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AQUATIC TOXICITY OF PINACOLYL ALCOHOL

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REPORT DOCUMENTATION PAGE				
1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED		1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.		
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE				
4. PERFORMING ORGANIZATION REPORT NUMBER(S) CRDEC-TR-86080		5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION CRDEC	6b. OFFICE SYMBOL (If applicable) SMCCR-RST	7a. NAME OF MONITORING ORGANIZATION		
6c. ADDRESS (City, State, and ZIP Code) Aberdeen Proving Ground, MD 21010-5423		7b. ADDRESS (City, State, and ZIP Code)		
8a. NAME OF FUNDING/SPONSORING ORGANIZATION CRDEC	8b. OFFICE SYMBOL (If applicable) SMCCR-RST	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER		
8c. ADDRESS (City, State, and ZIP Code) Aberdeen Proving Ground, MD 21010-5423		10. SOURCE OF FUNDING NUMBERS		
		PROGRAM ELEMENT NO.	PROJECT NO. 1L162622	TASK NO. A554
				WORK UNIT ACCESSION NO.
11. TITLE (Include Security Classification) Aquatic Toxicity of Pinacolyl Alcohol				
12. PERSONAL AUTHOR(S) Haley, Mark V., Johnson, Dennis W., Muse, William T., and Landis, Wayne G., Ph.D.				
13a. TYPE OF REPORT Technical	13b. TIME COVERED FROM 83 Jun TO 84 Jun	14. DATE OF REPORT (Year, Month, Day) 1986 September	15. PAGE COUNT 22	
16. SUPPLEMENTARY NOTATION				
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD 06	GROUP 01	SUB-GROUP	Pinacolyl alcohol <u>Ankistrodesmus falcatus</u> <u>Daphnia magna</u> <u>Pimephales promelas</u> Bioassay	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) The aquatic toxicity of a secondary alcohol, 3,3-Dimethyl-2-butanol [Pinacolyl Alcohol (PA)], was determined. Acute tests were conducted on <u>Pimephales promelas</u> , 48 hr EC50 = 443.1 mg PA/l, and <u>Daphnia magna</u> , 24 hr EC50 = 513.2 mg PA/l. Growth inhibition tests (96 hr) were conducted using <u>Ankistrodesmus falcatus</u> , EC50 = 257.7 mg PA/l. In general, the toxicity of straight-chained alcohols increases proportionally with the molecular weights. Using the structure activity equation ($\text{Log EC50} = 1.10 (\text{LogP}) + 0.05 (\text{LogP})^2 - 1.22$) for <u>P. promelas</u> , the calculated toxicity of PA (EC50 = 547.0 mg/l) agrees with experimental determinations.				
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED		
22a. NAME OF RESPONSIBLE INDIVIDUAL TIMOTHY E. HAMPTON		22b. TELEPHONE (Include Area Code) (301) 671-2914	22c. OFFICE SYMBOL SMCCR-SPD-R	

DD FORM 1473, 84 MAR

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PREFACE

The work described in this report was authorized under Project 1L162622A554, Deterrent Systems. This work was started in June 1983 and completed in June 1984.

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AQUATIC TOXICITY OF PINACOLYL ALCOHOL

1. INTRODUCTION

The toxicity of the secondary alcohol, 3,3-Dimethyl-2-butanol [pinacolyl alcohol (PA)], was determined. In order to examine the toxicity of this alcohol on aquatic life, acute tests were run on two aquatic organisms: Pimephales promelas (fathead minnow) and Daphnia magna (water flea). Growth inhibition and degradation tests were run on Ankistrodesmus falcatus, a single-celled, green algae. The physicochemical properties of PA are listed in Table 1.

Table 1. Physicochemical Properties of 3,3-Dimethyl-2-butanol^{1,2,3}

synonyms	Pinacolyl Alcohol PA
molecular weight	101.05 g/mole
solubility in water	2.44×10^{-1} M
density	0.812 g/ml
boiling point	120 °C
melting point	4.8 °C
log P	1.48

A structure-activity equation⁴ used to estimate the toxicity of many alcohol compounds was also used to compare the theoretical and the measured toxicities of PA.

