

ACUTE TOXICITY TO AQUATIC PLANTS (ALGAE)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, FC-109-X or R1904. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-129 referred to by the test laboratory as R1904. Current information indicates it is a mixture of 42% test substance, 32% water, 14% 2-butoxyethanol, 4% ethanol, and 8% C₄-C₇ fluoroalkyl carboxylate compounds. Test material was from 3M production lot 930014, mfg. date 3/97.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD:

Methods: OECD 201

Test type: Static acute

GLP: No

Year Completed: 1997

Species: *Selenastrum capricornutum*

Analytical monitoring: Temperature, pH, and conductivity

RESULTS

Nominal concentrations: Bk control, 129.6, 216, 360, 600, 1000 mg/L (4 replicates per concentrations, 6 per blank controls)

Element values:

Endpoint	Data (based on nominal concentrations)		95% Confidence Interval (mg/L)
	Time	(mg/L)	
Cell Count	24-hour EC ₅₀	431	385-484
	48-hour EC ₅₀	395	356-439
	72-hour EC ₁₀	113	94-130
	72-hour EC ₅₀	248	225-272
	96-hour EC ₁₀	163	54-228
	96-hour EC ₅₀	298	278-319
	96-hour NOEC	216	Not Applicable

Endpoint	Data (based on nominal concentrations)		95% Confidence Interval (mg/L)
	Time	(mg/L)	
Average Specific Growth Rate (μ)	24-hour EC ₅₀	586	524-656
	48-hour EC ₅₀	859	777-950
	72-hour EC ₁₀	234	197-268
	72-hour EC ₅₀	610	551-676
	96-hour EC ₁₀	287	248-322
	96-hour EC ₅₀	604	550-664
	96-hour NOEC	216	Not Applicable

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The values reported apply to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvents or what portion of the toxicity can be contributed to the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

The FC-129 96-hour EC₅₀ for *Selenastrum capricornutum* was determined to be 298 mg/L (C.I.: 278-319 mg/L) by Cell Count and 604 mg/L (C.I.: 550-664 mg/L) by Average Specific Growth Rate. The 96-hour no observed effect concentration for both Cell Count and Average Specific Growth Rate was 216 mg/L. Effect determined to be algistatic based on recovery of cells previously exposed to the 1000 mg/L concentration.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 2. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample.

REFERENCES

This study was conducted at AScl Corporation of Duluth, Minnesota at the request of the 3M Company.

OTHER

Last changed: 5/17/00

STUDY TITLE

GROWTH INHIBITION OF R1904 FOR
GREEN ALGA (*Selenastrum capricornutum*)

DATA STANDARD

OECD GUIDELINE 201

STUDY COMPLETED

June 18, 1997

TESTING FACILITY

ASCI Corporation/ASCI-Duluth
Environmental Testing Division
4444 Airpark Boulevard
Duluth, MN 55811

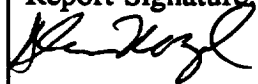
Tel. No. (218) 722-4040
Fax No. (218) 722-2592

STUDY IDENTIFICATION NUMBERS

ASCI Study ID# 5010-068

3M Company Lab Request# R1904

1.0 GENERAL INFORMATION

Study Title	Growth Inhibition of R1904 for Green Alga (<i>Selenastrum capricornutum</i>).	
Data Standard	OECD Guideline 201.	
Sponsor	3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (612) 778-7452.	
Sponsor's Representative	Susan Beach, 3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (612) 778-7452.	
Sponsor's Lab Request#	R1904.	
Testing Facility	ASCI Corporation/ASCI-Duluth Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.	
Principal Investigator	Todd McGuire	
Project Director	Joe Amato	
Testing Facility Director	Donald Mount	
Definitive Test Dates	June 9-13, 1997.	
Data Validation and Report Review	Alan Mozol	Report Signature: 
Retention of Raw Data and Final Report	Two years from the date study is completed.	
Location of Raw Data and Final Report	ASCI Corporation/ASCI-Duluth Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.	

2.0 OBJECTIVE(S)

To determine the 3-d and 4-d effect concentrations (EC_{10} 's and EC_{50} 's) and the 4-d no observable effect concentration (NOEC) of the test substance, R1904, for green alga (*Selenastrum capricornutum*) under static test conditions.

