8EHQ-0395-13372

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TSCA §8(e) Notification

(CONFIDENTIAL)

Dear Sir or Madam:

This report is being submitted in accordance with the provisions of TSCA §8(e). has completed aquatic toxicity testing on an alkyl biphenyl, (CONFIDENTIAL) . (CONFIDENTIAL) The trade name for is

(CONFIDENTIAL) is manufactured at:

(CONFIDENTIAL)

The aquatic toxicity study, "Chronic Toxicity of (CONFIDENTIAL) to Ceriodaphnia dubia", was performed by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO. The results from the study show lethality and reproductive effects. The EC_{50} for reproductive impairment could not be determined in the chronic study because the lowest concentration tested (25 μg/L) caused a significant decrease in measures of reproductive performance compared to untreated and vehicle-exposed controls. Please see the enclosed toxicological report for the supporting study data.

We are unable to judge the extent of risk involved.

This report has been compiled based on the information available as of the date of filing. We do not yet know how credible the test and the reported data are. Accordingly, the company, its agents and employees reserve the right to supplement and to revise and amend the data contained in this report and any conclusions drawn therefrom.

Confidential handling is requested for this submission. A non-confidential version of this information is attached for the public file

Respectfully submitted,

8EHQ-95-13372
86950000161

(CONFIDENTIAL)
Corporate TSCA Coordinator
(CONFIDENTIAL)
(CONFIDENTIAL)

nm
3/27/95

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STUDY TITLE

Chronic Toxicity of to *Ceriodaphnia dubia*

DATA REQUIREMENT

U.S. EPA-TSCA 40 CFR, Part 792
Guideline 797.1300

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AUTHOR

Dorothy E. England, M.S.
Biologist II/Study Director

STUDY COMPLETED ON

March 7, 1995

PERFORMING LABORATORY

ABC Laboratories, Inc.
Environmental Toxicology Division
7200 East ABC Lane
Columbia, Missouri 65202

ABC LABORATORY PROJECT ID

Final Report #42201

STUDY COMPLIANCE STATEMENT

Study Compliance Statement for ABC Laboratories' Final Report #42201, entitled "Chronic Toxicity of to *Ceriodaphnia dubia*," for

ABC's study director herein confirms that the study was conducted in compliance with the TSCA Good Laboratory Practice Standards (40 CFR, Part 792).

All original raw data were provided to along with the final report and a copy was retained by ABC Laboratories, Inc.

Dorothy C. England 3/7/95

Dorothy C. England, M.S. Date
ABC Laboratories' Study Director

QUALITY ASSURANCE STATEMENT

ABC Laboratories' Quality Assurance Unit reviewed Study #42201, "Chronic Toxicity of to *Ceriodaphnia dubia*," for The following inspections/audits were conducted on this study.

Date of Inspection	Phase Inspected	Date Reported to Study Director	Date Reported To Management
11-16-94	Weigh Out of Test Compound	11-16-94	01-23-95
12-13-94	Day 7 Biological Observations	12-13-94	01-23-95
01-16-95	Draft Report and Raw Data	01-16-95	01-23-95
03-06-95	Final Report	03-06-95	03-06-95

The undersigned conducted the draft and final report audits. These audits indicate the report is an accurate reflection of the study as it was conducted by ABC Laboratories, Inc.

Kimberly S. Frazier 03/06/95

Kimberly S. Frazier
Quality Assurance Officer I

Date

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SUMMARY/INTRODUCTION

Subject: "Chronic Toxicity of to *Ceriodaphnia dubia*", ABC Laboratories' Report Number 42201.

Sponsor:

Testing Facility:

ABC Laboratories, Inc.
Environmental Toxicology Division
7200 E. ABC Lane
Columbia, Missouri 65202

Location of Original Raw Data and Final Report:

Test Material:

Nominal Test Concentrations: Control, DMF Vehicle Blank, 25, 50, 100, 200, and 400 μg/L

Dilution Water: Hardness 150-158 mg/L as $CaCO_3$, Alkalinity 160-172 mg/L as $CaCO_3$, pH 8.4-8.6, and Conductivity 300-320 μmhos/cm

Experimental Test Dates: Initiation - December 6, 1994
Termination - December 13, 1994

Length of Study: 7-Days

Results: The 7-day LC_{50} value was calculated to be 117 μg/L (95% C.I. = 50-200 μg/L).

Mortality	Reproduction
7-Day NOEC = 50 μg/L	7-Day NOEC = <25 μg/L
7-Day LOEC = 100 μg/L	7-Day LOEC = 25 μg/L
7-Day CV = 70.7 μg/L	7-Day CV = could not be determined

Test Species: *Ceriodaphnia dubia* neonates (<24-hours old)

Source of Organisms: ABC Laboratories in-house culture

METHODS AND MATERIALS

The static *Ceriodaphnia dubia* bioassay was conducted following the procedures outlined in ABC Laboratories' protocol 8606. The protocol was signed by a representative of on October 7, 1994, and by ABC's study director on November 1, 1994. The test methods used are basically those described in *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (1), *Standard Methods for the Examination of Water and Wastewater* (2), and *Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians* (3).

I. Test Compound

The was received from on August 26, 1994, as a clear liquid. This sample was assigned ABC reference number TS-7604. A 4.0-mg/mL primary stock solution and secondary stock solutions were prepared in dimethyl formamide (DMF) on November 29, 1994. Secondary stocks were prepared by dilution of the primary stock. Test solutions were prepared on days 0-6 of the definitive test from aliquots of the primary and secondary stocks.

II. Test Organisms

Test specimens of *Ceriodaphnia dubia* were obtained from an in-house daphnid culture at ABC Laboratories. The primary culture was obtained from Aquatic Biosystems, Inc., on July 27, 1990, and was maintained "in-house" at ABC Laboratories. The organisms were identified to species by the supplier as *Ceriodaphnia dubia*.

All daphnids were cultured and tested in a temperature controlled area at 25 ± 2°C. The lighting was maintained at 50-70 footcandles on a 16-hour daylight photoperiod, with 30 minute dawn and dusk transition periods. The daphnid cultures were maintained in 1-L glass containers. Daphnids were cultured in hard blended water (Table I). Hard blended water is a combination of well water and reverse-osmosis water blended to a hardness of 160-180 mg/L as $CaCO_3$. During the holding period, the daphnids were fed daily with a suspension of at least one algae species cultured at ABC Laboratories: *Selenastrum capricornutum* and/or *Ankistrodesmus falcatus*. Along with the algae, the daphnids were fed a supplement consisting of trout chow, supplied by Zeigler Brothers, Gardners, PA, and Fleischmann's active dry yeast. Only first-instar daphnids (<24 hours old) were selected for testing. To provide test daphnids which were <24 hours old, the adult daphnids were transferred and isolated the previous day.

Neonates for the definitive test were provided by ABC culture CD-76 on December 6, 1994. The adults from this culture had been cultured for 7 days and were considered acceptable with no signs of stress, disease, or physical damage.

Since the culturing and testing parameters of temperature, dilution water and lighting were the same, no acclimation period was necessary.

III. Range-Finding Tests

A 48-hour preliminary exposure was conducted from November 2 to November 4, 1994, at concentrations of 0.010, 0.050, 0.10, 0.50, and 1.0 mg/L, based on results of previous testing with *Daphnia magna*. Control and vehicle blank (0.10 mL/L) chambers were included. Definitive test concentrations of 6.25, 12.5, 25.0, 50.0, and 100 μg/L were selected based on this preliminary exposure. However, an initial attempt at the definitive test, conducted in polystyrene test vessels, failed due to absence of mortality at the highest test concentrations. The 48-hour range-finding test was repeated at the initial concentrations, comparing glass and polystyrene test chambers. Based on the results of this test, definitive test concentrations were changed to 25, 50, 100, 200, and 400 μg/L.

IV. Test Method

The static *Ceriodaphnia dubia* bioassay was conducted in 50-mL glass beakers containing 30 mL of test solution. Two sets of test chambers were used, with fresh solutions added to alternating sets on renewal days (days 1-6). Ten beakers were prepared at each test concentration; 10 hard blended water control chambers and 10 vehicle blank chambers (0.10 mL/L DMF) were also prepared. The test chambers were randomized in styrofoam test boards held in a temperature-controlled waterbath at 25 $\pm$ 1°C. Lighting was maintained at 66-70 footcandles on a 16-hour light/8 hour dark photoperiod with 30-minute simulated dawn and dusk transition periods.

Test solutions were prepared daily in 1-L volumetrics. Test solution was added to labeled test cups and brought to test temperature in a 25°C waterbath. The remaining test solution was transferred to 1-L beakers which were also placed in the 25°C waterbath. This solution was used for water chemistry measurements of "new" solutions on the day of preparation and on the following day for water chemistry measurements of "old" solutions.

A. Biological

One *Ceriodaphnia dubia* (first instar less than 24 hours old) was added to each replicate. Daphnids were added impartially to test chambers as soon as test solutions reached test temperature. Daphnids were added (day 0) and transferred from old to new solutions (days 1-6) using a smooth-tipped plastic disposable pipet. Food was added to each test chamber prior to addition/transfer of the test organisms. All cups with new solution received 0.1 mL of algae (3.1×10^7 cells/mL) plus 0.1 mL of yeast cereal leaves/fish food (YCT, 1.7 mg/mL) per day, for a final concentration of

approximately 1.0×10^6 algal cells/mL and 0.01 mg/mL YCT suspended solids per cup.

All test chambers were observed daily for production of instar, mortality, and other abnormal effects such as surfacing, paleness, small size, daphnids trailing extraneous material, quiescence, and daphnids tending to the bottom of the test chambers. The number of neonates produced was recorded daily beginning with the first day of reproduction.

B. Chemical and Physical

Water chemistry measurements of temperature, dissolved oxygen (DO), conductivity, alkalinity, hardness, and pH were performed daily in all concentrations having live test organisms. Measurements were made on all test levels and controls of the "new" replacement solutions. Temperature, DO, and pH were measured in the 24-hour old test solutions.

V. Statistics

Statistical analysis of the *Ceriodaphnia dubia* survival data was performed using a computerized LC_{50}/EC_{50} program developed by Stephan et al. (4,5). This program calculated the LC_{50}/EC_{50} statistic and its 95-percent confidence limits using the binomial, moving average, and probit tests, if data permitted. Three different methods of analyzing the data were used since no one method of analysis is appropriate for all possible sets of data that may be obtained. The method of calculation selected for presentation in this report was that which gave the narrowest confidence limits for the LC_{50}/EC_{50} (3, 4) although all three models are valid.

The NOEC (no-observed effect concentration) and LOEC (lowest-observed effect concentration) endpoints were determined using SAS (6). The control and vehicle blank treatments were compared using chi-square and Fisher's exact test to determine suitability for pooling. Levene's test for homogeneity of variance was used to evaluate the necessity of data transformation using arcsine/square root transformation. A one-way analysis of variance (ANOVA) was performed to determine if statistical differences existed between mean survival values among the treatment levels, followed by a Dunnett's test to compare mean survival values of each concentration with that of the mean control values. Reproduction data, including mean young per adult (MYA), days to first brood (DFB), and young per adult reproduction day (YAD), were analyzed in a similar manner, except that no transformation of data was performed.

DISCUSSION AND RESULTS

A preliminary exposure of *Ceriodaphnia dubia* to at nominal concentrations of 0.010, 0.050, 0.10, 0.50, and 1.0 mg/L gave complete mortality after 48 hours at the three highest test levels, with a single mortality at 0.050 mg/L and no mortality at 0.010 mg/L or in the control or vehicle blank. An initial definitive test at concentrations of 6.25 to 100 μg/L failed due to insufficient mortality in the highest test concentrations. When the initial range-finding test was repeated comparing different types of test vessels, complete mortality was observed at 0.50 and 1.0 mg/L in both polystyrene and glass, no mortality or adverse effects were observed at 0.10 mg/L or below in polystyrene, and one mortality and two affected organisms were observed at 0.10 mg/L in the glass chambers. No mortality or adverse effects were observed in controls or vehicle blanks. A second definitive was attempted on November 29, 1994, at concentrations of 25-400 μg/L, but failed due to unexplained control mortality (40%).

The definitive 7-day chronic static renewal exposure of *Ceriodaphnia dubia* to was conducted from December 6, 1994, to December 13, 1994. All results were based on the nominal concentrations of 25, 50, 100, 200, and 400 μg/L. Table II summarizes survival during the 7-day chronic survival and reproduction test. Controls and vehicle blanks, with 100% survival, were not statistically different and were pooled for comparison with treatments. Mortality was 30% in the 100 μg/L treatment and 100% in the 200 and 400 μg/L treatments, with no mortality in the 25 and 50 μg/L treatments.

Reproduction for the 7-day exposure is summarized in Table III. Controls and vehicle blanks, which were not statistically different with respect to MYA, DFB, AND YAD, were pooled for comparison with treatments. Although mean reproductive values are reported for the 100 μg/L treatment, this level was excluded from statistical analyses on the basis of significantly reduced survival. The MYA was 35 $\pm$ 6 for pooled controls, with a mean of 28, 20, and 4 young per adult for 25, 50, and 100 μg/L treatments, respectively. Days to first brood was 3.2 $\pm$ 0.4 and young per adult reproduction day was 7.3 $\pm$ 1.2 for pooled controls. The 50 μg/L treatment was statistically lower than pooled controls in DFB. For the MYA and YAD analyses, both the 25 and 50 μg/L treatments were statistically lower than pooled controls.

Table IV summarizes the water quality parameters measured during the test. Temperature was 23-25°C for new solutions and 25-26°C for old solutions. Dissolved oxygen concentrations ranged between 7.4 and 8.5 mg/L for the new solutions and 6.5 to 7.9 mg/L for the old solutions. The values for the test solutions represented approximately 85-101% saturation at 23-26°C (corrected for local altitudinal pressure at ABC Labs) and were considered adequate for testing. The pH values ranged from 8.3 to 8.6 for the new solutions and 8.1 to 8.8 for the old solutions. Table V summarizes ranges in alkalinity, hardness, and conductivity values for new test solutions. Hardness ranged from 144 to 162 mg/L and alkalinity from 152 to 180 mg/L, as $CaCO_3$. Conductivity ranged from 300 to 340 μmhos/cm.

CONCLUSIONS

The NOEC (no-observed-effect concentration) for survival was established at 50 μg/L. The LOEC (lowest-observed effect concentration) for survival was 100 μg/L. The geometric mean

of these values or chronic value (CV) (equivalent to a maximum acceptable toxicant concentration or MATC), was calculated at 70.7 μg/L. The 7-day LC_{50} was calculated to be 117 μg/L (95% CI = 50-200 μg/L).

Although statistically reduced survival was observed at 50 μg/L, survival does not appear to be the most sensitive indicator of toxicant stress for *Ceriodaphnia dubia* exposed to
A significant reduction in number of young per adult reproduction days was observed at 25 μg/L, the lowest concentration tested. Therefore, an NOEC for reproduction was not obtained, but could be stated to be less than 25 μg/L. The LOEC was 25 μg/L. A chronic value for reproduction could not be calculated.

TABLE I

Chemical Characteristics of Hard Blended Water Used by ABC Laboratories' Environmental Toxicology Division

Dilution Water Screen[a]		Monthly Screens (mg/L)[b]	
Hardness	150-158 mg/L (as $CaCO_3$)	Total Organic Carbon	<1.0
Alkalinity	160-172 mg/L (as $CaCO_3$)	Suspended Solids	0.6
pH	8.4-8.6		
Conductivity	300-320 μmhos/cm		

Biyearly Screens[c]

Elements (mg/L)		Chlorinated Hydrocarbons (μg/L)	
Aluminum	<0.20	DDE	<0.01
Arsenic	<0.010	DDD	<0.01
Boron	0.273	DDT	<0.01
Cadmium	<0.0005	Dieldrin	<0.01
Chromium	<0.003	Endrin	<0.01
Cobalt	<0.050	α-BHC	<0.01
Copper	<0.025	β-BHC	<0.01
Fluoride	0.6	γ-BHC-Lindane	<0.01
Iron	<0.10	Δ-BHC	<0.01
Lead	<0.003	Heptachlor	<0.01
Mercury	<0.0002	Heptachlor Epoxide	<0.01
Nickel	<0.050	Aldrin	<0.01
Selenium	<0.005	Chlordane	<0.3
Silver	<0.0005	Methoxychlor	<0.05
Zinc	<0.025	Toxaphene	<4.0
		Endosulfan Sulfate	<0.03
		Endosulfan I	<0.01
		Endosulfan II	<0.01
		HCB	<0.01
		Mirex	<0.01
		Telodrin	<0.01
		PCBs	<1.0

Organophosphate (μg/L)		Miscellaneous (mg/L)	
Diazinon	<0.10	Chemical Oxygen Demand	<7.0
Methyl Parathion	<0.02	Un-Ionized Ammonia	<0.05
Ethyl Parathion	<0.02	Nitrite N	<0.02
Malathion	<0.05	Nitrate N	0.19
Ronnel	<0.01	Total Phosphorus as P	<0.05
Ethion	<0.02	Chlorine Residual	ND
Trithion	<0.05	Ammonia Nitrogen	<0.05
Vapona	<0.05		

[a] Represents the values measured on dilution water used for the test
[b] Represents the values obtained from the screens of December 1994
[c] Represents the values obtained from the screen of July 1994
ND = None Detected
Note: Results of the chemical analyses supporting these data are on file at ABC Laboratories.

TABLE II

Summary of Survival of Brood *Ceriodaphnia dubia*
During the 7-Day Exposure to

Nominal Test Concentration (μg/L)	Number of Live Brood *Ceriodaphnia dubia*								% Survival Per Conc.
	Study Days								
	0	1	2	3	4	5	6	7	
Control	10	10	10	10	10	10	10	10	100
Vehicle Blank	10	10	10	10	10	10	10	10	100
Pooled Controls	10	10	10	10	10	10	10	10	100
25	10	10	10	10	10	10	10	10	100
50	10	10	10	10	10	10	10	10	100
100	10	10	10	10	9	8	7	7	70[a]
200	10	10	5	4	3	0	0	0	0[a]
400	10	5	0	0	0	0	0	0	0[a]

[a] Significantly different from pooled controls by Dunnett's one-tailed test ($\alpha = 0.05$)

TABLE III

Summary of Reproduction for *Ceriodaphnia dubia*
During the 7-Day Exposure to

Nominal Test Conc. (μg/L)	Number of Young Produced per Concentration								Total # of Young	Mean # of Young Produced Per Adult	Days to First Brood	Young per Adult Reproduction Day
	Study Day											
	0	1	2	3	4	5	6	7				
Control	0	0	0	37	46	103	118	44	348	35±7	3.2±0.4	7.3±1.4
Vehicle Blank	0	0	0	38	38	106	115	60	357	36±6	3.1±0.3	7.3±1.1
Pooled Controls	-	-	-	-	-	-	-	-	--	35±6[a]	3.2±0.4[a]	7.3±1.2[a]
25	0	0	0	32	15	86	108	39	280	28±6[b]	3.2±0.4	5.9±1.4[b]
50	0	0	0	6	35	69	91	0	201	20±8[b]	4.0±0.7[b]	5.6±0.7[b]
100	0	0	0	19	1	18	0	0	38	4±2[c]	3.4±0.7[c]	1.1±0.4[c]
200	0	0	0	0	0	0	0	0	0	0	--	--
400	0	0	0	0	0	0	0	0	0	0	--	--

[a] Mean of two sets of controls
[b] Significantly different from pooled controls by Dunnett's one-tailed test
[c] Excluded from analysis or not considered on the basis of survival effects

TABLE IV

Ranges for Water Quality Measurements During the 7-Day Exposure of *Ceriodaphnia dubia* to

Nominal Test Conc. (μg/L)	New Solutions[a]			Old Solutions[a]		
	Temp[b] (°C)	D.O.[c] (mg/L)	pH[d]	Temp.[b] (°C)	D.O.[c] (mg/L)	pH[d]
Control	23-25	8.1-8.4	8.4-8.5	25-26	7.5-7.9	8.5-8.6
Vehicle Blank	23-25	8.1-8.4	8.4-8.6	25-26	6.7-7.6	8.2-8.7
25	23-25	8.0-8.5	8.4-8.6	25-26	6.7-7.7	8.1-8.8
50	23-25	8.0-8.4	8.4-8.6	25-26	6.7-7.8	8.1-8.8
100	23-25	7.4-8.4	8.4-8.6	25-26	6.5-7.5	8.2-8.6
200	23-25	8.0-8.4	8.3-8.6	25	7.3-7.7	8.5-8.7
400	23	8.1-8.3	8.3-8.5	25	6.9-7.4	8.6

[a] Water quality measurements for the new solutions were made on freshly prepared test solutions at each renewal. Water quality measurements for the old solutions were made on 24-hour old test solutions.
[b] Temperature (°C) - Monitored using a mercury thermometer.
[c] Dissolved Oxygen Concentrations - YSI Model 54 ARC Dissolved Oxygen Meter, ABC Material # 1905-730. Dissolved oxygen concentrations at the test temperatures of 23-26°C are 8.2-7.8 mg/L, respectively (corrected for altitudinal pressure at ABC Labs).
[d] pH - Beckman Φ34 pH Meter, ABC Material #163-640 with a Model 39841 probe.

TABLE V

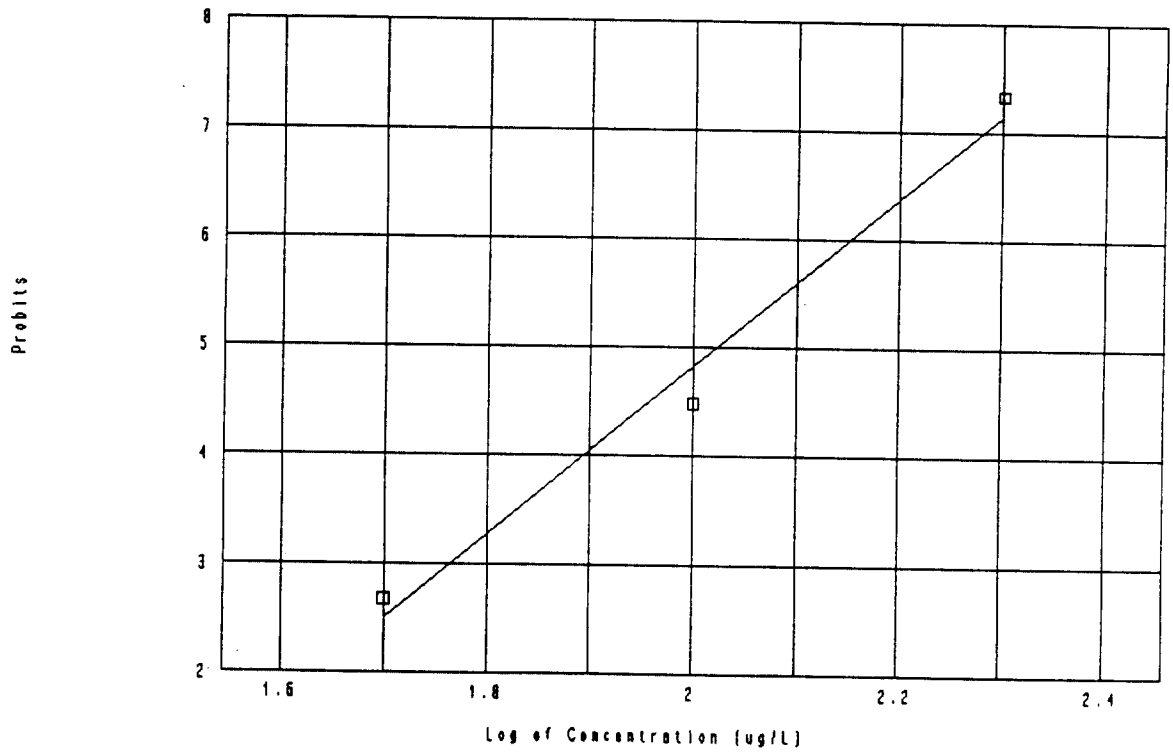
Alkalinity, Hardness, and Conductivity Measurements During the 7-Day Exposure of *Ceriodaphnia dubia* to

Nominal Test Conc. (μg/L)	Hardness[a] (mg/L $CaCO_3$)	Alkalinity[a] (mg/L $CaCO_3$)	Conductivity[b] (μmhos/cm)
Control	150-160	164-178	300-340
Vehicle Blank	154-160	160-172	300-340
25	156-160	164-178	300-340
50	144-160	152-178	300-340
100	150-160	170-180	300-340
200	154-162	158-180	300-340
400	148-154	168-170	300-320

[a] Determined by titrimetric procedures adapted from APHA Standard Methods
[b] Measured using a YSI Model 33 salinity/conductivity/temperature meter

FIGURE 1

The 7-Day Concentration-Response Slope for *Ceriodaphnia dubia* Exposed to



Slope = 7.74

REFERENCES

(1) Weber, C.I. and others. 1989. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, 2nd ed. U.S. Environmental Protection Agency, EPA/600/4-89/001, March 1989.

(2) American Public Health Association. 1980. *Standard Methods for the Examination of Water and Wastewater*. 15th ed. Washington, DC. 1134 p.

(3) Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. *Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians*. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April 1975. 61 p.

(4) Stephan, C. E., K. A. Busch, R. Smith, J. Burke and R. W. Andrews. 1978. A Computer Program for Calculating an EC_{50}. U. S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August, 1978.

(5) Stephan, C. E. 1977. Methods for Calculating an EC_{50}, p. 65-84. In F. L. Mayer and J. L. Hamelink (eds.). Aquatic Toxicology and Hazard Evaluation. ASTM Special Technical Publication 634. ASTM. Philadelphia.

(6) PC DOS SAS/STAT Release 6.04 copyright 1985, 1987, 1988 by SAS Institute Inc., Cary, NC 27512-8000, USA.

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Note: Some of the records which appear in this raw data appendix have been provided as exact copies of original records on file at ABC Laboratories. This has been done by necessity for certain data which are used commonly in several studies at ABC Laboratories. Such records may include analytical standard solution preparation sheets, organism culture logs and hard blended water chemical screen results.

The following symbols may appear on the raw data pages included in this report.

C = Error in calculations
E = Entry error
S = Spelling error
W = Writeover
R = Recording error
F = Form change
T = Transcription error
D = Date error

PROJECT PERSONNEL

This is a listing of the personnel who participated in various phases of this study. For official signatures of these individuals, please refer to the following pages.

Name	Title
Dorothy England, M.S.	Study Director/Research Scientist
Janelle Downing	Associate Scientist
Christina Gardner	Technical Scientist
Jane Bowman	Scientist
Marc Sword	Scientist
Ryan Warbritton	Associate Scientist
Tranà Houf	Assistant Technician
Brett Geger	Technical Scientist

ENVIRONMENTAL TOXICOLOGY PERSONNEL SIGNATURE & INITIAL IDENTIFICATION

NAME	SIGNATURE	INITIAL	DATE
D. Abram	David Abram	DA	8·16·94
Amy Adams	Amy Adams	AA	8·17-94
William J. Adams	William J. Adams	WJA	8-19-94
Jane H. Bowman	Jane Bowman	JHB	8-16-94
John Bucksath	John Bucksath	JDB	8/16/94
David Burgess	David Burgess	DB	8-16-94
James B. Bussard	Jim Bussard	JBB	8-16-94
Paul Cohle	Paul Cohle	PC	8-16-94
Janelle L. Downing	Janelle L. Downing	JLD	8-16-94
Dorothy C. England	Dorothy C. England	DCE	8/16/94
Alan D. Forbis	Alan D. Forbis	AF	8/22/94
Christina Gardner	Christina Gardner	CG	8-16-94
Brett Geger	Brett Geger	BEG	8-17-94
Doug Gledhill	Doug Gledhill	DG	8-16-94
Edward Harper	Edward Harper	EDH	8/17/94
Stephen L. Hicks	Stephen L. Hicks	SLH	8-22-94
Donna S. Hoek	Donna S. Hoek	DSH	8/17/94
Bret Hurshman	Bret A. Hurshman	BAH	8-17-94
John Ingersoll	[illegible]	JI	8·23·94
Charles E. Jameson	Charles E. Jameson	CEJ	8-17-94
Debbie Jameson	Debbie Jameson	dlj	8/16/94
Anita M. Klick	Anita M. Klick	AMK	8/16/94
Kathryn Konering	Kathryn Konering	KK	8-17-94

Note: This list includes all personnel of the Environmental Toxicology division and is not study specific. Primary project personnel will be identified separately for each project.

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ENVIRONMENTAL TOXICOLOGY PERSONNEL SIGNATURE & INITIAL IDENTIFICATION

NAME	SIGNATURE	INITIAL	DATE
Tom Leak	Tom Leak	TL	8-16-94
Jianping Liu	Jianping Liu	JL	8/16/94
Timothy J. Madsen	Timothy J. Madsen	TJM	08-17-94
Karen March	Karen March	KM	8-19-94
William A. McAllister	William A. McAllister	WAM	8-17-94
Michelle A. Muckerman	Michelle A. Muckerman	MM	8/16/94
Hugh Murrell	Hugh Murrell	HM	8-17-94
Gerald A. Nothdurft	Gerald A Nothdurft	GAN	8-23-1994
Robert G. Pezold	Robert G Pezold	RGP	8-17-94
Christopher J. Pope	Christopher J Pope	CJP	8/16/94
Jon E. Rhodes	Jon Rhodes	JR	8-17-94
Scot Stewart	Scot Stewart	SS	8-23-94
Luke Stuerman	Luke Stuerman	LMS	8/17/94
Marc Sword	Marc Sword	MCS	8-16-94
Jamie L. Veltri	Jamie L Veltri	JLV	8/17/94
Ryan Warbritton	Ryan Warbritton	RW	8-17-94
John Wilkes	John Wilkes	JDW	8-17-94
Yuan Yang	Yuan Yang	YY	8-16-94
Rick Northup	Rick Northup	RCN	9-14-94
Robert Gurley	RA Gurley	RSG	9-28-94
Trana Houf	Trana Houf	TNH	10-5-94
Kayne O'Brien	Kayne O'Brien	KOB	10-5-94

Note: This list includes all personnel of the Environmental Toxicology division and is not study specific. Primary project personnel will be identified separately for each project.

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APPENDIX I - RAW DATA

A. Test Material

COMPOUND RECEIPT

Lab Form No. 352

ANALYTICAL BIO-CHEMISTRY LABORATORIES, INC.
P.O. Box 1097 • Columbia, MO 65205
Shipping Address: 7200 East ABC Lane, Columbia, MO 65202
(314) 474-8579 • Answer Back (ABCLAB UD)
FAX (314) 443-9033

Compound _____

ABC SUPPLIED INFORMATION

Date Received: 08/26/94 Logged In By: RMF ABC Ref # TS-7604

Storage: Room Temp Total Weight: 676.7g

Physical Description: Clear liquid

Remarks: _____

SUPPLIER PROVIDED INFORMATION

Firm: _____

Address: _____

City/State/Zip _____ Phone: _____

Batch/Lot No. 940519+01 CAS # 81846-81-3 Purity: N/G

Amount Declared: 1 pt. Expiration Date: N/G

Storage Instructions N/G

Other _____

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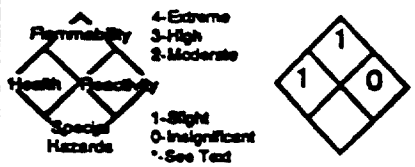
By Brett Cegan date 12-15-94

IRR (EYE)

replacement sheets

MATERIAL SAFETY DATA SHEET

24-HO ... ASSISTANCE	GENERAL ASSISTANCE	NFPA FIRE HAZARD SYMBOL*
CHEMTREC Assistance:		
MSDS Number > 5125		

MANUFACTURER/SUPPLIER:
ADDRESS:

TRADE NAME:

CAS NUMBER:
NONYM(S):
CHEMICAL FAMILY: HYDROCARBON MIXTURE
MOLECULAR FORMULA: NA
MOLECULAR WEIGHT: ND
PRODUCT CODE: 6022 **HIERARCHY:**

HEALTH

CAUTION!

ASPIRATION HAZARD IF SWALLOWED-CAN ENTER LUNGS AND CAUSE DAMAGE
OVEREXPOSURE MAY CAUSE CNS DEPRESSION
MAY BE IRRITATING TO THE SKIN, EYES AND RESPIRATORY TRACT

FLAMMABILITY

CAUTION!

COMBUSTIBLE

REACTIVITY

STABLE

ABC 42201 PG 00027

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This reprinted material is not the complete and official position of the NFPA on the referenced subject, which is represented

BEST COPY AVA

INGESTION:

PRACTICALLY NON-TOXIC. May cause irritation of the mouth, throat and gastrointestinal tract. Symptoms may include salivation, pain, nausea, vomiting and diarrhea. Aspiration into lungs may cause chemical pneumonia and lung damage.

Exposure may also cause central nervous system symptoms similar to those listed under "Inhalation" (see Inhalation section).

Overexposure to this material may cause systemic damage including target organ effects listed under "Special Toxic Effects."

SKIN:

MODERATELY IRRITATING. Repeated or prolonged skin contact may cause drying, reddening, itching and cracking.