2. MATERIALS AND METHODS

Static tests were conducted using guidelines set forth by the Organization of Economic Cooperation and Development,⁵ U.S. Environmental Protection Agency (EPA),⁶ American Society for Testing and Materials, and publications by Goulden et al.^{7,8}

D. magna, obtained from the Academy of Natural Sciences, Philadelphia, PA, were used in the acute immobilization tests. The algae obtained from U.S. Army Medical Bioengineering Research and Development Laboratory, Ft. Detrick, MD, were used in the 96-hr growth inhibition tests and also as the primary food

source for *Daphnia*. *P. promelas* from Kurtz's Fish Hatchery in Elverson, PA, were used in the 48-hr acute static testing.

2.1 Fish Bioassays.

P. promelas of 2 + 1 cm in length were conditioned in holding tanks for 10 days and examined for disease prior to testing. Fish were fed Tetramin fish food once a day during the acclimation period. Feeding was discontinued 24 hr prior to the start of testing.

Water was conditioned by passing it through two sets of particulate filters, a set of activated charcoal filters, and then placed in a holding tank for 24 hr. The proper amount of test water was then placed into glass test chambers without toxicant and aerated an additional 24 hr. Water temperature was regulated by placing the experimental containers into a water bath maintained at 22 ± 0.5 °C. Dissolved oxygen measurements were taken with a YSI oxygen meter after calibrating the probe in the air. An Orion pH meter was used to measure pH at the start and end of each experiment.

A stock solution of 3,3-Dimethyl-2-butanol (1000 mg/l) was prepared in a volumetric flask. Chemical concentrations were selected based on the estimated EC50 from preliminary range finding tests. Loading did not exceed 1 g of fish per liter.⁵ Immobilization, the effect studied, was considered to be the time at which the fish no longer responded to probing.

2.2 Daphnia Bioassays.

Stocks of *D. magna* were grown in 2-liter flasks using the same conditioned water described above. Animals used for testing were acclimated to test conditions by rearing the organisms to the F₂ generation.⁸ Experimental cultures were derived from first instars (*Daphnia* less than 24 hr old) isolated from acclimated adults.⁹ *Daphnia* were fed vitamin enriched *A. falcatus*, grown in american standard medium,¹⁰ at an approximate concentration of 2.4×10^5 algal cell per milliliter.

Ten organisms were placed in each 250-ml beaker filled with 100 ml of test solution and covered with aluminum foil to prevent excessive evaporation. All cultures and test vessels were maintained at 19.5 ± 0.5 °C with a dark/light cycle of 16/8 hr. Control organisms were observed an additional 24 hr beyond the conclusion of testing, and their continuing survival demonstrated that a sufficient lipid supply was present in the *Daphnia* body, eliminating starvation as a stress factor. Immobilization was the endpoint at which *Daphnia* were considered dead if they failed to swim actively for a minimum of 15 seconds after probing.

2.3 Algal Bioassays.

A. falcatus was grown in american standard medium and contained in a semicontinuous sterile culture apparatus.

Erlenmeyer flasks (250 ml) with ground glass stoppers were used as test chambers. Algal medium was placed in each flask (100 ml) and inoculated with approximately 2.5×10^4 algal cells per milliliter. Algae were in log phase

(exponential) growth before samples were taken to inoculate test flasks. Incubation temperature was maintained at 25 ± 0.5 °C with a dark/light cycle of 16/8 hr at 315 foot candles. Cell counts were made at 0, 24, 48, 72, and 96 hr into the experiments. Counting of algal cells was conducted using an MHR Coulter Counter with an aperture size of 100 micrometers. Microscopic observations were made at the end of each test for any morphological change in algal structure.

2.4 Degradation Assays.

The possible oxidation of PA into pinacolone was analyzed in degradation tests. A stock of 100 mg of PA per liter was prepared and stored under algal test conditions for 144 hr. The ability of A. falcatus to degrade PA was also examined. Algae were inoculated into test containers at a concentration of approximately 5×10^4 cells per milliliter. Each consecutive test container had two times the concentration of algae over the preceding chamber. The toxicant concentration remained the same in each chamber (100 mg PA/l). Samples were withdrawn every 24 hr and analyzed.

