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APPENDIX A

Apparatus and equipments

A. Facilities for holding and acclimating test organisms

B. Reservior unit

C. Sea bass culture unit

D. Brine shrimp, *Artemia* sp. ------for feeding test organisms

E. Drying oven -----for drying glassware

F. Air pump-----for oil-free air supply

G. Air line, and air stones-----for aerating water containing fish, or for supplying air to test solutions with low DO.

H. pH and DO meters-----for routine physical and chemical measurements

I. Desiccator-----for holding and dried glassware

J. Refractometer-----for determining salinity

K. Thermometers-----for measuring water temperatures

L. Thermobarometer-----for measuring barometric pressure

M. Test chambers-----18 glass aquaria, capacity of 14 L for acute toxicity tests
12 glass aquaria, capacity of 54 L for sublethal toxicity tests.

N. Beakers, Volumetric flasks, graduated cylinders, pipets, droppers, and other glassware-----for making test solutions and laboratory using

O. Siphon with bulb and clamp-----for cleaning test chambers

P. Data sheets-----for data recording

Q. Tape, colored-----for labelling test chambers

R. Markers, water-proof-----for marking containers, etc.

S. Gilson differential respirometer------for measuring oxygen consumption

T. Chemicals------for preparing stock solution and laboratory testtings

U. Rulers------for measuring the length

V. Electronic balances------for measuring the weight

Methods and instruments used for water quality analysis

Hardness	:	EDTA Tritimetric method	(APHA, 1992)
Alkalinity	:	Titration method	(APHA, 1992)
DO	:	Yellow Spring Instrument Co., Inc. Oxygen meter	YSI Model 57
Conductivity	:	Yellow Spring Instrument Co., Inc. S-C-T meter	YSI Model 23
Temperature	:	Mercury thermometer and YSI Model 23	
Salinity	:	Hand refractometer	
pH	:	pH meter	
Ammonia	:	Ammonia indicator, Merck Chemicals	

APPENDIX B

Table B.1 Percent mortality of *L. calcalifer* at various TBTO concentrations in the first range-finding test

TBTO concentations (μg/L)	Number of fish in experiment	Number of Replication	Percent mortality							
			6-hour		12-hour		24-hour		48-hour	
			Number of dead	death(%)	Number of dead	death(%)	Number of dead	death(%)	Number of dead	death(%)
Control	30	3	0	0	0	0	0	0	0	0
2.5	30	3	0	0	0	0	27	90	30	100
5.0	30	3	0	0	30	100				
10.0	30	3	30	100						
20.0	30	3	30	100						
40.0	30	3	30	100						

Table B.2 Percent mortality of *L. calcalifer* at various TBTO concentrations in the second range-finding test

TBTO concentations (μg/L)	Number of fish in experiment	Number of Replication	Percent mortality							
			24-hour		48-hour		72-hour		96-hour	
			Number of dead	death(%)	Number of dead	death(%)	Number of dead	death(%)	Number of dead	death(%)
Control	30	3	0	0	0	0	0	0	0	0
1.0	30	3	0	0	0	0	0	90	12	40
1.5	30	3	0	0	3	10	13	43.33	29	96.67
2.0	30	3	8	26.67	22	73.33	30	100		
2.5	30	3	19	63.33	30	100				
5.0	30	3	30	100						

Table B.3 Percent mortality of *L. calcalifer* at various TBTO concentrations in the third range-finding test

TBTO concentations (μg/L)	Number of fish in experiment	Number of Replication	Percent mortality							
			24-hour		48-hour		72-hour		96-hour	
			Number of dead	death(%)	Number of dead	death(%)	Number of dead	death(%)	Number of dead	death(%)
Control	30	3	0	0	0	0	0	0	0	0
0.25	30	3	0	0	0	0	0	0	0	0
0.50	30	3	0	0	0	0	0	0	0	0
1.00	30	3	0	0	0	0	0	0	10	33.33
1.50	30	3	1	3.33	5	16.67	22	73.33	30	100
2.00	30	3	6	20	27	90	30	100		

24hr-LC50

********** PROBIT ANALYSIS **********

DATA Information

5 unweighted cases accepted.
0 cases rejected because of missing data.
0 cases are in the control group.
0 cases rejected because LOG-transform can't be done.

MODEL Information
ONLY Normal Sigmoid is requested.

Parameter estimates converged after 16 iterations.
Optimal solution found.

Parameter Estimates (PROBIT model: (PROBIT(p)) = Intercept + BX):

	Regression Coeff.	Standard Error	Coeff./S.E.
CONC	3.62369	1.01404	3.57352

Intercept	Standard Error	Intercept/S.E.
-.76612	.12012	-6.37801

Pearson Goodness-of-Fit Chi Square = .247 DF = 3 P = .970

Since Goodness-of-Fit Chi square is NOT significant, no heterogeneity factor is used in the calculation of confidence limits.