PRACTICALLY NON-TOXIC. No significant effects are expected to occur following short term exposure. Repeated or prolonged contact with large amounts of this material may result i absorption through the skin to produce toxic effects.

EYE:

MODERATELY IRRITATING. Exposure to vapors, fumes or mists may cause irritation. Direct contact may cause irritation, redness, tearing and blurred vision. Prolonged or repeated exposure may cause irritation and conjunctivitis.

INHALATION:

SLIGHTLY TOXIC. Breathing of the mists, vapors or fumes may irritate the nose, throat a lungs. Symptoms may include sore throat, coughing, labored breathing, sneezing and burning sensation, depending on the concentration and duration of exposure.

May cause central nervous system depression or effects. Symptoms may include headache, excitation, euphoria, dizziness, incoordination, drowsiness, light-headedness, blurred vision, fatigue, tremors, convulsions, loss of consciousness, coma, respiratory arrest an death, depending on the concentration and duration of exposure.

SPECIAL TOXIC EFFECTS:

Acute or chronic overexposure to this material or its components may cause systemic toxicity, including adverse effects to the following: central nervous system and liver.

This product was not mutagenic in the Ames Assay . This product did not damage chromosomes using the CHO Chromosomal Aberration Assay.

This product was non-sensitizing to the skin using the Buehler Guinea Pig Assay.

Pre-existing medical conditions which may be aggravated by exposure include disorders of the skin and respiratory system .

FIRST AID

INGESTION:

Do not induce vomiting because of danger of aspirating liquid into lungs, causing seriou damage and chemical pneumonitis. If spontaneous vomiting occurs keep head below hips to prevent aspiration and monitor for breathing difficulty. Keep affected person warm and rest. GET IMMEDIATE MEDICAL ATTENTION.

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SKIN CONTACT:

Immediately wash skin with plenty of soap and water while removing contaminated clothing and shoes. GET IMMEDIATE MEDICAL ATTENTION.

Place contaminated clothing in closed container for storage until laundered or discarded. If clothing is to be laundered, inform person performing operation of contaminant's hazardous properties. Discard contaminated leather goods.

EYE CONTACT:

Flush immediately with large amounts of water for at least 15 minutes. Eyelids should be held away from the eyeball to ensure thorough rinsing. GET IMMEDIATE MEDICAL ATTENTION.

INHALATION:

Remove to fresh air. If not breathing, institute cardiopulmonary resuscitation (CPR). If breathing is difficult, ensure clear airway and give oxygen. GET IMMEDIATE MEDICAL ATTENTION.

Gastric lavage may be indicated if ingested. If spontaneous vomiting has occurred after ingestion, the patient should be monitored for difficult breathing, as adverse effects of aspiration into the lungs may be delayed up to 48 hours.

EYE PROTECTION:

Wear chemical safety goggles and face shield. Have eye washing facilities readily available where eye contact can occur.

SKIN PROTECTION:

Avoid skin contact with this material. Use appropriate chemical protective gloves when handling.

Use good personal hygiene.

RESPIRATORY PROTECTION:

Ventilation and other forms of engineering controls are the preferred means for controlling exposures.

A NIOSH/MSHA approved air purifying respirator with an organic vapor cartridge or canister may be permissible under certain circumstances where airborne concentrations are expected to exceed exposure limits. Protection provided by air purifying respirators is limited. Use a positive pressure air supplied respirator if there is any potential for an uncontrolled release, exposure levels are not known, or any other circumstances where air purifying respirators may not provide adequate protection.

BOILING POINT:	570 F (290 C)
SPECIFIC GRAVITY:	0.973
MELTING POINT:	-55 F (-48 C)
VOLATILE:	ND
VAPOR PRESSURE:	0.74 TORR @ 200 F (93 C)

ND = No Data
NA = Not Applicable

ABC 42201 PS 00029

EVAPORATION RATE (WATER=1): ND
VAPOR DENSITY (AIR=1): ND
VISCOSITY: 7.2 CST @ 100 F (38 C)
% SOLUBILITY IN WATER: ND
OCTANOL/WATER PARTITION COEFFICIENT: ND
POUR POINT: -55 F (-48 C)
pH: ESSENTIALLY NEUTRAL
APPEARANCE/ODOR: CLEAR, COLORLESS LIQUID WITH A MODERATE, NONOFFENSIVE AROMATIC ODOR

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FLASH POINT: 300 F (149 C) CLEVELAND OPEN CUP
AUTOIGNITION TEMPERATURE: 865 F (463 C)
FLAMMABILITY LIMITS IN AIR (% BY VOL.) LOWER: ND
FLAMMABILITY LIMITS IN AIR (% BY VOL.) UPPER: ND

BASIC FIREFIGHTING PROCEDURES:
Use water spray, dry chemical, alcohol foam, all purpose AFFF or carbon dioxide to extinguish fire. Evacuate area and fight fire from a safe distance.

If leak or spill has not ignited, ventilate area and use water spray to disperse gas or vapor and to protect personnel attempting to stop a leak.

Use water spray to cool adjacent structures and to protect personnel.

Firefighters must wear MSHA/NIOSH approved positive pressure breathing apparatus (SCBA) with full face mask and full protective equipment.

UNUSUAL FIRE AND EXPLOSION HAZARDS:
Vapors may form explosive mixture with air. Vapors can travel to a source of ignition an flash back.

Explosion hazard if exposed to extreme heat or to physical or thermal shock.

Fires involving this product may release carbon monoxide, carbon dioxide, reactive hydrocarbons and irritating vapors.

STABILITY/INCOMPATIBILTY:
Incompatible with oxidizing agents. See precautions under Handling/Storage.

HAZARDOUS REACTIONS/DECOMPOSITION PRODUCTS:
Combustion may produce CO, NOx, SOx and reactive hydrocarbons.

SPILL OR RELEASE TO THE ENVIRONMENT:
If product is released to the environment, take immediate steps to stop and contain release. Caution should be exercised regarding personnel safety and exposure to the released product. Notify local authorities and the National Response Center, if require

ND = No Data
NA = Not Applicable

ABC 42281 P6 00030

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Emergency Action:

Keep unnecessary people away; isolate hazard area and deny entry. Stay upwind. (See ersonal Protection Information Section.)

Spill or Leak Procedure:

Keep ignition sources out of area and shut off all ignition sources. Absorb spill with inert material (e. g. dry sand or earth) then place in a chemical waste container. Large Spills: Dike far ahead of liquid spill for later disposal. Shut off leak if safe to do so.

See Personal Protection Information section.

Notification:

This material, as supplied, contains no hazardous substances regulated under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302), or any extremely hazardous substances regulated under the Superfund Amendments and Reauthorization Act (SARA) (40 CFR 355), and thus a release of this material as supplied has no reporting requirements under these regulations. There may be other specific reporting requirements at the local, regional or state level pertaining to releases of this material.

Failure to report may result in substantial civil and criminal penalties.

WASTE DISPOSAL:

The transportation, storage, treatment and disposal of this waste material must be conducted in compliance with 40 CFR 262, 263, 264, 268 and 270. Disposal can occur only in properly permitted facilities. Check state and local regulations for any additional requirements as these may be more restrictive than federal laws and regulations. Chemical additions, processing or otherwise altering this material may make the waste management nformation presented in this MSDS incomplete,inaccurate or otherwise inappropriate. isposal of this material must be conducted in compliance with all federal, state and ocal regulations.

SARA TITLE III INFORMATION:

Listed below are the hazard categories for the Superfund Amendments and Reauthorization Act (SARA) Section 311/312 (40 CFR 370):

Immediate Hazard: X Delayed Hazard: X Fire Hazard: __ Pressure Hazard: __ Reactivity Hazard: __

This product does not contain toxic chemicals (in excess of the applicable de minimis concentration) that are subject to the annual toxic chemical release reporting requirements of the Superfund Amendments and Reauthorization Act (SARA) Section 313 (40 CFR 372).

ADDITIONAL ENVIRONMENTAL REGULATORY INFORMATION:

There may be specific regulations at the local, regional or state level that pertain to this material.

ND = No Data
NA = Not Applicable

ABC 42201 P6 00031

Based on available information this material does not contain any components or chemicals currently known to the State of California to cause cancer, birth defects or reproductive harm at levels which would be subject to Proposition 65. Reformulation, use or processing of this material may affect its composition and require re-evaluation.

All major components of this product are listed on the TSCA Inventory.

HANDLING/STORAGE:
Store in tightly closed containers in a cool, dry, isolated, well-ventilated area away from heat, sources of ignition and incompatibles. Avoid contact with strong oxidizers. Use non-sparking tools. Do not eat, drink or smoke in areas of use or storage.

EMPTY CONTAINERS:
Do not cut, grind, drill, weld or reuse containers unless adequate precautions are taken against these hazards. Empty containers may contain product residue. Do not reuse without adequate precautions.

D.O.T. PROPER SHIPPING NAME (49 CFR 172.101):	NON REGULATED
D.O.T. HAZARD CLASS (49 CFR 172.101):	NA
UN/NA CODE (49 CFR 172.101):	NA
PACKING GROUP (49 CFR 172.101):	NA
BILL OF LADING DESCRIPTION (49 CFR 172.202):	Alkyl Biphenyl
D.O.T. LABELS REQUIRED (49 CFR 172.101):	NA
D.O.T. PLACARDS REQUIRED (49 CFR 172.504):	NA

COMPONENT	CAS NO.	%	EXPOSURE LIMITS - REF.
		100	ND

REVISION DATE: 15-APR-1994 REPLACES SHEET DATED: 07-JAN-1994
COMPLETED BY:

NOTICE: The information presented herein is based on data considered to be accurate as of the date of preparation of this Material Safety Data Sheet. However, no warranty or representation, express or implied, is made as to the accuracy or completeness of the foregoing data and safety information, nor is any authorization given or implied to practice any patented invention without a license. In addition, no responsibility can be assumed by vendor for any damage or injury resulting from abnormal use, from any failure to adhere to recommended practices, or from any hazards inherent in the nature of the product.

ND = No Data
NA = Not Applicable

B. Preliminary Testing

ABC LABORATORIES, INC.

DAPHNID ACUTE TOXICITY BIOASSAY – IMMOBILIZATION

☐Definitive ☒Preliminary ☐Screen - ☐Flow-Through ☒Static

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Test Species: [ ] *Daphnia magna* [X] Ceriodaphnia dubia (Lot # CD 67-① 0407) Waterbath #: Effluent
Dilution Water: [X] Hard Blended [ ] _____ # Instar/Vessel: 5
Date Initiated: 11-2-94 Time: 1:45pm By: Brett Geger Date Terminated: 11-4-94

Test Conc. (mg/L)	Immobility and Behavioral Observations					
	0-Hour		24-Hour		48-Hour	
	Imm.	Obs.	Imm.	Obs.	Imm.	Obs.
Control	0	① ~~10~~ 5N, CLR	0	5N, CLR	0	5N, CLR
v Blank	0	5N, CLR	0	5N, CLR	0	5N, CLR
0.01	0	5N, CLR	0	5N, CLR	0	5N, CLR
0.05	0	5N, CLR	0	5N, CLR	1	4N, CLR
0.1	0	5N, CLR	2	3N, CLR	5	—
0.5	0	5N, CLR	5	—	5	—
1.0	0	5N, CLR	5	—	5	—
ID/Date/Time	2:30pm/11-2-94/BEG		2:00pm/11-3-94/BEG		9:00am/11-4-94/BEG	
Anal. ID Date/Time						

Comments: N = Normal, SUR = Surfacing, C = Clumping, CT = Coated with Extraneous Material, ERR = Erratic Movement, TR = Trailing Extraneous Material, Q = Quiescent, OB = On Bottom, P = Precipitate, CLR = Clear, CLD = Cloudy/Colored Solution, SF = Surface Film
① EBEG 11-2-94

Reviewed by: Dorothy England Date: 11/21/94

Study Director: Dorothy England Date: 11/21/94

FORM AQ106 (3/25/93)

ABC LABORATORIES, INC.

Form 27b (03/11/94)

COMPOUND PREPARATIONS

Test Material: _____ Protocol No.: 86060

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 9405194-01 Purity N/C % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: Brett Geger Date: 11-2-94

Target Weight 0.100 g

Final Gr. Weight 0.100 g

Tare Weight 0.000 g

Net Weight 0.100 g

Adj. Net Weight ~~0.100~~ ② g*

*Corrected for purity of primary standard

Dilution Volume 10 mL of DMF

Concentration 10 mg/mL (lot # ~~E2-67-MT~~ BE-440 ②)

Balance calibrated with Class C weights:

0.200 g (Class C) + 0.0000 g ① (tare) = 0.200 g (final wt)

Balance Used: Mettler ~~AE~~ PM③ 4600 ABC Material Control #: 1905-1020

PREPARATION OF WORKING STANDARD

Transferred by: ③ ~~Francisco~~ Brett Geger Date: 11-2-94

Conc. of Parent Sol. mg/mL	Aliquot Volume mL	Dilution Volume (L)	Dilution Carrier	Final Concentration mg/mL
10	0.01	0.100	HB Water	0.001

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Geger Date: 11-2-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc.* (mg/L)
Control	HB Water	50	0.050	Control 0
Vehicle Blank	DMF	0.0~~10~~05②	0.050	~~0.1~~③ 0
1	0.001	0.5	0.050	0.010
2	0.001	2.5	0.050	0.050
3	0.001	5.0	0.050	0.10
4	0.001	25	0.050	0.50
5	0.001	50	0.050	1.0
6				
7				

☐ * Aliquot volume was stirred into the dilution volume with a glass or stainless steel stirrer

Comments: ① DW BEG 11-2-94 ② C BEG 11-2-94 ③ E BEG 11-2-94

Prepared by: Brett Geger Date: 11-2-94

Calculations checked by: Dorothy England Date: 11/2/94

Reviewed by: Dorothy England Date: 11/2/94

ABC LABORATORIES, INC.

INVERTEBRATE CULTURE RECORD

Species: *Ceriodaphnia dubia* Lot #: CD-67-AQT

Date Initiated: 10-19-94 # Used to Initiate: 30

Lot # Initiated from: CD-61-AQT Culture Volume: ~1000 mL

Food Lot # 94-A50 S50 A51 S51 A52 S52 A53 S53, 94-A55 S55 ①

Date/ID	Temp.* °C	Changed Over	Fed	No. Adult Live	~Neonates Present	Comments
11-2-94 KJ	25	3:00pm	✓	25	150	
11-3 KJ	25	—	✓	—	—	
11-4 KJ	25	1:00pm	✓	23	200	
11-5 CJP	25	—	✓	—	—	
11-6 CJP	25	—	✓	—	—	
11-7 KJ	25	3:00pm	✓	23	350	
11-8 KJ	25	—	✓	—	—	
11-9 CJP	25	—	✓	—	—	
11-10 KJ	25	3:45pm	✓	23	300	
11-11 TH	25	—	✓	—	—	
11-12-CJP	25	—	✓	—	—	
11-13-94 PC	25	—	✓	—	—	① Added. PC 11-13-94
11-14 TH	25	11:25 A.M.	✓	23	>250	
11-15 TH	25	10:30 A.M.	✓	23	<20	Just beginning to crop

* Temperature measured with a mercury thermometer

Note: Cultures may be fed one or more of the following:
- ~0.25-0.5 mL of a 5.0 mg/mL suspension of trout chow yeast
- ~1.0 mL suspension of dried algae (spirulina and/or chlorella)
- ~1.0-3.0 mL of a 1.0 * 10^8 cells/mL concentration of live algae (The algae consists of Selenastrum capricornutum, Ankistrodesmus falcatus, *Chlamydomonas reinhardtii* and/or *Chlorella pyrenoidosa*.)

"This is an exact copy of The original document"

By [signature] date 1/4/95

Prepared by: [signature] Date: 11-2-94

Reviewed by: [signature] Date: 11-28-94

FORM # 396b (04/18/94)

ABC LABORATORIES, INC.

INVERTEBRATE CULTURE RECORD

Species: *Ceriodaphnia dubia* Lot #: CD-67-AQT

Date Initiated: 10-19-94 # Used to Initiate: 30

Lot # Initiated from: CD-61-AQT Culture Volume: ~1000 mL

Food Lot # 94-A47 S47 A48 S48 A49 S49 A50 S50

Date/ID	Temp.* °C	Changed Over	Fed	No. Adult Live	~Neonates Present	Comments
10-19-94 RJ	25	2:45 pm	✓	—	30+	will thin to 30 during 1st changeover
10-20 TMM	25	—	✓	—	—	
10-21 TH	25	11:45 a.m.	✓	30	—	
10-22 CJP	25	—	✓	—	—	
10-23 SLH	25	—	✓	—	—	
10-24 RJ	25	3:45 pm	✓	27	150	
10-25 RJ	25	—	✓	—	—	
10-26 RJ	25	2:00 pm	✓	27	200	
10-27 TH	25	—	✓	—	—	
10-28 RJ	25	9:45 am	✓	27	250	
10-29 RJ	25	—	✓	—	—	
10-30 RJ	25	—	✓	—	—	
10-31 RJ	25	4:00 pm	✓	27	300	
11-1 RJ	25	4:30 pm	✓	27	—	Removed 40 for study 42246

"This is an exact copy of The original document"
By D. England date 1/4/95

* Temperature measured with a mercury thermometer

Removed ~40 for study # 42201 11-2-94 BEG

Note: Cultures may be fed one or more of the following:
- ~0.25-0.5 mL of a 5.0 mg/mL suspension of trout chow yeast
- ~1.0 mL suspension of dried algae (spirulina and/or chlorella)
- ~1.0-3.0 mL of a 1.0 * 10^8 cells/mL concentration of live algae (The algae consists of Selenastrum capricornutum, Ankistrodesmus falcatus, *Chlamydomonas reinhardtii* and/or *Chlorella pyrenoidosa*.)

Prepared by: [signature] Date: 10-19-94

Reviewed by: Trina Haub Date: 11-2-94

FORM # 396b (04/18/94)

ABC LABORATORIES, INC.

FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # CD-71-AQT)

Date Initiated: 11-16-94 Vessel Size: 30 mL Test Vol.: 20 mL Temperature: 25 ± 1 °C Test Conc.: Control = 0 µg/L

Day	11-16-94 0 BEG		11-17-94 1 BEG		11-18-94 2 BEG		11-19-94 3 BEG		11-20-94 4 BEG		5		6		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	—	1N	1D	—												
B	—	1N	1N	—	1N	—	1N	5N	1N	7N						
C	—	1N	1N	—	1N	5N	1N	—	1N	8N						
D	—	1N	1N	—	1N	—	1N	5N	1N	4N						
E	—	1N	1N	—	1N	4N	1N	—	1N	3N						
F	—	1N	1N	—	1N	—	1N	3N	1N	~~5~~ ① —						
G	—	1N	1N	—	1N	—	1N	6N	1N	7N						
H	—	1N	1N	—	1N	—	1N	6N	1N	5N						
I	—	1N	1N	—	1N	—	1N	5N	1N	9N						
J	—	1N	1N	—	1N	—	1N	7N	1N	8N						

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead

① E BEG 11-20-94

Reviewed By: Janelle Drury Date: 12-13-94

ABC 42201 PG 00038

ABC LABORATORIES, INC.

FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # CD-11 AQ1)

Date Initiated: 11-16-94 Vessel Size: 30 mL Test Vol.: 20 mL Temperature: 25 ± 1 °C Test Conc.: Vehicle Blank (0.1 mL/L DMF) = 0µg/L

Day	11-16-94 0 BEG		11-17-94 1 BEG		11-18-94 2 BEG		11-19-94 3 BEG		11-20-94 4 BEG		5		6		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	—	1N	1N	—	1N	—	1N	—	1N	—						
B	—	1N	1N	—	1N	—	1N	4N	1N	—						
C	—	1N	1N	—	1N	—	1N	—	1N	5N						
D	—	1N	1N	—	1N	—	1N	2N	1N	—						
E	—	1N	1N	—	1N	—	1N	3N	1N	9N						
F	—	1N	1N	—	1N	—	1N	—	1N	6N						
G	—	1N	1N	—	1N	—	1N	3N	1N	7N						
H	—	1N	1N	—	1N	—	1N	—	1N	—						
I	—	1N	1N	—	1N	—	1N	3N	1N	—						
J	—	1N	1N	—	1N	—	1N	5N	1N	7N						

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead

Reviewed By: Janeece Downey Date: 12-13-94

ABC 42201 P5 00039

ABC LABORATORIES, INC.

FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot #CD-71-AQ1)

Date Initiated: 11-16-94 Vessel Size: 30 mL Test Vol.: 20 mL Temperature: 25 ± 1 °C Test Conc.: Level 1 = 6.25 µg/L

Day	11-16-94 0 BEG		11-17-94 1 BEG		11-18-94 2 BEG		11-19-94 3 BEG		11-20-94 4 BEG		5		6		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	–	1N	1N	–	1N	–	1N	–	1N	4N						
B	–	1N	1N	–	1N	–	1N	3N	1N	4N						
C	–	1N	1N	–	1N	–	1N	–	1N	–						
D	–	1N	1N	–	1N	–	1N	–	1N	4N						
E	–	1N	1N	–	1N	–	1N	5N	1N	–						
F	–	1N	1N	–	1N	–	1N	3N	1N	–						
G	–	1N	1N	–	1N	–	1N	2N	1N	–						
H	–	1N	1N	–	1N	–	1N	3N	1N	–						
I	–	1N	1N	–	1N	–	1N	2N	1N	–						
J	–	1N	1N	–	1N	–	1N	4N	1N	5N						

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead

Reviewed By: Janelle Young Date: 12-13-94

ABC 42201 35 00040

ABC LABORATORIES, INC.

FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot #CD 71-AGT)

Date Initiated: 11-16-94 Vessel Size: 30 mL Test Vol.: 20 mL Temperature: 25 ± 1 °C Test Conc.: Level 2 = 12.5 µg/L

Day	11-16-94 0 BEG		11-17-94 1 BEG		11-18-94 2 BEG		11-19-94 3 BEG		11-20-94 4 BEG		5		6		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	–	1N	1N	–	1N	–	1N	–	1N	1N						
B	–	1N	1N	–	1N	–	1N	–	1N	4N						
C	–	1N	1N	–	1N	–	1N	–	1N	–						
D	–	1N	1N	–	1D											
E	–	1N	1N	–	1N	–	1N	3N	1N	5N						
F	–	1N	1N	–	1N	–	1N	–	1N	3N						
G	–	1N	1N	–	1N	–	1N	–	1N	5N						
H	–	1N	1N	–	1N	–	1N	–	1N	4N						
I	–	1N	1N	–	1N	–	1N	–	1N	–						
J	–	1N	1N	–	1N	–	1N	2N	1N	2N						

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead

Reviewed By: Janette Downing Date: 12-13-94

ABC 42201 PG 00041

ABC LABORATORIES, INC.

FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # [illegible])

Date Initiated: 11-16-94 Vessel Size: 30 mL Test Vol.: 20 mL Temperature: 25 ± 1 °C Test Conc.: Level 3 = 25 µg/L

Day	11-16-94 0 BEG		11-17-94 1 BEG		11-18-94 2 BEG		11-19-94 3 BEG		11-20-94 4 BEG		5		6		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	–	1N	1N	–	1N	–	1N	2N	1N	~~3~~ 3N						
B	–	1N	1N	–	1N	–	1N	–	1N	–						
C	–	1N	1N	–	1N	–	1N	3N	1N	3N						
D	–	1N	1N	–	1N	–	1N	–	1N	–						
E	–	1N	1N	–	1N	–	1N	4N	1N	–						
F	–	1N	1N	–	1N	–	1N	–	1N	2N						
G	–	1N	1N	–	1N	–	1N	–	1N	~~3~~ 3N						
H	–	1N	1N	–	1N	–	1N	–	1N	5N						
I	–	1N	1N	–	1N	–	1N	2N	1N	–						
J	–	1N	1N	–	1N	–	1N	–	1N	–						

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead

(1) W BEG 11-20-94
(2) E BEG 11-20-94

Reviewed By: Janelle Dennis Date: 12-13-94

ABC 42201 PG 00042

ABC LABORATORIES, INC.

FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # CD 11-AQ1)

Date Initiated: 11-16-94 Vessel Size: 30 mL Test Vol.: 20 mL Temperature: 25 ± 1 °C Test Conc.: Level 4 = 50 µg/L

Day	11-16-94 0 BEG		11-17-94 1 BEG		11-18-94 2 BEG		11-19-94 3 BEG		11-20-94 4 BEG		5		6		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	-	1N	1N	-	1N	-	1N	-	1N	-						
B	-	1N	1N	-	1N	-	1N	-	1N	3N						
C	-	1N	1N	-	1N	-	1N	-	1S	-						
D	-	1N	1N	-	1N	-	1N	-	1N	-						
E	-	1N	1N	-	1N	-	1N	2N	1N	-						
F	-	1N	1N	-	1N	-	1N	-	1N	2N						
G	-	1N	1N	-	1N	-	1N	-	1N	4N						
H	-	1N	1N	-	1N	-	1N	-	1N	3N						
I	-	1N	1N	-	1N	-	1N	-	1N	3N						
J	-	1N	1N	-	1N	-	1N	-	1N	5N						

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead, S - SMALL

Reviewed By: Janelle Oberegg Date: 12/14/94

ABC 42201 PG 00043

ABC LABORATORIES, INC.

FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # CD-71-AQT)

Date Initiated: 11-16-94 Vessel Size: 30 mL Test Vol.: 20 mL Temperature: 25 ± 1 °C Test Conc.: Level 5 = 100 µg/L

Day	11-16-94 0 BEG		11-17-94 1 BEG		11-18-94 2 BEG		11-19-94 3 BEG		11-20-94 4 BEG		5		6		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	–	1N	1N	–	1N	–	1N	–	1N	–						
B	–	1N	1N	–	1N	–	1N	1N	1N	–						
C	–	1N	1N	–	1N	–	1N	–	1N	2N						
D	–	1N	1N	–	1N	–	1N	2N	1N	–						
E	–	1N	1N	–	1N	–	1N	–	1N	3N						
F	–	1N	1N	–	1N	–	1N	–	1N	1N						
G	–	1N	1N	–	1N	–	1N	–	1N	2N						
H	–	1N	1N	–	1N	–	1N	3N	1N	–						
I	–	1N	1N	–	1D	—										
J	–	1N	1N	–	1N	–	1N	–	1N	3N						

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead

Reviewed By: Janice Oanong Date: 12-17-94

ABC 42201 PG 00044

ABC LABORATORIES, INC.

FORM # 85 (1/7/92)

DAILY OBSERVATIONS, SAMPLE AND FEEDING SCHEDULE FOR CERIODAPHNIA DUBIA STATIC RENEWAL STUDIES

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Day of Week		WED	THURS	FRI	SAT	SUN	MON	
Date		11-16-94	11-17-94	11-18-94	11-19-94	11-20-94	11-21-94	
Study Day		0	1	2	3	4	5	
Observer ID		BEG	BEG	BEG	BEG	BEG	BEG	
Light Reading[b] (Lux/Footcandles*)		518.8 / 76.1	740.3 / 68.8	742.4 / 70.8	791.8 / 73.6	784.5 / 72.9	*	
Temperature (°C)[a] (Therm/Datalogger)		23.0 / 22.8	24.8 / 24.4	25.0 / 25.0	25.0 / 24.5	25.0 / 24.5	25.0 / 24.5	
Water Chemistry	Old Soln	—	BEG	BEG	BEG	BEG	BEG	
	New Soln	BEG	BEG	BEG	BEG	BEG		
Analytical Samples		—	—	—	—	—		
Feeding (ID & time)		BEG 12:00	BEG 12:00	BEG 11:30	BEG 10:00	BEG 11:30		
Algal Lot #:		94-S55	94-S55	94-S55	94-S55	94-S55		
Algal Concentration: (cells/mL)		94-A55 3.1 ③ 4.5 x10^7 cells/mL	94-A55 3.1 ③ 4.5 x10^7 cell/mL	94-A55 3.1 ③ 4.5 x10^7 cell/mL	94-A55 3.1 ③ 4.5 x10^7 cell/mL	94-A55 3.1 ③ 4.5 x10^7 cell/mL		
mL Algae Fed/Cup:		0.1mL	0.1mL	0.1mL	0.1mL	0.1mL		
YCT Lot #:		94-43 ②	94-43	94-43	94-43	94-43		
YCT Concentration: (mg/mL)		2.5 mg/mL 1.7 ③	2.5 mg/mL 1.7 ③	2.5 mg/mL 1.7 ③	2.5 mg/mL 1.7 ③	2.5 mg/mL 1.7 ③		
mL YCT Fed/Cup:		0.1mL	0.1mL	0.1mL	0.1mL	0.1mL		
Inspection								

[a] Mercury thermometer / datalogger readings ①

[b] Light Reading measured with a LI-COR Model LI-189 Quantum/Radiometer/Photometer (ABC Material #163-341) and LI-COR Model Photometric Sensor (Serial #PH-4447) *Footcandles = LUX x 0.0929

Comments: ① ABC control # 905-415 BEG 11-16-94
② E BEG 11-16-94
* Study terminated due to absence of mortality in highest treatment DCE 11/21/94 Natural as definitive date DCE 12/15/94
③ C BEG 1-5-95

Reviewed By: [signature] Date: 11/20/95 12-15-94

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 0

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD ⊕

Weighed by: Dorothy England Date: 11/16/94

Target Weight 0.100 g

Final Gr. Weight 0.1000 g

Tare Weight 0.0000 g

Net Weight 0.1000 g

Adj. Net Weight NA g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# BG-440)

Concentration 1.00 mg/mL

Balance calibrated with class C weights:

0.1000 g (class C) + 0.0000 g (tare) = 0.1000 g (final wt.)

Balance Used: Sartorius Research ABC Material Control No.: 1905-475

PREPARATION OF WORKING STANDARD ⊕

Transferred By: Dorothy England Date: 11/16/94

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
1.00	0.625	10.0	DMF	0.0625
1.00	1.25	10.0	DMF	0.125
1.00	2.50	10.0	DMF	0.250
1.00	5.00	10.0	DMF	0.500

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brit Geger Date: 11-16-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.00	0
Vehicle Blank	0 (DMF)	0.100	1.00	0
1	0.0625	0.100	1.00	0.00625
2	0.125	0.100	1.00	0.0125
3	0.250	0.100	1.00	0.0250
4	0.500	0.100	1.00	0.0500
5	1.00	0.100	1.00	0.100

Comments: ⊕ These solutions will be used for subsequent days preparation of definitive test concentrations. DCE 11/16/94

Prepared by: Dorothy England Date: 11/16/94

Calculations checked by: Jan Bowman Date: 11-16-94

Final Review by: Yantilla Downing Date: 12-13-94

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 1

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.100 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 1.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
1.00	0.625	10.0	DMF	0.0625
1.00	1.25	10.0	DMF	0.125
1.00	2.50	10.0	DMF	0.250
1.00	5.00	10.0	DMF	0.500

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Geiger Date: 11-18-94 170

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.00	0
Vehicle Blank	0 (DMF)	0.100	1.00	0
1	0.0625	0.100	1.00	0.00625
2	0.125	0.100	1.00	0.0125
3	0.250	0.100	1.00	0.0250
4	0.500	0.100	1.00	0.0500
5	1.00	0.100	1.00	0.100

Comments: DEBEG 11-18-94

Prepared by: Dorothy England Date: 11/16/94

Calculations checked by: Jan Bowne Date: 11-16-94

Final Review by: Janice Feeney Date: 12-13-94

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 2

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.100 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 1.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
1.00	0.625	10.0	DMF	0.0625
1.00	1.25	10.0	DMF	0.125
1.00	2.50	10.0	DMF	0.250
1.00	5.00	10.0	DMF	0.500

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Vegas Date: 11-18-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.00	0
Vehicle Blank	0 (DMF)	0.100	1.00	0
1	0.0625	0.100	1.00	0.00625
2	0.125	0.100	1.00	0.0125
3	0.250	0.100	1.00	0.0250
4	0.500	0.100	1.00	0.0500
5	1.00	0.100	1.00	0.100

Comments:

Prepared by: Dorothy England Date: 11/16/94

Calculations checked by: John Brown Date: 11-16-94

Final Review by: Janice Denny Date: 12-13-94

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 3

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.100 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 1.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
1.00	0.625	10.0	DMF	0.0625
1.00	1.25	10.0	DMF	0.125
1.00	2.50	10.0	DMF	0.250
1.00	5.00	10.0	DMF	0.500

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Begin Date: 11-19-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.00	0
Vehicle Blank	0 (DMF)	0.100	1.00	0
1	0.0625	0.100	1.00	0.00625
2	0.125	0.100	1.00	0.0125
3	0.250	0.100	1.00	0.0250
4	0.500	0.100	1.00	0.0500
5	1.00	0.100	1.00	0.100

Comments:

© D-JL 12-13-94

Prepared by: Dorothy England Date: 11/16/94

Calculations checked by: Jeff Bowman Date: 11-16-94

Final Review by: Jennifer Conway Date: 12/13/94

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 4

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.100 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 1.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
1.00	0.625	10.0	DMF	0.0625
1.00	1.25	10.0	DMF	0.125
1.00	2.50	10.0	DMF	0.250
1.00	5.00	10.0	DMF	0.500

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Beth Hegel Date: 11-20-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.00	0
Vehicle Blank	0 (DMF)	0.100	1.00	0
1	0.0625	0.100	1.00	0.00625
2	0.125	0.100	1.00	0.0125
3	0.250	0.100	1.00	0.0250
4	0.500	0.100	1.00	0.0500
5	1.00	0.100	1.00	0.100

Comments:

Prepared by: Dorothy England Date: 11/16/94

Calculations checked by: Jan Bowman Date: 11-16-94

Final Review by: Janice Bowman Date: 12-13-94

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 5

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.100 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 1.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
1.00	0.625	10.0	DMF	0.0625
1.00	1.25	10.0	DMF	0.125
1.00	2.50	10.0	DMF	0.250
1.00	5.00	10.0	DMF	0.500

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: _____ Date: _____

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.00	0
Vehicle Blank	0 (DMF)	0.100	1.00	0
1	0.0625	0.100	1.00	0.00625
2	0.125	0.100	1.00	0.0125
3	0.250	0.100	1.00	0.0250
4	0.500	0.100	1.00	0.0500
5	1.00	0.100	1.00	0.100

Comments:

Prepared by: Dorothy England Date: 11/16/94

Calculations checked by: John Burma Date: 11-16-94

Final Review by: James Downing Date: 12-[illegible]

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 6

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.100 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 1.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
1.00	0.625	10.0	DMF	0.0625
1.00	1.25	10.0	DMF	0.125
1.00	2.50	10.0	DMF	0.250
1.00	5.00	10.0	DMF	0.500

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: _____ Date: _____

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.00	0
Vehicle Blank	0 (DMF)	0.100	1.00	0
1	0.0625	0.100	1.00	0.00625
2	0.125	0.100	1.00	0.0125
3	0.250	0.100	1.00	0.0250
4	0.500	0.100	1.00	0.0500
5	1.00	0.100	1.00	0.100

Comments:

Prepared by: Dorothy England Date: 11/16/94

Calculations checked by: [illegible] Date: 11-16-94

Final Review by: [illegible] Date: 12-13-94

ABC LABORATORIES, INC.