2.5 Analytical Determinations of Pinacolyl Alcohol in Aqueous Media.

The concentration of PA in the algal medium was determined by gas chromatography. Samples were injected into a 3-foot, Porapak Q Column (100/120 mesh). The column separated PA from water within a relatively short retention time (2.6 min) and provided a very symmetrical peak (Figure 1). The column also allowed separation between PA and its principal breakdown product, pinacolone (Figure 2). The separation of PA and pinacolone was enhanced by altering column temperature and carrier flow conditions. Instrument and column conditions for routine analysis are listed in Table 2. Sample quantitation was based on peak area comparison to external PA standards:

$$\text{Sample Amt} = \frac{(\text{PA Standard Amt})}{\text{PA Standard Area}} \times (\text{Sample Area}) \quad (1)$$

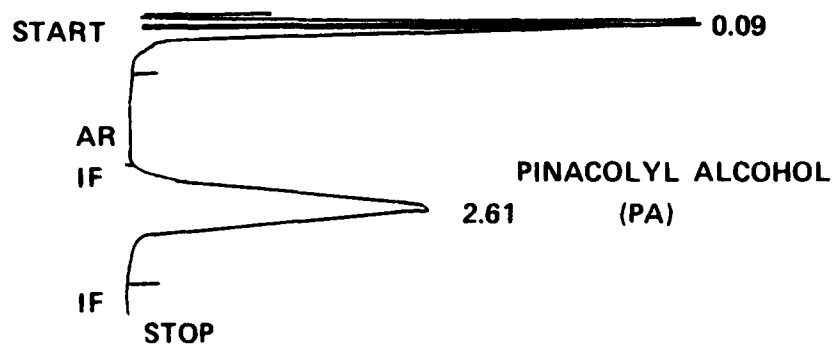
or

$$\text{Sample Amt} = (\text{PA Response Factor}) \times (\text{Sample Area}) \quad (2)$$

Linearity checks were conducted between the PA standard concentration levels of 130 to 400 mg of PA per liter. The sample concentration range of 180 to 200 mg of PA per liter fell within the linear range of the calibration curve.

2.6 Data Analysis.

The percent mortality and toxicant concentration for D. magna and P. promelas were plotted in log format to estimate the EC50. Probit analysis was employed using the Bliss11 method to compute the EC50 values.

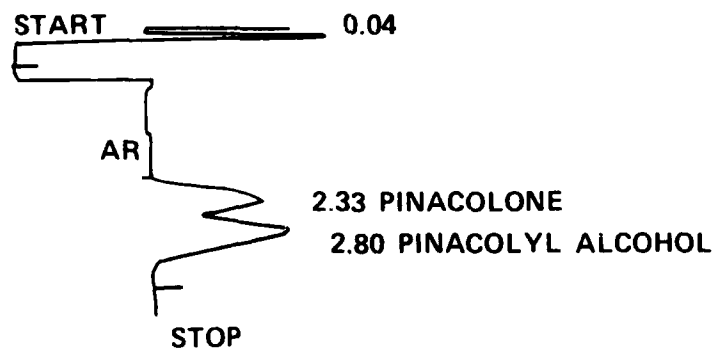


HP RUN # 238
AREA %

RT	AREA	AREA %
2.61	232300	100.000

DIL FACTOR: 1.0000 E+ 0

Figure 1. Pinacolyl Alcohol Separation from Water



HP RUN # 90
AREA %

RT	AREA	AREA %
2.33	57780	40.214
2.80	85900	59.786

DIL FACTOR 1.000

Figure 2. Separation of Pinacolyl Alcohol and Pinacolone

Table 2. Gas Chromatography Instrument Conditions

Model	Hewlett-Packard 5840A
Detector	Flame Ionization
Column Packing	Porapak Q 100/120 mesh
Column Size	3-ft x 4-mm Borosilicate glass
Column Temperature	220 °C
Detector Temperature	250 °C
Attenuation	8
Slope Sensitivity	0.05
Detector Flow (Air)	240 ml/min
Detector Flow (H ₂)	55 ml/min
Column Flow (N ₂)	55 ml/min