Observed and Expected Frequencies

CONC	Number of Subjects	Observed Responses	Expected Responses	Residual	Prob
-.22	30.0	2.0	1.746	.254	.05820
-.10	30.0	4.0	3.958	.042	.13193
.00	30.0	6.0	6.654	-.654	.22180
.08	30.0	9.0	9.477	-.477	.31590
.15	30.0	13.0	12.195	.805	.40648

Confidence Limits for Effective CONC

		95% Confidence Limits	
Prob	CONC	Lower	Upper
.01	.37105	.10377	.54532
.02	.44123	.15173	.61214
.03	.49248	.19293	.65927
.04	.53493	.23098	.69756
.05	.57214	.26726	.73075
.06	.60585	.30243	.76065
.07	.63703	.33688	.78829
.08	.66631	.37085	.81432
.09	.69410	.40448	.83920
.10	.72071	.43787	.86328
.15	.84218	.60216	.98012
.20	.95316	.75812	1.10927
.25	1.05995	.89406	1.27450
.30	1.16602	1.00435	1.49040
.35	1.27376	1.09596	1.75866
.40	1.38518	1.17775	2.08020
.45	1.50225	1.25541	2.46132
.50	1.62712	1.33239	2.91418
.55	1.76238	1.41115	3.45758
.60	1.91133	1.49383	4.11945
.65	2.07852	1.58275	4.94206
.70	2.27057	1.68085	5.99213
.75	2.49778	1.79236	7.38182
.80	2.77764	1.92415	9.31715
.85	3.14367	2.08888	12.22903
.90	3.67350	2.31495	17.22901
.91	3.81433	2.37294	18.71757
.92	3.97345	2.43752	20.48139
.93	4.15607	2.51049	22.61392
.94	4.36998	2.59447	25.26001
.95	4.62741	2.69358	28.65899
.96	4.94930	2.81474	33.24277
.97	5.37587	2.97099	39.89704
.98	6.00036	3.19192	50.85351
.99	7.13517	3.57333	74.55697