FORM # 55 (11/25/91)

OBSERVATIONS AND/OR REMARKS FORM

Test Material: _____ Protocol No. 8606

Study Director: Dorothy England Study #: 42201

FOOD PREPARATION

11-16-94 / BG
(date) (ID)

100 mL of Yeast/Trout Chow (YTC or TCY)), lot #94-43 TCY, at 5.0 mg/mL suspended solids were diluted with 200 mL of hard blended water to produce a dilution of ~~2.5~~ 1.7② mg/mL suspended solids.

90 mL of a mixture of *Selenastrum capricornutum* and *Ankistrodesmus falcatus*, lot #94-S55 94-A55, at 1.0 X 10^8 cells/mL were diluted with 200 mL of LabConCo water to get a dilution of ~~1.5x10^8~~ ① ② 3.1×10^7 cells/mL.

~~11-16-94~~ / BG①
(date) (ID)

____ mL of Yeast/Trout Chow (YTC or TCY)), lot #_____ TCY, at 5.0 mg/mL suspended solids were diluted with ____ mL of hard blended water to produce a dilution of _____ mg/mL suspended solids.

____ mL of a mixture of *Selenastrum capricornutum* and *Ankistrodesmus falcatus*, lot #_____, at 1.0 X 10^8 cells/mL were diluted with ____ mL of LabConCo water to get a dilution of _____ cells/mL.

① EBEG 11-16-94 ② CBEG 1-4-95

NOTE: Individual entries must be dated and initialed.

Reviewed by: Dorothy England Date: 1/4/95

Study Director: Dorothy England Date: 1/4/95

ABC LABORATORIES, INC.

INVERTEBRATE CULTURE RECORD

Species: *Ceriodaphnia dubia*

Lot #: ~~10-66~~ ③ CD-71-AQT

Date Initiated: 11-7-94

Used to Initiate: 30

Lot # Initiated from: 94-CD-67-AQT

Culture Volume: ~1000 mL

Food Lot # 94-A51S51, A52S52, A53S53, 94-A55S55 ①

Date/ID	Temp.* °C	Changed Over	Fed	No. Adult Live	~Neonates Present	Comments
11.7.94 RW	25	—	✓	—	30	
11.8 RW	25	—	✓	—	—	
11-9 CJP	25	—	✓	—	—	
11-10 RW	25	3:45pm	✓	30	<100	
11-11 TH	25	—	✓	—	—	
11-12 CJP	25	—	✓	—	—	
11-13 PC	25	—	✓	—	—	② Added. PC 11-13-94
11-14 TH	25	11:30 A.M.	✓	30	150	
11-15 TH	25	10:30AM	✓	30	<100	
11-16 TH	25	11:15AM	✓	30	~~~~ ~50	removed ~150 for study #4220 BCC
11-17 RW	25	—	✓	—	—	
11-18 AH	25	1:15pm	✓	28	100	
11-19 CJP	25	—	✓	—	—	
11-20 CJP	25	—	✓	—	—	

* Temperature measured with a mercury thermometer

③ ERW corrected 12-5-94

Note: Cultures may be fed one or more of the following:
- ~0.25-0.5 mL of a 5.0 mg/mL suspension of trout chow yeast
- ~1.0 mL suspension of dried algae (spirulina and/or chlorella)
- ~1.0-3.0 mL of a 1.0 * 10^8 cells/mL concentration of live algae (The algae consists of Selenastrum capricornutum, Ankistrodesmus falcatus, *Chlamydomonas reinhardtii* and/or *Chlorella pyrenoidosa*.)

Prepared by: [signature] Date: 11-7-94

Reviewed by: [signature] Date: 12-7-94

④ E TH 11-16-94

FORM # 396b (04/18/94)

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ABC 42281 PG 00054

ABC LABORATORIES, INC.

FORM # AQ141 (01/11/94)

WATER QUALITY

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

New SOLN'S Concentration (µg/L)	11-16-94 Day 0 BEG						11-17-94 Day 1 BEG						11-18-94 Day 2 BEG					
	Temp[a]	DO[b]	pH[c]	Total Hard[d]	Total Alk[d]	Cond[e]	Temp	DO	pH	Total Hard	Total Alk	Cond	Temp	DO	pH	Total Hard	Total Alk	Cond
Control	23	8.5	8.5	150	160	320	23	8.4	8.5	142	162	340	23	8.4	8.4	150	158	340
Vehicle Blank	23	8.7	8.5	148	164	320	23	8.3	8.6	144	162	320	23	8.3	8.4	150	162	340
Level 1 = 6.25	23	8.6	8.5	158	170	320	23	8.2	8.6	146	170	320	23	8.2	8.4	152	164	340
Level 2 = 12.5	23	8.8	8.5	148	166	320	23	8.2	8.6	148	168	320	23	8.1	8.4	158	166	340
Level 3 = 25	23	8.7	8.6	150	164	320	23	8.2	8.6	150	164	320	23	8.1	8.4	158	164	340
Level 4 = 50	23	9.0	8.6	156	164	320	23	8.2	8.6	150	164	320	23	8.2	8.4	148	162	340
Level 5 = 100	23	8.9	8.6	156	162	320	23	8.2	8.6	154	168	320	23	8.1	8.4	152	164	340

a Temperature (°C) measured with a mercury thermometer
b Dissolved Oxygen (mg/L) measured using a YSI Dissolved Oxygen System Model 54 ARC) ABC material control #1905-730
c pH measured using a Beckman Φ34 pH meter, ABC material code 163-640 used with a Beckman Model 39841 probe
d Hardness and total alkalinity (mg/L as $CaCo_3$) analyzed using titrimetric method adopted from Standard Methods
e Conductivity (µmhos/cm) measured using a YSI 33 SCT Meter, ABC Material Control #1905-520

Remarks: ① EBEG 11-17-94

Prepared by: Brett Geyer Date: 11-16-94

Reviewed by: [illegible] Date: 12-14-94

ABC 42281 PG 00055

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ABC LABORATORIES, INC.

FORM # AQ141 (01/11/94)

WATER QUALITY

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

New Solns	11-19-94 Day 3 BEG						11-20-94 Day 4 BEG						Day					
Concentration (µg/L)	Temp[a]	DO[b]	pH[c]	Total Hard[d]	Total Alk[d]	Cond[e]	Temp	DO	pH	Total Hard	Total Alk	Cond	Temp	DO	pH	Total Hard	Total Alk	Cond
Control	23	8.4	8.0	148	160	320	23	8.6	8.4	158	172	320						
Vehicle Blank	23	8.4	8.1	152	166	320	23	8.5	8.5	156	170	320						
Level 1 = 6.25	23	8.4	8.1	152	164	320	23	8.5	8.5	154	164	320						
Level 2 = 12.5	23	8.4	8.1	152	162	320	23	8.5	8.5	154	166	320						
Level 3 = 25	23	8.4	8.2	150	162	320	23	8.5	8.5	158	170	320						
Level 4 = 50	23	8.4	8.2	150	160	320	23	8.5	8.5	156	168	320						
Level 5 = 100	23	8.4	8.2	150	166	320	23	8.5	8.5	158	170	320						

a Temperature (°C) measured with a mercury thermometer
b Dissolved Oxygen (mg/L) measured using a YSI Dissolved Oxygen System Model 54 ARC) ABC material control #1905-730
c pH measured using a Beckman Φ34 pH meter, ABC material code 163-640 used with a Beckman Model 39841 probe
d Hardness and total alkalinity (mg/L as $CaCo_3$) analyzed using titrimetric method adopted from Standard Methods
e Conductivity (µmhos/cm) measured using a YSI 33 SCT Meter, ABC Material Control #1905-520

Remarks: ① EBEG 11-19-94 ② EBEG 11-20-94

Prepared by: Brett Leger Date: 11-19-94

Reviewed by: [illegible] Date: 12-14-94

ABC 42201 96 00856

ABC LABORATORIES, INC.

FORM # 28b (08/26/92)

WATER QUALITY

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

OLD SOL'NS Concen. (µg/L)	11-17-94 Day 1 BEG				11-18-94 Day 2 BEG				11-19-94 Day 3 BEG				11-20-94 Day 4 BEG			
	Temp[a] °C	DO[b] mg/L	pH[c]		Temp[a] °C	DO[b] mg/L	pH[c]		Temp[a] °C	DO[b] mg/L	pH[c]		Temp[a] °C	DO[b] mg/L	pH[c]	
Control	24	7.9	8.7		25	7.7	8.5		25	7.7	8.1		25	7.7	8.5	
Vehicle	24	8.0	8.8		25	7.7	8.5		25	7.4	8.1		25	6.5	8.3	
6.25	24	8.0	8.8		25	7.6	8.6		25	7.6	8.2		25	6.5	8.3	
12.5	24	8.0	8.8		25	7.7	8.6		25	7.7	8.2		25	6.8	8.3	
25	24	8.0	8.8		25	7.8	8.6		25	7.4	8.2		25	6.3	8.3	
50	24	8.0	8.8		25	7.7	8.6		25	7.7	8.2		25	6.5	8.3	
100	24	8.0	8.8		25	7.7	8.6		25	7.0	8.2		25	6.4	8.3	

[a] Temperature measured with ✓ mercury thermometer ___ digital probe

[b] Dissolved Oxygen Probe (YSI Dissolved Oxygen System Model 54 ARC), ABC material control #~~1900-700~~ 1905-730①

[c] Beckman Φ 34 pH meter, ABC Material Control #163-640, with Beckman model 39841 pH probe

Remarks: ①F BEG 11-17-94

Prepared by: Beth Geger Date: 11-17-94

Reviewed by: Jennifer Deering Date: 12-14-94

ABC 42201 PG 99857

ABC LABORATORIES, INC.

FORM # 28b (08/26/92)

WATER QUALITY

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

OLD SOLNS Concen. (µg/L)	11-21-94 Day 5 BEG- Temp[a] °C	DO[b] mg/L	pH[c]		Day Temp[a] °C	DO[b] mg/L	pH[c]		Day Temp[a] °C	DO[b] mg/L	pH[c]		Day Temp[a] °C	DO[b] mg/L	pH[c]	
Control	25	7.7	8.5													
Vehicle	25	3.0	8.1													
6.25	25	3.0	8.3													
12.5	25	2.3	8.1													
25	25	3.2	8.2													
50	25	2.2	8.2													
100	25	2.3	8.1													

[a] Temperature measured with ✓ mercury thermometer ___ digital probe

[b] Dissolved Oxygen Probe (YSI Dissolved Oxygen System Model 54 ARC), ABC material control #~~1900-700~~ 1905-730①

[c] Beckman Φ 34 pH meter, ABC Material Control #163-640, with Beckman model 39841 pH probe

Remarks: ① FBEG 11-21-94

Prepared by: Brett Geiger Date: 11-21-94

Reviewed by: Janice Young Date: 12-14-94

ABC 42201 PG 00058

ABC LABORATORIES, INC.

DAPHNID ACUTE TOXICITY BIOASSAY – IMMOBILIZATION

☐Definitive ☒Preliminary ☐Screen ☐Flow-Through ☒Static

Test Material: _____ Protocol No.: 8606

Study Director: 940519+01 Study #: 42201

Test Species: [ ] *Daphnia magna* [X] Ceriodaphnia dubia (Lot # CD-69) Waterbath #: Effluent
Dilution Water: [X] Hard Blended [ ] _____ # Instar/Vessel: 5
Date Initiated: 11-22-94 Time: 1:50pm By: Brett Geger Date Terminated: 11/24/94

Test Conc. (mg/L)	Immobility and Behavioral Observations					
	0-Hour		24-Hour		48-Hour	
	Imm.	Obs.	Imm.	Obs.	Imm.	Obs.
Control A①	0	5N CLR	0	5N CLR	0	5N CLR
V Blank A	0	5N CLR	0	5N CLR	0	5N CLR
0.01 A	0	5N CLR	0	5N CLR	0	5N CLR
0.05 A	0	5N CLR	0	5N CLR	0	5N CLR
0.1 A	0	5N CLR	1	4N CLR	1	2Q; 2N CLR
0.5 A	0	5N CLR	3	2OB.Q CLR	5	— CLR
1.0 A	0	5N CLR	5	— CLR	5	— CLR
Control B①	0	5N CLR	0	5N CLR	0	5N CLR
V Blank B	0	5N CLR	0	5N CLR	0	5N CLR
0.01 B	0	5N CLR	0	5N CLR	0	5N CLR
0.05 B	0	5N CLR	0	5N CLR	0	5N CLR
0.1 B	0	5N CLR	0	5N CLR	0	5N CLR
0.5 B	0	5N CLR	3	2OB.Q CLR	5	— CLR
1.0 B	0	5N CLR	5	— CLR	5	— CLR
ID/Date/Time	BEG 11-22-94 3:00pm		JAW 11-23-94 11:45AM		JHB 11-24-94 3.00PM	
Anal. ID Date/Time						

Comments: N = Normal, SUR = Surfacing, C = Clumping, CT = Coated with Extraneous Material, ERR = Erratic Movement, TR = Trailing Extraneous Material, Q = Quiescent, OB = On Bottom, P = Precipitate, CLR = Clear, CLD = Cloudy/Colored Solution, SF = Surface Film
①A replicates were done in glass test chambers; B replicates were done in plastic test chambers. 11-22-94 BEG

Reviewed by: Dorothy Eigler Date: 11/28/94

Study Director: Dorothy Eigler Date: 11/28/94

FORM AQ106 (3/25/93)

ABC LABORATORIES, INC.

Form 27b (03/11/94)

COMPOUND PREPARATIONS

Test Material: _____ Protocol No.: 8666

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519 FC1 Purity NG % ABC Ref #: TJ-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: Brett Geger Date: 11-22-94

Target Weight 0.100 g

Final Gr. Weight 0.100 g

Tare Weight 0.000 g

Net Weight 0.100 g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 10 mL of DMF

Concentration 10 mg/mL (lot # BE-440)

Balance calibrated with Class C weights: BEG 11-22-94

0.200 g (Class C) + 0.000 g (tare) = 0.200 g (final wt)

Balance Used: Mettler PM460 ABC Material Control #: 1905-1020

PREPARATION OF WORKING STANDARD

Transferred by: Brett Geger Date: 11-22-94

Conc. of Parent Sol. mg/mL	Aliquot Volume mL	Dilution Volume (L)	Dilution Carrier	Final Concentration mg/mL
10	0.01	0.100	HB water	0.001

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Geger Date: 11-22-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc.* (mg/L)
Control	HB water	50	0.05	0
Vehicle Blank	DMF	0.005	0.05	0
1	0.001	0.5	0.05	0.010
2	0.001	2.5	0.05	0.050
3	0.001	5.0	0.05	0.10
4	0.001	25	0.05	0.50
5	0.001	50	0.05	1.0
6				
7				

☐ * Aliquot volume was stirred into the dilution volume with a glass or stainless steel stirrer

Comments:

Prepared by: Brett Geger Date: 11-22-94

Calculations checked by: Dorothy England Date: 11/28/94

Reviewed by: Dorothy England Date: 11/28/94

ABC LABORATORIES, INC.

INVERTEBRATE CULTURE RECORD

Species: *Ceriodaphnia dubia* Lot #: CD - 69

Date Initiated: 11-2-94 # Used to Initiate: 30

Lot # Initiated from: CD - 63 Culture Volume: ~1000 mL

Food Lot # 94 A55S55 A56S56 A57S57

Date/ID	Temp.* °C	Changed Over	Fed	No. Adult Live	~Neonates Present	Comments
11-16-94 TH	25	11:15 A.m.	✓	30	<50	
11-17 RJ	25	11:15 am	✓	30	175	Removed all neonates for study # 42166
11-17 RJ	25					
11-18 AA	25	1:00pm	✓	30	100	
11-19 CJP	25	–	✓	–	–	
11-20 CJP	25	–	✓	–	–	
11-21 RJ	25	3:00pm	✓	30	300	
11-22 RJ	25	–	✓	–	–	Removed 150 for study # 42201
11-23 RJ	25	2:30pm	✓	30	<100	
11-24 THM	25	–	✓	–	–	
11-25 THM	25	11:15 AM	✓	30	300	
11-26 CJP	19①	–	✓	–	–	Aerator was off, turned back on 3:45pm., CJP/11-26-94
11-27 CJP	25	–	✓	–	–	
11-28 RJ	25	12:15pm	✓	28	200	Terminated used 30 to start CD-76 RJ 11-29-94

* Temperature measured with a mercury thermometer

Note: Cultures may be fed one or more of the following:
- ~0.25-0.5 mL of a 5.0 mg/mL suspension of trout chow yeast
- ~1.0 mL suspension of dried algae (spiruline and/or chlorella)
- ~1.0-3.0 mL of a 1.0 * 10^8 cells/mL concentration of live algae (The algae consists of Selenastrum capricornutum, Ankistrodesmus falcatus, *Chlamydomonas reinhardtii* and/or *Chlorella pyrenoidosa*.)

Prepared by: Trina Hoff Date: 11-16-94

Reviewed by: Robert J Reynolds Date: 11-28-94

FORM # 396b (04/18/94)

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ABC 42201 PG 00061

ABC LABORATORIES, INC.

INVERTEBRATE CULTURE RECORD

Species: *Ceriodaphnia dubia* Lot #: CD-69

Date Initiated: 11-2-94 # Used to Initiate: 30

Lot # Initiated from: CD-63 Culture Volume: ~1000 mL

Food Lot # 94-A50S50 A51S51 A52S52 A53S53, 94-A55S55 ①

Date/ID	Temp.* °C	Changed Over	Fed	No. Adult Live	~Neonates Present	Comments
11-2-94 RJ	25	—	✓	—	30+	
11-3 RJ	25	—	✓	—	—	
11-4 RJ	25	1:00pm	✓	30	—	
11-5 CJP	25	—	✓	—	—	
11-6 CJP	25	—	✓	—	—	
11-7 RJ	25	3:30pm	✓	30	150	
11-8 RJ	25	—	✓	—	—	
11-9 CJP	25	—	✓	—	—	
11-10 RJ	25	3:45pm	✓	30	250	
11-11 TH	25	—	✓	—	—	
11-12 CJP	25	—	✓	—	—	
11-13 PC	25	—	✓	—	—	① Added. PC 11-13-94
11-14 TH	25	11:30 A.M.	✓	30	<100	
11-15 TH	25	10:30 A.M.	✓	30	<20	Just bagging to drop

* Temperature measured with a mercury thermometer

Note: Cultures may be fed one or more of the following:
- 0.25-0.5 mL of a 5.0 mg/mL suspension of trout chow yeast
- 1.0 mL suspension of dried algae (spirulina and/or chlorella)
- 1.0-3.0 mL of a 1.0 * 10^8 cells/mL concentration of live algae (The algae consists of Selenastrum capricornutum, Ankistrodesmus falcatus, *Chlamydomonas reinhardtii* and/or *Chlorella pyrenoidosa*.)

Prepared by: [signature] Date: 11-2-94

Reviewed by: Robert H Peyold Date: 11-29-94

FORM # 396b (04/18/94)

"This is an exact copy of The original document" By [signature]

ABC LABORATORIES, INC.

FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # CD-74)

Date Initiated: 11-29-94 Vessel Size: 50 mL Test Vol.: 30 mL Temperature: 25 ± 1 °C Test Conc.: Control

Day	11-29-94 0 BEG		11-30-94 1 BEG		12-1-94 2 BEG		12-2-94 3 BEG		12-3-94 1:45pm 4 RW		12-4-94 5 RW		12-5-94 6 BEG		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	–	1N	1N	–	1N	–	1N	4N	1N	5N	1N	7N	1N	–		
B	–	1N	1N	–	1N	–	1N	5N	1N	10N	1N	13N	1N	–		
C	–	1N	1N	–	1N	–	1N	5N	1N	6N	1N	0	1N	5N		
D	–	1N	1N		1N	–	1N	3N	1D	0	–					
E	–	1N	1N		1N	–	1N	3N	1N	0	1N	11N	1N	8N		
F	–	1N	1N		1N	–	1N	4N	1N	9N	1N	12N	1N			
G	–	1N	1N	–	1N	–	1N	5N	1N	7N	1N	0	1N			
H	–	1N	1N	–	1D	–										
I	–	1N	1N	–	1N	–	1N	4N	1N	5N	1N	0	1D	–		
J	–	1N	1N	–	1N	–	1N	2N	1N	0	1N	0	1D	–		

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead
① W BEG 12-2-94

Reviewed By: Janelle Downing Date: 12-14-94

ABC 42201 PG 00063

ABC LABORATORIES, INC.

FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # [illegible])

Date Initiated: 11-30-94 Vessel Size: 50 mL Test Vol.: 30 mL Temperature: 25 ± 1 °C Test Conc.: Vehicle Blank

Day	11-30-94 0 BEG		12-1-94 1 BEG		12-2-94 2 BEG		12-3-94 3 BEG		12-4-94 4		12-5-94 5		12-6-94 (1) 6 BEG		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	–	1N	1N	–	1N	–	1N	–	1N	0	1N	8N				
B	–	1N	1N	–	1N	–	1N	–	1N	4N	1N	11N				
C	–	1N	1N	–	1N	–	1N	–	1N	5N	1N	10N				
D	–	1N	1N	–	1N	–	1N	5N	1N	5N	1N	11N				
E	–	1N	1N	–	1N	–	1N	4N	1N	10N	1N	0				
F	–	1N	1N	–	1N	–	1N	3N	1N	10N	1N	0				
G	–	1N	1N	–	1N	–	1N	2N	1N	0	1N	7N				
H	–	1N	1N	–	1N	–	1N	3N	1N	10N	1N	16N				
I	–	1N	1N	–	1N	–	1N	–	1N	5N	1N	0				
J	–	1N	1N	–	1N	–	1N	–	1N	1N	1N	6N				

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead

(1) E BEG 12-5-94 (1)5

Reviewed By: [signature] Date: 12-14-94

ABC 42201 PG 00064

ABC LABORATORIES, INC. FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # CD-94)

Date Initiated: 11-29-94 Vessel Size: 50 mL Test Vol.: 30 mL Temperature: 25 ± 1 °C Test Conc.: Level 1 = 25 µg/L

Day	11-29-94 0 BEC		11-30-94 1 BEC		12-1-94 2 BEC		12-2-94 3 BEC		12-3-94 1:45pm 4 AV		12-4-94 5 AV		~~12-5-94~~ ② 6 ~~BEC~~		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	-	1N	1N	-	1N	-	1N	3N	1N	0	1N	9N				
B	-	1N	1N	-	1N	-	1N	-	1N	6N	1N	10N				
C	-	1N	1N	-	1N	-	1N	-	1N	5N	1N	10N				
D	-	1N	1N	-	1N	-	1N	-	1N	5N	1N	12N				
E	-	1N	1N	-	1N	-	1N	-	1N	6N	1N	11N				
F	-	1N	1N	-	1N	-	1N	-	1N	3N	1N	8N				
G		1N	1N	-	1N	-	1N	-	1N	5N	1N ~~10~~ ①	10N				
H	-	1N	1N	-	1N	-	1N	-	1N	6N	1N ~~12~~ ①	8N				
I	-	1N	1N	-	1N	-	1N	-	1N	5N	1N ~~11~~ ①	11N				
J	-	1N	1N	-	1N	-	1N	-	1N	7N	1N	11N				

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead

② 1 Rep 12-4-94 ① E AV 12-4-94

Reviewed By: Janelle Downs Date: 12-14-94

ABC 42201 PG 00065

ABC LABORATORIES, INC. FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # CD 74)

Date Initiated: 11-30-94 Vessel Size: 50 mL Test Vol.: 30 mL Temperature: 25 ± 1 °C Test Conc.: Level 2 = 50 µg/L

Day	11-30-94 0 BEG		12-1-94 1 BEG		12-2-94 2 BEG		12-3-94 3 BEG		12-4-94 4 RN		12-5-94 5 RN		~~12-6-94~~ 6 ~~BEG~~		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	-	1N	1N	-	1N	-	1N	-	1N	6N	1N	9N				
B		1N	1N	-	1N	-	1N	-	1N	5N	1N	10N				
C	-	1N	1N	-	1N	-	1N	-	1N	4N	1N	6N				
D	-	1N	1N	-	1N	-	1N	-	1N	4N	1N	8N				
E	-	1N	1N	-	1N	-	1N	-	1N	4N	1N	8N				
F		1N	1N	-	1N	-	1N	-	1N	5N	1N	9N				
G	-	1N	1N	-	1N	-	1N	-	1N	7N	1N	10N				
H	-	1N	1N	-	1N	-	1N	-	1N	5N	1N	7N				
I	-	1N	1N	-	1N	-	1N	-	1N	5N	1N	8N				
J	-	1N	1N	-	1N	-	1N	-	1N	0	1N	7N				

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead
① DEBEG 12-5-94

Reviewed By: Janelle Young Date: 12-14-94

ABC 42201 PS 00066

ABC LABORATORIES, INC. FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # CD 74)

Date Initiated: 11-29-94 Vessel Size: 50 mL Test Vol.: 30 mL Temperature: 25 ± 1 °C Test Conc.: Level 3 = 100 µg/L

Day	11-29-94 0 BEG		11-30-94 1 BEG		12-1-94 2 BEG		12-2-94 3 BEG		12-3-94 4 RJ		12-4-94 5 RJ		~~12-5-94~~ 6 ~~BEG~~ (1)		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	-	1N	1N	-	1N	-	1N	-	1N	5N	1N	3N				
B	-	1N	1N	-	1N	-	1N	-	1N	4N	1N	3N				
C	-	1N	1N	-	1N	-	1N	-	1N	5N	1N	5N				
D	-	1N	1N	-	1N	-	1N	-	1N	2N	1P	6N				
E	-	1N	1N	-	1N	-	1N	-	1N	3N	1N	2N				
F		1N	1N	-	1N	-	1N	-	1N	5N	1N	4N				
G	-	1N	1N	-	1N	-	1N	-	1N	1N	1N	1N				
H	-	1N	1N	-	1N	-	1N	-	1N	3N	1N	2N				
I	-	1N	1N	-	1N	-	1N	-	1N	2N	1Sm P	4N				
J	-	1N	1N	-	1N	-	1N	-	1N	2N	1N	4N				

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead P. Pat RJ 12-4-94
(1) BEG 12-5-94
Sm = Small

Reviewed By: Janelle Downey Date: 12-14-94

ABC 42201 PG 00067

ABC LABORATORIES, INC. FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # CD-24)

Date Initiated: 11-29-94 Vessel Size: 50 mL Test Vol.: 30 mL Temperature: 25 ± 1 °C Test Conc.: Level 4 = 200 µg/L

Day	11-29-94 0 BEG		11-30-94 1 BEG		12-1-94 2 BEG		12-2-94 3 BEG		12-3-94 4 KW		12-4-94 5 KW		6		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A		1N	1N	—	1D	—										
B		1N	1N	—	1D	—										
C		1N	1N	—	1D	—										
D		1N	1N	—	1D	—										
E		1N	1N	—	1D	—										
F		1N	1N	—	1N	—	1P	—	1P	0	~~1N~~ D ①	0				
G		1N	1N	—	1D	—										
H		1N	1N	—	1D	—										
I		1N	1D													
J		1N	1N	—	1P	—	1D	—								

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead, P = PALE
① EKW 12-4-94

Reviewed By: Jennifer Tomney Date: 12-14-94

ABC 42201 PG 00068

ABC LABORATORIES, INC.

FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # CD 74)

Date Initiated: 11-29-94 Vessel Size: 50 mL Test Vol.: 30 mL Temperature: 25 ± 1 °C Test Conc.: Level 5 = 400 µg/L

Day	11-29-94 0 BEG		11-30-94 1 BEG		12-1-94 2 BEG		3		4		5		6		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	–	1N	1D	–												
B	–	1N	1P	–	1D	–										
C	–	1N	1D	–												
D	–	1N	1D	–												
E	–	1N	1D	–												
F	–	1N	1D	–												
G	–	1N	1D	–												
H	–	1N	1D	–												
I	–	1N	1D	–												
J	–	1N	1D	–												

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead, P = Pale

Reviewed By: [signature] Date: 12-14-94

ABC 42201 PG 00069

ABC LABORATORIES, INC.

FORM # 85 (1/7/92)

DAILY OBSERVATIONS, SAMPLE AND FEEDING SCHEDULE FOR CERIODAPHNIA DUBIA STATIC RENEWAL STUDIES

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Day of Week		TUES	WED	Thurs	Fri	Sat	Sun	Mon*
Date		11-29-94	11-30-94	12-1-94	12-2-94	12-3-94	12-4-94	12-5-94
Study Day		0	1	2	3	4	5	6
Observer ID		BEG	BEG	BEG	BEG	RW	RW	BEG
Light Reading[b] (Lux/Footcandles*)		728.3 / 67.7	748.1 / 69.5	736.8 / 68.4	753.9 / 70.0	752.9 ③ / 70.0 / 69.9	741.2 / 68.9	757.4 / 70.4
Temperature (°C)[a] (Therm/Datalogger)		24.0 / 23.5	23.0 / 22.6	25.0 / 24.9	25.05 ① / 25.3	25.0 / 24.7	25.0 / 24.7	25.0 / 25.0
Water Chemistry	Old Soln	—	BEG	BEG	BEG	RW	RW	BEG
	New Soln	BEG	BEG	BEG	BEG	RW	RW	BEG
Analytical Samples								
Feeding (ID & time)		2:00pm BEG	2:00pm BEG	1:45pm BEG	1:45pm BEG	1:45pm RW	1:30pm RW	2:00pm BEG
Algal Lot #:		94S57 94A57	94S57 94A57	94S57 94A57	94S57 94A57	94-A57 94S57	94-A57 94-S57	94-S57 94-A57
Algal Concentration: (cells/mL)		~~2.25x10^5~~ cells/mL 3.1x10^7 ②	~~2.25x10^5~~ cells/mL 3.1x10^7 ②	~~2.25x10^5~~ cells/mL 3.1x10^7 ②	~~2.25x10^5~~ cells/mL 3.1x10^7 ②	~~[illegible]~~ cells/mL 3.1x10^7 ②	~~2.25x10^5~~ ② cells/mL 3.1x10^7	~~2.25x10^5~~ ② cells/mL 3.1x10^7
mL Algae Fed/Cup:		0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL
YCT Lot #:		94-44 TCY	94-44 TCY	94-44 TCY	94-44 TCY	94-44 TCY	94-44 TCY	94-44 TCY
YCT Concentration: (mg/mL)		~~1.8~~ mg/mL 1.7 ②	~~1.8~~ mg/mL 1.7 ②	~~1.8~~ mg/mL 1.7 ②	~~1.8~~ mg/mL 1.7 ②	~~1.8~~ mg/mL 1.7 ②	~~1.8~~ mg/mL 1.7 ②	~~1.8~~ mg/mL 1.7 ②
mL YCT Fed/Cup:		0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL
Inspection					DCE			

[a] Mercury thermometer / datalogger readings

[b] Light Reading measured with a LI-COR Model LI-189 Quantum/Radiometer/Photometer (ABC Material #183-341) and LI-COR Model Photometric Sensor (Serial #PH-4447) *Footcandles = LUX x 0.0929

Comments: ① BEG 12-2-94 ② C BEG 12-5-94 ③ C - DCE 1/2/95

* study terminated due to control mortality BEG 12-5 ..
Not used as definitive death. DCE 12/15/94

Reviewed By: [signature] Date: 1/2/95

ABC LABORATORIES, INC.