In estimating the EC50 for the algal tests, slightly different methods for data analysis were used. Relative growth rates (RGR) were calculated at the time interval when the control went into log-phase growth:

$$RGR = \frac{N_2 - N_1}{t_2 - t_1} \quad (3)$$

where,

N_1 = starting cell count

N_2 = ending cell count

t_1 = starting time

t_2 = ending time

After RGR was calculated for each toxicant and control, percent inhibition (%I) was calculated by using the following equation:

$$\%I = \frac{G_c - G_t}{G_c} \times 100 \quad (4)$$

where,

G_c = RGR of the control

G_t = RGR of the treatment

Derived probit ratios were calculated and analyzed using the Bliss method for calculating EC50 results. Hypothesis testing using a one-way analysis of variance for fixed effects was performed. Scheffe's procedure¹² was applied to compare individual sample means.

3. RESULTS

All the minnows exposed to 400 mg of PA per liter were anesthetized. After 48 hr of exposure, 40% of the anesthetized fish had revived. The 48-hr EC50 for P. promelas was 443.1 mg of PA per liter (Table A-1 in the appendix).

The 96-hr EC50 for A. falcatus was 257.7 mg of PA per liter (Table A-2 in the appendix). Algae exposed to 200 mg of PA per liter and above showed RGR to be significantly different from the control at $p \leq 0.05$. Figure 3 illustrates algal growth curves during PA exposure.

D. magna exposed to PA yielded a 24-hr EC50 of 513.2 mg/l. At 930 mg of PA per liter, 100% of the exposed D. magna were immobilized (Table A-3 in the appendix).

Pinacolyl alcohol was very stable in an aqueous medium and was not light sensitive throughout the duration of the degradation experiments (144 hr). During log-phase growth and at carrying capacity (the point at which algal growth slows to produce a maximum standing crop), A. falcatus did not degrade pinacolyl alcohol (Table A-4 in the appendix).

4. DISCUSSION

In general, the toxicity of straight-chained alcohols increases proportionally with the molecular weights and log P values (Table 3). Branched-chained alcohols do not have the same linearity between toxicity and log P values.

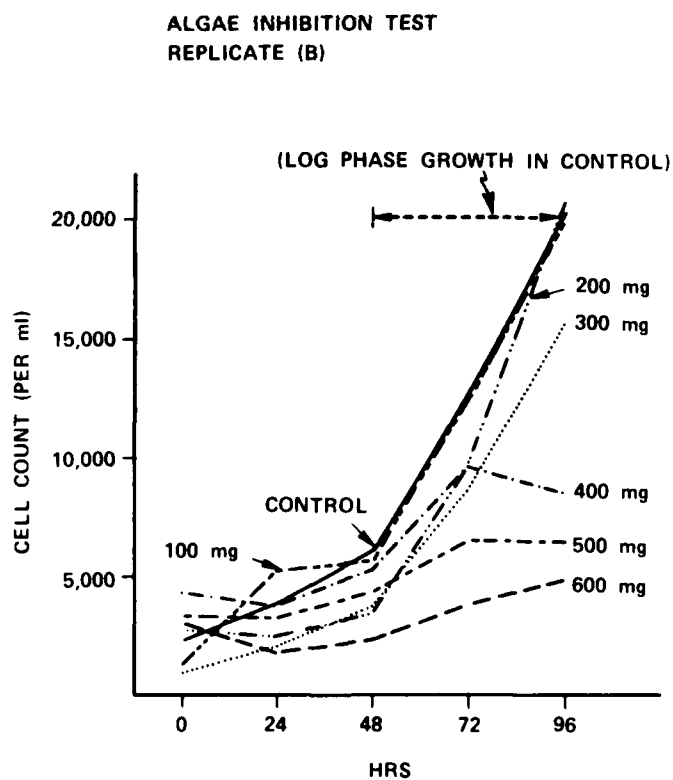
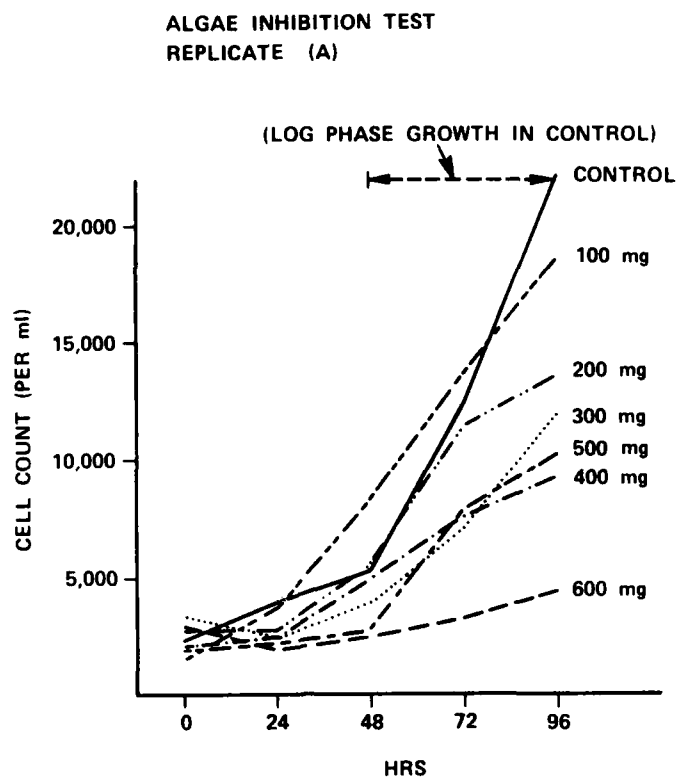


