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Henkel Corporation

Law Department

4EHP-95-13390
A
962950000169/s

March 21, 1995

Document Processing Center (7407)
Attention: Section 8(e) Coordinator
Office of Pollution Prevention and Toxics, Room E-G99
U.S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

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Subject: 8(e) Ecotoxicity

COMPANY SANITIZED

Dear Sir/Madam:

The following submission is made by Henkel Corporation to comply with the requirements of TSCA 8(e), Substantial Risk reporting.

Although we believe it is unlikely that a situation meeting the definition of Substantial Risk exists, the available data is insufficient to permit us to conclude that reporting is not required.

The following ecotoxicity results, prepared by Biological Monitoring, Inc., Blacksburg, VA on February 28, 1995, were received by Henkel on March 6, 1995.

Chemical Substance: Alcohols, C₁₆₋₁₈, ethoxylated (5 moles), propoxylated (13 moles)

48 hour LC50 Fathead Minnow: 0.319 mg/L

As you know, many chemicals with related structures produce similar effects in fish. Such chemicals have been in use for many years and are included in some EPA SAR classifications. However, we are not aware that EPA has specific knowledge of the results we are now reporting. A copy of the complete report is enclosed for your review.

The product in question is sold to the paper industry for various purposes. Different trade names are used to distinguish among applications. We intend to apprise our customers of the ecotoxicity data through its listing on the MSDS for the trade name products.

4/3/95

Section 8(e) Coordinator

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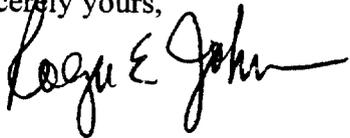
There is no release to the environment when the product is used in a pulp mill. However, when used as a drainage aid or rewetter, it is released to the environment, albeit at low concentrations. The production dosage rate does not exceed 0.75 lbs./ton of pulp, i.e., less than 0.04%. Considering the large amount of water used in the manufacture of paper, the concentrations found in the mill effluent are substantially lower than the percentage referenced above, and further reduced when it reaches the receiving stream.

Based on chemicals with related structures, it is reasonable to conclude that the chemicals at issue are biodegradable over time. Therefore, we would not anticipate bioaccumulation.

In conclusion, we are not aware of any environmental harm caused by the use of this product.

A sanitized version of this letter and its enclosures has been provided to ensure the confidentiality of proprietary information.

Sincerely yours,

A handwritten signature in black ink, appearing to read "Roger E. Johnson". The signature is written in a cursive style with a long horizontal stroke at the end.

Roger E. Johnson
Manager, Regulatory Affairs

REJ/dgm

Enclosures: Acute Toxicity Study

**ACUTE TOXICITY STUDY
ON PRODUCT
Oncorhynchus mykiss**

COMPANY SANITIZED

Submitted to:

Henkel Corporation
11709 Fruehauf Blvd.
Charlotte, NC 28273

Submitted by:

Biological Monitoring, Inc.
P.O. Box 184
Blacksburg, VA 24063

Phone: 703-953-2821
Fax: 703-951-1481

February 28, 1995

EXECUTIVE SUMMARY

Henkel Corporation contracted Biological Monitoring, Inc. (BMI), Blacksburg, Virginia to perform a static acute toxicity study on the product. The purpose of this study was to determine an LC50 (the nominal concentration which is lethal to 50% of the test population) for the freshwater indicator species Oncorhynchus mykiss (Rainbow Trout). The procedures used in this study followed the U.S. EPA guidelines for static acute testing (EPA/600/4-90/027) and followed BMI's Standard Operating Procedures (SOP). The dilution water for this study was Blacksburg, Virginia municipal tapwater dechlorinated, deionized (Milli-Pure, Milli-Q UV Plus System) 0.2 micron filtered and reconstituted to a hardness of 80-100 mg/L (Ca as CaCO₃) (BMI MHRW). This is BMI's standard dilution water which is used to culture and maintain rainbow trout and other freshwater species. The product was received on February 21, 1995 and was observed to be a clear, viscous liquid. It was readily soluble in water. Results from this study are summarized below.

Product	Experiment ID#	48h LC50 mg/L	95% Confidence Limits 96h	Method 48h
	HEN022295-1	0.319	0.285 and 0.356	Spearman

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1.0 INTRODUCTION

Henkel Corporation contracted Biological Monitoring, Inc. (BMI) of Blacksburg, Virginia to perform a static acute toxicity study on the product. The purpose of this study was to determine LC50s (the nominal concentration which is lethal to 50% of the test population) for the freshwater indicator species Oncorhynchus mykiss (Rainbow trout). This indicator species is commonly used for toxicity testing and water quality assessment, and is sensitive to a wide variety of toxicants. The species is also quite amenable to laboratory culturing methods. Test methods followed U.S. EPA guidelines for static acute exposures in accordance with Good Laboratory Practices. Since these methods are environmentally simplistic, actual product toxicity may be different depending on actual product use. This study did not address the effects of fish age, higher test temperatures, or dilution water characteristics on potential product toxicity. The environmental fate and bioavailability of the product are also not addressed in this study and would require additional testing.