INVERTEBRATE CULTURE RECORD

Species: *Ceriodaphnia dubia* Lot #: CD-79

Date Initiated: 11-21-94 # Used to Initiate: 30

Lot # Initiated from: CD-68 Culture Volume: ~1000 mL

Food Lot # 94-A56S56 A57S57

Date/ID	Temp.* °C	Changed Over	Fed	No. Adult Live	~Neonates Present	Comments
11-21-94 RJ	25	—	✓	—	30	<72hr young used to start
11-22 RJ	25	—	✓	—	—	
11-23 RJ	25	2:30pm	✓	30	100	
11-24 HM	25	✓	✓	—	—	
11-25 HM	25	11:15AM	✓	30	300	
11-26 CJP	25	—	✓	—	—	CJP/11-26-94 Heater was off turned on 3:45pm. CJP/11-26-94
11-27 CJP	25	—	✓	—	—	
11-28 RJ	25	12:15pm	✓	27	200	
11-28 RJ	25	—	—	—	—	Removed 100 for study # 42204 and 41038 prelim
11-29 RJ	25	—	✓	—	—	
11-30 TH	25	12:00 P.m.	✓	27	200	
12-1 RJ	25	—	✓	—	—	
12-2 RJ	25	2:15pm	✓	27	350	
12-3 CJP	25	—	✓	—	—	

* Temperature measured with a mercury thermometer

Note: Cultures may be fed one or more of the following:
- ~0.25-0.5 mL of a 5.0 mg/mL suspension of trout chow yeast
- ~1.0 mL suspension of dried algae (spirulina and/or chlorella)
- ~1.0-3.0 mL of a 1.0 * 10^8 cells/mL concentration of live algae (The algae consists of Selenastrum capricornutum, Ankistrodesmus falcatus, *Chlamydomonas reinhardtii* and/or *Chlorella pyrenoidosa*.)

Prepared by: [signature] Date: 11-21-94

Reviewed by: Robert H Peyold Date: 12-14-94

FORM # 396b (04/18/94)

"This is an exact copy of The original document" By [signature] date 1/4/95

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 0

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: Dorothy England Date: 11/29/94

Target Weight 0.400 g

Final Gr. Weight 0.400 g

Tare Weight 0.000 g

Net Weight 0.400 g

Adj. Net Weight NA g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# BG 628)

Concentration 4.00 mg/mL

Balance calibrated with class C weights:

0.200 g (class C) + 0.000 g (tare) = 0.200 g (final wt.)

Balance Used: Mettler PM460 ABC Material Control No.: 1905-1020

PREPARATION OF WORKING STANDARD

Transferred By: Dorothy England Date: 11/29/94

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
4.00	0.625	10.0	DMF	0.250
4.00	1.25	10.0	DMF	0.500
4.00	2.50	10.0	DMF	1.00
4.00	5.00	10.0	DMF	2.00

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Geiger Date: 11-29-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.0	0
Vehicle Blank	0 (DMF)	0.100	1.0	0
1	0.250	0.100	1.0	0.025
2	0.500	0.100	1.0	0.050
3	1.00	0.100	1.0	0.10
4	2.00	0.100	1.0	0.20
5	4.00	0.100	1.0	0.40

Comments:

Prepared by: Dorothy England Date: 11/29/94

Calculations checked by: Brett Geiger Date: 11-29-94

Final Review by: Jamie Young Date: 12-14-94

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 1

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.400 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 4.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
4.00	0.625	10.0	DMF	0.250
4.00	1.25	10.0	DMF	0.500
4.00	2.50	10.0	DMF	1.00
4.00	5.00	10.0	DMF	2.00

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Geiger Date: 11-30-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.0	0
Vehicle Blank	0 (DMF)	0.100	1.0	0
1	0.250	0.100	1.0	0.025
2	0.500	0.100	1.0	0.050
3	1.00	0.100	1.0	0.10
4	2.00	0.100	1.0	0.20
5	4.00	0.100	1.0	0.40

Comments:

Prepared by: Brett Geiger Date: 11-30-94

Calculations checked by: Janelle Downing Date: 12-14-94

Final Review by: Janelle Downing Date: 12-14-94

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 2

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.400 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 4.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
4.00	0.625	10.0	DMF	0.250
4.00	1.25	10.0	DMF	0.500
4.00	2.50	10.0	DMF	1.00
4.00	5.00	10.0	DMF	2.00

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Pegan Date: 12-1-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.0	0
Vehicle Blank	0 (DMF)	0.100	1.0	0
1	0.250	0.100	1.0	0.025
2	0.500	0.100	1.0	0.050
3	1.00	0.100	1.0	0.10
4	2.00	0.100	1.0	0.20
5	4.00	0.100	1.0	0.40

Comments:

Prepared by: Brett Pegan Date: 12-1-94

Calculations checked by: Janelle Ewing Date: 12-14-94

Final Review by: Janelle Ewing Date: 12-14-94

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 3

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.400 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 4.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g

(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
4.00	0.625	10.0	DMF	0.250
4.00	1.25	10.0	DMF	0.500
4.00	2.50	10.0	DMF	1.00
4.00	5.00	10.0	DMF	2.00

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Vegel Date: 12-2-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.0	0
Vehicle Blank	0 (DMF)	0.100	1.0	0
1	0.250	0.100	1.0	0.025
2	0.500	0.100	1.0	0.050
3	1.00	0.100	1.0	0.10
4	2.00	0.100	1.0	0.20
5	4.00	0.100	1.0	0.40

Comments:

Prepared by: Brett Vegel Date: 12-2-94

Calculations checked by: Janelle Downing Date: 12-14-94

Final Review by: Janelle Downing Date: 12-14-94

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 4

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.400 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 4.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
4.00	0.625	10.0	DMF	0.250
4.00	1.25	10.0	DMF	0.500
4.00	2.50	10.0	DMF	1.00
4.00	5.00	10.0	DMF	2.00

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: [signature] Date: 12-3-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.0	0
Vehicle Blank	0 (DMF)	0.100	1.0	0
1	0.250	0.100	1.0	0.025
2	0.500	0.100	1.0	0.050
3	1.00	0.100	1.0	0.10
4	2.00	0.100	1.0	0.20
5	4.00	0.100	1.0	0.40

Comments:

Prepared by: [signature] Date: 12-3-94

Calculations checked by: [signature] Date: 12-14-94

Final Review by: [signature] Date: 12-14-94

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 5

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.400 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 4.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
4.00	0.625	10.0	DMF	0.250
4.00	1.25	10.0	DMF	0.500
4.00	2.50	10.0	DMF	1.00
4.00	5.00	10.0	DMF	2.00

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: [signature] Date: 12-4-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.0	0
Vehicle Blank	0 (DMF)	0.100	1.0	0
1	0.250	0.100	1.0	0.025
2	0.500	0.100	1.0	0.050
3	1.00	0.100	1.0	0.10
4	2.00	0.100	1.0	0.20
5	4.00	0.100	1.0	0.40

Comments:

Prepared by: [signature] Date: 12-4-94

Calculations checked by: [signature] Date: 12-[illegible]

Final Review by: [signature] Date: 12-[illegible]

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 6

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.400 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 4.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
4.00	0.625	10.0	DMF	0.250
4.00	1.25	10.0	DMF	0.500
4.00	2.50	10.0	DMF	1.00
4.00	5.00	10.0	DMF	2.00

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Cogan Date: 12-5-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.0	0
Vehicle Blank	0 (DMF)	0.100	1.0	0
1	0.250	0.100	1.0	0.025
2	0.500	0.100	1.0	0.050
3	1.00	0.100	1.0	0.10
4	2.00	0.100	1.0	0.20
5	4.00	0.100	1.0	0.40

Comments:

Prepared by: Brett Cogan Date: 12-5-94

Calculations checked by: Janice Young Date: 12-14-94

Final Review by: Janice Young Date: 12-14-94

ABC LABORATORIES, INC.

FORM # AQ141 (01/11/94)

WATER QUALITY

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

New Solns	11-29-94				Day 0	BEG	11-30-94			Day 1		BEG	12-1-94			Day 2		BEG
Concentration (µg/L)	Temp[a]	DO[b]	pH[c]	Total Hard[d]	Total Alk[d]	Cond[e]	Temp	DO	pH	Total Hard	Total Alk	Cond	Temp	DO	pH	Total Hard	Total Alk	Cond
Control	23	8.5	8.3	160	162	340	23	8.4	8.6	160	182	320	23	8.5	85	160	180	340
Vehicle Blank	23	8.5	8.3	160	174	320	23	8.4	8.6	156	162	320	23	85	8.5	158	170	320
Level 1 = 25	23	8.5	8.3	160	178	320	23	8.4	8.6	158	180	300	23	8.4	85	154	172	320
Level 2 = 50	23	8.5	8.3	150	170	320	23	8.5	8.6	154	164	300	23	8.4	8.5	160	180	320
Level 3 = 100	23	8.5	8.3	158	178	320	23	8.5	8.6	160	180	300	23	8.4	8.5	158	168	320
Level 4 = 200	23	8.5	8.3	156	174	320	23	8.5	8.6	154	164	300	23	8.5	8.6	158	178	320
Level 5 = 400	23	8.5	8.3	156	178	320	23	8.5	8.6	160	180	300	—	—	—	—	—	—

a Temperature (°C) measured with a mercury thermometer
b Dissolved Oxygen (mg/L) measured using a YSI Dissolved Oxygen System Model 54 ARC) ABC material control #1905-730
c pH measured using a Beckman Φ34 pH meter, ABC material code 163-640 used with a Beckman Model 39841 probe
d Hardness and total alkalinity (mg/L as $CaCo_3$) analyzed using titrimetric method adopted from Standard Methods
e Conductivity (µmhos/cm) measured using a YSI 33 SCT Meter, ABC Material Control #1905-520

Remarks:

Prepared by: Brett Geger Date: 11-29-94

Reviewed by: Jennie Downey Date: 12-14-94

ABC 42201 PG 00079

ABC LABORATORIES, INC.

FORM # AQ141 (01/11/94)

WATER QUALITY

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

New Solns	12-2-94 Day 3 BEG / RW						12-3-94 Day 4 BW / RW						12-4-94 Day 5 RW					
Concentration (µg/L)	Temp[a]	DO[b]	pH[c]	Total Hard[d]	Total Alk[d]	Cond[e]	Temp	DO	pH	Total Hard	Total Alk	Cond	Temp	DO	pH	Total Hard	Total Alk	Cond
Control	23	8.5	8.5	150	180	340	23	8.3	8.4	156	① ~~180~~ 190	300	23	8.2	8.5	150	170	310
Vehicle Blank	23	8.5	8.5	156	180	320	23	8.4	8.5	156	170	300	23	8.2	8.5	154	168	310
Level 1 = 25	23	8.5	8.6	156	180	320	23	8.4	8.5	154	170	300	23	8.2	8.5	158	162	310
Level 2 = 50	23	8.5	8.6	154	180	320	23	8.4	8.5	150	170	300	23	8.1	8.5	154	162	310
Level 3 = 100	23	8.4	8.6	156	180	320	23	8.5	8.5	152	170	300	23	8.2	8.5	158	164	310
Level 4 = 200	23	8.4	8.6	158	170	320	23	8.4	8.6	154	170	300	23	8.1	8.5	158	168	310
Level 5 = 400	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

a Temperature (°C) measured with a mercury thermometer
b Dissolved Oxygen (mg/L) measured using a YSI Dissolved Oxygen System Model 54 ARC) ABC material control #1905-730
c pH measured using a Beckman Φ34 pH meter, ABC material code 163-640 used with a Beckman Model 39841 probe
d Hardness and total alkalinity (mg/L as $CaCo_3$) analyzed using titrimetric method adopted from Standard Methods
e Conductivity (µmhos/cm) measured using a YSI 33 SCT Meter, ABC Material Control #1905-520

Remarks: ① ERW 12-3-94

Prepared by: Brett Creger Date: 12-2-94

Reviewed by: [illegible] Date: 12-14-94

ABC 42201 55 00080

ABC LABORATORIES, INC.

FORM # AQ141 (01/11/94)

WATER QUALITY

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

New Solns Concentration (µg/L)	12-5-94 Day 6 BEG Temp[a]	DO[b]	pH[c]	Total Hard[d]	Total Alk[d]	Cond[e]	Day Temp	DO	pH	Total Hard	Total Alk	Cond	Day Temp	DO	pH	Total Hard	Total Alk	Cond
Control	23	8.2	8.5	160	180	320												
Vehicle Blank	23	8.2	8.5	154	170	320												
Level 1 = 25	23	8.2	8.5	148	160	320												
Level 2 = 50	23	8.1	8.5	150	168	320												
Level 3 = 100	23	8.1	8.5	160	188	320												
Level 4 = 200	—	—	—	—	—	—												
Level 5 = 400	—	—	—	—	—	—												

a Temperature (°C) measured with a mercury thermometer
b Dissolved Oxygen (mg/L) measured using a YSI Dissolved Oxygen System Model 54 ARC) ABC material control #1905-730
c pH measured using a Beckman Φ34 pH meter, ABC material code 163-640 used with a Beckman Model 39841 probe
d Hardness and total alkalinity (mg/L as $CaCo_3$) analyzed using titrimetric method adopted from Standard Methods
e Conductivity (µmhos/cm) measured using a YSI 33 SCT Meter, ABC Material Control #1905-520

Remarks:

Prepared by: Brett Geyer Date: 12-5-94

Reviewed by: [illegible] Date: 12-14-94

ABC 42201 55 00081

ABC LABORATORIES, INC.

FORM # 28b (08/26/92)

WATER QUALITY

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

OLD Solns Concen. (µg/L)	11-30-94 Day 1 BEG Temp[a] °C	DO[b] mg/L	pH[c]		12-1-94 Day 2 BEG Temp[a] °C	DO[b] mg/L	pH[c]		12-2-94 Day 3 RW Temp[a] °C	DO[b] mg/L	pH[c]		12-3-94 Day 4 DL Temp[a] °C	DO[b] mg/L	pH[c]	
Control	23	8.1	8.7		26	7.7	8.6		25	7.7	8.6		25	6.3	8.3	
Vehicle	23	8.2	8.7		26	7.8	8.7		25	7.5	8.7		25	7.5	8.6	
25	23	8.2	8.7		26	7.9	8.7		25	7.5	8.6		25	7.5	8.6	
55	23	8.2	8.8		26	7.8	8.7		25	7.7	8.7		25	7.7	8.6	
100	23	8.3	8.8		26	7.7	8.7		25	6.9	8.7		25	7.6	8.6	
200	23	8.3	8.8		26	7.7	8.7		25	7.4	~~8.7~~ 8.8		25	7.5	8.6	
400	23	8.3	8.8		26	7.8	8.7		~~25~~[D]	—	~~8.8~~		—	—	—	

[a] Temperature measured with ✓ mercury thermometer ___ digital probe

[b] Dissolved Oxygen Probe (YSI Dissolved Oxygen System Model 54 ARC), ABC material control #~~1900-700~~ 1905-730 ⊕

[c] Beckman Φ 34 pH meter, ABC Material Control #163-640, with Beckman model 39841 pH probe

Remarks: DEW 12-2-94 ⊕F BEG 12-3-94

Prepared by: [signature] Date: 11-30-94

Reviewed by: [signature] Date: 12-15-94

ABC 42201 P5 20082

ABC LABORATORIES, INC.

FORM # 28b (08/26/92)

WATER QUALITY

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

WLD Solns Concen. (µg/L)	12-4-94 Day 5 RW Temp[a] °C	DO[b] mg/L	pH[c]		12-5-94 Day 6 BFL Temp[a] °C	DO[b] mg/L	pH[c]		Day Temp[a] °C	DO[b] mg/L	pH[c]		Day Temp[a] °C	DO[b] mg/L	pH[c]	
Control	25	7.6	8.5		25	7.7	8.6									
Vehicle	25	7.9	8.6		25	7.4	8.6									
25	25	7.9	8.6		25	7.6	8.6									
55	25	7.9	8.6		25	7.6	8.6									
100	25	7.9	8.6		25	7.5	8.6									
200	25	7.7	8.5		25	7.5	8.5									
400	—	—	—		—	—	—	—								

[a] Temperature measured with ✓ mercury thermometer ___ digital probe
[b] Dissolved Oxygen Probe (YSI Dissolved Oxygen System Model 54 ARC), ABC material control #1900-700
[c] Beckman Φ 34 pH meter, ABC Material Control #163-640, with Beckman model 39841 pH probe

Remarks:

Prepared by: [signature] Date: 12-4-94

Reviewed by: [signature] Date: 12-14-94

ABC 42201 PG 00083

C. Definitive Testing

1. Biological Observations and Summaries
2. Changeover Procedure, System Observations, Food and Culture Records
3. Solution Water Chemistry

1. Biological Observations and Summaries

RANDOM GROUP ASSIGNMENT

TEST MATERIAL:
STUDY NUMBER : 42201
TEST GROUP : Daphnid placement
DATE : 11-16-1994

Analytical BioChemistry Labs
7200 East ABC Lane
P.O. Box 1097
Columbia, MO 65205

Group # 1

n	ID
1	VBJ
2	2G
3	VBH
4	5C
5	5B
6	4J
7	CE
8	VBB
9	CG
10	1B
11	3H
12	4D
13	1F
14	3E
15	CH
16	2J
17	2E
18	CB
19	5D
20	CF
21	2I
22	2A
23	4F
24	5F
25	CA
26	3A
27	1I
28	4A
29	CI
30	5I
31	2C
32	3F
33	4H
34	4I

DATA ENTERED BY: Brett Geger DATE: 11-16-94
REVIEWED BY: Dorothy England DATE: 11/16/94
STUDY DIRECTOR: Dorothy England DATE: 11/16/94

RANDOM GROUP ASSIGNMENT

TEST MATERIAL:
STUDY NUMBER : 42201
TEST GROUP : Daphnid placement
DATE : 11-16-1994

Analytical BioChemistry Labs
7200 East ABC Lane
P.O. Box 1097
Columbia, MO 65205

35	VBC
36	VBE
37	1H
38	3C
39	1A
40	CJ
41	5J
42	2B
43	CD
44	3G
45	VBF
46	VBA
47	2D
48	5G
49	1D
50	1C
51	2F
52	3D
53	4G
54	5A
55	3I
56	VBD
57	3B
58	VBG
59	5E
60	CC
61	VBI
62	3J
63	4C
64	1G
65	1J
66	4B
67	1E
68	4E
69	2H
70	5H

DATA ENTERED BY: Brett Geger DATE: 11-16-94
REVIEWED BY: [signature] DATE: 11/16/94
STUDY DIRECTOR: [signature] DATE: 11/16/94

ABC LABORATORIES, INC.

FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # CD-76)

Date Initiated: 12-6-94 Vessel Size: 50 mL Test Vol.: 30 mL Temperature: 25 ± 1 °C Test Conc.: Control

Day	12-6-94 0 BEG		12-7-94 1 BEG		12-8-94 2 BEG		12-9-94 3 BEG		12-10-94 4 BEG		12-11-94 5 BEG		12-12-94 6 BEG		12-13-94 7 BEG	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	–	1 N	1 N	–	1 N	–	1 N	4 N	1 N	8 N	1 N	8 N	1 N	~~15~~ 13 N ②	1 N	–
B	–	1 N	1 N	–	1 N	–	1 N	6 N	1 N	–	1 N	11 N	1 N	~~10~~ 12 N ②	1 N	9 N
C	–	1 N	1 N	–	1 N	–	1 N	6 N	1 N	–	1 N	14 N	1 N	13 N	1 N	10 N
D	–	1 N	1 N	–	1 N	–	1 N	D	1 N	10 N ①	1 N	9 N	1 N	~~8~~ 16 N ②	1 N	–
E	–	1 N	1 N	–	1 N	–	1 N	3 N	1 N	–	1 N	8 N	1 N	9 N	1 N	–
F	–	1 N	1 N	–	1 N	–	1 N	4 N	1 N	7 N	1 N	10 N	1 N	~~12~~ 15 N ②	1 N	–
G	–	1 N	1 N	–	1 N	–	1 N	D	1 N	6 N	1 N	9 N	1 N	~~10~~ 9 N ②	1 N	–
H	–	1 N	1 N	–	1 N	–	1 N	5 N	1 N	–	1 N	13 N	1 N	~~11~~ 10 N ②	1 N	10 N
I	–	1 N	1 N	–	1 N	–	1 N	5 N	1 N	8 N	1 N	10 N	1 N	~~10~~ 8 N ②	1 N	8 N
J	–	1 N	1 N	–	1 N	–	1 N	4 N	1 N	7 N	1 N	11 N	1 N	~~8~~ 13 N ②	1 N	7 N

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead

① E BEG 12-10-94 ② E BEG 12-12-94

Reviewed By: Janelle Dorney Date: 12-14-94

ABC 42201 PG 00033

ABC LABORATORIES, INC.

FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # CD-26)

Date Initiated: 12-6-94 Vessel Size: 50 mL Test Vol.: 30 mL Temperature: 25 ± 1 °C Test Conc.: Vehicle Blank

Day	12-6-94 0 BEG		12-7-94 1 BEG		12-8-94 2 BEG		12-9-94 3 BEG		12-10-94 4 BEG		12-11-94 5 BEG		12-12-94 6 BEG		12-13-94 7 BEG	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	–	1 N	1N	–	1N	–	1N	5N	1N	–	1N	15N	1N	16N	1N	11N
B	–	1 N	1N	–	1N	–	1N	5 N	1N	7N	1N	10 N	1N	12N	1N	–
C	–	1 N	1N	–	1N	–	1N	3 N	1N	–	1N	13 ③ +N	1N	14 N	1N	–
D	–	1 N	1N	–	1N	–	1N	4N+N ①	1N	8 N	1N	8 ③ +N	1N	9 N	1N	–
E	–	1 N	1N	–	1N	–	1N	–	1N	8 N	1N	9 ③ +N	1N	–	1N	15N
F	–	1 N	1N	–	1N	–	1N	6 N	1N	–	1N	12 N	1N	15N	1N	–
G	–	1 N	1N	–	1N	–	1N	3 N	1N	6 N	1N	10 N	1N	11 N	1N	9N
H	–	1 N	1N	–	1N	–	1N	3 N	1N	–	1N	11 N	1N	14 N	1N	7N
I	–	1 N	1N	–	1N	–	1N	5 N	1N	– + ②	1N	10 ③ +N	1N	10N	1N	11N
J	–	1 N	1N	–	1N	–	1N	4N	1N	9N	1N	8 N	1N	14N	1N	7N

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead

① E BEG 12-9-94 ② E BEG 12-10-94 ③ E BEG 12-11-94

Reviewed By: Jennifer Downey Date: 12-14-94

ABC 42201 PG 00089

ABC LABORATORIES, INC.

FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # CD-26)

Date Initiated: 12-6-94 Vessel Size: 50 mL Test Vol.: 30 mL Temperature: 25 ± 1 °C Test Conc.: Level 1 = 25 µg/L

Day	12-6-94 0 BEG		12-7-94 1 BEG		12-8-94 2 BEG		12-9-94 3 BEG		12-10-94 4 BEG		12-11-94 5 BEG		12-12-94 6 BEG		12-13-94 7 BEG	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	—	1 N	1N	—	1N	—	1N	5N	1N	—	1N	10N	1N	13N	1N	10N
B	—	1 N	1N	—	1N	—	1N	—	1N	6N	1N	9N	1N	10N	1N	1N
C	—	1 N	1N	—	1N	—	1N	5N	1N	—	1N	11N	1N	12N	1N	—
D	—	1 N	1N	—	1N	—	1N	4N	1N	—	1N	7N	1N	9N	1N	—
E	—	1 N	1N	—	1N	—	1N	4N	1N	—	1N	9N	1N	9N	1N	13N
F	—	1 N	1N	—	1N	—	1N	3N	1N	—	1N	7N	1N	15N	1N	8N
G	—	1 N	1N	—	1N	—	1N	3N	1N	—	1N	8N	1N	9N	1N	—
H	—	1 N	1N	—	1N	—	1N	—	1N	9N	1N	7N	1N	14N	1N	—
I	—	1 N	1N	—	1N	—	1N	4N	1N	—	1N	7N	1N	10N	1N	7N
J	—	1 N	1N	—	1N	—	1N	4N	1N	—	1N	11N	1N	7N	1N	—

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead

Reviewed By: [illegible] Date: 12-14-94

ABC 42201 35 00898

ABC LABORATORIES, INC. FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # CD-76)

Date Initiated: 12-6-94 Vessel Size: 50 mL Test Vol.: 30 mL Temperature: 25 ± 1 °C Test Conc.: Level 2 = 50 µg/L

Day	12-6-94 0 BEG		12-7-94 1 BEG		12-8-94 2 BEG		12-9-94 3 BEG		12-10-94 4 BEG		12-11-94 5 BEG		12-12-94 6 BEG		12-13-94 7 BEG	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	-	1N	1N	-	1N	-	1N	3N	1N	—	1N	7N	1N	15N	1N	—
B	-	1N	1N	-	1N	-	1N	—	1N	6N	1N	8N	1N	12N	1N	—
C	-	1N	1N	-	1N	-	1S	—	1S	—	1S	—	1S	—	1S	—
D	-	1N	1N	-	1N	-	1N	—	1N	8N	1N	8N	1N	8N	1N	—
E	-	1N	1N	-	1N	-	1N	—	1N	—	1N	7N	1N	9N	1N	—
F	-	1N	1N	-	1N	-	1N	—	1N	—	1N	9N	1N	10N	1N	—
G	-	1N	1N	-	1N	-	1N	—	1N	5N	1N	7N	1N	9N	1N	—
H	-	1N	1N	-	1N	-	1N	3N	1N	6N	1N	10N	1N	9N	1N	—
I	-	1N	1N	-	1N	-	1N	—	1N	5N	1N	5N	1N	7N	1N	—
J	-	1N	1N	-	1N	-	1N	—	1N	5N	1N	8N	1N	12N	1N	—

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead, S = SMALL
① E BEG 12-10-94

Reviewed By: [signature] Date: 12-14-94

ABC 42281 PG 00091

ABC LABORATORIES, INC. FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # [illegible])

Date Initiated: 12-6-94 Vessel Size: 50 mL Test Vol.: 30 mL Temperature: 25 ± 1 °C Test Conc.: Level 3 = 100 µg/L

Day	12-6-94 0 BEC		12-7-94 1 BEC		12-8-94 2 BEC		12-9-94 3 BEC		12-10-94 4 BEC		12-11-94 5 BEC		12-12-94 6 BEC		12-13-94 7 BEC	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	–	1 N	1N	–	1N	–	1N	3 N	1N	–	1N	–	1N	–	1N	–
B	–	1 N	1N	–	1N	–	1N	–	1N	–	1N	4 N	1N	–	1N	–
C	–	1 N	1N	–	1N	–	1N	4 N	1N	–	1D					
D	–	1 N	1N	–	1N	–	1N	–	1S	–	1S	–	1N	–	1N	–
E	–	1 N	1N	–	1N	–	1N	3 N	1N	–	1N	1P	1N	–	1N	–
F	–	1 N	1N	–	1N	–	1N	–	1D							
G	–	1 N	1N	–	1N	–	1N	2 N	1N	–	1N	4 N	1D			
H	–	1 N	1N	–	1N	–	1N	3 N	1N	–	1N	4 N	1N	–	1N	–
I	–	1 N	1N	–	1N	–	1N	–	1N	1N	1N	2 N	1N	–	1N	–
J	–	1 N	1N	–	1N	–	1N	4 N	1N	–	1N	3 N	1N	–	1N	–

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead, S = SMALL, P = Pale

Reviewed By: Janelle Downing Date: 12-14-94

ABC 42201 PG 00092

ABC LABORATORIES, INC. FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # 60-26)

Date Initiated: 12-6-94 Vessel Size: 50 mL Test Vol.: 30 mL Temperature: 25 ± 1 °C Test Conc.: Level 4 = 200 µg/L

Day	12-6-94 0 BEG		12-7-94 1 BEG		BEG 12-8-94 2		12-9-94 3 BEG		12-10-94 4 BEG		12-11-94 5 BEG		6		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	–	1 N	1N	–	1N	–	1N	–	1P	–	1D					
B	–	1 N	1N	–	1N	–	1N	–	1P	–	1D					
C	–	1 N	1N	–	1N	–	1S	–	1S,P	–	1D					
D	–	1 N	1N	–	1D											
E	–	1 N	1N	–	1N	–	1N	–	1D							
F	–	1 N	1N	–	1D											
G	–	1 N	1N	–	1D											
H	–	1 N	1N	–	1D											
I	–	1 N	1N	–	1D											
J	–	1 N	1N	–	1P	–	1D	–								

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead, P = Pale, S = Small

Reviewed By: [signature] Date: 12-14-94

ABC 42201 PG 00093

ABC LABORATORIES, INC. FORM # 84 (8/27/91)

CERIODAPHNIA CHRONIC STUDY OBSERVATIONS

Test Material: _____ Protocol No.: 8606 Study #: 42201

Study Director: Dorothy England Test Species: Ceriodaphnia dubia (Lot # LD 76)

Date Initiated: 12-6-94 Vessel Size: 50 mL Test Vol.: 30 mL Temperature: 25 ± 1 °C Test Conc.: Level 5 = 400 µg/L

Day	12-6-94 0 BEG		12-7-94 1 BEG		12-8-94 2 BEG		3		4		5		6		7	
Rep	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar	Adult	# Instar
A	–	1 N	1D	–												
B	–	1 N	1D	–												
C	–	1 N	1D	–												
D	–	1 N	1P	–	1D	–										
E	–	1 N	1D	–												
F	–	1 N	1D	–												
G	–	1 N	1P	–	1D	–										
H	–	1 N	1P	–	1D	–										
I	–	1 N	1P	–	1D	–										
J	–	1 N	1P	–	1D	–										

Comments: The following abbreviations will be used for observations: N = Normal, D = Dead, P = Pale

Reviewed By: Jeanette Dunning Date: 12-11-94

ABC LABORATORIES, INC.