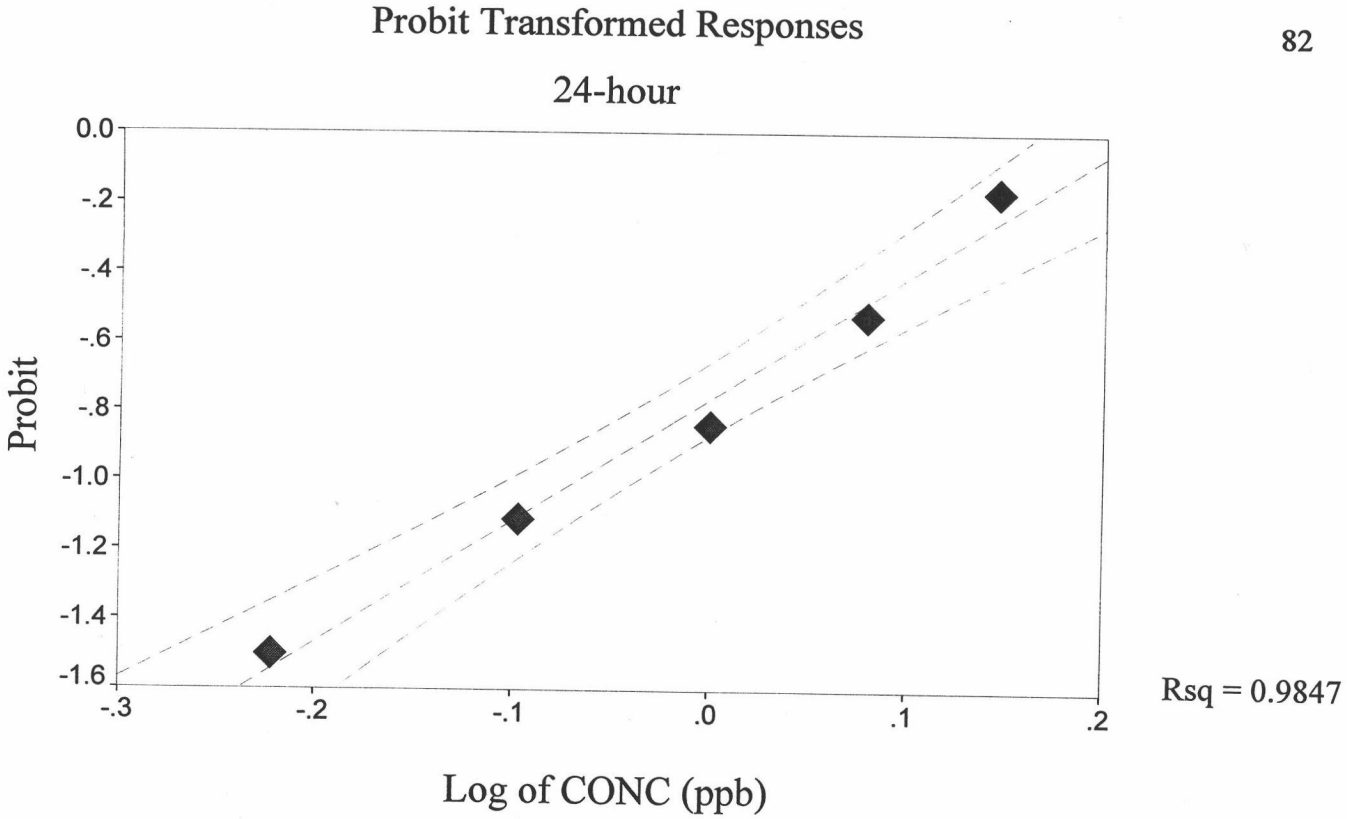

Figure B.1 24-hour LC50

48hr-LC50

********** PROBIT ANALYSIS **********

DATA Information

5 unweighted cases accepted.
0 cases rejected because of missing data.
0 cases are in the control group.
0 cases rejected because LOG-transform can't be done.

MODEL Information

ONLY Normal Sigmoid is requested.

Parameter estimates converged after 12 iterations.
Optimal solution found.

Parameter Estimates (PROBIT model: (PROBIT(p)) = Intercept + BX):

	Regression Coeff.	Standard Error	Coeff./S.E.
CONC	3.85690	.89590	4.30507

Intercept	Standard Error	Intercept/S.E.
-.29616	.10913	-2.71393

Pearson Goodness-of-Fit Chi Square = .477 DF = 3 P = .924

Since Goodness-of-Fit Chi square is NOT significant, no heterogeneity factor is used in the calculation of confidence limits.

Observed and Expected Frequencies

CONC	Number of Subjects	Observed Responses	Expected Responses	Residual	Prob
-.22	30.0	4.0	3.741	.259	.12470
-.10	30.0	8.0	7.544	.456	.25145
.00	30.0	10.0	11.507	-1.507	.38355
.08	30.0	15.0	15.111	-.111	.50368
.15	30.0	19.0	18.163	.837	.60544

Confidence Limits for Effective CONC

		95% Confidence Limits	
Prob	CONC	Lower	Upper
.01	.29759	.10642	.44133
.02	.35019	.14316	.49463
.03	.38828	.17273	.53195
.04	.41964	.19888	.56201
.05	.44701	.22300	.58784
.06	.47171	.24578	.61087
.07	.49448	.26761	.63191
.08	.51581	.28876	.65147
.09	.53600	.30938	.66989
.10	.55528	.32962	.68740
.15	.64278	.42755	.76662
.20	.72206	.52357	.83951
.25	.79783	.61949	.91262
.30	.87262	.71482	.99154
.35	.94816	.80726	1.08261
.40	1.02589	.89396	1.19260
.45	1.10715	.97384	1.32693
.50	1.19340	1.04851	1.48923
.55	1.28638	1.12089	1.68335
.60	1.38827	1.19389	1.91555
.65	1.50207	1.27031	2.19623
.70	1.63212	1.35309	2.54237
.75	1.78511	1.44605	2.98237
.80	1.97242	1.55499	3.56734
.85	2.21569	1.69036	4.40075
.90	2.56485	1.87535	5.73806
.91	2.65712	1.92271	6.11871
.92	2.76113	1.97543	6.56123
.93	2.88020	2.03495	7.08521
.94	3.01927	2.10343	7.72048
.95	3.18608	2.18420	8.51540
.96	3.39388	2.28289	9.55526
.97	3.66801	2.41010	11.01033
.98	4.06699	2.58987	13.29515
.99	4.78577	2.90008	17.90107