This study was conducted according to OECD Guideline 201 (OECD 1993).

3.0 TEST METHODS

Test Substance	<i>Properties:</i> R1904, amber liquid, specific gravity ca. 1.3 (water = 1), stable, and completely soluble in water. <i>Test concentrations:</i> 0 (algal medium control), 129.6, 216, 360, 600, and 1000 mg/L. A 2000 mg/L stock solution was prepared by mass addition to algal medium followed by mechanical stirring. Each test concentration was made by volume addition of the stock to algal medium.
Test Organisms	<i>Source:</i> ATCC 22662 <i>Inoculum preparation:</i> Algal stock was subcultured in OECD recommended medium for 4 days before test initiation. <i>Use in test:</i> Stock culture was diluted and spiked into test solutions to obtain an initial cell density of 1.0×10^4 cells per ml (1.0×10^4 actual concentration).
Algal Medium	<i>Preparation:</i> Deionized water was spiked with nutrient stocks (including Na_2EDTA) to OECD recommended concentrations. The most recent chemical analysis for deionized water is in Raw Data Package. <i>Characteristics:</i> At test time, the medium had a pH of 7.86. Sterilized via $0.22 \mu m$ filtration prior to use in the test.
Exposure Chambers	<i>Type:</i> Sterilized via autoclave 250-ml borosilicate-glass Erlenmeyer flasks sealed with foam stoppers. <i>Use in test:</i> 100 ml of algal medium or test solution/chamber. Six control replicates and four replicates per test substance concentration were employed. During the test, kept stoppered, except when experimental observations were made.

Incubation	<i>Duration:</i> 4 days. <i>Daily photoperiod:</i> Continuous at 740 ft-candles using cool-white fluorescent lamps. Test chambers held on shaker table set at 100 rpm. <i>Temperature:</i> 25.0 $\pm$ 2°C.
Observations	<i>Biological:</i> Daily cell counts using direct microscopic enumeration via Improved Neubauer hemacytometer. <i>Water chemistry:</i> (1) At test initiation and termination- pH, conductivity, and temperature, (2) daily- temperature. All determinations were made according to APHA methods (1995).
Endpoint Calculations	1-, 2-, 3-, and 4-d EC₅₀'s, 3- and 4-d EC₁₀'s, and 4-day NOEC's based on both cell counts and average specific growth rates (μ). Statistical analyses performed using Trimmed Spearman-Kärber Method (EC₅₀'s), Toxstat (NOEC's) and EPA Probit software (EC₁₀'s).

4.0 RESULTS

Test Substance Toxicity (Pertinent data are in Tables 1, 2, and 3)	<i>Cell Count 1-d EC₅₀</i>: 431 mg/L (385-484). <i>μ 1-d EC₅₀</i>: 586 mg/L (524-656). <i>Cell Count 2-d EC₅₀</i>: 395 mg/L (356-439). <i>μ 2-d EC₅₀</i>: 859 mg/L (777-950). <i>Cell Count 3-d EC₁₀</i>: 113 mg/L (94-130). <i>μ 3-d EC₁₀</i>: 234 mg/L (197-268). <i>Cell Count 3-d EC₅₀</i>: 248 mg/L (225-272). <i>μ 3-d EC₅₀</i>: 610 mg/L (551-676). <i>Cell Count 4-d EC₁₀</i>: 163 mg/L (54-228). <i>μ 4-d EC₁₀</i>: 287 mg/L (248-322). <i>Cell Count 4-d EC₅₀</i>: 298 mg/L (278-319). <i>μ 4-d EC₅₀</i>: 604 mg/L (550-664). <i>Cell Count 4-d NOEC</i>: 216 mg/L. <i>μ 4-d NOEC</i>: 216 mg/L. Effect was determined to be algistatic based on recovery of cells previously exposed to the 1000 mg/L test substance concentration.
Test Conditions	<i>Temperature (°C)</i>: 24.8-26.0. <i>Light intensity ft-cdls</i>: 740. <i>pH</i>: 6.78-9.46. <i>Specific conductivity (μmhos/cm)</i>: 123-189.

5.0 TEST QA/QC

QA/QC Criteria	Data
Algal counts in control must increase by at least a factor of 16 during the first 3 d of the exposure.	During the first 3 d of the test control algal counts increased by a factor of 348.
Test duration must be 4 d.	The test duration was 4 d.