FORM # 96 (1/7/92)

ADULT REPRODUCTION DAYS CALCULATION

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Test Level: Control	Days First Brood	# of Adults	X	Days # of Adults Reproduce	Adult Reproduction Days
Rep A	3	1	x	5	5
Rep B	3	1	x	5	5
Rep C	3	1	x	5	5
Rep D	4	1	x	4	4
Rep E	3	1	x	5	5
Rep F	3	1	x	5	5
Rep G	4	1	x	4	4
Rep H	3	1	x	5	5
Rep I	3	1	x	5	5
Rep J	3	1	x	5	5

Remarks:

Prepared By: Dorothy England Date: 1/3/95

Reviewed By: Brett Degel Date: 1-4-95

ADULT REPRODUCTION DAYS CALCULATION

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Test Level: Vehicle Blank	Days First Brood	# of Adults	X	Days # of Adults Reproduce	Adult Reproduction Days
Rep A	3	1	x	5	5
Rep B	3	1	x	5	5
Rep C	3	1	x	5	5
Rep D	3	1	x	5	5
Rep E	4	1	x	4	4
Rep F	3	1	x	5	5
Rep G	3	1	x	5	5
Rep H	3	1	x	5	5
Rep I	3	1	x	5	5
Rep J	3	1	x	5	5

Remarks:

Prepared By: Dorothy England Date: 1/3/95

Reviewed By: Brett Aegler Date: 1-4-95

ADULT REPRODUCTION DAYS CALCULATION

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Test Level: 25 µg/L	Days First Brood	# of Adults	X	Days # of Adults Reproduce	Adult Reproduction Days
Rep A	3	1	X	5	5
Rep B	4	1	X	4	4
Rep C	3	1	X	5	5
Rep D	3	1	X	5	5
Rep E	3	1	X	5	5
Rep F	3	1	X	5	5
Rep G	3	1	X	5	5
Rep H	4	1	X	4	4
Rep I	3	1	X	5	5
Rep J	3	1	X	5	5

Remarks:

Prepared By: Dorothy England Date: 1/3/95

Reviewed By: Brett Cogan Date: 1-4-95

ADULT REPRODUCTION DAYS CALCULATION

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Test Level: 50 µg/L	Days First Brood	# of Adults	X	Days # of Adults Reproduce	Adult Reproduction Days
Rep A	3	1	x	5	5
Rep B	4	1	x	4	4
Rep C	NA①	1	x	0	0
Rep D	4	1	x	4	4
Rep E	5	1	x	3	3
Rep F	5	1	x	3	3
Rep G	4	1	x	4	4
Rep H	3	1	x	5	5
Rep I	4	1	x	4	4
Rep J	4	1	x	4	4

Remarks: ① No broods produced. DCE 1/3/95

Prepared By: Dorothy England Date: 1/3/95

Reviewed By: Brett Geyer Date: 1-4-95

ADULT REPRODUCTION DAYS CALCULATION

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Test Level: 100 µg/L	Days First Brood	# of Adults	X	Days # of Adults Reproduce	Adult Reproduction Days
Rep A	3	1	x	5	5
Rep B	5	1	x	3	3
Rep C	3	1	x	3	3
Rep D	NA ①	1	x	0	0
Rep E	3	1	x	5	5
Rep F	NA ①	1	x	0	0
Rep G	3	1	x	4	4
Rep H	3	1	x	5	5
Rep I	4	1	x	4	4
Rep J	3	1	x	5	5

Remarks: ① No broods produced. DCE 1/3/95

Prepared By: Dorothy England Date: 1/3/95

Reviewed By: Brett Geger Date: 1-4-95

ABC LABORATORIES, INC. FORM # 96 (1/7/92)

ADULT REPRODUCTION DAYS CALCULATION

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Test Level: 200 µg/L	Days First Brood	# of Adults	X	Days # of Adults Reproduce	Adult Reproduction Days
Rep A	NA①	1	x	0	0
Rep B	↓	1	x	0	0
Rep C		1	x	0	0
Rep D		1	x	0	0
Rep E		1	x	0	0
Rep F		1	x	0	0
Rep G		1	x	0	0
Rep H		1	x	0	0
Rep I		1	x	0	0
Rep J		1	x	0	0

Remarks: ① No broods produced. DCE 1/3/95

Prepared By: Dorothy England Date: 1/3/95

Reviewed By: Brett Cegel Date: 1-4-95

ADULT REPRODUCTION DAYS CALCULATION

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Test Level: 400 µg/L	Days First Brood	# of Adults	X	Days # of Adults Reproduce	Adult Reproduction Days
Rep A	NA①	1	X	0	0
Rep B		1	X	0	0
Rep C		1	X	0	0
Rep D		1	X	0	0
Rep E		1	X	0	0
Rep F		1	X	0	0
Rep G		1	X	0	0
Rep H		1	X	0	0
Rep I		1	X	0	0
Rep J		1	X	0	0

Remarks: ① No broods produced. DCE 1/3/95

Prepared By: Dorothy England Date: 1/3/95

Reviewed By: Brett Legard Date: 1-4-95

ABC LABORATORIES, INC. FORM # 55 (11/25/91)

OBSERVATIONS AND/OR REMARKS FORM

Test Material: _____ Protocol No. 8606

Study Director: Dorothy England Study #: 42201

1/3/95 DCE

Instar Summary by Replicate

conc. (µg/L)	A	B	C	D	E	F	G	H	I	J	TOTAL
Control	33	38	43	35	20	36	24	38	39	42	348
V. Blank	47	34	30	29	32	33	37 ~~45~~ ①	35	36	42	357
25	38	26	28	20	35	33	20	30	28	22	280
50	25	26	0	24	16	19	21	28	17	25	201
100	3	4	4	0	4	0	6	7	3	7	38
200	0	0	0	0	0	0	0	0	0	0	0
400	0	0	0	0	0	0	0	0	0	0	0

Instar Summary by Day

conc (µg/L)	0	1	2	3	4	5	6	7	TOTAL
Control	0	0	0	37	46	103	118	44	348
V. Blank	0	0	0	38	38	106	115	60	357
25	0	0	0	32	15	86	108	39	280
50	0	0	0	6	35	69	91	0	201
100	0	0	0	19	1	18	0	0	38
200	0	0	0	0	0	0	0	0	0
400	0	0	0	0	0	0	0	0	0

① E. DCE 1/3/95

NOTE: Individual entries must be dated and initialed.

Reviewed by: Britt Geger Date: 1-4-95

Study Director: Dorothy England Date: 1/6/95

2. Changeover Procedure, System Observations, Food and Culture Records

OBSERVATIONS AND/OR REMARKS FORM

Test Material: _____ Protocol No. 8606

Study Director: Dorothy England Study #: 42201

DCE 11/16/94

Changeover Procedure for 7-Day Static Renewal with *Ceriodaphnia dubia*

1. Do daily observations on the test system (thermometer and datalogger temperatures, light reading, etc.).

2. On days 0-6, prepare new test solutions from stock solutions by transfer of stock aloquots to individual 1-L volumetric flasks. Bring the test solutions to volume using hard blended (HB) water. Mix thoroughly by inverting and swirling the flask.

3. Fill one of two sets of alternating test cups, place them in the test board, and place the test board in the 25°C waterbath. Transfer the remaining test solution into 1-L beakers, reserving enough solution to measure alkalinity, hardness, and conductivity on the new test solutions. Place the beakers into the waterbath. When new solutions are at test temperature (25 ± 1°C), record the temperature. Take dissolved oxygen (DO) and pH measurements on the new solutions, beginning with the control.

4. On days 1-7, do water chemistry on the old solutions after doing daily system observations. Do not pour out old solutions until all water chemistry is complete. When old solution water chemistry measurements are complete, empty the water chem beakers and refill them with new test solution. For all water chemistry measurements, take temperature before removing the beaker from the WB, then measure DO, then pH.

5. After new test solutions have reached test temperature, make biological observations and do changeover, beginning with the controls. a) Record adult obs. (Normal or Dead), and transfer adults to new solutions. b) Count neonates in old cups and record number N and D. c) Discard contents of cups after counting and briefly clean them with a brush and/or rinse them with d.i. water.

NOTE: Individual entries must be dated and initialed.

Reviewed by: Butt Cegan Date: 12-3-94

Study Director: Dorothy England Date: 1/5/95

DAILY OBSERVATIONS, SAMPLE AND FEEDING SCHEDULE FOR CERIODAPHNIA DUBIA STATIC RENEWAL STUDIES

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Day of Week		TUES	WED	THURS	FRI	SAT	SUN	MON
Date		12-6-94	12-7-94	12-8-94	12-9-94	12-10-94	12-11-94	12-12-94
Study Day		0	1	2	3	4	5	6
Observer ID		BEG	BEG	BEG	BEG	BEG	BEG	BEG
Light Reading[b] (Lux/Footcandles*)		734.5 / 68.2	748.3 / 69.5	708.3 / 65.8	714.7 / 66.4	728.3 / 67.7	707.6 / 65.7	750.5 / 69.7
Temperature (°C)[a] (Therm/Datalogger)		25.0 / 24.8	25.0 / 24.8	25.0 / 24.6	24.3 / 24.0	24.6 / 24.4	23.9 / 23.5	25.3 / 25.0
Water Chemistry	Old Soln	—	BEG	BEG	BEG	BEG	BEG	BEG
	New Soln	BEG	BEG	BEG	BEG	BEG	BEG	BEG
Analytical Samples		—	—	—	—	—	—	—
Feeding (ID & time)		11:40 AM BEG	11:30 AM BEG	11:15 AM BEG	11:00 AM BEG	10:00 AM BEG	10:00 AM BEG	11:00 AM BEG
Algal Lot #:		94-557 94-A57	94-557 94-A57	94-557 94-A57	94-557 94-A57	94-557 94-A57	94-557 94-A57	94-557 94-A57
Algal Concentration: (cells/mL)		~~2.25 x10^5~~ cells/mL 3.1 x10^7 ①	~~2.25 x10^5~~ cells/mL 3.1 x10^7 ①	~~2.25 x10^5~~ cells/mL 3.1 x10^7 ①	~~2.25 x10^5~~ cells/mL 3.1 x10^7 ①	~~2.25 x10^5~~ cells/mL 3.1 x10^7 ①	~~2.25 x10^5~~ cells/mL 3.1 x10^7 ①	~~2.25 x10^5~~ cells/mL 3.1 x10^7 ①
mL Algae Fed/Cup:		0.1 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL
YCT Lot #:		94-44 TCY	94-44 TCY	94-44 TCY	94-44 TCY	94-44 TCY	94-44 TCY	94-44 TCY
YCT Concentration: (mg/mL)		~~1.8 mg/mL~~ 1.7 ①	~~1.8 mg/mL~~ 1.7 ①	~~1.8 mg/mL~~ 1.7 ①	~~1.8 mg/mL~~ 1.7 ①	~~1.8 mg/mL~~ 1.7 ①	~~1.8 mg/mL~~ 1.7 ①	~~1.8 mg/mL~~ 1.7 ①
mL YCT Fed/Cup:		0.1 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL
Inspection								DCE

[a] Mercury thermometer / datalogger readings

[b] Light Reading measured with a LI-COR Model LI-189 Quantum/Radiometer/Photometer (ABC Material #163-341) and LI-COR Model Photometric Sensor (Serial #PH-4447) *Footcandles = LUX x 0.0929

Comments: ① C BEG 1-4-95

Reviewed By: Janelle Downing Date: [illegible]-94

DAILY OBSERVATIONS, SAMPLE AND FEEDING SCHEDULE FOR CERIODAPHNIA DUBIA STATIC RENEWAL STUDIES

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Day of Week		TUE						
Date		12-13-94						
Study Day		7						
Observer ID		BEG						
Light Reading[b] (Lux/Footcandles*)		723.1 / 67.5						
Temperature (°C)[a] (Therm/Datalogger)		26.1 / 25.8						
Water Chemistry	Old Soln	BEG						
	New Soln	—						
Analytical Samples		—						
Feeding (ID & time)		—						
Algal Lot #: Algal Concentration: (cells/mL) mL Algae Fed/Cup:		—						
YCT Lot #: YCT Concentration: (mg/mL) mL YCT Fed/Cup:		—						
Inspection		✓						

[a] Mercury thermometer / datalogger readings

[b] Light Reading measured with a LI-COR Model LI-189 Quantum/Radiometer/Photometer (ABC Material #163-341) and LI-COR Model Photometric Sensor (Serial #PH-4447) *Footcandles = LUX x 0.0929

Comments:

Reviewed By: Janelle Downing Date: 12-14-94

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 0

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: Dorothy England Date: 11/29/94

Target Weight 0.400 g

Final Gr. Weight 0.400 g

Tare Weight 0.000 g

Net Weight 0.400 g

Adj. Net Weight NA g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# BG-628)

Concentration 4.00 mg/mL

Balance calibrated with class C weights:

0.200 g (class C) + 0.000 g (tare) = 0.200 g (final wt.)

Balance Used: Mettler PM460 ABC Material Control No.: 1905-1020

PREPARATION OF WORKING STANDARD

Transferred By: Dorothy England Date: 11/29/94

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
4.00	0.625	10.0	DMF	0.250
4.00	1.25	10.0	DMF	0.500
4.00	2.50	10.0	DMF	1.00
4.00	5.00	10.0	DMF	2.00

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Weger Date: 11-29-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.0	0
Vehicle Blank	0 (DMF)	0.100	1.0	0
1	0.250	0.100	1.0	0.025
2	0.500	0.100	1.0	0.050
3	1.00	0.100	1.0	0.10
4	2.00	0.100	1.0	0.20
5	4.00	0.100	1.0	0.40

Comments:

Prepared by: Dorothy England Date: 11/29/94

Calculations checked by: Brett Weger Date: 11-29-94

Final Review by: Janelle Downing Date: 12-14-94

"This is an exact copy of The original document"

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 0

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: (1) Date: _____

Target Weight 0.400 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 4.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
4.00	0.625	10.0	DMF	0.250
4.00	1.25	10.0	DMF	0.500
4.00	2.50	10.0	DMF	1.00
4.00	5.00	10.0	DMF	2.00

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Cegar Date: 12-6-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.0	0
Vehicle Blank	0 (DMF)	0.100	1.0	0
1	0.250	0.100	1.0	0.025
2	0.500	0.100	1.0	0.050
3	1.00	0.100	1.0	0.10
4	2.00	0.100	1.0	0.20
5	4.00	0.100	1.0	0.40

Comments: (1) Same stock concentration used from the failed definitive made 11-29-94. DEC-12-6-94

Prepared by: Brett Cegar Date: 12-6-94

Calculations checked by: Janelle Browning Date: 12-14-94

Final Review by: Janelle Browning Date: 12-14-94

ABC LABORATORIES, INC. Form 27c (2/17/94)

COMPOUND PREPARATIONS DAY 1

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.400 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 4.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
4.00	0.625	10.0	DMF	0.250
4.00	1.25	10.0	DMF	0.500
4.00	2.50	10.0	DMF	1.00
4.00	5.00	10.0	DMF	2.00

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Cegan Date: 12-7-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.0	0
Vehicle Blank	0 (DMF)	0.100	1.0	0
1	0.250	0.100	1.0	0.025
2	0.500	0.100	1.0	0.050
3	1.00	0.100	1.0	0.10
4	2.00	0.100	1.0	0.20
5	4.00	0.100	1.0	0.40

Comments:

Prepared by: Brett Cegan Date: 12-7-94

Calculations checked by: Janice Downing Date: 12-14-94

Final Review by: Janice Downing Date: 12-14-94

ABC LABORATORIES, INC. Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 2

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.400 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 4.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
4.00	0.625	10.0	DMF	0.250
4.00	1.25	10.0	DMF	0.500
4.00	2.50	10.0	DMF	1.00
4.00	5.00	10.0	DMF	2.00

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Cryer Date: 12-8-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.0	0
Vehicle Blank	0 (DMF)	0.100	1.0	0
1	0.250	0.100	1.0	0.025
2	0.500	0.100	1.0	0.050
3	1.00	0.100	1.0	0.10
4	2.00	0.100	1.0	0.20
5	4.00	0.100	1.0	0.40

Comments:

Prepared by: Brett Cryer Date: 12-8-94

Calculations checked by: Janelle Lowery Date: 12-[illegible]

Final Review by: Janelle Lowery Date: 12-[illegible]

ABC LABORATORIES, INC.

Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 3

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.400 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 4.00 mg/mL

Balance calibrated with class C weights:

_____ g (class C) + _____ g (tare) = _____ g (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
4.00	0.625	10.0	DMF	0.250
4.00	1.25	10.0	DMF	0.500
4.00	2.50	10.0	DMF	1.00
4.00	5.00	10.0	DMF	2.00

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Geyer Date: 12-9-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.0	0
Vehicle Blank	0 (DMF)	0.100	1.0	0
1	0.250	0.100	1.0	0.025
2	0.500	0.100	1.0	0.050
3	1.00	0.100	1.0	0.10
4	2.00	0.100	1.0	0.20
5	4.00	0.100	1.0	0.40

Comments:

Prepared by: Brett Geyer Date: 12-9-94

Calculations checked by: Jennifer Lowing Date: 12-14-94

Final Review by: Jennifer Lowing Date: 12-14-94

ABC LABORATORIES, INC. Form 27c (2/17/94)

COMPOUND PREPARATIONS DAY 4

Test Material: $ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.400 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 4.00 mg/mL

Balance calibrated with class C weights:

_____ g (class C) + _____ g (tare) = _____ g (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
4.00	0.625	10.0	DMF	0.250
4.00	1.25	10.0	DMF	0.500
4.00	2.50	10.0	DMF	1.00
4.00	5.00	10.0	DMF	2.00

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Hegar Date: 12-10-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.0	0
Vehicle Blank	0 (DMF)	0.100	1.0	0
1	0.250	0.100	1.0	0.025
2	0.500	0.100	1.0	0.050
3	1.00	0.100	1.0	0.10
4	2.00	0.100	1.0	0.20
5	4.00	0.100	1.0	0.40

Comments:

Prepared by: Brett Hegar Date: 12-10-94

Calculations checked by: Jamie Downing Date: 12-[illegible]

Final Review by: Jamie Downing Date: 12-14-94

ABC LABORATORIES, INC. Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 5

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.400 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 4.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: ① ~~12-11-94~~

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
4.00	0.625	10.0	DMF	0.250
4.00	1.25	10.0	DMF	0.500
4.00	2.50	10.0	DMF	1.00
4.00	5.00	10.0	DMF	2.00

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Geiger Date: 12-11-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.0	0
Vehicle Blank	0 (DMF)	0.100	1.0	0
1	0.250	0.100	1.0	0.025
2	0.500	0.100	1.0	0.050
3	1.00	0.100	1.0	0.10
4	2.00	0.100	1.0	0.20
5	4.00	0.100	1.0	0.40

Comments: ① DEBEL 12-11-94

Prepared by: Brett Geiger Date: 12-11-94

Calculations checked by: Janelle Downing Date: 12-14-94

Final Review by: Janelle Downing Date: 12-14-94

ABC LABORATORIES, INC. Form 27c (2/17/94)

COMPOUND PREPARATIONS

DAY 6

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Lot/Batch #: 940519+01 Purity NG % ABC Ref #: TS-7604

PREPARATION OF PRIMARY STANDARD

Weighed by: _____ Date: _____

Target Weight 0.400 g

Final Gr. Weight _____ g

Tare Weight _____ g

Net Weight _____ g

Adj. Net Weight _____ g*

*Corrected for purity of primary standard

Dilution Volume 100 mL of DMF (lot# _____)

Concentration 4.00 mg/mL

Balance calibrated with class C weights:

_____ g + _____ g = _____ g
(class C) (tare) (final wt.)

Balance Used: _____ ABC Material Control No.: _____

PREPARATION OF WORKING STANDARD

Transferred By: _____ Date: _____

Conc. of Parent Sol. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)	Dilution Carrier	Final Concentration (mg/mL)
4.00	0.625	10.0	DMF	0.250
4.00	1.25	10.0	DMF	0.500
4.00	2.50	10.0	DMF	1.00
4.00	5.00	10.0	DMF	2.00

PREPARATION OF DEFINITIVE TEST CONCENTRATIONS

Transferred by: Brett Cegur Date: 12-12-94

	Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
Control	---	---	1.0	0
Vehicle Blank	0 (DMF)	0.100	1.0	0
1	0.250	0.100	1.0	0.025
2	0.500	0.100	1.0	0.050
3	1.00	0.100	1.0	0.10
4	2.00	0.100	1.0	0.20
5	4.00	0.100	1.0	0.40

Comments:

Prepared by: Brett Cegur Date: 12-12-94

Calculations checked by: [illegible] Date: 12-14-94

Final Review by: [illegible] Date: 12-14-94

ABC LABORATORIES, INC.

CHEMICAL/PHYSICAL MEASUREMENTS OF TEST SOURCE WATER

WATER SOURCE: AF Hard Blended A

TEMPERATURE: 21.6 °C

DO: 8.5 mg/L

pH: 8.5

ALKALINITY:* 160 mg/L

HARDNESS:* 150 mg/L

CONDUCTIVITY: 310 µmhos/cm

OTHER: —

INSTRUMENTS:

TEMPERATURE ☒ Mercury ☐ Digital

METER TYPE		BRAND	MODEL	MATERIAL CODE
DISSOLVED OXYGEN	☒	YSI	54ARC	1905-730
	☐			
pH Meter:	☒	BECKMAN	Φ34	163-640
Probe:		BECKMAN	39841	------
	☐			
CONDUCTIVITY:	☒	YSI	33	1905-520
	☐			

* Total alkalinity and hardness measured using a titrimetric method adapted from Standard Methods (mg/L as $CaCO_3$)

Analysis by: Jane Bowman Date: 12-6-94

Reviewed by: [signature] Date: 12 Dec 94

FORM # AQ-17 (9/15/93)

"This is an exact copy of The original document" By [signature] date 1/4/95

ABC LABORATORIES, INC.

CHEMICAL/PHYSICAL MEASUREMENTS OF TEST SOURCE WATER

WATER SOURCE: AF Hard Blended A

TEMPERATURE: 20.9 °C

DO: 8.3 mg/L

pH: ② ~~8.4~~ 8.4

ALKALINITY:* 164 mg/L

HARDNESS:* ① ~~104~~ 152 mg/L

CONDUCTIVITY: ① ~~152~~ 320 µmhos/cm

OTHER: —

INSTRUMENTS:

TEMPERATURE ☒ Mercury ☐ Digital

METER TYPE		BRAND	MODEL	MATERIAL CODE
DISSOLVED OXYGEN	☒	YSI	54ARC	1905-730
	☐			
pH Meter:	☒	BECKMAN	Φ34	163-640
Probe:		BECKMAN	39841	------
	☐			
CONDUCTIVITY:	☒	YSI	33	1905-520
	☐			

* Total alkalinity and hardness measured using a titrimetric method adapted from Standard Methods (mg/L as $CaCO_3$)

① E.J. 12.7.94

② E.J. 12.9.94

Analysis by: [signature] Date: 12.7.94

Reviewed by: Jane Bowman Date: 12-10-94

FORM # AQ-17 (9/15/93)

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ABC LABORATORIES, INC.

CHEMICAL/PHYSICAL MEASUREMENTS OF TEST SOURCE WATER

WATER SOURCE: AF Hard Blended A

TEMPERATURE: 21.1 °C

DO: 8.4 mg/L

pH: 8.6

ALKALINITY:* 160 mg/L

HARDNESS:* 150 mg/L

CONDUCTIVITY: 320 µmhos/cm

OTHER: —

INSTRUMENTS:

TEMPERATURE ☒ Mercury ☐ Digital

METER TYPE		BRAND	MODEL	MATERIAL CODE
DISSOLVED OXYGEN	☒	YSI	54ARC	1905-730
	☐			
pH Meter:	☒	BECKMAN	Φ34	163-640
Probe:		BECKMAN	39841	------
	☐			
CONDUCTIVITY:	☒	YSI	33	1905-520
	☐			

* Total alkalinity and hardness measured using a titrimetric method adapted from Standard Methods (mg/L as $CaCO_3$)

Analysis by: Jane Bowman Date: 12-8-94

Reviewed by: Brett Gepe Date: 12.21.94

FORM # AQ-17 (9/15/93)

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ABC 42281 PG 00117

ABC LABORATORIES, INC.

CHEMICAL/PHYSICAL MEASUREMENTS OF TEST SOURCE WATER

WATER SOURCE: AF Hard Blended A

TEMPERATURE: 20.6 °C

DO: 8.5 mg/L

pH: 8.6

ALKALINITY:* 170 mg/L

HARDNESS:* 158 mg/L

CONDUCTIVITY: 300 µmhos/cm

OTHER: —

INSTRUMENTS:

TEMPERATURE ☒ Mercury ☐ Digital

METER TYPE		BRAND	MODEL	MATERIAL CODE
DISSOLVED OXYGEN	☒	YSI	54ARC	1905-730
	☐			
pH Meter:	☒	BECKMAN	Φ34	163-640
Probe:		BECKMAN	39841	------
	☐			
CONDUCTIVITY:	☒	YSI	33	1905-520
	☐			

* Total alkalinity and hardness measured using a titrimetric method adapted from Standard Methods (mg/L as $CaCO_3$)

Analysis by: [signature] Date: 12.9.94

Reviewed by: Jane Bowman Date: 12-10-94

"This is an exact copy of The original document" By [signature] date 4/95

FORM # AQ-17 (9/15/93)

ABC LABORATORIES, INC.

CHEMICAL/PHYSICAL MEASUREMENTS OF TEST SOURCE WATER

WATER SOURCE: AF Hard Blended A

TEMPERATURE: 20.5 °C

DO: 8.5 mg/L

pH: 8.5

ALKALINITY:* 162 mg/L

HARDNESS:* 150 mg/L

CONDUCTIVITY: 300 µmhos/cm

OTHER: —

INSTRUMENTS:

TEMPERATURE ☒ Mercury ☐ Digital

METER TYPE		BRAND	MODEL	MATERIAL CODE
DISSOLVED OXYGEN	☒	YSI	54ARC	1905-730
	☐			
pH Meter:	☒	BECKMAN	Φ34	163-640
Probe:		BECKMAN	39841	------
	☐			
CONDUCTIVITY:	☒	YSI	33	1905-520
	☐			

* Total alkalinity and hardness measured using a titrimetric method adapted from Standard Methods (mg/L as $CaCO_3$)

Analysis by: Jane Bowman Date: 12-10-94

Reviewed by: Brett Wegner Date: 12-23-94

FORM # AQ-17 (9/15/93)

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ABC LABORATORIES, INC.

CHEMICAL/PHYSICAL MEASUREMENTS OF TEST SOURCE WATER

WATER SOURCE: AF Test Blended A

TEMPERATURE: 20.2 °C

DO: 8.5 mg/L

pH: 8.5

ALKALINITY:* 160 mg/L

HARDNESS:* 150 mg/L

CONDUCTIVITY: 300 µmhos/cm

OTHER: —

INSTRUMENTS:

TEMPERATURE ☒ Mercury ☐ Digital

METER TYPE		BRAND	MODEL	MATERIAL CODE
DISSOLVED OXYGEN	☒	YSI	54ARC	1905-730
	☐			
pH Meter:	☒	BECKMAN	Φ34	163-640
Probe:		BECKMAN	39841	------
	☐			
CONDUCTIVITY:	☒	YSI	33	1905-520
	☐			

* Total alkalinity and hardness measured using a titrimetric method adapted from Standard Methods (mg/L as $CaCO_3$)

Analysis by: Jan Bowman Date: 12-11-94

Reviewed by: Brett Legg Date: 12 21 94

FORM # AQ-17 (9/15/93)

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ABC LABORATORIES, INC.

CHEMICAL/PHYSICAL MEASUREMENTS OF TEST SOURCE WATER

WATER SOURCE: AF Hard Blended A

TEMPERATURE: 19.6 °C

DO: 8.5 mg/L

pH: 8.5

ALKALINITY:* 172 mg/L

HARDNESS:* 156 mg/L

CONDUCTIVITY: 300 µmhos/cm

OTHER: —

INSTRUMENTS:

TEMPERATURE ☒ Mercury ☐ Digital

METER TYPE		BRAND	MODEL	MATERIAL CODE
DISSOLVED OXYGEN	☒	YSI	54ARC	1905-730
	☐			
pH Meter:	☒	BECKMAN	Φ34	163-640
Probe:		BECKMAN	39841	------
	☐			
CONDUCTIVITY:	☒	YSI	33	1905-520
	☐			

* Total alkalinity and hardness measured using a titrimetric method adapted from Standard Methods (mg/L as $CaCO_3$)

"This is an exact copy of The original document"

Analysis by: [signature] Date: 12.12.94

Reviewed by: Brett Hegan Date: 12.23.94

FORM # AQ-17 (9/15/93)

ABC LABORATORIES, INC. FORM # 55 (11/25/91)

OBSERVATIONS AND/OR REMARKS FORM

Test Material: _____ Protocol No. 8606

Study Director: Dorothy England Study #: 42201

FOOD PREPARATION

11-29-94/BEG
(date) (ID)

100 mL of Yeast/Trout Chow (YTC or TCY)), lot #94-~~43~~ 44 ① TCY, at 5.0 mg/mL suspended solids were diluted with 200 mL of hard blended water to produce a dilution of ~~2.5~~ 1.7 ② mg/mL suspended solids.

90 mL of a mixture of *Selenastrum capricornutum* and *Ankistrodesmus falcatus*, lot #94-557, 94A57, at 1.0 X 10^8 cells/mL were diluted with 200 mL of LabConCo water to get a dilution of ~~4.5 x 10~~ 3.1 x 10^7 ② cells/mL.

_____/____
(date) (ID)

____ mL of Yeast/Trout Chow (YTC or TCY)), lot #_____ TCY, at 5.0 mg/mL suspended solids were diluted with ____ mL of hard blended water to produce a dilution of _____ mg/mL suspended solids.

____ mL of a mixture of *Selenastrum capricornutum* and *Ankistrodesmus falcatus*, lot #_____, at 1.0 X 10^8 cells/mL were diluted with ____ mL of LabConCo water to get a dilution of _____ cells/mL.

① DEBEG 11-29-94 ② CBEG 1-4-95

NOTE: Individual entries must be dated and initialed.

Reviewed by: Dorothy England Date: 1/4/95

Study Director: Dorothy England Date: 1/4/95

ABC LABORATORIES, INC.

INVERTEBRATE CULTURE RECORD

Species: *Ceriodaphnia dubia* Lot #: CD-76

Date Initiated: 11-29-94 # Used to Initiate: 30

Lot # Initiated from: 94-CD-69 Culture Volume: ~1000 mL

Food Lot # 94-A57 S57 94-A58 S58 A59 S59 A60 S60

Date/ID	Temp.* °C	Changed Over	Fed	No. Adult Live	~Neonates Present	Comments
11-29-94 RJ	25	3:00pm	✓	—	30	
11-30 FH	25	—	✓	—	—	
12-1 RJ	25	—	✓	—	—	
12-2 RJ	25	2:15pm	✓	30	150	
12-3 CJP	25	—	✓	—	—	
12-4 SUH	25	—	✓	—	—	
12-5 RJ	25	1:45pm	✓	30	350	
12-6 FH	25	—	✓	—	—	Removed ~80 for Study # 42201
12-7 RJ	25	2:30pm	✓	30	350	
12-8 RJ	25	—	✓	—	—	
12-9 RJ	25	2:30pm	✓	30	350	
12-10 CJP	25	—	✓	—	—	
12-11 CJP	25	—	✓	—	—	
12-12 RJ	25	2:30pm	✓	30	350	

* Temperature measured with a mercury thermometer

Note: Cultures may be fed one or more of the following:
- ~0.25-0.5 mL of a 5.0 mg/mL suspension of trout chow yeast
- ~1.0 mL suspension of dried algae (spirulina and/or chlorella)
- ~1.0-3.0 mL of a 1.0 * 10^8 cells/mL concentration of live algae (The algae consists of Selenastrum capricornutum, Ankistrodesmus falcatus, *Chlamydomonas reinhardtii* and/or *Chlorella pyrenoidosa*.)