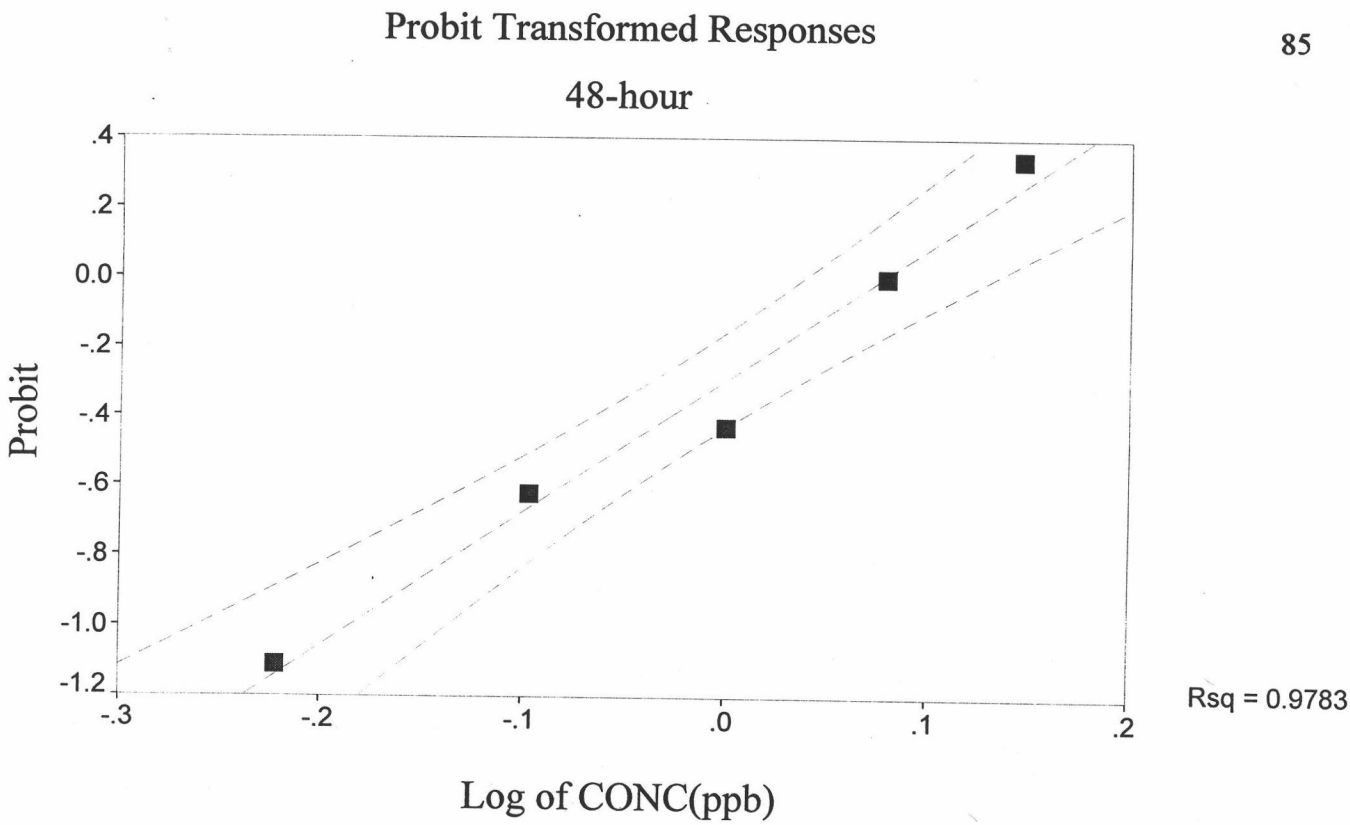

Figure B.2 48-hour LC50

72hr-LC50

********** PROBIT ANALYSIS **********

DATA Information

5 unweighted cases accepted.
0 cases rejected because of missing data.
0 cases are in the control group.
0 cases rejected because LOG-transform can't be done.

MODEL Information

ONLY Normal Sigmoid is requested.

Parameter estimates converged after 7 iterations.
Optimal solution found.

Parameter Estimates (PROBIT model: (PROBIT(p)) = Intercept + BX):

	Regression Coeff.	Standard Error	Coeff./S.E.
CONC	4.70707	.90188	5.21920

Intercept	Standard Error	Intercept/S.E.
-.07708	.10978	-.70215

Pearson Goodness-of-Fit Chi Square = 2.396 DF = 3 P = .494

Since Goodness-of-Fit Chi square is NOT significant, no heterogeneity factor is used in the calculation of confidence limits.

Observed and Expected Frequencies

CONC	Number of Subjects	Observed Responses	Expected Responses	Residual	Prob
-.22	30.0	5.0	3.932	1.068	.13107
-.10	30.0	9.0	8.908	.092	.29693
.00	30.0	11.0	14.078	-3.078	.46928
.08	30.0	18.0	18.487	-.487	.61624
.15	30.0	24.0	21.880	2.120	.72932

Confidence Limits for Effective CONC

		95% Confidence Limits	
Prob	CONC	Lower	Upper
.01	.33278	.17114	.45334
.02	.38025	.21150	.50040
.03	.41382	.24184	.53290
.04	.44101	.26746	.55884
.05	.46444	.29024	.58095
.06	.48537	.31111	.60054
.07	.50449	.33060	.61832
.08	.52224	.34906	.63475
.09	.53894	.36670	.65013
.10	.55477	.38370	.66468
.15	.62545	.46228	.72937
.20	.68798	.53493	.78689
.25	.74659	.60481	.84192
.30	.80347	.67321	.89738
.35	.86003	.74053	.95583
.40	.91739	.80656	1.01993
.45	.97652	.87083	1.09253
.50	1.03843	.93315	1.17644
.55	1.10426	.99405	1.27430
.60	1.17543	1.05477	1.38894
.65	1.25382	1.11709	1.52417
.70	1.34209	1.18325	1.68595
.75	1.44434	1.25613	1.88419
.80	1.56738	1.34007	2.13650
.85	1.72410	1.44267	2.47758
.90	1.94373	1.58050	2.98986
.91	2.00085	1.61541	3.12929
.92	2.06480	1.65411	3.28836
.93	2.13748	1.69762	3.47284
.94	2.22168	1.74743	3.69143
.95	2.32177	1.80588	3.95790
.96	2.44513	1.87685	4.29609
.97	2.60581	1.96769	4.75229
.98	2.83587	2.09493	5.43557
.99	3.24041	2.31163	6.71949