2.0 METHODS AND MATERIALS

2.1 General

The procedures used in this study conformed to U.S. EPA guidelines for static acute testing (EPA/600/4-90/027F) and followed BMI's Standard Operating Procedures (SOP), presented in Appendix A. In general, 1 hour elapsed between test concentration preparation and organism exposure for the test. Standard procedures for assessing test water physiochemical parameters were followed. These included measurements of dissolved oxygen (YSI model 58), pH (Orion pH meter, model 720), conductivity (YSI Model 33) and temperature (calibrated thermometer) as well as initial determinations of diluent alkalinity and hardness (titrametric technique, Standard Methods, 16th ed.). All chemical monitoring equipment was calibrated daily prior to testing as per Standard Good Laboratory Practices and was recorded in a

special notebook for this purpose.

2.2 Dilution Water

The dilution water for this study was Blacksburg, Virginia Municipal tapwater dechlorinated (activated carbon filtration), deionized (Milli-Pore, Milli-Q UV Plus system), 0.2 micron filtered and reconstituted to a hardness of 80-100 mg/L (Ca as CaCO₃) as per U.S. EPA guidelines (BMI-MHRW). This is BMI's standard dilution water which is used to culture and maintain rainbow trout and other freshwater species. No total residual chlorine was detected in the dilution water by amperometric titration (detection limit = 0.01 mg/L).

2.3 Product Sample

The product was received February 21, 1995, in a 500 mL polyethylene container labeled and was stored at room temperature until used. The product was in liquid form, clear and viscous.

2.4 Test Organisms - *Oncorhynchus mykiss*

Rainbow Trout

Test fish were obtained from Mt. Lassen Trout Farms, California. Reference toxicant tests, using copper sulfate, were performed on this culture in order to confirm the health of these test organisms. The batch of fish used for the present study was MLT020795-1. The fish were 15 days old at the time of testing. Fish were maintained under a constant 16:8 light-dark photoperiod at 12°C ± 1°C and were fed Trout Chow and TetraMin brand food until the day of testing. No significant mortalities or abnormalities were observed with this batch of fish prior to this test.

2.5 Range Finding Toxicity Test

In order to establish toxicity levels to be used as a guideline in the Static Acute Trout Test, a Range Finding Toxicity Test was performed on product. The results of this Range Finding Test are in Appendix B. The definitive test concentrations were then chosen based on these results.

2.6 Test Method - Static Acute Toxicity Test

The methods used in the rainbow trout toxicity tests conformed to the recommended guidelines specified by the U.S. EPA for acute fish tests (EPA/600/4-90/027/F). The test was initiated on February 22, 1995.

The test consisted of five test concentrations and a control. For the test, concentrations were 0.0625, 0.125, 0.25, 0.5, and 1 mg/L. Twenty organisms were exposed to each concentration and the control; ten organisms in each of two replicate test chambers. 4.5 liter glass bowls were used as test chambers. The test solution volume was 4.0 liters and the test temperature was $12 \pm 1^\circ\text{C}$. Fish were not fed during the test. Temperature ($^\circ\text{C}$), pH, and dissolved oxygen (mg/L) were measured in one replicate of all concentrations daily (APHA et. al., 1988). Conductivity was measured at the beginning and end of the test. Alkalinity and hardness (mg/L as CaCO_3) of the diluent were measured at test initiation.

Dead organisms and those which did not respond to gentle prodding were recorded and removed from each chamber daily. Behavioral or anatomical aberrations (listlessness, sounding, sporadic movement, lesions, etc.) elicited by the fish were also recorded. The U.S. EPA computer program for analysis of acute toxicity test data was used to generate an LC50.

3.0 RESULTS

Oncorhynchus mykiss Static Acute Toxicity Test

The acute toxicity test data summary is presented in Table 1. Physiochemical parameters were within acceptable limits for the test conducted. Appendix C contains all the raw data sheets for the definitive test.

The 48 hour LC50 for the rainbow trout and was 0.319 mg/L. The long-term effects of this product can not be extrapolated from this study. Sub-acute or chronic toxicity testing would be necessary to evaluate this potential.

4.0 QUALITY ASSURANCE/QUALITY CONTROL

BMI maintains a comprehensive in-house Quality Assurance Plan that includes complete documentation of organism culture and maintenance, daily equipment calibration, a detailed SOP manual, and quarterly performance audits of laboratory personnel.

BMI performs monthly reference toxicant tests to ensure the health of in-house indicator test organisms and on every batch of organisms received from outside suppliers. The results of the reference test performed on the batch of fish used for this study indicated that the organisms were healthy (Appendix D).

5.0 LITERATURE CITED

APHA et al., 1985. Standard methods for the examination of water and wastewater. 16th edition. APHA. Washington, D.C.

Biological Monitoring, Inc., 1993. Quality Assurance/Quality Control manual. Biological Monitoring, Inc. Blacksburg, Virginia.

C. I. Weber, et al., 1991. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fourth edition. EPA/600/4-90/027F. Environmental Monitoring and Support Laboratory, U.S. EPA, Cincinnati, Ohio 45268.

U.S. Environmental Protection Agency. 1990. Good Laboratory Practices for Toxicology Studies, Final Riche. Federal register CFR 840, Washington, D.C.