6.0 REFERENCES

American Public Health Association (APHA). 1995. Standard Methods for the Examination of Water and Wastewater. APHA, Washington, D.C.

Organization for Economic Cooperation and Development (OECD). 1993. OECD Guidelines for Testing of Chemicals. OECD Publication Information Center, Washington, D.C.

Hamilton, M.A., R.C. Russo, and R.V. Thurston, 1977. Trimmed Spearman-Kärber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays. Environ. Sci. Technol. 11(7): 714-719; Correction 12 (4): 417 (1978).

U.S. Environmental Protection Agency (EPA). 1988. Computer Program Probit. Biological Methods Branch, EMSL, U.S. EPA, Cincinnati, OH 45268.

TABLE 1. EC₁₀'s EC₅₀'S, 95% Confidence Intervals, and 4-d NOEC'S from Algal Growth Inhibition Test with R1904

Endpoint	Data ^a		95 % Confidence Interval (mg/L)
	Time	(mg/L)	
Cell Count	1-d EC ₅₀	431	385-484
	2-d EC ₅₀	395	356-439
	3-d EC ₁₀	113	94-130
	3-d EC ₅₀	248	225-272
	4-d EC ₁₀	163	54-228
	4-d EC ₅₀	298	278-319
	4-d NOEC	216	Not Applicable

Endpoint	Data ^a		95 % Confidence Interval (mg/L)
	Time	(mg/L)	
Average Specific Growth Rate (μ)	1-d EC ₅₀	586	524-656
	2-d EC ₅₀	859	777-950
	3-d EC ₁₀	234	197-268
	3-d EC ₅₀	610	551-676
	4-d EC ₁₀	287	248-322
	4-d EC ₅₀	604	550-664
	4-d NOEC	216	Not Applicable

^a Values Based on Nominal Test Concentrations

TABLE 2. Cells per ml and Percentage Difference from Mean Control Value for *Selenastrum capricornutum* Exposed to R1904

Test Conc. (mg/L)	Replicate	Cells/ml (10^4)			
		1 d	2 d	3 d	4 d
Control	A	11	52	342	605
	B	8	48	306	704
	C	8	60	348	616
	D	9	51	336	572
	E	7	64	390	627
	F	10	48	366	726
	Mean	8.8 (-)	53.8 (-)	348.0 (-)	641.7 (-)
129.6	A	9	50	264	506
	B	6	43	282	528
	C	7	50	318	627
	D	7	52	300	671
	Mean	7.3 (-17)	48.8 (-9)	291.0 (-16)	583.0 (-9)
216	A	10	37	240	517
	B	7	45	204	561
	C	6	44	198	583
	D	8	39	234	594
	Mean	7.8 (-11)	41.3 (-23)	219.0 (-37)	563.8 (-12)
360	A	3	24	72	143
	B	5	27	72	154
	C	6	27	96	165
	D	3	28	84	165
	Mean	4.3 (-51)	26.5 (-51)	81.0 (-77)	156.8 (-76)
600	A	3	24	33	37
	B	3	24	27	32
	C	4	17	30	32
	D	4	18	25	30
	Mean	3.5 (-60)	20.8 (-61)	28.8 (-92)	32.8 (-95)
1000	A	2	4	4	2
	B	1	6	2	3
	C	2	3	2	3
	D	1	6	3	4
	Mean	1.5 (-83)	4.8 (-91)	2.8 (-99)	3.0 (-100)

* Parenthetic Values = % Difference from Control Mean Value

* 0-d Mean Algal Count = 1.0×10^4

TABLE 3. Average Specific Growth Rates (μ) and Percentage Difference from Mean Control Value for *Selenastrum capricornutum* Exposed to R1904

Test Conc. (mg/L)	Average Specific Growth Rate (μ) ^a			
	1 d	2 d	3 d	4 d
Control	2.175 (-)	1.993 (-)	1.951 (-)	1.616 (-)
129.6	1.988 (-9)	1.944 (-2)	1.891 (-3)	1.592 (-1)
216	2.054 (-6)	1.860 (-7)	1.796 (-8)	1.584 (-2)
360	1.495 (-33)	1.639 (-18)	1.465 (-25)	1.264 (-22)
600	1.253 (-42)	1.517 (-24)	1.120 (-43)	0.873 (-46)
1000	0.405 (-81)	0.784 (-61)	0.343 (-82)	0.275 (-83)