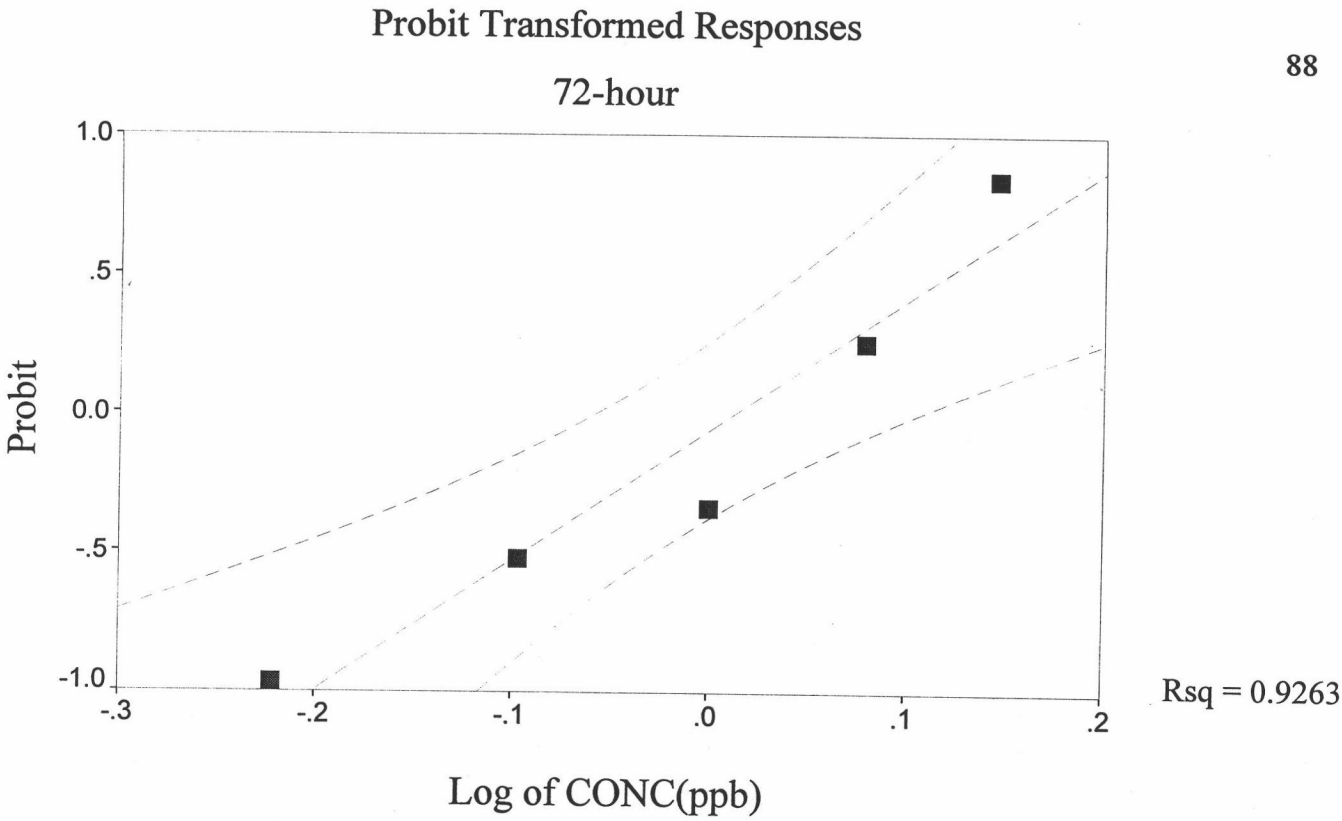

Figure B.3 72-hour LC50

96hr-LC50

********** PROBIT ANALYSIS **********

DATA Information

5 unweighted cases accepted.
0 cases rejected because of missing data.
0 cases are in the control group.
0 cases rejected because LOG-transform can't be done.

MODEL Information

ONLY Normal Sigmoid is requested.

Parameter estimates converged after 13 iterations.
Optimal solution found.

Parameter Estimates (PROBIT model: (PROBIT(p)) = Intercept + BX):

	Regression Coeff.	Standard Error	Coeff./S.E.
CONC	5.15613	.91703	5.62267

Intercept	Standard Error	Intercept/S.E.
.02737	.11114	.24629

Pearson Goodness-of-Fit Chi Square = 3.235 DF = 3 P = .357

Since Goodness-of-Fit Chi square is NOT significant, no heterogeneity factor is used in the calculation of confidence limits.