TABLE 1
BIOLOGICAL MONITORING, INC.
Toxicity Test Condition Summary

Client: Henkel Corporation

Prepared by: Jennifer Maloney

VPDES Permit #: N/A

Experiment ID#: HEN022295-1

Test Organism: Oncorhynchus mykiss

Test Type: Static Acute

Organism Age at Start of Test: 15d

Sample Tested:

Sample Type: Compound

Sample Collection Frequency and Dates and Times: N/A

Sample Collector: N/A

Delivered by: Overnight Courier

Test Solution Renewal Frequency: N/A

Dilution Water Used: MHRW

Test Temperature: 12 ± 1°C

No. of Replicates per conc.: 2

No. of Organisms per Replicate: 10

Chamber Size: 4500 mL glass

Feeding prior to test: Normal

Feeding Regime: N/A

Test Volume: 4000 mL

Photo Period: 16h light/8h dark

Test Duration: 48h

Start of Test: Date: 02/22/95

Time: 1745

End of Test: Date: 02/24/95

Time: 1700

Equipment:

pH Meter: SA 720 (c)

DO Meter: YSI 58 (A)

SCT Meter: YSI 33 (c)

°C Measurement: Calibrated Thermometer

Salinity: SCT Meter

Chlorine: Fisher/Porter Amperometric Titrator

Test Method Reference: U.S. EPA. 1991. Methods for measuring the Acute toxicity of effluents and receiving waters to freshwater and marine organisms.

EPA/600/4-90/027.

TABLE 1 (cont.)

BIOLOGICAL MONITORING, INC.
Acute Toxicity Test Data Summary

Client	Henkel Corporation	VVOSS PERMIT #NA		
Test Organism	<u>Oncorhynchus mykiss</u>		Date	Time
Experiment ID	HEN022295-1	Start Test	02/22/95	1745
Sample Tested		End Test	02/24/95	1700

RESULTS

Water Chemistry Analyses							
(Range)							
Conc. mg/L	Temp. (°C)	D.O. (mg/l)	pH	Initial Alkalinity mg/l as CaCO ₃	Initial Hardness mg/l as CaCO ₃	Cond. (µmhos)	Survival (#) 48h
0	12 - 13	7.4 - 8.0	7.0 - 7.4	60	84	240 - 245	100
0.06	12 - 13	7.0 - 7.8	7.0 - 7.5			240 - 250	100
0.13	12 - 13	7.5 - 7.9	7.1 - 7.5			250 - 255	100
0.25	12 - 13	7.4 - 8.0	7.1 - 7.5			250	85
0.5	12 - 13	7.9 - 8.2	7.3 - 7.5			240 - 250	0
1	12 - 13	8.0 - 8.3	7.3 - 7.6			245 - 250	0

STATISTICAL ANALYSES

Test Method	LC50 (mg/L)	95% Fiducial (Confidence) Limits
Spearman	0.319	0.285 to 0.356

COMMENTS:

* = See stock preparation log sheet.

Appendix
A

**BIOLOGICAL MONITORING, INC.
STANDARD OPERATING PROCEDURES**

SOP Number: C.4

Title: Obtaining Fish From an Outside Supplier

Date: May 2, 1991

Procedures:

1. At least 24 hours (but preferably 1 week) before receiving fish, set up a clean holding tank with appropriate water (fresh, salt) with or without a filter system.
2. Monitor the presence of chlorine, temperature, and salinity to comply with supplier's recommendations for culturing. Use a chiller or heater, if necessary.
3. Aerate using airstones or filter system.
4. When the fish arrive, open the plastic bag and add an active airstone. After the water temperature in the bag reaches temperature of the holding tank, place bag containing fish and water into holding tank. Make a small opening in the bag to allow the fish to swim out on their own. (If this is impossible because of the size of the holding tank, gently transfer fish from bag to tank with a clean net.)
5. Enter fish batch number in the fish log file and fill out a Fish Culture Tank Log Sheet (figure 20) for the new holding tank.
6. Make frequent checks at first to make sure fish are not under stress. (Stress may be indicated by abnormal behavior in swimming and gill movements.) Check temperature.
7. If disease is evident or suspect by unusual fish appearance or movements, a prophylactic treatment using Maracyn may be in order. Follow directions for treatment included with Maracyn.
8. Feed appropriately as directed by supplier or testing guidelines.

BMI/SOP4.0

**BIOLOGICAL MONITORING, INC.
STANDARD OPERATING PROCEDURES**

SOP Number: G.1

Title: General Laboratory Acute Toxicity Testing Procedures
(Conforming to Weber et al., 1991; EPA/600/4-90/027)

Date: October 13, 1992; revised December 3, 1992; revised December 8, 1992

Procedures:

1. Prior to mixing toxicity test dilutions, measure and record dissolved oxygen (DO), pH, total residual chlorine, conductivity, salinity, and a visual description of the sample (effluent) or stock solution (compound or product). If the DO of an effluent sample is less than 4.0 mg/L for warm water species or 6.0 mg/L for cold water species, aerate the sample with single-bubble aeration until the DO is above the minimum required. Record the aeration rate and duration. Excessive aeration must be avoided. If the pH of an effluent sample is below 6.0 or above 9.0, or for a North Carolina test below 6.5 or above 8.5, the pH is generally adjusted to an acceptable pH with base or acid (See SOP A.16). If it is necessary to adjust the pH of a sample, two extra sets of controls are required as follows: (1) adjusted diluent with the amount of acid or base used to adjust the effluent pH; and, (2) unadjusted 100% effluent. The Laboratory Manager or Supervisor must always be consulted prior to adjusting a sample. The type, strength, and volume of acid or base added is recorded. For saltwater tests, artificial sea salts are added to the effluent sample if its salinity is less than the desired test salinity. If the sample contains chlorine, it may or may not be desirable to dechlorinate the sample prior to testing. The Laboratory Manager should be consulted. If it is determined that dechlorination is necessary, two extra sets of controls are required: (1) dilution water treated with a quantity of dechlorination agent equal to that used in the 100% effluent; and, (2) 100% effluent without dechlorination agent. For any test done for North Carolina, the dilution water must meet the following parameters: Hardness must be between 30 and 50 mg/L as CaCO₃ and the pH must be between 6.5 and 8.5.
2. If reconstituted (see SOP A.4.3) water is being used as a diluent, the total residual chlorine must be measured as nondetectable prior to using a given batch for testing. If synthetic seawater is used, be sure the salinity is that of the test solution (typically 20 ppt).
3. All containers, glassware, and materials coming into contact with the test solution are cleaned as per glassware washing SOP (A.2.1) and air dried. Each test container and other glassware is labeled as to the test concentration, replicate number, and test identifier or experiment ID number.
4. Appropriate data sheets are completed for all toxicity tests (see Figures following each SOP in Sections G and H of this document). These data sheets are completed as the test is being conducted. All test conditions, pertinent information, and comments are recorded on the data sheets.
5. For definitive tests at least 5 test concentrations and a control are tested. The proper range of concentrations depends on earlier test results, a range-finding test, or other information such as instream waste concentration (TWC). Ideally, the test concentration should produce extreme effects in the high concentrations (assuming the sample is toxic), moderate or partial effects in