Figure 3. *A. falcatus* Growth at Various Concentrations of PA

Table 3. Comparative Alcohol Toxicities

<u>Compound</u>	<u>Log P</u>	<u>P. promelas</u> ⁴	<u>C. pyrenoidosa</u> ¹³
		<u>96-hr EC50 (mg/l)</u>	<u>96-hr EC50 (mg/l)</u>
Methanol	-0.66	28200	32500
Ethanol	-0.16	14700	NR
2-Propanol	0.14	9820	3500
1-Butanol	0.88	1740	2000
1-Hexanol	2.03	97.2	NR
1-Octanol	3.03	13.4	250
1-Nanol	3.53	5.7	NR
3-Furanmethanol	0.32	508	
3,3-Dimethyl-2-butanone	0.94	87	NR

The P. promelas results were from flow-through tests conducted at the EPA in Duluth.

NR - Not Reported

Table 3 presents comparative toxicities of alcohol compounds to P. promelas and Chlorella pyrenoidosa. C. pyrenoidosa is a single-celled, green alga that is spherical in shape and much smaller than A. falcatus. Comparing the toxicity results of C. pyrenoidosa to A. falcatus is difficult. The authors have learned through personal experience that when conducting bioassays on dissimilar algal species with considerable size difference, a toxicity differential of one order in magnitude may be seen. Fathead minnow data presented in Table 4 are from 96-hr flow-through conditions conducted at the EPA in Duluth. The studies presented in this report are under static conditions.

Table 4. EC50 Comparison of the Various Test Organisms Exposed to Pinacolyl Alcohol

<u>Species</u>	<u>EC50 (mg PA/l)</u>
<u>Ankistrodesmus falcatus</u>	257.7 (96 hr)
<u>Pimephales promelas</u>	443.1 (48 hr)
<u>Daphnia magna</u>	513.2 (24 hr)

G. D. Veith published work on the structure-activity relationship of various industrial chemicals (such as alcohols, ketones, and aldehydes) to fathead minnows. Using the N-octanol/water partition coefficient (log P), Veith et al⁴ were able to develop the following structure activity equation:

$$\text{Log EC50} = -1.1 \log P + 0.05 (\log P)^2 - 1.22 \quad (5)$$

Equation (5) estimates toxicity values which consistently fall within one order of magnitude of the observed value. The calculated EC50 value for PA toxicity is 547.6 mg of PA per liter. The difference between the 48-hr observed EC50 and predicted toxicity is 100 mg of PA per liter. The above equation was developed for 96 hr of flow-through conditions. These studies ran for 48 hr under static conditions. If the EC50 for fathead minnows presented in this report were projected to 96 hr, there may be smaller discrepancies between observed and theoretical EC50 values.