^a $\mu = \frac{\ln \text{Cells per ml } t_n - \ln \text{Cells/ml } t_0}{t_n - t_0}$

^b Parenthetic Values = % Difference from Control Mean Value

* 0-d Mean Algal Count = 1.0×10^4

ACTIVATED SLUDGE RESPIRATION INHIBITION

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, FC-109-X or R1904. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-129 referred to by the test laboratory as R1904. Current information indicates it is a mixture of 42% test substance, 32% water, 14% 2-butoxyethanol, 4% ethanol, and 8% C₄-C₇ fluoroalkyl carboxylate compounds. Test material was from 3M production lot 930014, mfg. date 3/97.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD:

Methods: OECD 209

Test type: Static acute

GLP: No

Year Completed: 1997

Test organism source: Activated sludge collected from the City of Superior, Wisconsin, Wastewater Treatment Plant.

RESULTS

Nominal concentrations: Bk control, Reference substance, 3.75, 7.5, 15.0, 30 mg/L

Element values: 30-minute EC₅₀ = >1000 mg/L
3-hour EC₅₀ = >1000 mg/L (No inhibition exhibited)

All element values based on nominal concentrations.

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The value reported applies to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvents or what portion of the toxicity can be contributed to the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

The FC-129 3-hour EC₅₀ for activated sludge respiration inhibition was determined to be >1000 mg/L.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 2. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample.

REFERENCES

This study was conducted at AScl Corporation of Duluth, Minnesota at the request of the 3M Company.

OTHER

Last changed: 5/17/00

STUDY TITLE

INHIBITION OF R1904 FOR ACTIVATED SLUDGE RESPIRATION

DATA STANDARD

OECD GUIDELINE 209

STUDY COMPLETED

JULY 10, 1997

TESTING FACILITY

ASCI Corporation
ASCI-Duluth Environmental Testing Division
4444 Airpark Boulevard
Duluth, MN 55811

Tel. No. (218) 722-4040

PROJECT IDENTIFICATION NUMBERS

ASCI Study ID# 5010-068

3M Company Lab Request# R1904

1.0 GENERAL INFORMATION

Study Title	Inhibition of R1904 For Activated Sludge Respiration	
Data Standard	OECD Guideline 209.	
Sponsor	3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (612) 778-7452.	
Sponsor's Representative	Susan Beach, 3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (612) 778-7452.	
Sponsor's Lab Request#	R1904	
Testing Facility	ASCI Corporation/ASCI-Duluth Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.	
Principal Investigator	Joe Dierkes	
Project Director	Joe Amato	
Testing Facility Director	Donald Mount	
Definitive Test Date	July 1, 1997	
Data Validation and Report Review	Alan Mozol	Report Signature: 
Retention of Raw Data and Final Report	Five years from the date study is completed.	
Location of Raw Data and Final Report	ASCI Corporation/ASCI-Duluth Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.	

2.0 OBJECTIVE

To determine if possible, the 30-minute and 3-h (contact times) EC₅₀'s of the test substance R1904 for activated sludge respiration.

This study was conducted according to the ASCI study plan retained by the Sponsor and OECD Guideline 209 (OECD 1993).

