection system:

Indicator	0.56 mM Chlorpromazine
Indicator Flow	0.4 mL/min
Acid	2.1 M HCl
Acid Flow	2.0 mL/min
Mixing Coils	M1=60 cm, M2=120 cm
Pathlength	10 mm flow cell
Wavelength	530 nm
Detector Settings	0.002 AUFS/10 sec rise time
6. Equilibrate the system by allowing the IC and post column reagents to flow for a minimum of 30 minutes. Set the auto offset on IC to zero. Check the system backpressure and background conductivity.

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Chlorite Ion
Chlorpromazine Post Column

NOTE

We have noticed that fluorescent lights seem to effect the post column chemistry sensitivity. Precautions should be taken to shield reagents from flouorescent lights.

7. Check the post-column system for leaks and air bubbles.
8. Set the integrators according to the instructions provided by the manufacturer.
9. Place the injection valve on the IC in the load position. Fill the sample loop with the most concentrated sample.
10. Inject the sample. A normal anion profile will be recorded by the IC integrator. The ClO_2^- typically is detected ca. 2.5 minutes. The post-column tracing can be expected in an additional 30 - 45 seconds (Figure 2).
11. The high standard should be injected until the calculated peak area values are within $\pm 5\%$.
12. Prepare a calibration curve by injecting standards in random order.
13. Analyze the check standard. The calculated value for the standard from the calibration curve should be within ± 0.02 mg/L of the known concentration. If the value is outside this range, the instrument is not properly calibrated.

NOTE

Calibration curves should exhibit a correlation coefficient better than 0.999. If this level cannot be attained, check your reagents, standards and the instrument for potential causes that prevent adequate calibration. Make adjustments as necessary. Do not analyze samples until a suitable calibration curve is recorded.

STANDARDIZATION AND QUANTITATION

The quantitation of bromate ion separated by this method is performed by injecting standard solutions and integrating the detector response at a defined retention time to calculate a peak area. A calibration curve is developed from a series of standard injections (concentration vs. peak area) and used to quantitate injected samples.

1. Establish the IC and post-column operating parameters.

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Chlorpromazine Post Column

2. Prepare calibration standards at a minimum of four concentration levels and a blank by adding accurately measured volumes of the dilute chlorite ion standard solution to a volumetric flask and diluting to volume.
3. Prepare a calibration curve. This curve should be developed each day and checked every 10 samples. If the response or retention time for any analyte varies from the expected value by more than 10%, the test should be repeated using fresh calibration standards.
4. Calculate the sample concentration using a statistics program. Enter the data into the program to generate a worksheet.

NOTE

Data quality should be evaluated on multiple levels:

1. The correlation coefficient should be ≥ 0.999 .
2. Calculated standard peak area values should be within 5% of the measured area.
3. Calculated standard concentration values should be within 0.01 mg/L of the analyte being calibrated. This test indicates the error associated with the calculated regression line.
4. The concentration of the check sample should be within 0.02 mg/L of the theoretical concentration. This test indicates the accuracy of the calibration.

SAFETY

The chlorite ion measurement requires the careful handling of solutions. Refer to the appropriate Material Safety Data Sheet for reagent handling instructions. For added protection, the laboratory should be well ventilated and safety goggles should be worn at all times.

REFERENCES

1. USEPA Method 300.0
2. Hautman/Bolyard personal communication.

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Chlorpromazine Post Column

3. Gordon, G.; Cooper, W.J.; Rice, R.G.; Pacey, G.E. Disinfectant Residual Measurement Methods, American Water Works Association - Research Foundation (AWWA-RFISBN0 -89867-408-5) Denver Colorado, 1987, 815pp
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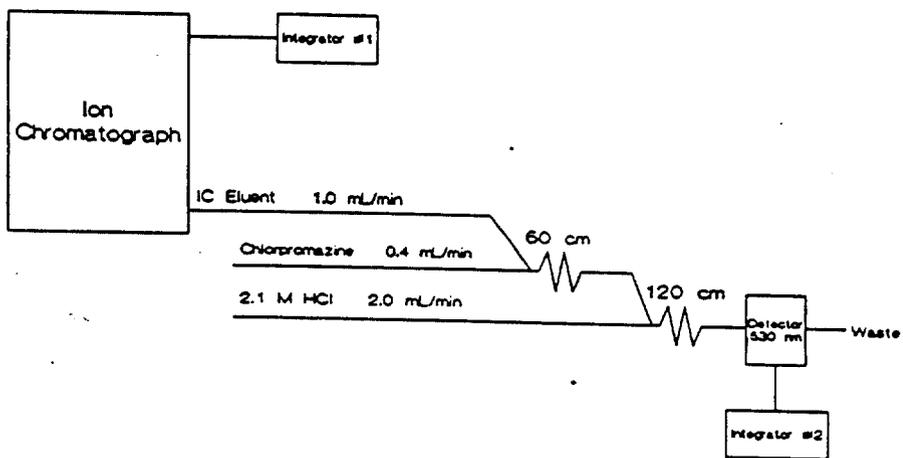


Figure 1. IC post-column diagram.

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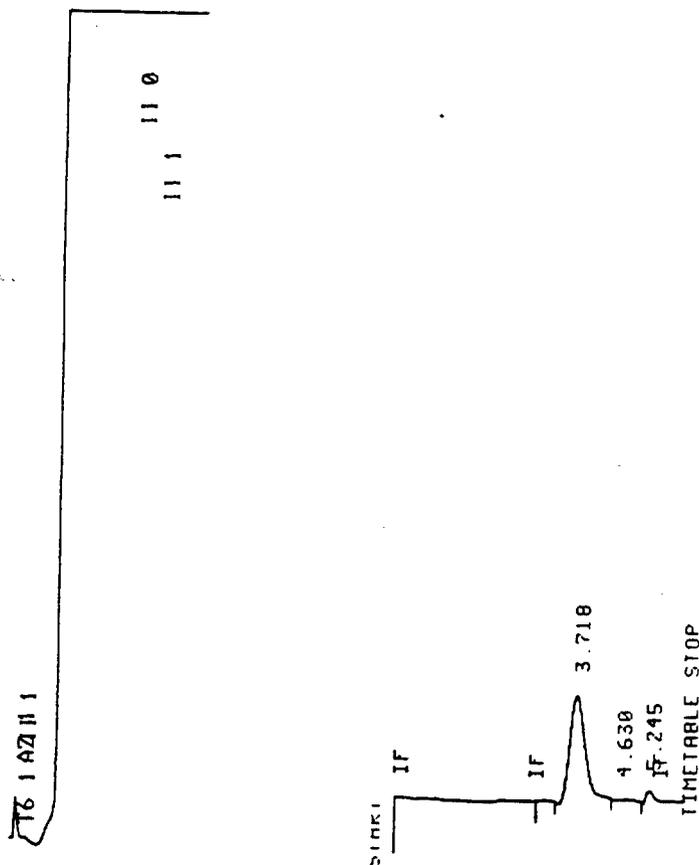


Figure 2. US EPA Method 300.0 conductivity and post-column detection of ClO_2^- .