5. CONCLUSIONS

An estimate of the potential impact of PA on aquatic organisms should include chronic as well as acute toxicity. A safety factor should be applied to the results to ensure that transient lethal concentrations do not occur due to uneven dilutions and to possibly prevent chronic effects that may become apparent at lower concentrations of the toxicant. With acute toxicity data, a safety factor of 10 is commonly used. Averaging the EC50 results reported in this report yields a combined figure of 390 mg of PA per liter. One-tenth of this number is 39 mg of PA per liter which is a suggested average concentration that should not be exceeded. On a volume-to-volume basis, 39 mg of PA per liter corresponds to .048 ml of PA per liter. Although preliminary, the not-to-exceed average of 39 mg/l may be used as an interim figure when initially estimating the potential threat of PA to aquatic environments.

LITERATURE CITED

1. Yalkowsky, S.H., and Valvani, S.C. Solubility and Partitioning. I. Solubility of Nonelectrolytes in Water. *J. of Pharmac. Sci.* 69(8), 912-920 (1980).
2. Amidon, G.L., Yalkowsky, S.H., and Leung, S. Solubility of Nonelectrolytes in Polar Solvents. II. Solubility of Aliphatic Alcohols in Water. *J. of Pharmac. Sci.* 63, 1858-1866 (1974).
3. Valvani, S.C., Yalkowsky, S.H., and Roseman, T.J. Solubility and Partitioning. IV. Aqueous Solubility and Octanol - Water Partition Coefficients of Liquid Nonelectrolytes. *J. of Pharmac. Sci.* 70(5), 502-507 (1981).
4. Veith, G.D., Call, D.J., and Brooke, L.T. Structure-Toxicity Relationships for the Fathead Minnow, *Pimephales promelas*: Narcotic Industrial Chemicals. *Cana. J. Fish. Aqu. Sci.* 40(6), 743-748 (1983).
5. OECD Guidelines for Testing of Chemicals. Effects on Biotic Systems, Section 2, Artical 203. 1-12. 1981.
6. EPA-660/3-75-009. Environment Protection Agency. Methods For Acute Toxicity Tests with Fish, Macro-Invertebrates and Amphibians. 1975.
7. Goulden, C.E., and Henry, L. Daphnia Bioassay Workshop, Academy of Natural Sciences of Philadelphia, Philadelphia, PA. 1983.
8. Goulden, C.E., Comotto, R.M., Hendrickson, J.A., Jr., Hornig, L.L., and Johnson, K.L. ASTM Publication 766. Procedures and Recommendations for the Culture and Use of Daphnia in Bioassay Studies, pp 139-160. 1982.
9. Goulden, C.E., and Hornig, L.L. Population Oscillations and Energy Reserves in Planktonic Cladocera and Their Consequences to Competition. *Proc. Natl. Acad. Sci.* 77(3), 1716-1720 (1980).
10. Carmichael, W.W., and Gorham, P.R. An Improved Method of Obtaining Axenic Clones of Planktonic Blue Green Algae. *J. of Phycology* 10(2), 238-240 (1974).
11. Bliss, C.I. The Calculation of the Dose-Mortality Curve. *Ann. Appl. Biol.* 22, 134-167 (1937).
12. Gibra, I.N. One Way Classification; Fixed Effects Models. In *Probability and Statistical Inference for Scientists and Engineers*, pp 342-365. Prentice-Hall, Inc. 1967.
13. Gloyna, E.F., and Thirumurthi, D. Suppression of Photosynthetic Oxygenation. *Water Sewage Works* 114(3), 83-88 (1967).