3.0 TEST METHODS

Test Substance	<i>Properties:</i> R1904, yellow liquid, specific gravity ca. 1.3 (water = 1), stable, and completely soluble in water. <i>Test concentrations:</i> 0 and 1,000 mg/L prepared in duplicate by direct additions.																
Reference Substance	<i>Name:</i> 3,5-dichlorophenol, purity 97%, Aldrich Chemicals, Lot# 02110JX. <i>Test concentrations:</i> 3.75, 7.5, 15.0, and 30 mg/L prepared by direct addition of 500 mg/L stock solution in Millipore® water.																
Test Organisms	<i>Source:</i> Activated sludge from the City of Superior (Wisconsin) Wastewater Treatment Plant receiving approximately 95% domestic sewage. <i>Use in test:</i> (1) Centrifuged five minutes, three times (4°C, 7500 rpm), (2) diluted the sludge pellet with carbon-treated water to selected volume, final total solids concentration of 1.6 g/L was obtained by further dilution, and (3) added 50 ml of synthetic sewage feed per L of the diluted sludge, and aerated at 20°C until test initiation. The sludge was held for less than 24 hours between collection and test initiation.																
Synthetic Sewage Feed	<i>Composition:</i> <table> <tr> <th><u>Chemical</u></th><th>g dissolved/L of deionized water</th></tr> <tr> <td>Bacto. peptone</td><td>16.0</td></tr> <tr> <td>Beef extract</td><td>11.0</td></tr> <tr> <td>Urea</td><td>3.0</td></tr> <tr> <td>NaCl</td><td>0.7</td></tr> <tr> <td>CaCl₂·2H₂O</td><td>0.4</td></tr> <tr> <td>MgSO₄·7H₂O</td><td>0.2</td></tr> <tr> <td>K₂HPO₄</td><td>2.8</td></tr> </table>	<u>Chemical</u>	g dissolved/L of deionized water	Bacto. peptone	16.0	Beef extract	11.0	Urea	3.0	NaCl	0.7	CaCl ₂ ·2H ₂ O	0.4	MgSO ₄ ·7H ₂ O	0.2	K ₂ HPO ₄	2.8
<u>Chemical</u>	g dissolved/L of deionized water																
Bacto. peptone	16.0																
Beef extract	11.0																
Urea	3.0																
NaCl	0.7																
CaCl ₂ ·2H ₂ O	0.4																
MgSO ₄ ·7H ₂ O	0.2																
K ₂ HPO ₄	2.8																
Deionized Water	Deionized water used in the study was obtained from a Millipore® Milli-Q System. The most recent chemical analysis is in Raw Data Package.																

Test Setup	<i>Exposure vessels:</i> 8, 1-L borosilicate glass beakers; two inoculum controls, two test exposures, and four reference substance treatments. <i>Order of preparation:</i> At 10-minute time intervals: (1) first inoculum control, (2) test exposure concentration replicates, (3) from low to high reference substance exposure concentrations, and (4) second inoculum control. The final volume in each beaker was 800 ml. <i>Preparation:</i> (1) Added 284 ml of the sludge preparation in each beaker, (2) added 26 ml of synthetic sewage feed to each beaker, and (3) in test and reference exposures, added the required amounts of the test and reference substances, respectively, (4) and diluted each exposure to 800 ml with carbon-treated water. <i>Incubation and aeration:</i> 19.9° to 20.0°C and vigorous aeration.
Determination of Respiration	<i>Sequence:</i> Same as exposures were prepared. <i>Determination:</i> At 30-min and 3-h contact times: (1) filled one 300-ml BOD bottle with the exposure medium and continued to aerate the remaining medium, (2) using a YSI 54A oxygen meter with a self-stirring probe, determined the DO concentration in the exposure medium every 0.5 minutes for up to 5.5 minutes (the first determination was made after 0.5 minutes), and (3) the sub-sample was properly disposed. DO meter calibration checks were made between the sets of measurements for each control or treatment.
Calculation of Respiration Rate	For each exposure, correlated DO depletion and time interval, and calculated the slope of the regression line as the respiration rate.
Endpoint Calculation	<i>EC50:</i> Probit Analysis (EPA 1988).

3.0 RESULTS

Test Substance Toxicity (Pertinent data are in Table 1)	30-minute EC_{50} : >1,000 mg/L. 3-h EC_{50} : >1,000 mg/L.
Reference Substance Toxicity (Pertinent data are in Table 1)	30-minute EC_{50} : <3.75 mg/L. 3-h EC_{50} : <3.75 mg/L.

4.0 TEST QA/QC

QA/QC Criteria	Data
Respiration rates of two inoculum controls must not vary by more than 15%.	Variations after 30-minute and 3-h contact times were less than 3%.
3-h EC_{50} of reference substance must be between 5-30 mg/L.	3-h EC_{50} was <3.75 mg/L indicating the sludge had greater than normal sensitivity. The lack of effect due to R1904 is a valid result.
Test duration must be 3 h.	The test duration was 3 h.

5.0 REFERENCES

Organization for Economic Cooperation and Development (OECD). 1993. OECD Guidelines for Testing of Chemicals. OECD Publication Information Center, Washington, D.C.