BMI/SOP4.2

the intermediate concentrations, and no effects in the low concentrations. The minimum number of replicates for each test concentration is dependent upon the test species. Refer to the appropriate Test Summary Conditions Table for this information.

6. Appropriate glassware (Class A volumetric flasks, graduated cylinders, and pipets) is used for preparing the test concentrations with the total volume of the glassware used being not more than 2 times the volume being measured. The glassware used and volumes of effluent/stock solutions and diluent measured for each test concentration are recorded on the data sheet.
7. For static tests, prior to introducing organisms to the test solutions, and at test end, dissolved oxygen, pH, temperature, and conductivity (or salinity for saltwater testing) of one replicate of the control and all other concentrations are measured. Temperature and dissolved oxygen are measured in one replicate of all concentrations at least every 24 h for the test duration in fish tests. For invertebrates, dissolved oxygen, pH, and conductivity (or salinity for saltwater testing) are done in one replicate of all concentrations only at the beginning and end of the test (unless all organisms die in a concentration prior to test end), but temperature is measured every 24 hours in at least 1 test replicate from each concentration.

In the event the acute test is in the static renewal mode, water chemistry is performed two times on the days of renewal. The first water chemistry is performed on the freshly prepared solutions of the same concentrations before the organisms are exposed. This is the "after" renewal solution. After the organisms have been transferred to the fresh solutions, water chemistry is performed on the solutions that the organisms were exposed to during at least the previous 24 h period. This is the "before" renewal solution. Renewal is done \pm 2 hours from test start time.

If the initial DO of the high concentration is low or if it is suspected that the DO may drop, more frequent (once per hour) measurements should be made. If any parameter is outside the acceptable range for a given test, the Laboratory Manager or Supervisor should be notified. If the DO of any test solution falls below 4.0 mg/L for warm water species or 6.0 mg/L for cold water species, all test chambers must be provided single-bubble aeration (approx. 100 bubbles/min.) for the remainder of the test. Alkalinity and hardness are measured in the control and high concentration (typically 100% effluent) on each new effluent concentration.

8. Test chambers are placed in random order by using BMI's random number generating computer program or acceptable alternative. One-four organisms are sequentially placed in each container starting with the first assigned chamber and ending with the last assigned chamber. This is repeated until the appropriate number of test organisms are added to each container. (See individual SOPs for specifics.) When transferring organisms, it is important that they are released under the surface of the water and that the volume of culture water transferred with the organism is minimal (< 0.5 mL).
9. All containers are checked to make sure that each has the proper number of organisms and that the organisms were not harmed in the transfer process.
10. The number of live organisms in each test container is recorded every 24 hours for the test duration. Dead organisms are removed. General behavior of the organisms is recorded. Other data may be required depending on the test.

11. Tests are ended \pm 1 hour from test start time.
12. In a static renewal,
 - a) If the volume of water is not greater than 1 liter, a duplicate set of chambers is used and fresh solutions of the initial concentrations are prepared. All the organisms in one replicate are transferred to their fresh corresponding solution one by one with a wide bore pipet or a net. Each replicate is renewed completely before beginning to transfer organisms of another replicate to their fresh solution. Careful release of the replicate organism(s) is made under the surface of the water from the wide bore pipet. If the organism is accidentally injured or killed during transfer, an explanation is recorded on the logsheet.
 - b) If the volume in each replicate is greater than 1 liter, 90% of the original test solution is siphoned off using plastic tubing. This volume is replaced with freshly prepared solutions of the same concentration by gently pouring it into the chamber to avoid significant turbulence.
13. If there are significant mortalities, a second person counts the surviving organisms as a QC check and also initials the data sheet, leaving 2 sets of initials.
14. Acute toxicity tests will not be initiated with the first effluent sample used in a chronic test with the same test species.