APPENDIX

Table A-1. 48-hr LC50 Determination for P. promelas

Concentrations mg/l	M	Replicate 1	Replicate 2	Total
500	4.9×10^{-3}	9/10	6/10	15/20
400	3.9×10^{-3}	4/10	2/10	6/20
300	2.9×10^{-3}	0/10	0/10	0/20
200	1.9×10^{-3}	0/10	0/10	0/20
100	9.8×10^{-3}	0/10	0/10	0/20

5% Mortality in controls

Probit Analysis

	LC50 (mg/l)	Upper Limit	Lower Limit
R ₁	417.5	460.2	378.7
R ₂	474.0	557.1	403.4
Total	443.1	476.3	412.3

Dissolved Oxygen and pH of P. promelas Experiments (48 hr)

Concentrations mg/l	D.O. (ppm)		pH	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
	Start/End	Start/End	Start/End	Start/End
500	4.7/2.7	4.5/3.1	6.2/6.1	6.2/6.1
400	4.8/3.9	4.9/4.4	6.2/6.2	6.2/6.1
300	5.2/3.6	4.6/4.5	6.2/6.2	6.2/6.1
200	5.3/3.9	4.6/4.5	6.2/6.3	6.3/6.1
100	5.6/4.1	5.3/4.4	6.2/6.4	6.3/6.1
Control	5.5/4.8	5.5/4.8	6.2/6.1	6.2/6.0

Table A-2. Algal Inhibition Test with A. falcatus

Concentration mg/l	Replicate 1		Replicate 2	
	Relative Growth Rate	% Inhibition	Relative Growth Rate	% Inhibition
600	37.9	89.3	51.6	85.4
500	157.5	55.5	43.3	87.8
400	90.0	74.6	111.6	68.5
300	165.0	53.3	238.8	32.6
200	168.3	52.4	323.3	8.5
100	213.3	39.7	296.6	16.1
Control	353.3	-	353.3	-

Combined Results Replicate 1 and Replicate 2

Concentration mg/l	Relative Growth Rate	% Inhibition
600	45.0	87.3
500	100.4	71.6
400	100.8	71.5
300	201.6	42.9
200	245.8	30.4
100	255.0	27.9
Control	353.3	-

Probit Analysis

EC50	Upper Limit	Lower Limit
257.77 mg/l	338.25 mg/l	194.43 mg/l

Table A-3. 24-hr EC50 Determinations for D. magna

Concentrations mg/l	M	Replicate 1	Replicate 2	Replicate 3	Total
930	9.2×10^{-3}	10/10	11/11	11/11	32/32
740	7.3×10^{-3}	10/10	10/11	6/9	26/30
560	5.5×10^{-3}	7/10	6/10	6/11	19/31
460	4.5×10^{-3}	1/9	7/10	4/11	12/30
370	3.6×10^{-3}	2/10	0/10	1/9	3/30

No Mortality in Controls

Probit Analysis

	EC50 (mg/l)	Upper Limit	Lower Limit
R ₁	501.8	563.2	447.1
R ₂	490.2	556.5	431.8
R ₃	547.4	630.8	474.9
Total	513.3	551.09	478.0

pH Determinations for D. magna Experiments (24 hr)

Concentrations mg/l	pH		
	Replicate 1	Replicate 2	Replicate 3
930	7.1	6.2	6.3
740	7.2	6.2	6.3
560	7.2	6.2	6.3
460	7.1	6.3	6.3
370	7.2	6.2	6.3
Control	7.0	6.1	6.1

Table A-4. Degradation Test I During Carrying Capacity Growth

	GLC Readings				
	<u>0 hr</u>	<u>24 hr</u>	<u>48 hr</u>	<u>72 hr</u>	<u>96 hr</u>
PA Control	198	196	201	204	199
Medium	0	0	0	0	0
1x	-	196	201	196	199
2x	-	191	192	191	195
4x	-	190	182	187	195
8x	-	185	188	187	187
16x	-	187	182	183	186

Degradation Test II During Log-Phase Growth

	GLC Readings				
	<u>0 hr</u>	<u>24 hr</u>	<u>48 hr</u>	<u>72 hr</u>	<u>144 hr</u>
PA Control	185	187	185	190	191
Medium	0	0	0	0	0
1x	-	183	185	192	191
5x	-	187	182	190	190
10x	-	184	182	191	190

GLC Readings in mg/l $\pm$ 10 mg/l