Observed and Expected Frequencies

CONC	Number of Subjects	Observed Responses	Expected Responses	Residual	Prob
-.22	30.0	5.0	3.963	1.037	.13210
-.10	30.0	10.0	9.551	.449	.31835
.00	30.0	12.0	15.328	-3.328	.51092
.08	30.0	19.0	20.054	-1.054	.66845
.15	30.0	26.0	23.476	2.524	.78255

Confidence Limits for Effective CONC

		95% Confidence Limits	
Prob	CONC	Lower	Upper
.01	.34955	.19945	.46144
.02	.39480	.24003	.50588
.03	.42650	.26990	.53639
.04	.45202	.29474	.56065
.05	.47389	.31659	.58126
.06	.49335	.33641	.59946
.07	.51106	.35479	.61594
.08	.52746	.37206	.63114
.09	.54283	.38846	.64533
.10	.55737	.40417	.65873
.15	.62184	.47579	.71794
.20	.67837	.54078	.77002
.25	.73093	.60246	.81920
.30	.78160	.66236	.86794
.35	.83169	.72121	.91820
.40	.88218	.77922	.97187
.45	.93394	.83635	1.03100
.50	.98785	.89254	1.09775
.55	1.04487	.94802	1.17435
.60	1.10618	1.00352	1.26319
.65	1.17333	1.06030	1.36721
.70	1.24852	1.12018	1.49070
.75	1.33507	1.18562	1.64059
.80	1.43853	1.26039	1.82910
.85	1.56928	1.35105	2.08013
.90	1.75082	1.47182	2.44983
.91	1.79772	1.50226	2.54909
.92	1.85011	1.53594	2.66168
.93	1.90947	1.57371	2.79145
.94	1.97802	1.61687	2.94411
.95	2.05921	1.66737	3.12874
.96	2.15887	1.72854	3.36088
.97	2.28802	1.80657	3.67050
.98	2.47174	1.91541	4.12749
.99	2.79172	2.09968	4.96773