**BIOLOGICAL MONITORING, INC.
STANDARD OPERATING PROCEDURES**

SOP Number: G.5.1

Title: Static Acute (24, 48 or 96 h) Trout Toxicity Tests (And other cold water species)
(Conforming to Weber et al., 1991; EPA/600/4-90/027)

Date: March 26, 1991; revised December 3, 1992

Procedures:

1. All fish used in a test must be 15-30 days for rainbow trout and 30-60 days old for brook trout at the test start date.
2. Trout are obtained from a commercial supplier who certifies their age and species (e.g. Mt. Lassen Trout Farm, Mt. Lassen, CA). The trout are held in BMI MHRW at $12 \pm 2^{\circ}\text{C}$ for a minimum of 5 days prior to the testing date. During this time the fish are fed twice daily and observed for any abnormalities. A group of organisms must not be used for a test if they appear to be diseased, discolored, or otherwise stressed, or if more than 5% die during the 48-hour period immediately preceding the test. The fish are not fed 48 hours prior to testing if the testing is not for NPDES purposes.
3. The test temperature is $12 \pm 1^{\circ}\text{C}$.
4. The test volume is based on the loading (weight) of organisms per liter of test solution. Loading in the test chambers must not exceed 1.1 g/L of test solution.
5. The test chamber size is dependent on the test volume, and fish loading. The chambers hold a minimum of 4 liters and are usually glass aquaria.
6. The dilution water used is BMI MHRW unless otherwise stated by the sponsor or regulatory agency.
7. Follow steps 1 through 15 in the General Laboratory Acute Toxicity Testing Procedures (G.1). Record results on the Acute Toxicity Test Data Sheet and Toxicity Test Procedure Sheet (Figure 31A, B).
8. Analyze the test data using the U.S. EPA computer program for analysis of acute toxicity test data to generate an LC50 (see SOP K.4).
9. Test acceptability criterion: 90% or greater survival in controls.

**Test Conditions for Acute Rainbow Trout (*Oncorhynchus mykiss*) and Brook Trout
 (*Salvelinus fontinalis*) Toxicity Tests**
*(Conforming to Weber et al., 1991; EPA/600/4-90/027
 and TSCA, CFR 797, 1400)*
Revised December 3, 1992

1.	Test type:	Static non-renewal, static-renewal, or flow-through
2.	Test duration:	24, 48, or 96 h
3.	Temperature:	12°C ± 1°C
4.	Light quality:	Ambient laboratory illumination
5.	Light intensity:	10-20 uE/m ² /s (50-100 ft-c) (ambient laboratory levels)
6.	Photoperiod:	16 h light, 8 h darkness.
7.	Test chamber size:	5 L (minimum) (test chambers should be covered to prevent fish from jumping out)
8.	Test solution volume:	4 L (minimum)
9.	Renewal of test solutions:	Minimum, after 48 h
10.	Age of test organisms:	Rainbow Trout: 15-30 days (after yolk sac absorption to 30 days) Brook Trout: 30-60 days
11.	No. organisms per test chamber:	Minimum, 10 for effluent and receiving water tests
12.	No. replicate chambers per concentration:	Minimum, 2 for effluent tests Minimum, 4 for receiving water tests
13.	No. organisms per concentration:	Minimum, 20 for effluent tests Minimum, 40 for receiving water tests
14.	Feeding regime:	Feeding not required
15.	Test chamber cleaning:	Cleaning not required

BMI/SOP4.2

16. **Test solution aeration:** None, unless DO concentration falls below 6.0 mg/L; rate should not exceed 100 bubbles/min
17. **Dilution water:** Moderately hard synthetic water prepared using deionized water and reagent grade chemicals or 20% DMW receiving water, or synthetic water modified to reflect receiving water hardness.
18. **Test concentrations:** Effluents: Minimum of five effluent concentrations and a control
Receiving Waters: 100% receiving water and a control
19. **Dilution series:** Effluents: ≥ 0.5 dilution series
Receiving Waters: None, or ≥ 0.5 dilution series
20. **Endpoint:** Effluents: Mortality (LC50 or NOAEC)
Receiving Waters: Mortality (Significant difference from control)
21. **Sampling and sample holding requirements:** Effluents and Receiving Waters: Grab or composite samples are used within 36 h of completion of the sampling period
22. **Sample volume required:** 20 L for effluents
40 L for receiving water
23. **Test acceptability criterion:** 90% or greater survival in controls
-

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APPENDIX
B

BIOLOGICAL MONITORING, INC.
Toxicity Test Procedure Check Sheet

Test I.D.N: KEN022195.1 Test containers used: 4 250 ml

Specify below no. milliliters (mls) of diluent and effluent measured out per concentration in this test:

No. of replicates per concentration: 1

Are all test chambers properly labelled? YES

Specify vessel type and volume used to measure and deliver effluent and diluent to test chambers:

graduated cylinder(s) 2000, 1000, 1000 pipet(s) 10, 10, 10
volumetric flask(s) _____ other _____

Specify material(s) used to place test organisms into test chambers: slide bars pipet

Total Chlorine of sample upon arrival (mg/L): N/A

Total Chlorine of sample after dechlorination (mg/L): N/A

Pretest Treatment for organisms: Normal

Exposure Chamber
Total vessel capacity: 4500 ml
Test solution volume: 4000 ml
Water Depth Constant: 1
cyclic: _____

Feeding Schedule
Pretest feeding: None
Not fed: ✓
Fed Daily: _____
Fed irregularly (describe): _____

Aeration
Pretest: None
None: _____
Slow: _____ (bubbles/min)
Moderate: _____
Vigorous: _____

Type of food: _____
Beginning: _____ (hour)

Conditions of surviving organisms at end of test: Normal

Methods of randomization employed: Random #'s Table

Comments: See stock preparation log sheet

Concentration % (mg/L) other	Diluent ml/RW	Effluent * ml	Total
0	4000	520	4000
0.1	3999.92	0.08	
1	3999.2	0.8	
10	3992	8	
100	3920	80	
1000	4000	800	✓