U.S. Environmental Protection Agency (EPA). 1988. Computer Program Probit. Biological Methods Branch, EMSL, U.S. EPA, Cincinnati, OH 45268.

TABLE 1.0
OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
CALCULATION OF RESULTS

StdY N 5010-068

Date: 7/1/97

ASCI Corporation/ASCI-Duluth
Environmental Testing Division
ASCI Study ID# 5010-068

Test Substance: R1904

Analyst: DIERKES

30-Minute Exposure												Dissolved Oxygen Concentrations, mg/L			
mg/L	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp, °C	R (1)	Ra (2)	% Inh. (3)
BK1	6.8	6.5	6.0	5.6	5.1	4.6	4.2	3.7	3.2	2.7	2.2	19.9	0.9503	0.9569	
1000A	6.9	6.5	6.2	5.8	5.4	5.0	4.6	4.2	3.8	3.3	3.0	19.9	0.7976	0.8031	16
1000B	6.8	6.4	6.0	5.6	5.2	4.8	4.4	4.0	3.6	3.2	2.8	20.0	0.8000	0.8000	16
3.75ref	7.6	7.3	7.1	6.9	6.6	6.4	6.2	5.9	5.7	5.5	5.2	19.9	0.4655	0.4687	51
7.5ref	7.6	7.4	7.3	7.1	7.0	6.8	6.6	6.4	6.2	6.1	5.9	20.0	0.3442	0.3442	64
15ref	8.1	8.0	7.9	7.8	7.8	7.7	7.6	7.5	7.4	7.3	7.3	19.9	0.1636	0.1648	83
30ref	8.7	8.6	8.6	8.5	8.5	8.4	8.4	8.3	8.2	8.2	8.1	20.0	0.1139	0.1139	88.0
BK2	6.9	6.4	6.0	5.5	5.1	4.5	4.1	3.7	3.1	2.6	2.2	20.0	0.9479	0.9479	
% diff in controls(4) 1.0 EC50 (95% CI): >1000 mg/L (NC) RaCm (5): 0.9524															

3-Hour Exposure												Dissolved Oxygen Concentrations, mg/L			
mg/L	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp, °C	R (1)	Ra (2)	% Inh. (3)
BK1	7.5	7.0	6.7	6.2	5.8	5.4	5.0	4.6	4.1	3.7	3.2	20.0	0.8448	0.8448	
1000A	7.3	6.8	6.4	5.9	5.5	5.1	4.6	4.2	3.7	3.2	2.8	20.0	0.8945	0.8945	-5
1000B	7.0	6.6	6.2	5.7	5.3	4.8	4.3	3.9	3.4	2.9	2.5	19.9	0.9236	0.9301	-9
3.75ref	8.3	8.1	7.9	7.7	7.5	7.3	7.1	6.9	6.8	6.5	6.1	20.0	0.4158	0.4158	51
7.5ref	8.5	8.3	8.2	8.0	7.9	7.7	7.6	7.4	7.3	7.2	7.0	19.9	0.2885	0.2905	66
15ref	8.9	8.8	8.7	8.6	8.6	8.5	8.4	8.4	8.3	8.3	8.2	20.0	0.1261	0.1261	85
30ref	9.0	9.0	8.9	8.9	8.9	8.8	8.8	8.8	8.7	8.7	8.7	20.0	0.0655	0.0655	92.3
BK2	7.9	7.4	7.0	6.6	6.2	5.7	5.3	4.9	4.4	4.0	3.5	20.0	0.8655	0.8655	
% diff in controls(4) 2.4 EC 50 (95% CI): >1000 mg/L (NC) RaCm (5): 0.8552															

(1) R= Oxygen uptake rate (mg O₂/L-min)

(2) Ra= R adjusted to 20°C= [R/θ^{Ex}]

(3) % Inhibition= [(RaCm)-RaS/(RaCm)]100

(4) % difference in controls: [(RaBk1-RaBk2)/RaCm]100 (Two controls must be within 15% of each other.)

(5) Mean adjusted R, controls= [RaBk1+RaBk2]/2

θ= 1.072

RaCm= mean adjusted R, controls

Bk= blank control, S= sample

Tobs= sample temp

x= Tobs-20°C