Prepared by: [signature] Date: 11-29-94

Reviewed by: Dorothy England Date: 1/4/95

FORM # 396b (04/18/94)

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3. Solution Water Chemistry

Continuous Temperature Graph

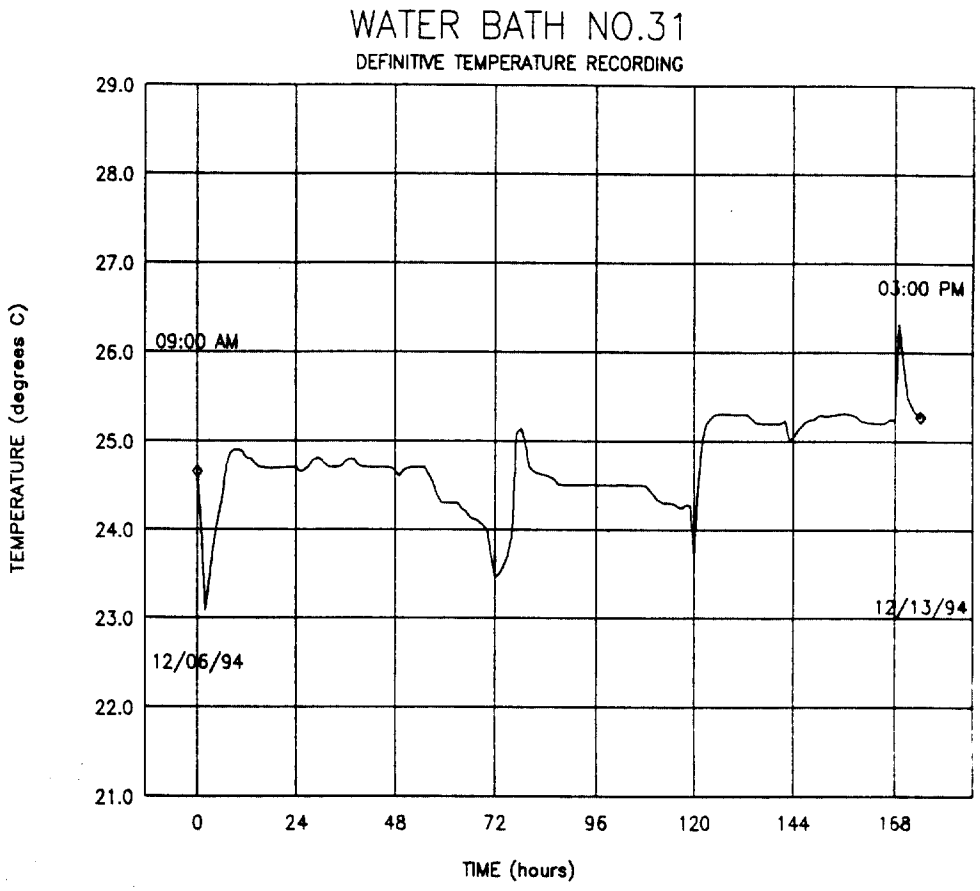
Test Material: _____

ABC Study Number: 42201

Protocol Number: 8606

Lab Form No: AQ9 (4/15/91)
ABC Laboratories
P.O.Box 1097, 7200 East ABC Lane
Columbia, Missouri 65205

Data Logger
Material Cont. No.: 1905-415



Comments: _____

Prepared By: Brett Ceger Date: 12-15-94

Reviewed By: Dorothy England Date: 1/3/95

Approved By: Dorothy England Date: 1/3/95

ABC LABORATORIES, INC. FORM # AQ141 (01/11/94)

WATER QUALITY

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

New solns	12-6-94				Day 0	BEG/III	12-7-94				Day 1	BEG	12-8-94				Day 2	BEG
Concentration (µg/L)	Temp[a]	DO[b]	pH[c]	Total Hard[d]	Total Alk[d]	Cond[e]	Temp	DO	pH	Total Hard	Total Alk	Cond	Temp	DO	pH	Total Hard	Total Alk	Cond
Control	23	8.4	8.4	158	170	300	23	8.1	8.5	158	178	300	25	8.2	8.5	160	164	340
Vehicle Blank	23	8.4	8.4	154	170	300	23	8.1	8.5	158	164	300	25	8.2	8.6	158	170	340
Level 1 = 25	23	8.4	8.4	160	178	300	23	8.0	8.5	158	172	300	25	8.2	8.6	158	164	340
Level 2 = 50	23	8.4	8.4	164	152	300	23	8.0	8.5	156	170	300	25	8.1	8.6	158	164	340
Level 3 = 100	23	8.3	8.4	156	170	300	23	8.0	8.5	160	180	300	25	7.4	8.6	150	172	340
Level 4 = 200	23	8.3	8.3	154	158	300	23	8.0	8.5	160	178	300	25	8.0	8.6	160	170	340
Level 5 = 400	23	8.3	8.3	156	168	300	23	8.1	8.5	154	170	300	~~25~~	—	—	—	—	—

a Temperature (°C) measured with a mercury thermometer
b Dissolved Oxygen (mg/L) measured using a YSI Dissolved Oxygen System Model 54 ARC) ABC material control #1905-730
c pH measured using a Beckman Φ34 pH meter, ABC material code 163-640 used with a Beckman Model 39841 probe
d Hardness and total alkalinity (mg/L as $CaCo_3$) analyzed using titrimetric method adopted from Standard Methods
e Conductivity (µmhos/cm) measured using a YSI 33 SCT Meter, ABC Material Control #1905-520

Remarks: (1) BEG 12-8-94

Prepared by: Brett Creger Date: 12-6-94

Reviewed by: [illegible] Date: 12-14-94

ABC 42201 PG 00126

ABC LABORATORIES, INC. FORM # AQ141 (01/11/94)

WATER QUALITY

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

New Solns	12-9-94			Day 3	BEG		12-10-94			Day 4	BEG		12-11-94			Day 5	BEG	
Concentration (µg/L)	Temp[a]	DO[b]	pH[c]	Total Hard[d]	Total Alk[d]	Cond[e]	Temp	DO	pH	Total Hard	Total Alk	Cond	Temp	DO	pH	Total Hard	Total Alk	Cond
Control	25	8.2	8.4	~~160~~ 153	~~180~~ 173	340	25	8.4	8.4	160	170	320	24	8.4	8.4	158	164	320
Vehicle Blank	25	8.2	8.4	160	168	340	25	8.4	8.4	160	170	320	24	8.4	8.4	160	160	320
Level 1 = 25	25	8.1	8.4	156	170	340	25	8.3	8.4	158	168	320	24	8.3	8.4	160	166	320
Level 2 = 50	25	8.2	8.4	160	178	340	25	8.3	8.4	158	168	320	24	8.4	8.4	154	168	320
Level 3 = 100	25	8.3	8.5	158	170	340	25	8.4	8.4	160	174	320	24	8.4	8.4	160	170	320
Level 4 = 200	25	8.1	8.5	162	180	340	25	8.4	8.5	160	178	320	—	—	—	—	—	—
Level 5 = 400	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

a Temperature (°C) measured with a mercury thermometer
b Dissolved Oxygen (mg/L) measured using a YSI Dissolved Oxygen System Model 54 ARC) ABC material control #1905-730
c pH measured using a Beckman Φ34 pH meter, ABC material code 163-640 used with a Beckman Model 39841 probe
d Hardness and total alkalinity (mg/L as $CaCo_3$) analyzed using titrimetric method adopted from Standard Methods
e Conductivity (µmhos/cm) measured using a YSI 33 SCT Meter, ABC Material Control #1905-520

Remarks: ①EBEG 12-9-94

Prepared by: Brett Lego Date: 12-9-94

Reviewed by: [illegible] Date: 12-14-94

ABC 42201 PG 00127

ABC LABORATORIES, INC. FORM # AQ141 (01/11/94)

WATER QUALITY

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

New Solns	12-12-94 Day 6 BEG						Day						Day					
Concentration (µg/L)	Temp[a]	DO[b]	pH[c]	Total Hard[d]	Total Alk[d]	Cond[e]	Temp	DO	pH	Total Hard	Total Alk	Cond	Temp	DO	pH	Total Hard	Total Alk	Cond
Control	23	8.3	8.5	160	170	300												
Vehicle Blank	23	8.3	8.5	154	172	300												
Level 1 = 25	23	8.5	8.5	158	164	300												
Level 2 = 50	23	8.4	8.5	150	170	300												
Level 3 = 100	23	8.4	8.5	160	170	300												
Level 4 = 200	—	—	—	—	—	—												
Level 5 = 400	—	—	—	—	—	—												

a Temperature (°C) measured with a mercury thermometer
b Dissolved Oxygen (mg/L) measured using a YSI Dissolved Oxygen System Model 54 ARC) ABC material control #1905-730
c pH measured using a Beckman Φ34 pH meter, ABC material code 163-640 used with a Beckman Model 39841 probe
d Hardness and total alkalinity (mg/L as $CaCo_3$) analyzed using titrimetric method adopted from Standard Methods
e Conductivity (µmhos/cm) measured using a YSI 33 SCT Meter, ABC Material Control #1905-520

Remarks:

Prepared by: Brett Geiger Date: 12-12-94

Reviewed by: James Bowling Date: 12-14-94

ABC LABORATORIES, INC. FORM # 28b (08/26/92)

WATER QUALITY

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

OLD Solns	12-7-94 Day 1 BEG				12-8-94 Day 2 BEG				12-9-94 Day 3 BEG				12-10-94 Day 4 BEG			
Concen. (µg/L)	Temp[a] °C	DO[b] mg/L	pH[c]		Temp[a] °C	DO[b] mg/L	pH[c]		Temp[a] °C	DO[b] mg/L	pH[c]		Temp[a] °C	DO[b] mg/L	pH[c]	
Control	25	7.5	8.6		25	7.9	8.6		25	7.8	8.5		25	7.7	8.5	
Vehicle	25	7.5	8.6		25	7.2	8.7		25	7.6	8.4		25	7.1	8.5	
25	25	7.4	8.6		25	7.7	8.8		25	7.7	8.5		25	7.6	8.5	
④ ~~50~~ 50	25	7.4	8.6		25	7.8	8.8		25	7.4	8.2		25	7.5	8.5	
100	25	7.3	8.6		25	6.5	8.3		25	6.9	8.4		25	~~7.7~~	~~8.6~~	7.7 ②
200	25	7.3	8.6		25	7.4	8.7		25	7.4	8.6		25	7.7	8.6	
400	25	7.4	8.6		25	6.9	8.6		—	—	—	—	—	—	—	—

[a] Temperature measured with ✓ mercury thermometer ___ digital probe

[b] Dissolved Oxygen Probe (YSI Dissolved Oxygen System Model 54 ARC), ABC material control ~~#1900-700~~ 1905-730 ①

[c] Beckman Φ 34 pH meter, ABC Material Control #163-640, with Beckman model 39841 pH probe

Remarks: ① F BEG 12-7-94 ② clarification BEG 1-4-94 ③ D-DCE 1/4/95 ④ F-DCE 1/6/95

Prepared by: Brett Beger Date: 12-7-94

Reviewed by: Jeanette Dinning DCE 1/6/95 Date: 12-14-94 1/4/95 ③

ABC 42201 PG 000129

ABC LABORATORIES, INC. FORM # 28b (08/26/92)

WATER QUALITY

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

OLD Solns Concen. (µg/L)	12-11-94 Day 5 BEG Temp[a] °C	DO[b] mg/L	pH[c]		12-12-94 Day 6 BEG Temp[a] °C	DO[b] mg/L	pH[c]		12-13-94 Day 7 BEG Temp[a] °C	DO[b] mg/L	pH[c]		Day Temp[a] °C	DO[b] mg/L	pH[c]	
Control	25	7.7	8.5		25	7.8	8.5		26	7.7	8.5					
Vehicle	25	6.7	8.5		25	7.0	8.2		26	7.3	8.2					
25	25	7.7	8.5		25	6.7	8.2		26	7.3	8.1					
② ~~55~~ 50	25	7.8	8.4		25	6.7	8.4		26	7.1	8.1					
100	25	7.5	8.4		25	7.1	8.3		26	7.2	8.2					
200	25	7.0	8.5		—	—	—	—	—	—	—	—				
400	—	—	—	—	—	—	—	—	—	—	—	—				

[a] Temperature measured with ✓ mercury thermometer ___ digital probe

[b] Dissolved Oxygen Probe (YSI Dissolved Oxygen System Model 54 ARC), ABC material control ~~#1900-700~~ 1905-730 ①

[c] Beckman Φ 34 pH meter, ABC Material Control #163-640, with Beckman model 39841 pH probe

Remarks: ① F BEG 12-11-94 ② F DCC 1/6/95

Prepared by: Brett Cooper Date: 12-11-94

Reviewed by: [illegible] Date: 12-14-94 DC 1/6/95

ABC 42201 PG 000138

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D. Statistical Analyses

1. LC_{50}, Concentration Response, and Chronic Value (MATC) Calculations
2. Survival
3. Mean Young per Adult
4. Days to First Brood
5. Young per Adult Reproduction Day

1. LC_{50}, Concentration Response, and Chronic Value (MATC) Calculations

LC_{50} Calculations

ABC Study No. : 42201 Compound Name : Species : Ceriodaphnia dubia Exposure Period: 7 days Study Director : Dorothy England	Analytical Bio-Chemistry Labs 7200 East ABC Lane P.O. Box 1097 Columbia, Missouri 65205 01-04-1995 Page # 1

——RESULTS CALCULATED USING THE BINOMIAL METHOD——

CONC. ug/L	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
400	10	10	100	9.765620529651642D-02
200	10	10	100	9.765620529651642D-02
100	10	3	30	17.18748994271619
50	10	0	0	9.765620529651642D-02
25	10	0	0	9.765620529651642D-02

THE BINOMIAL TEST SHOWS THAT 50 AND 200 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THIS LIMIT IS >95 PERCENT

AN APPROXIMATE LC_{50} OF 117.1433563232422 ug/L IS OBTAINED BY NONLINEAR INTERPOLATION BETWEEN 100 AND 200

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE OR PROBIT METHOD CAN GIVE STATISTICALLY SOUND RESULTS.

Method Reported: [✓] Binomial [ ] Moving Average [ ] Probit

Note: Method selected is that which gives the narrowest confidence limits for LC_{50}.

PREPARED BY: Dorothy England DATE: 04 Jan 95

REVIEWED BY: Jarred Downing DATE: 1/5/95

DOSE-RESPONSE CALCULATIONS

Test Material: _____

ABC Study Number: 42201

Protocol Number: 8606

Study Director: Dorothy England

Data at 7 days ~~Hours~~ **of Exposure.** DF-DCE 04JAN95

The following calculations provide a least squares estimate of the slope of the dose response line.

$$Y = m\ X + b$$

where: Y = Percent Mortality in Probits
b = Y-intercept
X = Log Concentration
m = Slope of Dose Response Line

Actual Conc.	Log Conc.	Percent Mort.	Probits	Calc. Probits
50	1.699	0.0	2.67	2.50
100	2.000	30.0	4.48	4.83
200	2.301	100.0	7.33	7.16

Regression Output:

Constant	-10.653
Std Err of Y Est	0.42457
R Squared	0.98366
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	7.74009
Std Err of Coef.	0.99731

Y = 7.740 X + (-10.654)

Dose Response Slope = 7.74

Correlation Coefficient (r) = 0.99

NOTE: These results are only intended to illustrate the dose-response line and determine the slope for this acute toxicity data. Probits for 0 and 100% mortality were set at 2.67 and 7.33, respectively. All other probits were obtained from: Finney, D. J. 1964. Statistical Methods in Biological Assay, 2nd Edition. Griffin, London.

Data Entered By: Dorothy England **Date:** 04JAN95

Reviewed By: Jewell Digby **Date:** 1-5-95

OBSERVATIONS AND/OR REMARKS FORM

Test Material:

ABC Study Number: 42201 - Survival

Protocol Number: 8606

ABC Study Director: Dorothy England

Lab Form No.: 55 (4/03/89)

Analytical Bio-Chemistry Labs
P. O. Box 1097, 7200 ABC Lane
Columbia, Missouri 65202

Date/Initials	Comments
01-02-1995 / DCE	Calculation of the Geometric Mean (GM) of the Maximum Acceptable Toxicant Concentration (MATC) Limits NOEC = No Observed Effect Concentration LOEC = Lowest Observed Effect Concentration $GM = \text{Antilog} \frac{(\log(NOEC) + \log(LOEC))}{2}$ Data Entry: NOEC = 50 ug/L LOEC = 100 ug/L Result: GM = 70.710678 ug/L

OD - DCE 1/4/95

Prepared By: Dorothy England Date: 01-~~02~~-1995 004

Checked By: Janelle Downey Date: 1/5/95

OBSERVATIONS AND/OR REMARKS FORM

Test Material:

ABC Study Number: 42201 - First Brood ③ and Mean Young per Adult

Protocol Number: 8606

ABC Study Director: Dorothy England

Lab Form No.: 55 (4/03/89)

Analytical Bio-Chemistry Labs
P. O. Box 1097, 7200 ABC Lane
Columbia, Missouri 65202

Date/Initials	Comments
01-02-1995 / DCE	Calculation of the Geometric Mean (GM) of the Maximum Acceptable Toxicant Concentration (MATC) Limits NOEC = No Observed Effect Concentration LOEC = Lowest Observed Effect Concentration $GM = \text{Antilog} \frac{(\log(NOEC) + \log(LOEC))}{2}$ Data Entry: NOEC = 25 ug/L LOEC = 50 ug/L Result: GM = 35.355339 ug/L

① D-DCE 1/4/95 ⑤ Entry for Clarification. DCE 1/6/95

Prepared By: Dorothy England Date: 01-~~02~~-1995 ① 04

Checked By: Janelle Deremy Date: 1/5/95 JD 1/6/95

2. Survival

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING
PRINTOUT OF RAW DATA

TREATMENT	REPLICATE	INITIAL NUMBER	FINAL NUMBER	PERCENT SURVIVAL
LEVEL-1	A	1	1	100
LEVEL-1	B	1	1	100
LEVEL-1	C	1	1	100
LEVEL-1	D	1	1	100
LEVEL-1	E	1	1	100
LEVEL-1	F	1	1	100
LEVEL-1	G	1	1	100
LEVEL-1	H	1	1	100
LEVEL-1	I	1	1	100
LEVEL-1	J	1	1	100
LEVEL-2	A	1	1	100
LEVEL-2	B	1	1	100
LEVEL-2	C	1	1	100
LEVEL-2	D	1	1	100
LEVEL-2	E	1	1	100
LEVEL-2	F	1	1	100
LEVEL-2	G	1	1	100
LEVEL-2	H	1	1	100
LEVEL-2	I	1	1	100
LEVEL-2	J	1	1	100
LEVEL-3	A	1	1	100
LEVEL-3	B	1	1	100
LEVEL-3	C	1	0	0
LEVEL-3	D	1	1	100
LEVEL-3	E	1	1	100
LEVEL-3	F	1	0	0
LEVEL-3	G	1	0	0
LEVEL-3	H	1	1	100
LEVEL-3	I	1	1	100
LEVEL-3	J	1	1	100
LEVEL-4	A	1	0	0
LEVEL-4	B	1	0	0
LEVEL-4	C	1	0	0
LEVEL-4	D	1	0	0
LEVEL-4	E	1	0	0
LEVEL-4	F	1	0	0
LEVEL-4	G	1	0	0
LEVEL-4	H	1	0	0
LEVEL-4	I	1	0	0
LEVEL-4	J	1	0	0
LEVEL-5	A	1	0	0
LEVEL-5	B	1	0	0
LEVEL-5	C	1	0	0
LEVEL-5	D	1	0	0
LEVEL-5	E	1	0	0

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: Janelle Downey DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING
PRINTOUT OF RAW DATA

TREATMENT	REPLICATE	INITIAL NUMBER	FINAL NUMBER	PERCENT SURVIVAL
LEVEL-5	F	1	0	0
LEVEL-5	G	1	0	0
LEVEL-5	H	1	0	0
LEVEL-5	I	1	0	0
LEVEL-5	J	1	0	0
CONTROL	A	1	1	100
CONTROL	B	1	1	100
CONTROL	C	1	1	100
CONTROL	D	1	1	100
CONTROL	E	1	1	100
CONTROL	F	1	1	100
CONTROL	G	1	1	100
CONTROL	H	1	1	100
CONTROL	I	1	1	100
CONTROL	J	1	1	100
VEHICLE BLANK	A	1	1	100
VEHICLE BLANK	B	1	1	100
VEHICLE BLANK	C	1	1	100
VEHICLE BLANK	D	1	1	100
VEHICLE BLANK	E	1	1	100
VEHICLE BLANK	F	1	1	100
VEHICLE BLANK	G	1	1	100
VEHICLE BLANK	H	1	1	100
VEHICLE BLANK	I	1	1	100
VEHICLE BLANK	J	1	1	100

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J Downey DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 05JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON OF CONTROL GROUPS FOR NUMBER SURVIVING
MEAN PERCENT - BY CONTROL GROUP

Analysis Variable : SURVIVAL

---------- LEVEL=CONTROL ----------

N Obs	Minimum	Maximum	Mean
10	100.0	100.0	100.0

---------- LEVEL=VEHICLE BLANK ----------

N Obs	Minimum	Maximum	Mean
10	100.0	100.0	100.0

ANALYSIS BY: Dorothy England DATE: 05JAN95

REVIEWED BY: DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 05JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON OF CONTROL GROUPS FOR NUMBER SURVIVING
COMPARISON OF CONTROL VS. VEHICLE BLANK
IF Prob IS LESS THAN .05 FOR CHI-SQUARE AND/OR FISHER'S EXACT (2-TAIL)
AND IF DIFFERENCE IS BIOLOGICALLY MEANINGFUL
THEN USE CONTROL OR VEHICLE BLANK AS CONTROL

TABLE OF LEVEL BY STATUS

LEVEL	STATUS ALIVE	Total
CONTROL Frequency Expected Percent Row Pct	10 10 50.00 100.00	10 50.00
VEHICLE BLANK Frequency Expected Percent Row Pct	10 10 50.00 100.00	10 50.00
Total	20 100.00	20 100.00

ANALYSIS BY: Dorothy England DCE DATE: 05JAN95

REVIEWED BY: J Downing DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING

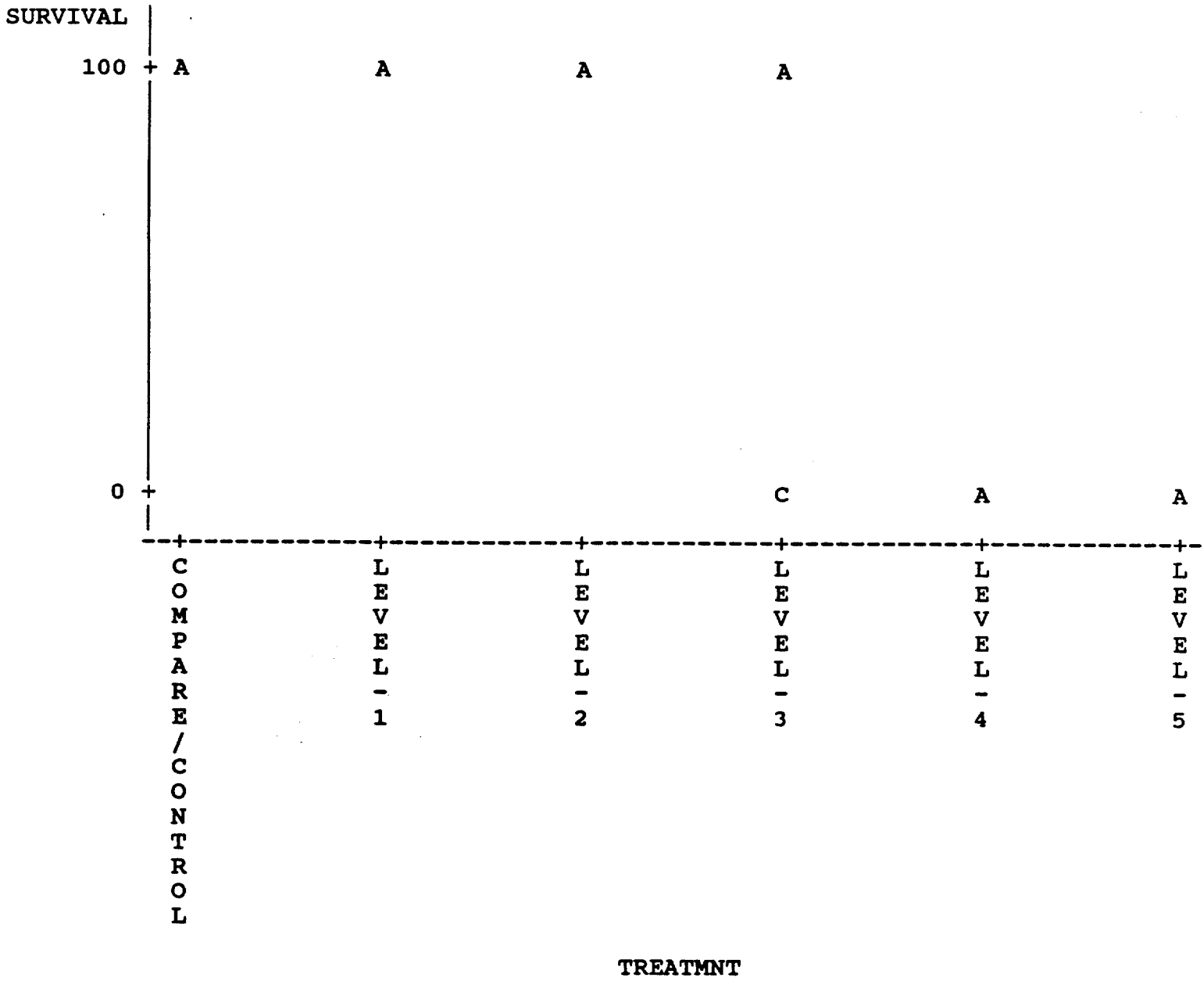
CONT_MSG	CONT_LEV
CONTROL USED FOR COMPARISONS TO OTHER LEVELS IS	POOLED

ANALYSIS BY: Dorothy England DE DATE: 04JAN95

REVIEWED BY: J Downing DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING
SCATTER PLOT OF REP VALUES FOR EACH TREATMENT
LOOK FOR UNUSUAL VALUES WHICH MAY BE INCORRECT REP DATA

Plot of SURVIVAL*TREATMNT. Symbol is value of REP.



NOTE: 63 obs hidden.

ANALYSIS BY: Dorothy England DE DATE: 04JAN95

REVIEWED BY: GDarling DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING
MEANS BY TREATMENT

MEAN OF SURVIVAL BY TREATMNT

TREATMNT		FREQ	SURVIVAL MEAN
COMPARE/CONTROL	**********	20	100.0000
LEVEL-1	**********	10	100.0000
LEVEL-2	**********	10	100.0000
LEVEL-3	**********	10	70.0000
LEVEL-4		10	0.0000
LEVEL-5		10	0.0000

```
----+---+---+---+---+---+---+---+---+---+
    10  20  30  40  50  60  70  80  90 100
```

SURVIVAL MEAN

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J Dang DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING
DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, AND CV)
FOR SURVIVAL - BY TREATMENT

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STND_DEV	CV
COMPARE/CONTROL	20	100	100	100.00	0.000	0.000
LEVEL-1	10	100	100	100.00	0.000	0.000
LEVEL-2	10	100	100	100.00	0.000	0.000
LEVEL-3	10	0	100	70.000	48.305	69.007
LEVEL-4	10	0	0	0.000	0.000	.
LEVEL-5	10	0	0	0.000	0.000	.

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J Doung DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING
COMPARISON OF CONTROL VS. TREATMENT LEVEL
FOR PERCENT SURVIVAL
A VALUE OF Prob LESS THAN .05
FOR THE CHI-SQUARE AND/OR FISHER'S EXACT (1-TAIL)
MAY INDICATE SIGNIFICANT DIFFERENCES FROM THE CONTROL

TABLE OF TREATMNT BY STATUS

TREATMNT Frequency Expected Percent Row Pct	STATUS ALIVE	Total
COMPARE/CONTROL	20 20 66.67 100.00	20 66.67
LEVEL-1	10 10 33.33 100.00	10 33.33
Total	30 100.00	30 100.00

ANALYSIS BY: Dorothy England DE DATE: 04JAN95

REVIEWED BY: J Downey DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING
COMPARISON OF CONTROL VS. TREATMENT LEVEL
FOR PERCENT SURVIVAL
A VALUE OF Prob LESS THAN .05
FOR THE CHI-SQUARE AND/OR FISHER'S EXACT (1-TAIL)
MAY INDICATE SIGNIFICANT DIFFERENCES FROM THE CONTROL

TABLE OF TREATMNT BY STATUS

TREATMNT Frequency Expected Percent Row Pct	STATUS ALIVE	Total
COMPARE/CONTROL	20 20 66.67 100.00	20 66.67
LEVEL-2	10 10 33.33 100.00	10 33.33
Total	30 100.00	30 100.00

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J Young DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING
COMPARISON OF CONTROL VS. TREATMENT LEVEL
FOR PERCENT SURVIVAL
A VALUE OF Prob LESS THAN .05
FOR THE CHI-SQUARE AND/OR FISHER'S EXACT (1-TAIL)
MAY INDICATE SIGNIFICANT DIFFERENCES FROM THE CONTROL

TABLE OF TREATMNT BY STATUS

TREATMNT	STATUS		
Frequency Expected Percent Row Pct	ALIVE	DEAD	Total
COMPARE/CONTROL	20 18 66.67 100.00	0 2 0.00 0.00	20 66.67
LEVEL-3	7 9 23.33 70.00	3 1 10.00 30.00	10 33.33
Total	27 90.00	3 10.00	30 100.00

STATISTICS FOR TABLE OF TREATMNT BY STATUS

Statistic	DF	Value	Prob
Chi-Square	1	6.667	0.010
Likelihood Ratio Chi-Square	1	7.288	0.007
Continuity Adj. Chi-Square	1	3.750	0.053
Mantel-Haenszel Chi-Square	1	6.444	0.011
Fisher's Exact Test (Left)			1.000
(Right)			2.96E-02
(2-Tail)			2.96E-02
Phi Coefficient		0.471	
Contingency Coefficient		0.426	
Cramer's V		0.471	

Sample Size = 30
WARNING: 50% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: JDoney DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING
COMPARISON OF CONTROL VS. TREATMENT LEVEL
FOR PERCENT SURVIVAL
A VALUE OF Prob LESS THAN .05
FOR THE CHI-SQUARE AND/OR FISHER'S EXACT (1-TAIL)
MAY INDICATE SIGNIFICANT DIFFERENCES FROM THE CONTROL

TABLE OF TREATMNT BY STATUS

TREATMNT Frequency Expected Percent Row Pct	STATUS ALIVE	DEAD	Total
COMPARE/CONTROL	20 13.333 66.67 100.00	0 6.6667 0.00 0.00	20 66.67
LEVEL-4	0 6.6667 0.00 0.00	10 3.3333 33.33 100.00	10 33.33
Total	20 66.67	10 33.33	30 100.00

STATISTICS FOR TABLE OF TREATMNT BY STATUS

Statistic	DF	Value	Prob
Chi-Square	1	30.000	0.000
Likelihood Ratio Chi-Square	1	38.191	0.000
Continuity Adj. Chi-Square	1	25.669	0.000
Mantel-Haenszel Chi-Square	1	29.000	0.000
Fisher's Exact Test (Left)			1.000
(Right)			3.33E-08
(2-Tail)			3.33E-08
Phi Coefficient		1.000	
Contingency Coefficient		0.707	
Cramer's V		1.000	

Sample Size = 30
WARNING: 25% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

ANALYSIS BY: Dorothy England DE DATE: 04JAN95

REVIEWED BY: J Downey DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING
COMPARISON OF CONTROL VS. TREATMENT LEVEL
FOR PERCENT SURVIVAL
A VALUE OF Prob LESS THAN .05
FOR THE CHI-SQUARE AND/OR FISHER'S EXACT (1-TAIL)
MAY INDICATE SIGNIFICANT DIFFERENCES FROM THE CONTROL

TABLE OF TREATMNT BY STATUS

TREATMNT	STATUS		
Frequency Expected Percent Row Pct	ALIVE	DEAD	Total
COMPARE/CONTROL	20 13.333 66.67 100.00	0 6.6667 0.00 0.00	20 66.67
LEVEL-5	0 6.6667 0.00 0.00	10 3.3333 33.33 100.00	10 33.33
Total	20 66.67	10 33.33	30 100.00

STATISTICS FOR TABLE OF TREATMNT BY STATUS

Statistic	DF	Value	Prob
Chi-Square	1	30.000	0.000
Likelihood Ratio Chi-Square	1	38.191	0.000
Continuity Adj. Chi-Square	1	25.669	0.000
Mantel-Haenszel Chi-Square	1	29.000	0.000
Fisher's Exact Test (Left)			1.000
(Right)			3.33E-08
(2-Tail)			3.33E-08
Phi Coefficient		1.000	
Contingency Coefficient		0.707	
Cramer's V		1.000	

Sample Size = 30
WARNING: 25% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

ANALYSIS BY: Dorothy England WCE DATE: 04JAN95

REVIEWED BY: [signature] DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING
RESULTS OF LEVENE'S TEST FOR HOMOGENEITY OF VARIANCE
ACROSS TREATMENTS
IF P IS LESS THAN .01 THEN THE ANALYSIS IS
PERFORMED ON THE TRANSFORMED VALUE (ARCSINE TRANSFORMATION)

OBS	NUM_DF	DEN_DF	F	P
1	5	64	57.6	2.1931E-22

ANALYSIS BY: Dorothy England DE DATE: 04JAN95

REVIEWED BY: J Downing DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING
ANOVA AND DUNNETT'S MEANS COMPARISON
ON ARCSIN TRANSFORMED VALUE
OR PERCENT SURVIVAL
DEPENDING ON RESULTS OF LEVENE'S TEST

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREATMNT	6	COMPARE/CONTROL LEVEL-1 LEVEL-2 LEVEL-3 LEVEL-4 LEVEL-5

Number of observations in data set = 70

ANALYSIS BY: Dorothy England DE DATE: 04JAN95

REVIEWED BY: J Dunning DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING
ANOVA AND DUNNETT'S MEANS COMPARISON
ON ARCSIN TRANSFORMED VALUE
OR PERCENT SURVIVAL
DEPENDING ON RESULTS OF LEVENE'S TEST

General Linear Models Procedure

Dependent Variable: SURVIVAL
Weight: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	8.23054510	1.64610902	81.33	0.0001
Error	64	1.29538558	0.02024040		
Corrected Total	69	9.52593068			

R-Square	C.V.	Root MSE	SURVIVAL Mean
0.864015	15.46336	0.142269	0.92003785

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMNT	5	8.23054510	1.64610902	81.33	0.0001

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: [signature] DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING
ANOVA AND DUNNETT'S MEANS COMPARISON
ON ARCSIN TRANSFORMED VALUE
OR PERCENT SURVIVAL
DEPENDING ON RESULTS OF LEVENE'S TEST

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: SURVIVAL

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 64 MSE= 0.02024
Critical Value of Dunnett's T= 2.329

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMNT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
LEVEL-1	- COMPARE/CONTROL	-0.1283	0.0000	0.1283	
LEVEL-2	- COMPARE/CONTROL	-0.1283	0.0000	0.1283	
LEVEL-3	- COMPARE/CONTROL	-0.3639	-0.2356	-0.1073	***
LEVEL-4	- COMPARE/CONTROL	-0.9137	-0.7854	-0.6571	***
LEVEL-5	- COMPARE/CONTROL	-0.9137	-0.7854	-0.6571	***

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J Dunn DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201SV1.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR NUMBER SURVIVING
ANOVA AND DUNNETT'S MEANS COMPARISON
ON ARCSIN TRANSFORMED VALUE
OR PERCENT SURVIVAL
DEPENDING ON RESULTS OF LEVENE'S TEST

General Linear Models Procedure

Level of TREATMNT	N	Sum of Weights	SURVIVAL Mean	SURVIVAL SD
COMPARE/CONTROL	20	20	1.17809725	0.00000000
LEVEL-1	10	10	1.17809725	0.00000000
LEVEL-2	10	10	1.17809725	0.00000000
LEVEL-3	10	10	0.94247780	0.37938336
LEVEL-4	10	10	0.39269908	0.00000000
LEVEL-5	10	10	0.39269908	0.00000000