Screened Animal Enclosures
Not used: ✓
Used: _____
Photoperiod: ✓
8h/16h: _____
other: _____

BIOLOGICAL MONITORING, INC.
SUMMARY OF TEST STOCK SOLUTION PREPARATION

Client: Henkel

Test ID Nos. HEA 02295-1

STOCK A - MASTER STOCK SOLUTION

ID #: _____
Compound Type: unknown
Weight of Compound: 5g
Diluent Type: MHW
Volume of Diluent (units): 1000mL
Final Concentration: 5g/L
Prepared By: JKE
Date/Time: 2/21 / 1500

Substock A - ID # _____

Volume of Stock A: _____
Diluent Type: _____
Volume of Diluent: _____
Final Concentration: _____
Prepared By: _____
Date/Time: _____

Substock A - ID # _____

Volume of Stock A: _____
Diluent Type: _____
Volume of Diluent: _____
Final Concentration: _____
Prepared By: _____
Date/Time: _____

Substock A - ID # _____

Volume of Stock A: _____
Diluent Type: _____
Volume of Diluent: _____
Final Concentration: _____
Prepared By: _____
Date/Time: _____

Substock A - ID # _____

Volume of Stock A: _____
Diluent Type: _____
Volume of Diluent: _____
Final Concentration: _____
Prepared By: _____
Date/Time: _____

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Appendix C

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BIOLOGICAL MONITORING, INC.

Toxicity Test Procedure Check Sheet

Test I.D.#: HEV022295.1 Test containers used: 4.5L glass Specify below no. milliliters (mls)
 No. of replicates per concentration: 2 of diluent and effluent measured out per concentration in this test:

Are all test chambers properly labelled? YES
 Specify vessel type and volume used to measure and deliver effluent and diluent to test chambers:
 graduated cylinder(s) 200 mL, 5.0, 25 pipet(s) 10, 1 mL
 volumetric flask(s) _____ other _____

Specify material(s) used to place test organisms into test chambers: Wide bore pipet
 Total Chlorine of sample upon arrival (mg/L): N/A

Total Chlorine of sample after dechlorination (mg/L): N/A

Exposure Chamber Feeding Schedule
 Total vessel capacity: 4.5L Not fed:
 Test solution volume: 4L Fed daily: _____
 Water Depth Constant: Fed irregularly (describe) _____
 cyclic: _____ Vigorous: _____ " _____
 Beginning: _____ (hour)

Condition of surviving organisms at end of test: NORMAL

Concentration % (mg/L) Other	Diluent	Effluent STOCK	Total
0	4000 mL	100 mg/L	4000 mL
0.0625	3999.75	3.5	
0.125	3995	5	
0.250	3990	10	
0.500	3980	20	
1.00	3960	40	

Aeration None: Slow: _____ (bubbles/min) Moderate: _____ Vigorous: _____ " _____
 Screened Animal Enclosures Not used: Used: _____
 Photoperiod: 8h/16h: other: _____

Comments: RANDOM #'S TABLE USED
& see stock preparation log sheet.

BIOLOGICAL MONITORING, INC.
SUMMARY OF TEST STOCK SOLUTION PREPARATION

Client: HENKEL

Test ID Nos. HEN022295.1

STOCK A - MASTER STOCK SOLUTION

ID #: A
Compound Type: _____
Weight of Compound: 100mg
Diluent Type: MARLW
Volume of Diluent (units): 999.9 ml
Final Concentration: 100mg/L
Prepared By: JPE
Date/Time: 02/22/95 ~ 1600

Substock A - ID # _____

Volume of Stock A: _____
Diluent Type: _____
Volume of Diluent: _____
Final Concentration: _____
Prepared By: _____
Date/Time: _____

Substock A - ID # _____

Volume of Stock A: _____
Diluent Type: _____
Volume of Diluent: _____
Final Concentration: _____
Prepared By: _____
Date/Time: _____

Substock A - ID # _____

Volume of Stock A: _____
Diluent Type: _____
Volume of Diluent: _____
Final Concentration: _____
Prepared By: _____
Date/Time: _____

Substock A - ID # _____

Volume of Stock A: _____
Diluent Type: _____
Volume of Diluent: _____
Final Concentration: _____
Prepared By: _____
Date/Time: _____

CT-TOX: BINOMIAL, MOVING AVERAGE, PROBIT, AND SPEARMAN METHODS

SPEARMAN-KARBER

TRIM: .00%
 LC50: .319
 95% LOWER CONFIDENCE: .285
 95% UPPER CONFIDENCE: .356

CONC. mg/L	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (%)
.06	20.	0.	.00	.9537D-04
.13	20.	0.	.00	.9537D-04
.25	20.	3.	15.00	.1288D+00
.50	20.	20.	100.00	.9537D-04
1.00	20.	20.	100.00	.9537D-04

THE BINOMIAL TEST SHOWS THAT .25 AND .50 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS 99.8711 PERCENT.
 AN APPROXIMATE LC50 FOR THIS DATA SET IS .319

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

DATE: 2/22/95 TEST NUMBER: HEN-1 DURATION: 48 h
 SAMPLE: Aquaquest SPECIES: Om

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
BINOMIAL	.319	.250	.500	.250
MAA	*****	*****	*****	*****
PROBIT	*****	*****	*****	*****
SPEARMAN	.319	.285	.356	.071

**** = LIMIT DOES NOT EXIST

BEST COPY AVAILABLE

Appendix
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BIOLOGICAL MONITORING, INC.
TOXICITY TEST DATA SHEET