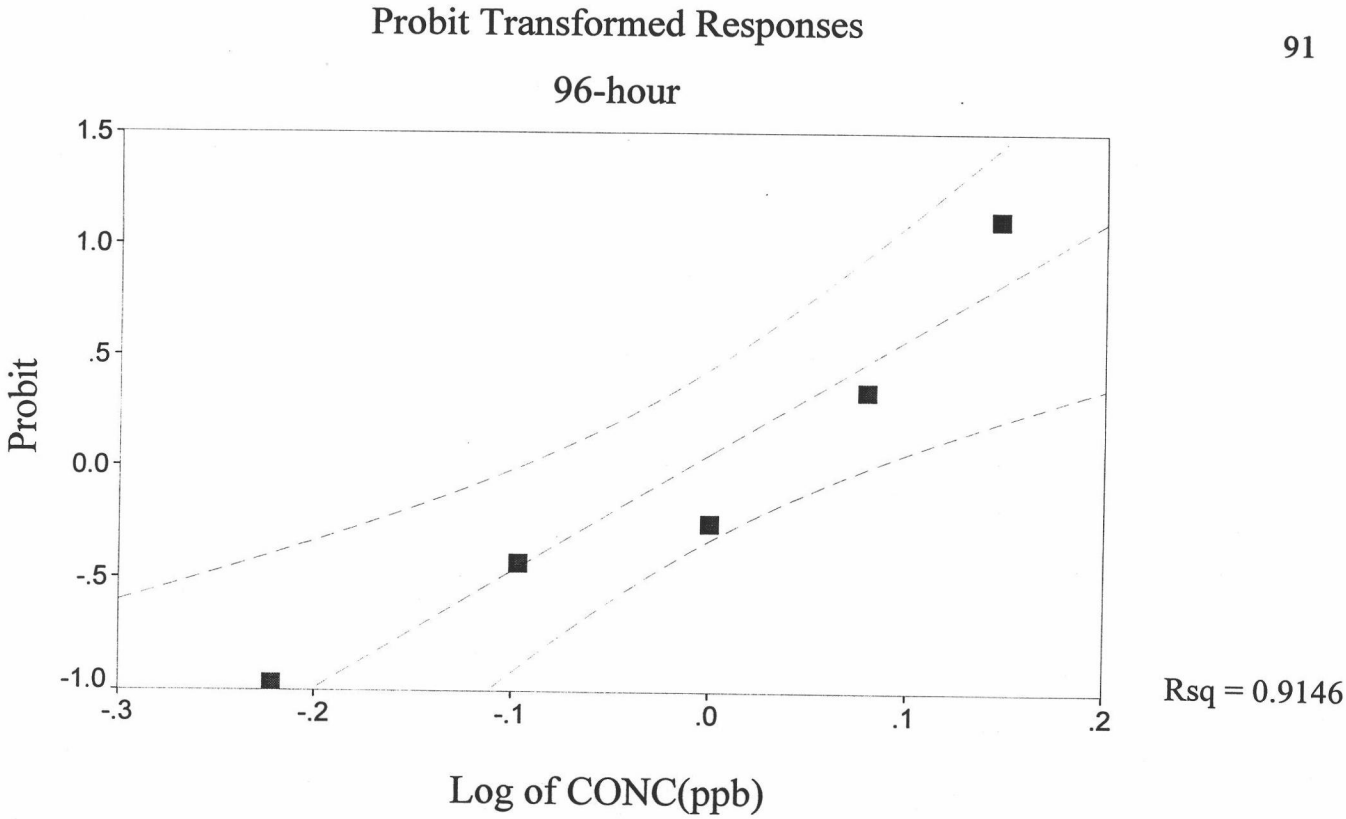

Figure B.4 96-hour LC50

APPENDIX C

Gilson differential respirometer

The respirometer is functioned based upon the principle that at constant temperature and constant gas volume, any changes in the amount of gas can be measured by changing in its pressure. Basicallly, when the animal consumes O_2, CO_2 is elevated, and taken up by the CO_2 absorbent, leaving a net reduction of gas pressure in the animal chamber. This causes the manometer fluid in the connecting tube move to the direction of animal chamber. After a period of time the balancing knob can be turned to introduce air and back to its original level. Accordingly, the micrometer measures the volume of gas injected. The amount of gas consumed can be calculated from the known operation temperature, the exchanged gas, and the barometric pressure.

Procedure employed

1. Turn on the Gilson differential respirometer, allow to equilibrate for 15 min.
2. Add 30 mL of newly prepared test solution to the flask.
3. Place the fish in the flask
4. Add 0.2 mL 10% NaOH to the sidearm
5. Grease attachment joint on manometer and grease and insert plug to sidearm.
6. Attach flask to manometer
7. Tighten flask with elastic
8. Place in constant temperature bath and acclimate the fish for 1 h
9. Adjust manometer fluid to reference point with stopcock open
10. Close stopcock
11. Begin readings

The respirometer was continuously operated for 1 h in each experiment. The reading datum was taken every one-quarter of an hour. However, the first quarter reading value was invalid, as the fish was stressed in a new environment. The data observed were recorded in a worksheet. After the end of the experiment, the fish were

gently blotted dry and weighed to obtain wet weight, and then placed into the test aquaria.

Computations

The micrometer readings were given in microliters. To convert to standard condition a constant , c , must be derived as follows :

$$c = \frac{(273)(Pb)}{(t+273)(760)}$$

where t = experimental temperature in degrees celsius at 1 ATM

Pb = operating barometric pressure in mm Murcury

Oxygen consumption, K (in μL O_2) was determined by

$$K = c \times h$$

;where h is the corrected manometer change, then the oxygen consumption in μL O_2 was converted to μg O_2.

The oxygen consumption was obtained, and the weight-specific consumption was calculated by dividing h by the wet weight of the fish.

Computation

Weight growth

$$(G) = W_{t+1} - W_t$$

G = Growth

t = measuring time (day)

W_t = mean weight at t

W_{t+1} = mean weight at t+1

Relative growth rate

$$G = \frac{W_{t+1} - W_t \times 100}{W_t}$$

Length growth

$$G = L_{t+1} - L_t$$

G = Growth

t = measuring time (day)

L_t = mean length at t

L_{t+1} = mean length at t+1

Relative growth rate

$$G = \frac{L_{t+1} - L_t \times 100}{L_t}$$

APPENDIX D

Terminology

Acclimate - to accustom test organisms to different environmental conditions, such as temperature, light, and water quality

Acute toxicity - relative short-term lethal or other effect, usually defined as occuring within 4 d for fish and macroinvertebrates and shorter times (2 d) for organisms with shorter life spans.

Chronic toxicity - toxicity involving a stimulus that lingers or continues for a relatively long period of time, often one-tenth of the life span or more. "chronic" should be considered a relative term depending on the life span of an organism. A chronic toxic effect can be measured in terms of reduced growth, reduced reproduction, etc., in addition to lethality.

Control - treatment in a toxicity test that duplicates all the conditions of the exposure treatment but contains no test material.

Definitive test - toxicity test designed to establish concentration at which a particular end points occurs. Exposures for these tests are longer than for screening or range-finding tests, incorporating multiple concentrations at closer intervals and multiple replicates.

Exposure time - time of exposure of test organism to test solution.

Flow-through test - test in which solution is replaced continuously in test chambers throughout the test duration.

Lethal concentration - toxicant concentration estimated to produce death in a specified proportion of test organisms. Usually defined as median (50%) lethal concentration, LC50, i.e., concentration killing 50% of exposed organisms at a specific time of observation, for example, 96-hLC50.

No-observed-effect concentration (NOEL) - in a full- or partially-life-cycle test, the highest toxicant concentration in which the values for the measured response are not statistically significant different from those in the control.

Range-finding test - preliminary test designed to establish approximate toxicity of solution. Test design incorporates multiple, widely spaced, concentrations with single replicates; exposure is usually 8 to 24 h.

Response - the measured biological effect of the variable tested. In acute toxicity tests the response usually is death or immobilization.