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: [signature] DATE: 1/5/95

3. Mean Young per Adult

7-Day Exposure of Ceriodaphnia dubia to

ABC Study #42201

Test Level (mg/L)	REP	Number Young
Control	A	33
Control	B	38
Control	C	43
Control	D	35
Control	E	20
Control	F	36
Control	G	24
Control	H	38
Control	I	39
Control	J	42
Vehicle*Blank	A	47
Vehicle*Blank	B	34
Vehicle*Blank	C	30
Vehicle*Blank	D	29
Vehicle*Blank	E	32
Vehicle*Blank	F	33
Vehicle*Blank	G	39
Vehicle*Blank	H	35
Vehicle*Blank	I	36
Vehicle*Blank	J	42

Analysis by: __Dorothy England__ DCE Date:__20_JAN_95___

Reviewed by: Janice Sewing Date: 1/23/95

7-Day Exposure of Ceriodaphnia dubia to

ABC Study #42201

Analysis Variable : YOUNG

TRTMT=C

N Obs	N	Minimum	Maximum	Mean	Std Dev
10	10	20	43	35	7

N Obs	CV
10	21

TRTMT=VB

N Obs	N	Minimum	Maximum	Mean	Std Dev
10	10	29	47	36	6

N Obs	CV
10	16

Analysis by: Dorothy England DCE Date: 20_JAN_95

Reviewed by: Janelle Doring Date: Jan 23, 1995

7-Day Exposure of Ceriodaphnia dubia to

ABC Study #42201

TTEST PROCEDURE

Variable: YOUNG

TRTMT	N	Mean	Std Dev	Std Error
C	10	34.80000000	7.43564986	2.35135895
VB	10	35.70000000	5.57872945	1.76414915

Variances	T	DF	Prob>|T|
Unequal	-0.3062	16.7	0.7633
Equal	-0.3062	18.0	0.7630

For H0: Variances are equal, F′ = 1.78 DF = (9,9) Prob>F′ = 0.4049

Analysis by: Dorothy England DCE Date: 20 JAN 95

Reviewed by: Jaulle Bonney Date: Jan 23, 95

7-Day Exposure of Ceriodaphnia dubia to

ABC Study #42201

Test Level (mg/L)	REP	Number Young
25ppb	A	38
25ppb	B	26
25ppb	C	28
25ppb	D	20
25ppb	E	35
25ppb	F	33
25ppb	G	20
25ppb	H	30
25ppb	I	28
25ppb	J	22
50ppb	A	25
50ppb	B	26
50ppb	C	0
50ppb	D	24
50ppb	E	16
50ppb	F	19
50ppb	G	21
50ppb	H	28
50ppb	I	17
50ppb	J	25
100ppb	A	3
100ppb	B	4
100ppb	C	4
100ppb	D	0
100ppb	E	4
100ppb	F	0
100ppb	G	6
100ppb	H	7
100ppb	I	3
100ppb	J	7
Control	A	33
Control	B	38
Control	C	43
Control	D	35
Control	E	20
Control	F	36
Control	G	24
Control	H	38
Control	I	39
Control	J	42
Vehicle*Blank	A	47
Vehicle*Blank	B	34

Analysis by: Dorothy England Date: 20 JAN 95

Reviewed by: Janelle Downey Date: Jan 23, 95

7-Day Exposure of Ceriodaphnia dubia to

ABC Study #42201

Test Level (mg/L)	REP	Number Young
Vehicle*Blank	C	30
Vehicle*Blank	D	29
Vehicle*Blank	E	32
Vehicle*Blank	F	33
Vehicle*Blank	G	39
Vehicle*Blank	H	35
Vehicle*Blank	I	36
Vehicle*Blank	J	42

Analysis by: Dorothy England DCE Date: 20_JAN_95

Reviewed by: Julie Deming Date: Jan 23 95

7-Day Exposure of Ceriodaphnia dubia to

ABC Study #42201

Analysis Variable : YOUNG

TRTMT=1

N Obs	N	Minimum	Maximum	Mean	Std Dev
10	10	20	38	28	6

N Obs	CV
10	22

TRTMT=2

N Obs	N	Minimum	Maximum	Mean	Std Dev
10	10	0	28	20	8

N Obs	CV
10	40

TRTMT=3

N Obs	N	Minimum	Maximum	Mean	Std Dev
10	10	0	7	4	2

N Obs	CV
10	65

Analysis by: Dorothy England Date: 20 JAN 95

Reviewed by: Jamie Doring Date: 1/23/95

7-Day Exposure of Ceriodaphnia dubia to

ABC Study #42201

Analysis Variable : YOUNG

TRTMT=C

N Obs	N	Minimum	Maximum	Mean	Std Dev
10	10	20	43	35	7

N Obs	CV
10	21

TRTMT=VB

N Obs	N	Minimum	Maximum	Mean	Std Dev
10	10	29	47	36	6

N Obs	CV
10	16

Analysis by: Dorothy England DCE Date: 20 JAN 95

Reviewed by: Janelle Dorning Date: 1/23/95

7-Day Exposure of Ceriodaphnia dubia to

ABC Study #42201

OBS	TRTMT	REP	YOUNG
1	1	A	38
2	1	B	26
3	1	C	28
4	1	D	20
5	1	E	35
6	1	F	33
7	1	G	20
8	1	H	30
9	1	I	28
10	1	J	22
11	2	A	25
12	2	B	26
13	2	C	0
14	2	D	24
15	2	E	16
16	2	F	19
17	2	G	21
18	2	H	28
19	2	I	17
20	2	J	25
21	3	A	3
22	3	B	4
23	3	C	4
24	3	D	0
25	3	E	4
26	3	F	0
27	3	G	6
28	3	H	7
29	3	I	3
30	3	J	7
31	P	A	33
32	P	B	38
33	P	C	43
34	P	D	35
35	P	E	20
36	P	F	36
37	P	G	24
38	P	H	38
39	P	I	39
40	P	J	42
41	P	A	47
42	P	B	34
43	P	C	30
44	P	D	29

Analysis by: __Dorothy England__ DCE Date:__20_JAN_95___

Reviewed by: Janice Downing Date: Jan 23, 95

7-Day Exposure of Ceriodaphnia dubia to

ABC Study #42201

OBS	TRTMT	REP	YOUNG
45	P	E	32
46	P	F	33
47	P	G	39
48	P	H	35
49	P	I	36
50	P	J	42

Analysis by: __Dorothy England_____ Date:__20_JAN_95___

Reviewed by: Janice Strong Date: Jan 23, 95

7-Day Exposure of Ceriodaphnia dubia to

ABC Study #42201

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRTMT	4	100ppb 25ppb 50ppb Pooled

Number of observations in data set = 50

Analysis by: __Dorothy England_____ Date:__20_JAN_95___

Reviewed by: _____ Date: Jan 23, 95

7-Day Exposure of Ceriodaphnia dubia to

ABC Study #42201

General Linear Models Procedure

Dependent Variable: YOUNG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	6912.2300	2304.0767	59.67	0.0001
Error	46	1776.2500	38.6141		
Corrected Total	49	8688.4800			

R-Square	C.V.	Root MSE	YOUNG Mean
0.795563	25.38410	6.2140	24.48000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRTMT	3	6912.2300	2304.0767	59.67	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRTMT	3	6912.2300	2304.0767	59.67	0.0001

Analysis by: __Dorothy England_____ DCE Date:__20_JAN_95___

Reviewed by: Janice Nourney Date: Jan 23, 95

7-Day Exposure of Ceriodaphnia dubia to

ABC Study #42201

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: YOUNG

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 46 MSE= 38.61413
Critical Value of Dunnett's T= 2.150

Comparisons significant at the 0.05 level are indicated by '***'.

TRTMT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
25ppb - Pooled	-12.425	-7.250	-2.075	***
50ppb - Pooled	-20.325	-15.150	-9.975	***
100ppb - Pooled	-36.625	-31.450	-26.275	***

Analysis by: Dorothy England DCE Date: 20 JAN 95

Reviewed by: Janell Dorney Date: Jan 23/95

4. Days to First Brood

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201DFB.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR TIME TO FIRST BROOD
PRINTOUT OF RAW DATA

TREATMENT	REPLICATE	TIME TO 1ST BROOD
LEVEL-1	A	3
LEVEL-1	B	4
LEVEL-1	C	3
LEVEL-1	D	3
LEVEL-1	E	3
LEVEL-1	F	3
LEVEL-1	G	3
LEVEL-1	H	4
LEVEL-1	I	3
LEVEL-1	J	3
LEVEL-2	A	3
LEVEL-2	B	4
LEVEL-2	D	4
LEVEL-2	E	5
LEVEL-2	F	5
LEVEL-2	G	4
LEVEL-2	H	3
LEVEL-2	I	4
LEVEL-2	J	4
LEVEL-3	A	3
LEVEL-3	B	5
LEVEL-3	C	3
LEVEL-3	E	3
LEVEL-3	G	3
LEVEL-3	H	3
LEVEL-3	I	4
LEVEL-3	J	3
CONTROL	A	3
CONTROL	B	3
CONTROL	C	3
CONTROL	D	4
CONTROL	E	3
CONTROL	F	3
CONTROL	G	4
CONTROL	H	3
CONTROL	I	3
CONTROL	J	3
VEHICLE BLANK	A	3
VEHICLE BLANK	B	3
VEHICLE BLANK	C	3
VEHICLE BLANK	D	3
VEHICLE BLANK	E	4
VEHICLE BLANK	F	3
VEHICLE BLANK	G	3
VEHICLE BLANK	H	3

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J. Dorney DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201DFB.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR TIME TO FIRST BROOD
PRINTOUT OF RAW DATA

TREATMENT	REPLICATE	TIME TO 1ST BROOD
VEHICLE BLANK	I	3
VEHICLE BLANK	J	3

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: JQ Dunning DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 05JAN95 USING DATA FILE M:\SAS\42201DFB.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON OF CONTROL GROUPS FOR TIME TO FIRST BROOD
LOOK FOR UNUSUAL VALUES WHICH MAY BE INCORRECT REP DATA

FREQUENCY OF TIM_1BRD

TIM_1BRD MIDPOINT		FREQ	CUM FREQ	PERCENT	CUM PERCENT
3.125	CCCCCCCCCCCCCCCCVVVVVVVVVVVVVVVVVV	17	17	85.00	85.00
3.375		0	17	0.00	85.00
3.625		0	17	0.00	85.00
3.875	CCCCVV	3	20	15.00	100.00

```
----+---+---+---+---+---+---+---+--
    2   4   6   8  10  12  14  16
```

FREQUENCY

SYMBOL	LEVEL	SYMBOL	LEVEL
C	CONTROL	V	VEHICLE BLANK

ANALYSIS BY: Dorothy England DE DATE: 05JAN95

REVIEWED BY: J Dong DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 05JAN95 USING DATA FILE M:\SAS\42201DFB.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON OF CONTROL GROUPS FOR TIME TO FIRST BROOD
DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, AND CV)
FOR TIM_1BRD - BY CONTROL GROUP

LEVEL	NO_REPS	MINIMUM	MAXIMUM	MEAN	STND_DEV	CV
CONTROL	10	3	4	3.2	0.42164	13.1762
VEHICLE BLANK	10	3	4	3.1	0.31623	10.2009

ANALYSIS BY: Dorothy England DCE DATE: 05JAN95

REVIEWED BY: J Dunn DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 05JAN95 USING DATA FILE M:\SAS\42201DFB.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON OF CONTROL GROUPS FOR TIME TO FIRST BROOD
STUDENT T-TEST COMPARING CONTROLS
IF PROB>|T| FOR EQUAL VARIANCES IS LESS THAN .05
AND IF DIFFERENCE IS BIOLOGICALLY MEANINGFUL
THEN USE CONTROL OR VEHICLE BLANK AS CONTROL

TTEST PROCEDURE

Variable: TIM_1BRD

LEVEL	N	Mean	Std Dev	Std Error
CONTROL	10	3.20000000	0.42163702	0.13333333
VEHICLE BLANK	10	3.10000000	0.31622777	0.10000000

Variances	T	DF	Prob>|T|
Unequal	0.6000	16.7	0.5566
Equal	0.6000	18.0	0.5560

For H0: Variances are equal, F′ = 1.78 DF = (9,9) Prob>F′ = 0.4043

ANALYSIS BY: Dorothy England DE DATE: 05JAN95

REVIEWED BY: J Dawing DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201DFB.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR TIME TO FIRST BROOD

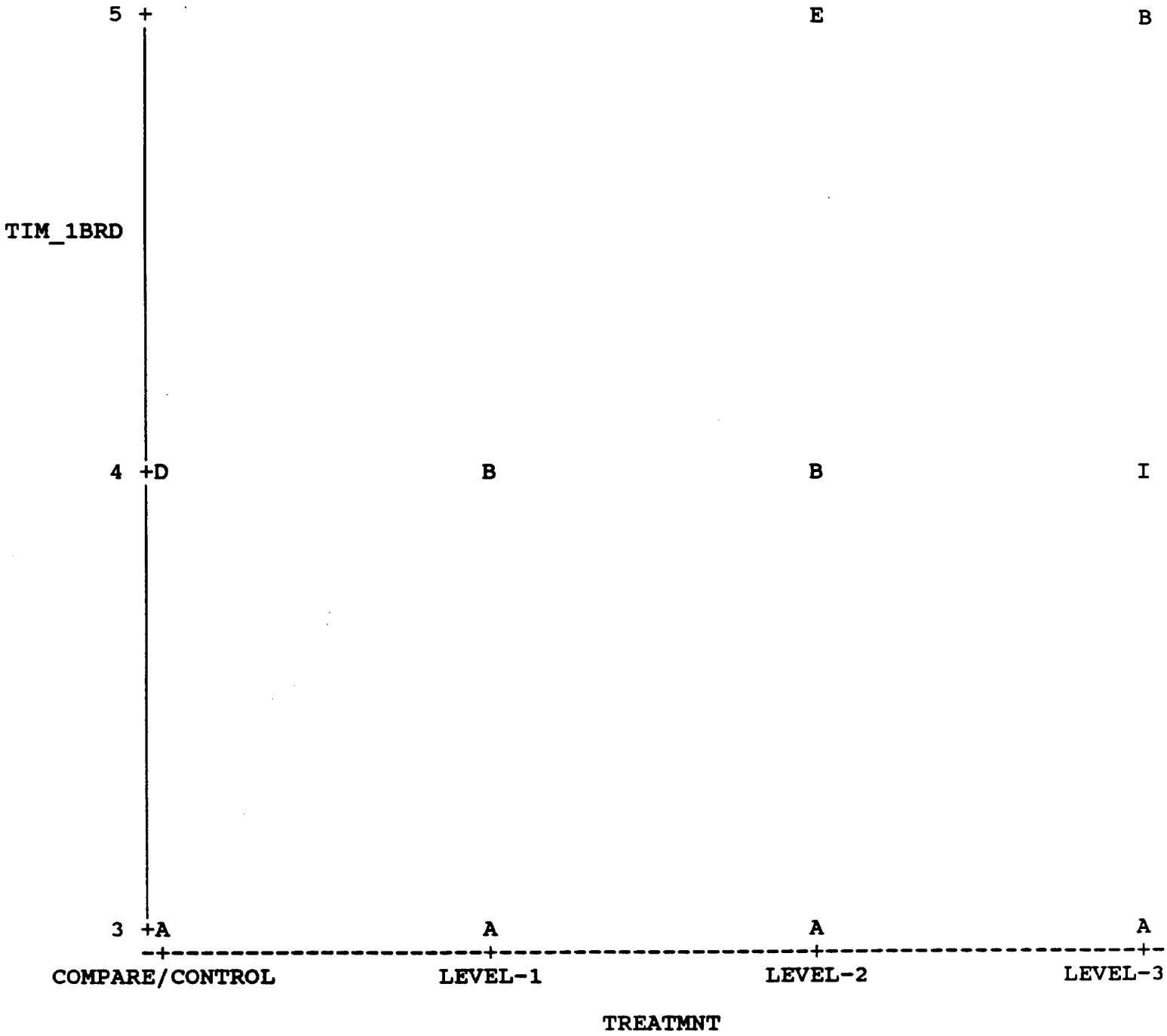
CONT_MSG	CONT_LEV
CONTROL USED FOR COMPARISONS TO OTHER LEVELS IS	POOLED

ANALYSIS BY: Dorothy England DATE: 04JAN95

REVIEWED BY: J Dang DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201DFB.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR TIME TO FIRST BROOD
SCATTER PLOT OF REP VALUES FOR EACH TREATMENT
LOOK FOR UNUSUAL VALUES WHICH MAY BE INCORRECT REP DATA

Plot of TIM_1BRD*TREATMNT. Symbol is value of REP.



NOTE: 37 obs hidden.

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J Dorney DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201DFB.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR TIME TO FIRST BROOD
MEANS BY TREATMENT

MEAN OF TIM_1BRD BY TREATMNT

```
TREATMNT                                                          TIM_1BRD
                                                        FREQ         MEAN
                 |
COMPARE/CONTROL  |*******************************        20      3.150000
                 |
LEVEL-1          |********************************       10      3.200000
                 |
LEVEL-2          |****************************************  9      4.000000
                 |
LEVEL-3          |**********************************      8      3.375000
                 ----------+---------+---------+---------+
                           1         2         3         4

                        TIM_1BRD MEAN
```

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: [signature] DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201DFB.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR TIME TO FIRST BROOD
DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, AND CV)
FOR TIM_1BRD - BY TREATMENT

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STND_DEV	CV
COMPARE/CONTROL	20	3	4	3.150	0.366	11.630
LEVEL-1	10	3	4	3.200	0.422	13.176
LEVEL-2	9	3	5	4.000	0.707	17.678
LEVEL-3	8	3	5	3.375	0.744	22.045

ANALYSIS BY: Dorothy England WCC DATE: 04JAN95

REVIEWED BY: [signature] DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201DFB.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR TIME TO FIRST BROOD
RESULTS OF LEVENE'S TEST FOR HOMOGENEITY OF VARIANCE
ACROSS TREATMENTS
IF P IS LESS THAN .01 THEN THE ANALYSIS IS
PERFORMED ON THE TRANSFORMED VALUE (LOG TRANSFORMATION)

OBS	NUM_DF	DEN_DF	F	P
1	2	36	0.99188	0.38079

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J Berry DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201DFB.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR TIME TO FIRST BROOD
ANOVA AND DUNNETT'S MEANS COMPARISON
ON LOG TRANSFORMED VALUE
OR TIM_1BRD
DEPENDING ON RESULTS OF LEVENE'S TEST

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREATMNT	3	COMPARE/CONTROL LEVEL-1 LEVEL-2

Number of observations in data set = 39

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J Berry DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201DFB.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR TIME TO FIRST BROOD
ANOVA AND DUNNETT'S MEANS COMPARISON
ON LOG TRANSFORMED VALUE
OR TIM_1BRD
DEPENDING ON RESULTS OF LEVENE'S TEST

General Linear Models Procedure

Dependent Variable: TIM_1BRD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	4.82435897	2.41217949	10.66	0.0002
Error	36	8.15000000	0.22638889		
Corrected Total	38	12.97435897			

R-Square	C.V.	Root MSE	TIM_1BRD Mean
0.371838	14.16514	0.475803	3.35897436

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMNT	2	4.82435897	2.41217949	10.66	0.0002

ANALYSIS BY: Dorothy England WCE DATE: 04JAN95

REVIEWED BY: [signature] DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201DFB.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR TIME TO FIRST BROOD
ANOVA AND DUNNETT'S MEANS COMPARISON
ON LOG TRANSFORMED VALUE
OR TIM_1BRD
DEPENDING ON RESULTS OF LEVENE'S TEST

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: TIM_1BRD

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 36 MSE= 0.226389
Critical Value of Dunnett's T= 1.999

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMNT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
LEVEL-2	- COMPARE/CONTROL	0.468	0.850	1.232	***
LEVEL-1	- COMPARE/CONTROL	-0.318	0.050	0.418	

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: [signature] DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201DFB.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR TIME TO FIRST BROOD
ANOVA AND DUNNETT'S MEANS COMPARISON
ON LOG TRANSFORMED VALUE
OR TIM_1BRD
DEPENDING ON RESULTS OF LEVENE'S TEST

General Linear Models Procedure

Level of TREATMNT	N	TIM_1BRD Mean	TIM_1BRD SD
COMPARE/CONTROL	20	3.15000000	0.36634755
LEVEL-1	10	3.20000000	0.42163702
LEVEL-2	9	4.00000000	0.70710678

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: _____ DATE: 1/5/95

5. Young per Adult Reproduction Day

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)
PRINTOUT OF RAW DATA

TREATMENT	REPLICATE	REPROD. DAYS	INSTAR1	INSTAR2	INSTAR3
LEVEL-1	A	5	5	0	10
LEVEL-1	B	4	0	6	9
LEVEL-1	C	5	5	0	11
LEVEL-1	D	5	4	0	7
LEVEL-1	E	5	4	0	9
LEVEL-1	F	5	3	0	7
LEVEL-1	G	5	3	0	8
LEVEL-1	H	4	0	9	7
LEVEL-1	I	5	4	0	7
LEVEL-1	J	5	4	0	11
LEVEL-2	A	5	3	0	7
LEVEL-2	B	4	0	6	8
LEVEL-2	C	0	0	0	0
LEVEL-2	D	4	0	8	8
LEVEL-2	E	3	0	0	7
LEVEL-2	F	3	0	0	9
LEVEL-2	G	4	0	5	7
LEVEL-2	H	5	3	6	10
LEVEL-2	I	4	0	5	5
LEVEL-2	J	4	0	5	8

TREATMENT	INSTAR4	INSTAR5	TOTAL INSTAR	YAD
LEVEL-1	13	10	38	7.60
LEVEL-1	10	1	26	6.50
LEVEL-1	12	0	28	5.60
LEVEL-1	9	0	20	4.00
LEVEL-1	9	13	35	7.00
LEVEL-1	15	8	33	6.60
LEVEL-1	9	0	20	4.00
LEVEL-1	14	0	30	7.50
LEVEL-1	10	7	28	5.60
LEVEL-1	7	0	22	4.40
LEVEL-2	15	0	25	5.00
LEVEL-2	12	0	26	6.50
LEVEL-2	0	0	0	.
LEVEL-2	8	0	24	6.00
LEVEL-2	9	0	16	5.33
LEVEL-2	10	0	19	6.33
LEVEL-2	9	0	21	5.25
LEVEL-2	9	0	28	5.60
LEVEL-2	7	0	17	4.25
LEVEL-2	12	0	25	6.25

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J Downing DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)
PRINTOUT OF RAW DATA

TREATMENT	REPLICATE	REPROD. DAYS	INSTAR1	INSTAR2	INSTAR3
LEVEL-3	A	5	3	0	0
LEVEL-3	B	3	0	0	4
LEVEL-3	C	3	4	0	0
LEVEL-3	D	0	0	0	0
LEVEL-3	E	5	3	0	1
LEVEL-3	F	0	0	0	0
LEVEL-3	G	4	2	0	4
LEVEL-3	H	5	3	0	4
LEVEL-3	I	4	0	1	2
LEVEL-3	J	5	4	0	3
LEVEL-4	A	0	0	0	0
LEVEL-4	B	0	0	0	0
LEVEL-4	C	0	0	0	0
LEVEL-4	D	0	0	0	0
LEVEL-4	E	0	0	0	0
LEVEL-4	F	0	0	0	0
LEVEL-4	G	0	0	0	0
LEVEL-4	H	0	0	0	0
LEVEL-4	I	0	0	0	0
LEVEL-4	J	0	0	0	0

TREATMENT	INSTAR4	INSTAR5	TOTAL INSTAR	YAD
LEVEL-3	0	0	3	0.60
LEVEL-3	0	0	4	1.33
LEVEL-3	0	0	4	1.33
LEVEL-3	0	0	0	.
LEVEL-3	0	0	4	0.80
LEVEL-3	0	0	0	.
LEVEL-3	0	0	6	1.50
LEVEL-3	0	0	7	1.40
LEVEL-3	0	0	3	0.75
LEVEL-3	0	0	7	1.40
LEVEL-4	0	0	0	.
LEVEL-4	0	0	0	.
LEVEL-4	0	0	0	.
LEVEL-4	0	0	0	.
LEVEL-4	0	0	0	.
LEVEL-4	0	0	0	.
LEVEL-4	0	0	0	.
LEVEL-4	0	0	0	.
LEVEL-4	0	0	0	.
LEVEL-4	0	0	0	.

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J Downing DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)
PRINTOUT OF RAW DATA

TREATMENT	REPLICATE	REPROD. DAYS	INSTAR1	INSTAR2	INSTAR3
LEVEL-5	A	0	0	0	0
LEVEL-5	B	0	0	0	0
LEVEL-5	C	0	0	0	0
LEVEL-5	D	0	0	0	0
LEVEL-5	E	0	0	0	0
LEVEL-5	F	0	0	0	0
LEVEL-5	G	0	0	0	0
LEVEL-5	H	0	0	0	0
LEVEL-5	I	0	0	0	0
LEVEL-5	J	0	0	0	0
CONTROL	A	5	4	8	8
CONTROL	B	5	6	0	11
CONTROL	C	5	6	0	14
CONTROL	D	4	0	10	9
CONTROL	E	5	3	0	8
CONTROL	F	5	4	7	10
CONTROL	G	4	0	6	9
CONTROL	H	5	5	0	13
CONTROL	I	5	5	8	10
CONTROL	J	5	4	7	11

TREATMENT	INSTAR4	INSTAR5	TOTAL INSTAR	YAD
LEVEL-5	0	0	0	.
LEVEL-5	0	0	0	.
LEVEL-5	0	0	0	.
LEVEL-5	0	0	0	.
LEVEL-5	0	0	0	.
LEVEL-5	0	0	0	.
LEVEL-5	0	0	0	.
LEVEL-5	0	0	0	.
LEVEL-5	0	0	0	.
LEVEL-5	0	0	0	.
CONTROL	13	0	33	6.60
CONTROL	12	9	38	7.60
CONTROL	13	10	43	8.60
CONTROL	16	0	35	8.75
CONTROL	9	0	20	4.00
CONTROL	15	0	36	7.20
CONTROL	9	0	24	6.00
CONTROL	10	10	38	7.60
CONTROL	8	8	39	7.80
CONTROL	13	7	42	8.40

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: [signature] DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)
PRINTOUT OF RAW DATA

TREATMENT	REPLICATE	REPROD. DAYS	INSTAR1	INSTAR2	INSTAR3
VEHICLE BLANK	A	5	5	0	15
VEHICLE BLANK	B	5	5	7	10
VEHICLE BLANK	C	5	3	0	13
VEHICLE BLANK	D	5	4	8	8
VEHICLE BLANK	E	4	0	8	9
VEHICLE BLANK	F	5	6	0	12
VEHICLE BLANK	G	5	3	6	10
VEHICLE BLANK	H	5	3	0	11
VEHICLE BLANK	I	5	5	0	10
VEHICLE BLANK	J	5	4	9	8

TREATMENT	INSTAR4	INSTAR5	TOTAL INSTAR	YAD
VEHICLE BLANK	16	11	47	9.40
VEHICLE BLANK	12	0	34	6.80
VEHICLE BLANK	14	0	30	6.00
VEHICLE BLANK	9	0	29	5.80
VEHICLE BLANK	0	15	32	8.00
VEHICLE BLANK	15	0	33	6.60
VEHICLE BLANK	11	9	39	7.80
VEHICLE BLANK	14	7	35	7.00
VEHICLE BLANK	10	11	36	7.20
VEHICLE BLANK	14	7	42	8.40

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J Downey DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 05JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON OF CONTROL GROUPS FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)
LOOK FOR UNUSUAL VALUES WHICH MAY BE INCORRECT REP DATA

FREQUENCY OF YOUNG_DY

YOUNG_DY MIDPOINT		FREQ	CUM FREQ	PERCENT	CUM PERCENT
4.2	CCCCC	1	1	5.00	5.00
5.4	VVVVV	1	2	5.00	10.00
6.6	CCCCCCCCCCVVVVVVVVVVVVVVVVVVVV	6	8	30.00	40.00
7.8	CCCCCCCCCCCCCCCCCCCCVVVVVVVVVVVVVVVV	7	15	35.00	75.00
9.0	CCCCCCCCCCCCCCCVVVVVVVVVV	5	20	25.00	100.00

```
-----+----+----+----+----+----+----+
     1    2    3    4    5    6    7
```

FREQUENCY

SYMBOL	LEVEL	SYMBOL	LEVEL
C	CONTROL	V	VEHICLE BLANK

ANALYSIS BY: Dorothy England DCE DATE: 05JAN95

REVIEWED BY: J Dang DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 05JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON OF CONTROL GROUPS FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)
DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, AND CV)
FOR YOUNG_DY - BY CONTROL GROUP

LEVEL	NO_REPS	MINIMUM	MAXIMUM	MEAN	STND_DEV	CV
CONTROL	10	4.0	8.75	7.255	1.43342	19.7577
VEHICLE BLANK	10	5.8	9.40	7.300	1.11255	15.2405

ANALYSIS BY: Dorothy England DE DATE: 05JAN95

REVIEWED BY: J. Dewing DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 05JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON OF CONTROL GROUPS FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)
STUDENT T-TEST COMPARING CONTROLS
IF PROB>|T| FOR EQUAL VARIANCES IS LESS THAN .05
AND IF DIFFERENCE IS BIOLOGICALLY MEANINGFUL
THEN USE CONTROL OR VEHICLE BLANK AS CONTROL

TTEST PROCEDURE

Variable: YOUNG_DY

LEVEL	N	Mean	Std Dev	Std Error
CONTROL	10	7.25500000	1.43342054	0.45328738
VEHICLE BLANK	10	7.30000000	1.11255462	0.35182066

Variances	T	DF	Prob>|T|
Unequal	-0.0784	17.0	0.9384
Equal	-0.0784	18.0	0.9384

For H0: Variances are equal, F' = 1.66 DF = (9,9) Prob>F' = 0.4619

ANALYSIS BY: Dorothy England DCE DATE: 05JAN95

REVIEWED BY: J Danny DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)

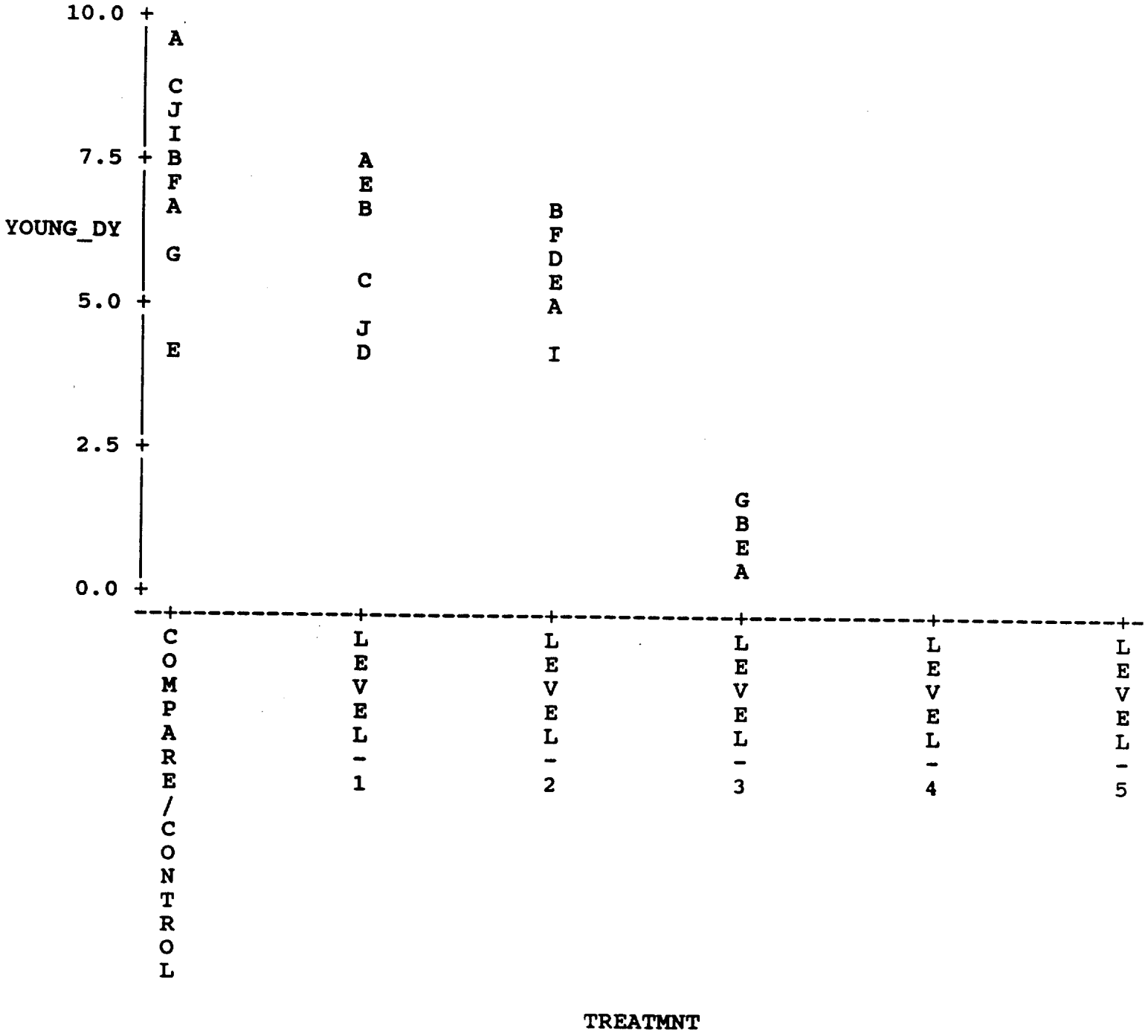
CONT_MSG	CONT_LEV
CONTROL USED FOR COMPARISONS TO OTHER LEVELS IS	POOLED

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J Denny DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)
SCATTER PLOT OF REP VALUES FOR EACH TREATMENT
LOOK FOR UNUSUAL VALUES WHICH MAY BE INCORRECT REP DATA

Plot of YOUNG_DY*TREATMNT. Symbol is value of REP.