Experiment I.D. #: MLT 022495-1 NPDES Permit #: NA Client: BMI Ref to X Outfall/Station No. NA
 Effluent/Sample: Sea in C-504 Sample Container: LL glass Project Scientist: SEA QC Officer: HE
 Sample Type: Compound Test Mode: Static Acute Test Duration: 48h
 Grab: _____ Test Start Date: 022495 Time: 1715
 Collection Date: _____ Test End Date: 022695 Time: 1615
 Composite: _____ Batch #: MLT 020795-1
 Collected from: Date: _____ Time: _____ Age: 17d
 Collected to: Date: _____ Time: _____ No. of organisms/conc.: 20
 Dilution Water Used: MHW 022195-DT Temp. of Org. Stock Solution: 12°

Conc. %	Time	Number of Live Organisms						Dissolved Oxygen (mg/L)						pH						Alkalinity						Hardness						Conductivity (umhos)						Temperature (°C)					
		0	24	48	72	96		0	24	48	72	96		0	24	48	72	96		0	24	48	72	96		0	24	48	72	96		0	24	48	72	96							
0	A	10	10	10			8.3	6.8	7.2			7.5	7.1	7.1			60						84					240					13	12	13								
12.5	A	10	10	10			8.5	6.8	7.0			7.6	7.1	6.9														240					13	12	13								
25	A	10	10	10			8.3	6.3	6.7			7.6	7.1	6.9									240					240					13	12	13								
50	A	10	10	7			8.3	6.3	6.7			7.6	7.1	6.9									240					235					13	12	13								
100	A	10	10	7			8.3	6.2	6.4			7.5	7.0	6.8									245					240					13	12	17								
200	A	10	6	3			8.3	6.5	7.4			7.4	7.1	7.0									240					245					13	12	13								
	B	10	7	5																																							

BIOLOGICAL MONITORING, INC.

Toxicity Test Procedure Check Sheet

Test I.D.#: MLT 022495-1 Test containers used: 4500 mL glassbottle Specify below no. milliliters (mls)

No. of replicates per concentration: 2 of diluent and effluent measured out per concentration in this test:

Are all test chambers properly labelled? yes
 Specify vessel type and volume used to measure and deliver effluent and diluent to test chambers:
 graduated cylinder(s) 2000 mL pipet(s) 10 mL
 volumetric flask(s) _____ other _____
 Specify material(s) used to place test organisms into test chambers: small mesh net
 Total Chlorine of sample upon arrival (mg/L): NA

Concentration mg/L	Diluent	Effluent	Total
0 or 5/L	HTLRW	0 mL	4000 mL
12.5	4000 mL	0.5	4000
25	3999.5	1	4000
50	3998	2	4000
100	3996	4	4000
200	3992	8	4000

Total Chlorine of sample after dechlorination (mg/L): NA

Exposure Chamber Feeding Schedule Aeration Screened Animal Enclosures

Total vessel capacity: 4500 mL Not fed: None: Not used:
 Test solution volume: 4000 mL Fed daily: _____ Slow: _____ (bubbles/min) Used: _____
 Water Depth Constant: Fed irregularly (describe) _____ Moderate: _____ " Photoperiod:
 cyclic: _____ Vigorous: _____ " 8h/16h: _____
 Beginning: _____ (hour) other: _____

Condition of surviving organisms at end of test: Normal

Comments: * See stock preparation log sheet.

BIOLOGICAL MONITORING, INC.
SUMMARY OF TEST STOCK SOLUTION PREPARATION

Client: BMI Ref tox

Test ID Nos. MLT 022495-1

STOCK A - MASTER STOCK SOLUTION

ID #: Cu COI
Compound Type: CuSO₄ · 5H₂O
Weight of Compound: Stock 100mg/L Cu previously mixed up
Diluent Type: MHRW
Volume of Diluent (units): _____
Final Concentration: 100mg/L Cu
Prepared By: Don Mackler
Date/Time: 2/8/95 - preserved.

Substock A - ID # _____

Volume of Stock A: _____

Diluent Type: _____

Volume of Diluent: _____

Final Concentration: _____

Prepared By: _____

Date/Time: _____

Substock A - ID # _____

Volume of Stock A: _____

Diluent Type: _____

Volume of Diluent: _____

Final Concentration: _____

Prepared By: _____

Date/Time: _____

Substock A - ID # _____

Volume of Stock A: _____

Diluent Type: _____

Volume of Diluent: _____

Final Concentration: _____

Prepared By: _____

Date/Time: _____

Substock A - ID # _____

Volume of Stock A: _____

Diluent Type: _____

Volume of Diluent: _____

Final Concentration: _____

Prepared By: _____

Date/Time: _____

CT-TOX: BINOMIAL, MOVING AVERAGE, PROBIT, AND SPEARMAN METHODS

SPEARMAN-KARBER

TRIM: 40.00%
 LC50: 158.740
 95% LOWER CONFIDENCE: 109.367
 95% UPPER CONFIDENCE: 230.402

CONC. ug/L	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (%)
12.50	20.	0.	.00	.9537D-04
25.00	20.	0.	.00	.9537D-04
50.00	20.	5.	25.00	.2069D+01
100.00	20.	6.	30.00	.5766D+01
200.00	20.	12.	60.00	.2517D+02

THE BINOMIAL TEST SHOWS THAT 50.00 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS 97.9305 PERCENT.
 AN APPROXIMATE LC50 FOR THIS DATA SET IS 159.220