Static renewal test - tests in which organisms are exposed to solutions of the same composition that are renewed periodically during the test period (with renewals usually at 24-h intervals). This is accomplished by transferring test organisms or replacing test solution.

Static test - test in which solutions and test organisms are placed in test chambers and kept there for the duration of the test.

Toxicity - potential or capacity of a test material to cause adverse effects on living organisms, generally a poison or mixture of poisons. Toxicity is a result of dose or exposure concentration and exposure time, modified by variables such as temperature, chemical form, and availability.

APPENDIX E

ANOVA for oxygen consumption at short-term exposure

----- ONEWAY -----

Variable Oxygen consumption
By Variable CONC

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	5	.4193	.0839	3.8700	.0057
Within Groups	42	.9101	.0217		
Total	47	1.3293			

Multiple Range Tests: Duncan test with significance level .05

The difference between two means is significant if
MEAN(J)-MEAN(I) >= .1041 * RANGE * SQRT(1/N(I) + 1/N(J))
with the following value(s) for RANGE:

Step	2	3	4	5	6
RANGE	2.85	3.00	3.10	3.17	3.22

(*) Indicates significant differences which are shown in the lower triangle

		G G G G G G
		r r r r r r
		p p p p p p
		1 2 4 6 3 5
Mean	CONC	
.4456	Grp 1	
.4460	Grp 2	
.6282	Grp 4	* *
.6305	Grp 6	* *
.6443	Grp 3	* *
.6664	Grp 5	* *

Group 1 = Control
Group 2 = Acetone
Group 3 = 0.03 ug/L
Group 4 = 0.05 ug/L
Group 5 = 0.10 ug/L
Group 6 = 1.00 ug/L

ANOVA for oxygen consumption at continuous exposure

----- O N E W A Y -----

Variable OXYGEN CONSUMPTION
By Variable WEEK

Analysis of Variance

Source	D.F.	Sum of Squares	Mean squares	F Ratio	F Prob.
Between Groups	4	3.7329	.9332	74.7272	.0000
Within Groups	155	1.9357	.0125		
Total	159	5.6686			

Multiple Range Tests: Duncan test with significance level .05

The difference between two means is significant if
MEAN(J)-MEAN(I) >= .0790 * RANGE * SQRT(1/N(I) + 1/N(J))
with the following value(s) for RANGE:

Step	2	3	4	5
RANGE	2.80	2.94	3.03	3.11

(*) Indicates significant differences which are shown in the lower triangle

```
                G G G G G
            r r r r r
            p p p p p

                5 3 4 2 1
  Mean    WEEK

 .1793  Grp 5
 .2296  Grp 3
 .2345  Grp 4
 .3930  Grp 2      * * *
 .5961  Grp 1      * * * *
```

Group 1 = Week 1
Group 2 = Week 2
Group 3 = Week 3
Group 4 = Week 4
Group 5 = Week 5

ANOVA for length growth during 8-week period

----- ONEWAY -----

Variable LENGTH
By Variable WEEK

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	4	258.0368	64.5092	412.7662	.0000
Within Groups	886	138.4686	.1563		
Total	890	396.5055			

Multiple Range Tests: Duncan test with significance level .05

The difference between two means is significant if
MEAN(J)-MEAN(I) >= .2795 * RANGE * SQRT(1/N(I) + 1/N(J))
with the following value(s) for RANGE:

Step	2	3	4	5
RANGE	2.78	2.93	3.01	3.09

(*) Indicates significant differences which are shown in the lower triangle

```
                    G G G G G
                    r r r r r
               p p p p p

                    1 2 3 4 5
Mean     WEEK

2.7378   Grp 1
3.2698   Grp 2      *
3.6983   Grp 3      * *
3.9657   Grp 4      * * *
4.2716   Grp 5      * * * *
```

Group 1 = Week 1
Group 2 = Week 2
Group 3 = Week 3
Group 4 = Week 4
Group 5 = Week 5

ANOVA for weight growth during 8-week period

----- ONEWAY -----

Variable WEIGHT
By Variable WEEK

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	4	196.8779	49.2195	339.7376	.0000
Within Groups	886	128.3592	.1449		
Total	890	325.2372			

Multiple Range Tests: Duncan test with significance level .05

The difference between two means is significant if
MEAN(J)-MEAN(I) >= .2691 * RANGE * SQRT(1/N(I) + 1/N(J))
with the following value(s) for RANGE:

Step	2	3	4	5
RANGE	2.78	2.93	3.01	3.09

(*) Indicates significant differences which are shown in the lower triangle

Mean	WEEK	G G G G G r r r r r p p p p p	1 2 3 4 5
.4804	Grp 1		
.8254	Grp 2	*	
1.1973	Grp 3	* *	
1.4446	Grp 4	* * *	
1.8303	Grp 5	* * * *	

Group 1 = Week 1
Group 2 = Week 2
Group 3 = Week 3
Group 4 = Week 4
Group 5 = Week 5

BIOGRAPHY

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