NOTE: 23 obs had missing values. 22 obs hidden.

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: gDorney DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)
MEANS BY TREATMENT

MEAN OF YOUNG_DY BY TREATMNT

TREATMNT		FREQ	YOUNG_DY MEAN
COMPARE/CONTROL	**********	20	7.277500
LEVEL-1	**********	10	5.880000
LEVEL-2	**********	9	5.612222
LEVEL-3	******	8	1.138750
LEVEL-4		0	0.000000
LEVEL-5		0	0.000000

```
-----+----+----+----+----+----+----+-
     1    2    3    4    5    6    7
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YOUNG_DY MEAN

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J Denny DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)
DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, AND CV)
FOR YOUNG_DY - BY TREATMENT

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STND_DEV	CV
COMPARE/CONTROL	20	4.00	9.4	7.277	1.249	17.163
LEVEL-1	10	4.00	7.6	5.880	1.381	23.481
LEVEL-2	9	4.25	6.5	5.612	0.733	13.066
LEVEL-3	8	0.60	1.5	1.139	0.358	31.423
LEVEL-4	0	.	.	.	.	.
LEVEL-5	0	.	.	.	.	.

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: _____ DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)
RESULTS OF LEVENE'S TEST FOR HOMOGENEITY OF VARIANCE
ACROSS TREATMENTS
IF P IS LESS THAN .01 THEN THE ANALYSIS IS
PERFORMED ON THE TRANSFORMED VALUE (LOG TRANSFORMATION)

OBS	NUM_DF	DEN_DF	F	P
1	2	36	1.79159	0.18124

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: [signature] DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)
ANOVA AND DUNNETT'S MEANS COMPARISON
ON LOG TRANSFORMED VALUE
OR YOUNG_DY
DEPENDING ON RESULTS OF LEVENE'S TEST

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREATMNT	3	COMPARE/CONTROL LEVEL-1 LEVEL-2

Number of observations in data set = 40

NOTE: Due to missing values, only 39 observations can be used in this analysis.

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: JDenno DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)
ANOVA AND DUNNETT'S MEANS COMPARISON
ON LOG TRANSFORMED VALUE
OR YOUNG_DY
DEPENDING ON RESULTS OF LEVENE'S TEST

General Linear Models Procedure

Dependent Variable: YOUNG_DY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	22.98004380	11.49002190	8.09	0.0013
Error	36	51.10033056	1.41945363		
Corrected Total	38	74.08037436			

R-Square	C.V.	Root MSE	YOUNG_DY Mean
0.310204	18.23155	1.191408	6.53487179

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMNT	2	22.98004380	11.49002190	8.09	0.0013

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J Dunn DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)
ANOVA AND DUNNETT'S MEANS COMPARISON
ON LOG TRANSFORMED VALUE
OR YOUNG_DY
DEPENDING ON RESULTS OF LEVENE'S TEST

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: YOUNG_DY

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 36 MSE= 1.419454
Critical Value of Dunnett's T= 1.999

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMNT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
LEVEL-1	- COMPARE/CONTROL	-2.320	-1.397	-0.475	***
LEVEL-2	- COMPARE/CONTROL	-2.621	-1.665	-0.709	***

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: [signature] DATE: 1/5/95

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 04JAN95 USING DATA FILE M:\SAS\42201YAD.DAT
7-DAY CHRONIC TOXICITY STUDY WITH Ceriodaphnia dubia EXPOSED TO
ABC STUDY NUMBER: 42201
COMPARISON TO CONTROL GROUP FOR YOUNG PER ADULT REPRODUCTIVE DAY (YAD)
ANOVA AND DUNNETT'S MEANS COMPARISON
ON LOG TRANSFORMED VALUE
OR YOUNG_DY
DEPENDING ON RESULTS OF LEVENE'S TEST

General Linear Models Procedure

Level of TREATMNT	N	YOUNG_DY Mean	YOUNG_DY SD
COMPARE/CONTROL	20	7.27750000	1.24904964
LEVEL-1	10	5.88000000	1.38066007
LEVEL-2	9	5.61222222	0.73331061

ANALYSIS BY: Dorothy England DCE DATE: 04JAN95

REVIEWED BY: J Dorney DATE: 1/5/95

APPENDIX II - PROTOCOL AND CORRESPONDENCE

7200 E. ABC Lane, Columbia, MO 65202
Tel: 314/474-8579 Fax: 314/443-9033

ABC LABORATORIES
DEC 5 1994

Working for You

PROTOCOL ALTERATION NOTIFICATION

PROTOCOL TITLE: "Procedure for Determining Chemical Effects on *Ceriodaphnia* sp. Survival and Reproduction During a 7-Day Exposure Period"	
PROTOCOL NO.: 8606	**ALTERATION NO.:** 1
LABORATORY: ABC Laboratories, Inc.	**LAB STUDY NO.:** 42201
SPONSOR:	**EFFECTIVE DATE:** 11\17\94

ALTERATIONS:

1. Protocol Section: 3.3 Test Procedure and 4.2 Test Organism

   Revise the first sentence of each of these sections to read, "Test daphnids (neonate *Ceriodaphnia* less than 24 hours old) will be selected from a subculture of neonates."

   Reason for the change: Culturing techniques used by ABC Laboratories do not permit verification of the age of the neonates, nor can the span of ages be verified.

   Effect on the study: Updates and clarifies the protocol

2. Protocol Section: 4.2 Test Organism

   Revise the last sentence of this section to read, "The species will be documented using a taxonomic key such as that presented by Pennek (2), or species will be documented by the supplier.

   Reason for the change: Elimination of the word "periodically", which is inappropriate.

   Effect on the study: Updates and clarifies the protocol

3. Protocol Section: 4.3 Culturing and Feeding

   Revise the second sentence of this section to read, "Cultures will be fed daily with an algal suspension (e.g., *Selenastrum capricornutum* and/or *Ankistrodesmus falcatus*) supplemented with a suspension of fish food and yeast (YTC)."

   Reason for the change: This change allows flexibility in the choice of algal species used to culture the test organism, and reflects nature of the current supplemental food suspension.

   Effect on the study: Updates and clarifies the protocol

4. Protocol Section: 4.4 Test Chambers

   Revise the first and second sentence of this section to read, "Chambers containing test solutions, consisting of 10 replicates of each of 5 treatments and a control (and a vehicle blank if appropriate), will be randomly assigned to a test board using a template or a table of random numbers. The test chambers will be 50 mL beakers filled with 30 mL of test solution."

   Reason for the change: The change incorporates a vehicle blank treatment into the test design and gives flexibility to the test by allowing the use of greater or lesser test solution volume.

   Effect on the study: Updates and clarifies the protocol

5. Protocol Section: 4.5 Test Solutions

Change the term "YCT" in the fourth sentence to read "YTC".

Reason for the change: This is the correct designation used for the supplemental food used during the test, as described in section 4.3 of the protocol.

Effect on the study: Updates and clarifies the protocol

6. Protocol Section: 4.6 Test Initiation

Revise the first sentence to read, "Neonates will be impartially selected from brood chambers and distributed one to each test chamber at test initiation."

Reason for the change: The process used to select test organisms does not necessitate a randomization procedure, and there is very little bias inherant in the process.

Effect on the study: Updates and clarifies the protocol

7. Protocol Section: 4.8 Test Procedure — Chemical/Physical

Revise the second sentence in this section to read, "These measurements will be carried out at least on the highest concentration having living brood daphnids and on low and mid-level concentrations and control water (plus vehicle blank, if used)."

Reason for the change: This change deletes the redundant term "low concentration," and allows additional water chemistry measurements at the descretion of the study director.

Effect on the study: Updates and clarifies the protocol

8. Protocol Section: Definitive Test Concentrations

Definitive test concentrations are changed to 25, 50, 100, 200, and 400 μg/L.

Reason for the change: In an initial exposure, no mortality was observed after 5 days at concentrations of 6.25, 12.5, 25, 50, or 100 μg/L. A 48-hour range-finding test comparing glass and plastic (polystyrene) test chambers gave complete mortality at concentrations of 500 and 1000 μg/L for both types of vessels. No mortality was observed in glass test vessels at or below 100 μg/L and only a single mortality was observed in the plastic vessels at 100 μg/L.

Effect on the study: The new range of concentrations selected is expected to give a concentration response, as well as no-effect, low-effect, and total-effect (complete mortality) concentrations.

STUDY DIRECTOR'S SIGNATURE:	Newton C. England	**DATE:**	12/1/94
STUDY SPONSOR'S SIGNATURE		**DATE:**	12/2/94

7200 E. ABC Lane, Columbia, MO 65202

Tel: 314/474-8579 Fax: 314/443-9033

ABC LABORATORIES

FEB 28 1995

Working for You

PROTOCOL ALTERATION NOTIFICATION

PROTOCOL TITLE: "Procedure for Determining Chemical Effects on *Ceriodaphnia* sp. Survival and Reproduction During a 7-Day Exposure Period"	
PROTOCOL NO.: 8606	**ALTERATION NO.:** 2
LABORATORY: ABC Laboratories, Inc.	**LAB STUDY NO.:** 42201
SPONSOR:	**EFFECTIVE DATE:** 20JAN95

DEVIATIONS:

1. Protocol Section: 3.3 Test Procedure and 4.4 Test Chambers

   The protocol specifies a test temperature of 25 ± 1°C for range-finding and definitive tests. During both range-finding and definitive tests the temperature of new solutions were recorded at 23°C.

   Reason for the change: Temperatures were measured in the extra replicate beaker reserved for water chemistry measurements. These were placed in the waterbath after the test cups were filled and placed in the waterbath, They had evidently not come up to test temperature at the time of the measurements.

   Effect on the study: There were no adverse effects observed in either survival or reproduction for controls or vehicle blanks during the study. Therefore, this deviation is considered to have no effect on the test's validity.

2. Protocol Section: 3.3 Test Procedure and 4.2 Test Organism

   The protocol states that test daphnids will be maintained under test conditions for at least two weeks. The daphnids used for two preliminary exposures and the definitive exposure did not technically meet these criteria.

   Reason for the change: The cultures used were less than two weeks of age.

   Effect on the study: Since the test organisms used were offspring of sexually mature, reproducing adults, and since control and vehicle blank replicates showed acceptable survival and reproduction, this deviation is not considered to be detrimental to the study.

STUDY DIRECTOR'S SIGNATURE:	Dorothy C. England	**DATE:**	1/23/95
STUDY SPONSOR'S SIGNATURE		**DATE:**	2/7/95

ABC LABORATORIES, INC.

DEFINITIVE CONCENTRATION NOTIFICATION

Test Material: _____ Protocol No.: 8606

Study Director: Dorothy England Study #: 42201

Sponsor's Representative: _____

Company: _____

Person Notified: Same (✓) Other: _____

(Voice Mail & FAX)

Test Species: Ceriodaphnia dubia

Definitive Test Concentrations: 100, 50, 25, 12.5, 6.25 µg/L

Vehicle: DMF

Notes: Prelim conducted at 0.01, 0.05, 0.1, 0.5, and 1.0 mg/L
100% mortality at 0.1, 0.5, and 1.0 mg/L in 48 hrs.
No mortality at 0.01 mg/L, Control or Vehicle Blank.
One mortality at 0.05 mg/L.

Notified by: Dorothy England Date: 11/7/94

Study Director: Dorothy England Date: 11/7/94

FORM # 186 (6/02/92) (FDII)

7200 E. ABC Lane, Columbia, MO 65202

Tel: 314/474-8579 Fax: 314/443-9033

"Working for You"

ABC LABORATORIES

OCT 1 ? 1994

ABC PROTOCOL NO. 8606

(Revised October 4, 1994 for)

Procedure for Determining Chemical Effects on *Ceriodaphnia sp.* Survival and Reproduction During a 7-Day Exposure Period

ABC Study # 42201

Test Substance _____

PROTOCOL APPROVAL

ABC Laboratories' Study Director

Name (signed): Dorothy C. England Date: 11/1/94

Name/Title (typed): Dorothy England/Biologist II

Sponsor Representative

Name (signed): _____ Date: 10/7/94

Name/Title (typed): _____/Corporate TSCA Cood., Environmental Health and Safety

ABC Laboratories' QAU Protocol Review

Name (signed): Kimberly S. Frazier Date: 11-1-94

(Other sponsor-required signatures may be added below.)

TEST-SPECIFIC INFORMATION

The following information is necessary to be in compliance with Good Laboratory Practice regulations and/or ABC Laboratories' policy.

The sponsor is responsible for providing a Material Safety Data Sheet (MSDS), if available, and any other information necessary for proper handling, shipping, and storage of the test substance. The sponsor also agrees to accept any and all of the test substance (except effluents) that remains unused at the end of testing and to assume responsibility for its proper disposal.

Testing Facility

ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202

Phone: (314) 474-8579
Fax: (314) 443-9089

Study Sponsor

Phone:
Fax:

Sample Identification Number

Name: _____

Sponsor Identification Number (if applicable) N/A

Purity Correction (Please check the appropriate statement.)

_____ Test concentrations will be based on purity or active ingredient.

X Test concentrations will be based on total product.

Analytical Confirmation (Please check the appropriate statement.)

_____ Analytical confirmation will be performed by ABC Laboratories, Inc.

__X__ Sponsor requests no analytical confirmation.

_____ Analytical samples will be collected and shipped to sponsor for analysis.

Special Instructions and/or Comments ☒ Check here if not applicable.

_____

_____

_____

_____

GLP Compliance (Please check appropriate statement.)

_____	EPA-FIFRA	_____	OECD
__X__	EPA-TSCA	_____	None
_____	FDA	_____	(Other (specify) _____

Test Concentrations To be listed in a protocol alteration after range-finding testing is complete.

Test Organisms

Species: *Ceriodaphnia dubia*

Supplier: ABC In-house culture

Experimental Dates (to be completed by testing facility)

Proposed starting date: October 1994

Proposed termination date: November 1994

1.0 INTRODUCTION

In evaluating the toxicity of a test substance to aquatic invertebrates, the first approach is normally the determination of the material's toxicity during short-term lethality tests. These tests are typically conducted under static conditions with neonate daphnids. However, in general, the sensitivity to toxic substances varies with the life cycle of the organism. To more accurately define the toxicity of test substances or effluents to aquatic invertebrates, the 7-day *Ceriodaphnia sp.* survival and reproduction test (1) was developed. This test is based on the premise that chemical exposure during the complete life cycle of *Ceriodaphnia sp.* presents chemical challenge to the most sensitive life stages and functions of the test organism. Thus, by defining a no-observed-effect concentration (NOEC) and a lowest-observed-effect concentration (LOEC) for a chemical at the most sensitive life stages of the organism and observing the effect on reproduction, an estimate of chronic toxicity can be made.

2.0 OBJECTIVE

The primary objective of the toxicity test described herein is to estimate the chronic toxicity of a test substance to *Ceriodaphnia sp.* at all life stages under a static-renewal exposure system. This is achieved by defining a chronic value (CV) for survival data (alive-dead) and a reproductive value (RV) for young produced (young per adult) after a 7-day exposure period. The chronic value is defined as the geometric mean of the lowest-observed-effect concentration (LOEC) and the no-observed-effect concentration (NOEC). At the request of the study sponsor, LC_{50} and concentration response slopes may be calculated for one or more exposure periods. This test is applicable for the toxicity evaluation of a chemical product or for a surface water or effluent sample.

3.0 RANGE-FINDING STUDY

3.1 General. For most test substances, the approximate toxic level to aquatic organisms is not known. Because this information is essential before a 7-day chronic estimate test can be conducted, ABC routinely performs range-finding tests for bioassays with aquatic invertebrates. In the case of effluents or surface water samples, however, the range-finding test is often omitted due to stability concerns, and immediate definitive testing proceeds using a standard concentration series. The information derived from a preliminary test may be used to set concentration levels for the definitive 7-day *Ceriodaphnia sp.* survival and reproduction test. However, if information exists that would aid in defining the test concentrations, such concentrations can be established with the consent and consultation of the study sponsor.

3.2 Test Substance. Specific information regarding the test substance is to be supplied by the sponsor and will be addressed at the time of protocol approval in

the test-specific information section of the protocol. For test substances other than effluents or surface waters, characterization, stability and solubility studies will be the sponsor's responsibility unless contracted to ABC Laboratories, Inc. The test substance will be returned to the study sponsor at study completion. Archival of retention samples will be the sponsor's responsibility. The test concentrations will be prepared on a weight/volume basis unless otherwise specified. A record of all sample weights and dilutions will be kept, checked by a second party, and furnished in the final report. In the case of effluents, the sponsor must furnish ABC Laboratories with a planned sample receipt date at least 7 days in advance to allow for priming of the cultures for neonate production.

3.3 Test Procedure. The range-finding procedure is as follows:

Test Daphnids (neonate *Ceriodaphnia sp.* 2- to 24-hours old and released over an 8-hour period) will be selected from a subculture of neonates. These individuals will have been spawned from an ABC in-house culture of sexually mature *Ceriodaphnia sp.* adults which has been maintained at the test temperature (25 ± 1°C) for at least 2 weeks.

The range-finding test will be initiated by exposing groups of five neonate *Ceriodaphnia sp.* (2- to 24-hours old) to 2 or more widely spaced test substance concentrations, usually spaced by a factor of 10.

After 24 hours of exposure, the test chambers will be observed for mortality and/or adverse behavioral effects. A record will be maintained of mortality and abnormal behavior at each observation. Dependent upon these observations, additional test concentrations may be added at levels above or below the initial concentrations. This procedure will be followed until a toxic range is determined. For example, if the 24 hour exposure results in total mortality, new solutions will be prepared at a factor of 10 below the lowest initial concentration until no mortality or partial mortality is reached. In the converse situation, if no mortality is observed after 24 hours, new solutions will be prepared at concentrations spaced by a factor of 10 above the highest initial level until mortality is noted to a maximum of 100 ppm. In this manner, a bracket is formed for the toxic range of the compound.

The preliminary test will be conducted for a period of at least 24 hours. In most cases, a preliminary test for 24 hours with at least three test substance concentrations is sufficient to determine the toxic range.

Unless otherwise requested by the study sponsor, results of the range-finding study will be used to set the concentration range of the definitive study. At least

five toxicant concentrations will generally be selected for the 7-day *Ceriodaphnia sp.* survival and reproduction test.

4.0 CERIODAPHNIA sp. 7-DAY SURVIVAL AND REPRODUCTION TEST

4.1 General. Neonate *Ceriodaphnia sp.* will be exposed in a static renewal system for seven days to at least five concentrations of test substance. The test concentrations for chemical products will be based on the range-finding results (if performed) and will follow a numbered series with concentrations differing by a dilution factor of 0.30-0.50. Effluents will generally be tested using a five-concentration series of either 50% dilutions (6.25, 12.5, 25, 50, and 100%) or a series of 1.0, 3.0, 10, 30 and 100%. If the effluent is known or suspected to be highly toxic, a lower range of concentrations can be substituted (such as 0.10, 0.30, 1.0, 3.0 and 10%). Maximum exposure levels will be established at 1000 ppm for a chemical product evaluation or 100% for an effluent or surface water sample. Other concentrations will be tested at the request of the study sponsor. The results will be based on the survival and reproduction of *Ceriodaphnia sp.*

4.2 Test Organism. Neonate *Ceriodaphnia sp.* (2- to 24-hours old and released over an 8-hour period) will be used for testing. The test lot of neonates will have been spawned from an in-house culture of *Ceriodaphnia sp.* that has been maintained for at least 2 weeks under the conditions outlined in section 4.3 below. The taxonomic key presented by Pennek (2) will be used to periodically document the species or the supplier of the organisms will document species.

4.3 Culturing and Feeding. Brood organisms will be cultured in ~900 mL of culture medium in 1-L glass beakers. Cultures will be fed daily with an algal suspension of *Selenastrum capricornutum* and *Ankistrodesmus falcatus* supplemented with a suspension of fish food, cereal leaves, and yeast (YCT). Fish food will be analyzed for contaminants before use with the results kept on file at ABC Laboratories. The algal species fed and the source of the fish food will be provided in the report text. The dilution water used in toxicity testing at ABC Laboratories will be ABC well water prepared to a total hardness of between 130 to 160 mg/L as $CaCO_3$. Cultures will be maintained at a temperature of 25 ± 1°C on a 16-hour day/8-hour night photoperiod with 15-30 minute dusk/dawn transition periods. Dissolved oxygen levels in the cultures will be maintained at greater than 5.0 mg/L by aeration if necessary.

4.4 Test Chambers. Chambers containing test solutions, consisting of 10 replicates of each of 5 treatments and a control, will be randomly assigned to a test board using a computer generated random numbers table. The test chambers will be 30 mL cups or beakers filled with 15 mL of test solution. Individual chambers

will be labeled with a black marking pen. Temperature will be controlled by partially submerging the test chambers in a thermostatically controlled waterbath held at 25 ± 1°C or by placing the test board in an environmental chamber at 25 ± 1°C.

4.5 Test Solutions. A volume of the test solution will be prepared for daily renewal and analysis (if required) for each test concentration. The test organisms will be transferred to clean test vessels containing fresh food-enriched test solution prepared on a daily basis. The water used in culturing (section 4.3) will serve as dilution water for the test. Test vessels will receive 0.1 mL of YCT (~1.7 - 1.9 mg/mL suspended solids) and 0.1 mL of algal (3.0-3.5 X 10^7 cells/mL) per 15 mL of test solution.

4.6 Test Initiation. Neonates will be randomly selected from brood chambers and distributed one to each test chamber at test initation to eliminate bias. Each test concentration will have ten replicate test chambers for a total of 10 daphnids per test concentration and control. A vehicle blank, containing a solvent concentration equal to the highest amount of solvent used in the test solutions, will be prepared if necessary. Recommended solvents are acetone, triethylene glycol and dimethylformamide. Solvent use will be restricted to a maximum concentration of 0.10 mL per liter of solution. The replicate test chambers will be randomized within the waterbath at the beginning of the test.

4.7 Test Procedure – Biological. During daily renewal of test solutions, a record of adult survival (or mortality) and production of young will be kept. The adult test organisms will be transferred to the new test solution and the young will be counted. If necessary, the young may be killed by adding a small amount of acid or solvent to the test vessel. The immobilized young will then be counted with the aid of a microscope. The number of any dead young should be noted before adding the acid (solvent). Death of any adult with young present is assumed to have occurred just prior to observation for the purpose of determining the mean number of young per adult. The test will be terminated when 60% or more of surviving females in the controls have produced their third brood.

4.8 Test Procedure – Chemical/Physical. Dissolved oxygen, pH, and temperature measurements will be conducted daily on the new replacement solutions and the 24-hour old test solutions. These measurements will be carried out on the highest concentration having living brood daphnids and on low and mid-level concentration, low concentration, and control water (plus vehicle blank, if used). Measurements may be made in an extra beaker replicate of the test solution prepared for this purpose. If dissolved oxygen deviates more than 2.0 ppm, or if pH deviates more than 1.0 unit between the control and test solutions, these parameters will be measured in all test solutions to further document the impact

of the test substance on these parameters. In addition, alkalinity, hardness, and conductivity will be measured in each new test solution and the control. A 16 hour light-8 hour dark photoperiod will be maintained throughout the study. Light intensity will be maintained at ~50-100 ft. cd and daily light readings will be recorded.

4.9 Statistical Analyses of Test Results.

Survival data from each replicate will be transformed by converting the square root of the proportion of surviving daphnids to arcsin (2). An analysis of variance (one-way ANOVA) will be used to determine whether or not statistical differences exist between mean survival among the treatment levels. This will be followed by a Dunnett's test (3) which compares each mean survival from each toxicant concentration with that of the control mean. From this data, a NOEC and a LOEC will be determined and a chronic value (CV) calculated as the geometric mean of the NOEC and the LOEC. LC_{50} and concentration response slopes may be calculated by regressing analysis for one or more exposure periods if requested by the study sponsor.

Reproductive data from each replicate will be analyzed in a manner similar to the survival data, except that no transformation will be done. A comparison of the CV analyses for survival and reproduction will indicate whether survival or reproduction was a more sensitive indicator of the effects of the test substance.

For test results to be acceptable, survival in controls must be at least 80% and reproduction in the controls must average 15 or more young per surviving female.

5.0 DATA MAINTENANCE/REPORTING

5.1 Records to be Maintained. Records to be maintained will include, but not be limited to, compound receipts; solution preparations and dilutions; instrument logbooks detailing calibration and maintenance; facility records (kept at ABC); material control identification numbers for all instruments used; storage of test substance, solutions, and samples; and weights and volumes. All original raw data collected during this study will be maintained at ABC Laboratories until finalization of the study. Upon completion of the project, all raw data specifically for this study will be submitted to the sponsor as part of the final report.

5.2 Report. A final report containing all original raw data and/or certified copies of certain raw data records will be submitted to the sponsor. A copy of the report and associated raw data will be kept on file in ABC Laboratories' archives. The final report will include, but not be limited to, the following:

5.2.1 Study dates, name, and address of test facility.

5.2.2 Objectives and test procedures as stated in approved protocol.

5.2.3 A description of the experimental design along with a description of and reference to any statistical methods used for data analysis.

5.2.4 Description of test substance (date of receipt, storage conditions, purity, physical characteristics, and method of preparing stock and/or test solutions).

5.2.5 Description of methods used during the study.

5.2.6 Description of test organisms (source, culture techniques, etc.).

5.2.7 Summary of all data and a statement of the conclusions drawn from any data analyses, if appropriate.

5.2.8 Location of raw data.

5.2.9 List and signatures of study personnel.

5.2.10 GLP compliance statement by study director and a statement by ABC Laboratories' Quality Assurance Unit.

5.2.11 An appendix or separate raw data package will contain the original raw data or certified copies of raw data, letter of test authorization (if available), protocol alterations, the approved protocol, and the analytical method appendix (if analytical confirmation is performed by ABC Laboratories).

6.0 PROTOCOL ALTERATIONS

The study director, upon approval of the sponsor representative, may make other changes to this protocol. All changes will take the form of a written protocol alteration describing the change, the reason for the change, and the effect on the study, if any. All alterations will be signed and dated by both the study director and the sponsor representative. The signed Protocol Alteration Notification form will be maintained with the protocol.

Should a significant problem develop while the study is in progress, the study director will notify the sponsor representative as soon as practical to discuss the problem and any corrective actions taken. Upon verbal authorization from the sponsor representative, the study director will proceed with any further actions deemed appropriate. If the sponsor

representative cannot be reached, the study director will proceed with the appropriate modifications and will notify the sponsor representative as soon as possible.

In the event of protocol deviations, an attempt will be made to notify the sponsor representative within a reasonable period of time. A written description of the deviation(s) will be submitted on a Protocol Alteration Notification form to the sponsor representative. All deviations will be signed and dated by both the study director and the sponsor representative.

7.0 QUALITY ASSURANCE

ABC's Quality Assurance Unit will inspect one or more critical phases to assure that equipment, personnel, procedures, and records conform to the guidelines listed in this protocol. The results of these inspections will be reported to the study director and ABC management. The draft and final reports will be reviewed for protocol and GLP compliance, as well as to assure that the methods and standard operating procedures used were followed. A signed statement will be included in the report specifying types of inspections made, the dates inspections were made, and the dates inspections were reported to the study director and management.

8.0 GLP COMPLIANCE

This study will be conducted in accordance with one of the following GLPs (4). The report will contain a statement attesting to that fact.

1. U.S. FDA Good Laboratory Practice Regulations (21 CFR, Part 58).
2. U.S. EPA Good Laboratory Practice Standards; Toxic Substance Control (40 CFR, Part 792).
3. U.S. EPA Good Laboratory Practice Standards; Pesticide Programs (40 CFR, Part 160).
4. OECD Principles of Good Laboratory Practice; Annex 2 C(81) 30 (Final).
5. Any state or local GLP which may apply.

The specific GLP regulation, with which this study specific protocol and resultant study must be in compliance, is to be designated by the study sponsor.

9.0 REFERENCES

(1) U.S. Environmental Protection Agency. 1989. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*. EPA/600/4-89/001. Environmental Monitoring Systems Laboratory, Cincinnati, OH.

(2) Pennak, R.W. 1978. Freshwater Invertebrates of the United States, 2nd ed. 365 p.

(3) Systat, Inc. 1985. Systat, The System for Statistics, Systat, Inc., Evanston, Illinois, 417 p.

(4) Dunnett, C.W. 1985. A Multiple Comparison Procedure for Comparing Several Treatments with a Control. *J. Amer. Stat. Assoc.* 50:1096-1121.

(5) (a) U.S. Food and Drug Administration. Nonclinical Laboratory Studies, Good Laboratory Practice Regulations (21 CFR, Part 58). *Federal Register*.

(b) U.S. Environmental Protection Agency. Pesticide Programs; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). *Federal Register*.

(c) U.S. Environmental Protection Agency. Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 792). *Federal Register*.

(d) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals, Principles of Good Laboratory Practice Annex 2, C(81) 30 (Final):7-28.

Triage of 8(e) Submissions

Date sent to triage: MAY 0 9 1995

NON-CAP CAP

Submission number: 13372H

TSCA Inventory: Y N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO AQUATO

Group 2 - E nie 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.): [redacted]

Notes:

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

For Contractor Use Only

entire document: 0 1 2 **pages** — **pages** 4 TTB

Notes:

Contractor reviewer : PJ **Date:** 4/21/95

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ- 0395 -13372 (5) SEQ. A

TYPE: (INT) SUPP FLWP

SUBMITTER NAME: Confidential

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
(0639) REFER TO CHEMICAL SCREENING
0678 CAP NOTICE

VOLUNTARY ACTIONS:
(0401) NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKERS/OTHERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP/USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

SUB. DATE: 03/09/95 OTS DATE: 03/13/95 CSRAD DATE: 03/27/95

CHEMICAL NAME: Alkyl Biphenyl

CAS#: Confident

INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04
0202	ONCO (ANIMAL)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04
0204	MUTA (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04
(0207)	REPRO/TERATO (ANIMAL)	01 02 04
0208	NEURO (HUMAN)	01 02 04
0209	NEURO (ANIMAL)	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04
0212	ACUTE TOX. (ANIMAL)	01 02 04
0213	SUB ACUTE TOX (ANIMAL)	01 02 04
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04
0215	CHRONIC TOX (ANIMAL)	01 02 04

INFORMATION TYPE:		P F C
0216	EPI/CLIN	01 02 04
0217	HUMAN EXPOS (PROD CONTAM)	01 02 04
0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0219	HUMAN EXPOS (MONITORING)	01 02 04
(0220)	ECO/AQUA TOX	01 02 04
0221	ENV. OCCC/REL/FATE	01 02 04
0222	EMER INCI OF ENV CONTAM	01 02 04
0223	RESPONSE REQEST DELAY	01 02 04
(0224)	PROD/COMP/CHEM ID	01 02 04
0225	REPORTING RATIONALE	01 02 04
0226	CONFIDENTIAL	01 02 04
0227	ALLERG (HUMAN)	01 02 04
0228	ALLERG (ANIMAL)	01 02 04
0239	METAB/PHARMACO (ANIMAL)	01 02 04
0240	METAB/PHARMACO (HUMAN)	01 02 04

INFORMATION TYPE:		P F C
0241	IMMUNO (ANIMAL)	01 02 04
0242	IMMUNO (HUMAN)	01 02 04
(0243)	CHEM/PHYS PROP	01 02 04
0244	CLASTO (IN VITRO)	01 02 04
0245	CLASTO (ANIMAL)	01 02 04
0246	CLASTO (HUMAN)	01 02 04
0247	DNA DAM/REPAIR	01 02 04
0248	PROD/USE/PROC	01 02 04
(0251)	MSDS	01 02 04
0299	OTHER	01 02 04

TRIAGE DATA:	NON-CBI INVENTORY	ONGOING REVIEW	SPECIES	TOXICOLOGICAL CONCERN:	USE:	PRODUCTION:
	YES	YES (DROP/REFER)	Daphnids	LOW		
CAS SR	NO	NO (CONTINUE)		MED		
	(IN TRANSIT)	REFER		HIGH		

COMMENTS: Non-Cap