RESULTS USING MOVING AVERAGE

SPAN	G	LC50	95% CONFIDENCE LIMIT
1	1.102	159.22	68.59 + INFINITY

***** RESULTS CALCULATED BY PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT
5	.184	1.00	.36

SLOPE = 2.29

95% CONFIDENCE LIMITS: 1.31 AND 3.27

LC50= 148.90

95% CONFIDENCE LIMITS: 106.79 AND 262.36

LC1 = 14.33

95% CONFIDENCE LIMITS: 3.43 AND 26.39

DATE: 2/24/95
 SAMPLE: Cu

TEST NUMBER: MLT-1
 SPECIES: Om

DURATION: 48 h

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
BINOMIAL	159.220	50.000	*****	*****
MAA	159.220	68.593	*****	*****
PROBIT	148.902	106.794	262.358	155.563
SPEARMAN	158.740	109.367	230.402	121.034

**** = LIMIT DOES NOT EXIST

Triage of 8(e) Submissions

Date sent to triage: MAR 15 1996

NON-CAP

CAP

Submission number: 13380A

TSCA Inventory:

Y

N

D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO

AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX

SBTOX

SEN

w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX

CTOX

EPI

RTOX

GTOX

STOX/ONCO

CTOX/ONCO

IMMUNO

CYTO

NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY

For Contractor Use Only

entire document: **0** 1 2 pages 1 pages _____

Notes:

Contractor reviewer : JW Date: 1/24/96

CECATS/TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA
 Submission # REQD: 0395-13380 ⁵ SEQ. A

TYPE INT SUPP FLWP
 SUBMITTER NAME: Henkel Corporation

DEPOSIT#
 REFER TO CHEMICAL SCREENING
 678 CAP NOTICE

INFORMATION REQUESTED: FLWP DATE
 6591 NO INFO REQUESTED
 6592 INFO REQUESTED (TECI)
 6593 INFO REQUESTED (VOL ACTIONS)
 6594 INFO REQUESTED (REPORTING RATIONALE)
 DEPOSIT#

03/21/95 OTH DATE 03/27/95 CSRAD DATE: 04/03/95

CASE
68002-96-0

VOLUNTARY ACTIONS
 6401 NO ACTION REPORTED
 6402 STUDIES PLANNED (IN HUMAN)
 6403 INTERACTION WITH MATERIALS
 6404 LABELS/MSDS (HUMAN)
 6405 PROFESSIONAL INFO (HUMAN)
 6406 APP USE DISCONTINUED
 6407 PRODUCTION DISCONTINUED
 6408 CONFIDENTIAL

CHEMICAL NAME

INFORMATION TITLE	L.F.C.	INFORMATION TITLE	P.F.C.	INFORMATION TITLE	P.F.C.
0201 ONCO (HUMAN)	01 02 04	0216 EPICLIN	01 02 04	0201 BAKLINO (ANIMAL)	01 02 04
0202 ONCO (ANIMAL)	01 02 04	0217 HUMAN EXPOS (PROD CONTAM)	01 02 04	0202 BAKLINO (HUMAN)	01 02 04
0203 CELL TRANS (IN VITRO)	01 02 04	0218 HUMAN EXPOS (ACCIDENTAL)	01 02 04	0203 CHEMOPHYS PROF	01 02 04
0204 MUTA (IN VITRO)	01 02 04	0219 HUMAN EXPOS (MONITORING)	01 02 04	0204 CLASTO (IN VITRO)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	0220 BOOJAQUA TOX	01 02 04	0205 CLASTO (ANIMAL)	01 02 04
0206 REPROTERATO (HUMAN)	01 02 04	0221 ENV. OCCUREL/FATE	01 02 04	0206 CLASTO (HUMAN)	01 02 04
0207 REPROTERATO (ANIMAL)	01 02 04	0222 EMER INC OF ENV CONTAM	01 02 04	0207 DNA DAMAGE/REPAIR	01 02 04
0208 NEURO (HUMAN)	01 02 04	0223 RESPONSE REQUEST DELAY	01 02 04	0208 PRODUSE/PROC	01 02 04
0209 NEURO (ANIMAL)	01 02 04	0224 PRODCOMP/CHEM ID	01 02 04	0209 MSDS	01 02 04
0210 ACUTE TOX. (HUMAN)	01 02 04	0225 REPORTING RATIONALE	01 02 04	0210 OTHER	01 02 04
0211 CHR. TOX. (HUMAN)	01 02 04	0226 CONFIDENTIAL	01 02 04		
0212 ACUTE TOX. (ANIMAL)	01 02 04	0227 ALLERG (HUMAN)	01 02 04		
0213 SUB ACUTE TOX (ANIMAL)	01 02 04	0228 ALLERG (ANIMAL)	01 02 04		
0214 SUB CHRONIC TOX (ANIMAL)	01 02 04	0229 METAPHARMACOD (ANIMAL)	01 02 04		
0215 CHRONIC TOX (ANIMAL)	01 02 04	0240 METAPHARMACOD (HUMAN)	01 02 04		

USE: paper industry PRODUCTION: dosage rate = .75/ton p4p or L.04%

TOXICOLOGICAL CONCERN

LOW
 MED
 HIGH

SPECIES

Fish

ONGOING REVIEW

YES (DROP/REFER)
 NO (CONTINUE)
 BEST

NON-CELL INVENTORY

YES
 NO

CAS SR

IN HUMAN

Non-Cap