A Department of Ecology Report

WASHINGTON STATE DEPARTMENT OF ECOLOGY

Seawater Challenge of Chinook Salmon Smolts (Oncorhynchus tshawytscha) Exposed to the Aquatic Herbicide Hydrothol 191®

Abstract

A seawater challenge was used to determine if low concentrations of the aquatic herbicide Hydrothol 191® affect the osmoregulatory performance of chinook salmon smolts (*Oncorhynchus tshawytscha*). Chinook smolts were exposed to 0, 50, 100, or 200 µg/L Hydrothol 191 (endothall acid equivalent) for 96 hours, followed by a 24-hour seawater challenge.

Fish exposed to 200 µg/L Hydrothol 191 suffered 45% mortality following the seawater challenge. There was 5% mortality among fish exposed to either 50 or 100 µg/L Hydrothol 191. Condition factors, plasma sodium levels, and gill ATPase activities were not significantly different among groups (one-way ANOVA, $p<0.05$) following exposure to seawater. Plasma sodium concentrations in all groups of fish, except those exposed to 200 µg/L of the herbicide, were consistent with normal seawater adaptation for chinook smolts (≤170 meq). Smolts exposed to 200 µg/L Hydrothol 191 had slightly higher plasma sodium levels (173.0 meq/L), but the difference from normal levels was not significant (student's *t*, $p<0.025$).

Results of this study indicate that low concentrations of Hydrothol 191 do not cause a decrease in the osmoregulatory capacity of chinook smolts. The cause of mortality is unclear, but may have been respiratory distress due to gill inflammation.

by Dave Serdar, Art Johnson
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Ecology Report 95-358
November 1995

Introduction

Background

Hydrothol 191®[1] has been shown to be a highly effective aquatic algicide and herbicide yet it is not permitted for use in Washington because of its toxicity to fish at low concentrations (Ecology, 1992). However, Hydrothol 191 is currently being considered as an alternative to copper compounds for controlling algae in lakes where repeated use has led to unacceptable levels of copper in bottom sediments.

The acute toxicity of Hydrothol to fish has been reasonably well documented in the Final Supplemental Environmental Impact Statement for Ecology's Aquatic Plants Management Program (Ecology, 1992). Lethal concentrations (LC_{50}s) are generally less than 1,000 µg/L (range of 320-1,600 µg/L) for a variety of species exposed to Hydrothol 191 from 4 to 120 hours.

One important aspect of aquatic herbicide use in Washington is the potential to impact migrating salmonids. Data have shown that certain herbicidal chemicals, including copper, decrease the ability of out-migrating (smolting) juvenile salmon to physiologically adapt to the marine environment (Lorz and McPherson, 1976; Bouck and Johnson, 1979). This effect may be seen at concentrations well below those recommended for herbicide application. Therefore, assessing an herbicide's effect on the osmoregulatory performance of smolting salmon can be a sensitive indicator of that herbicide's suitability for use in salmon-bearing waters.

In 1992, Ecology used a procedure similar to the one described here to assess the effects of endothall on smolting coho salmon (*Oncorhynchus kisutch*). Endothall is the herbicidal ingredient in Hydrothol 191. Results indicated that endothall alone did not adversely affect the ability of coho smolts to adapt for marine survival (Serdar and Johnson, 1993).

Objectives

The primary objective of this study was to assess the effects of Hydrothol 191 on osmoregulatory performance of smolting chinook salmon (*O. tshawytscha*). Results of this study may be used by Ecology's Water Quality Program to decide on the suitability of using Hydrothol 191 for algae control in Washington.

Chinook smolts were exposed to low levels of Hydrothol 191 in fresh water for 96 hours, followed by a 24-hour seawater challenge. Plasma sodium and gill ATPase levels were measured to determine if herbicide exposure interfered with the smolts' osmoregulatory capacity.

[1] Hydrothol 191® is the mono(N,N,-dimethylalkylamine) salt of endothall (7-oxabicyclo [2,2,1]heptane-2,3-dicarboxylic acid).

Methods

Sampling Procedures

The chinook smolt bioassay/seawater challenge was conducted at the Ecology/EPA Manchester Environmental Laboratory. Fall-run chinook smolts from the Minter Creek State Hatchery near Purdy, Washington were provided through the assistance of Dennis Popochock of the Washington Department of Fish and Wildlife during early May. Smolts were transported to Manchester in two 30-gallon plastic barrels which had been scrubbed with hot water and detergent, disinfected with clorox, and rinsed. Approximately 95% of the smolts in one of these barrels died within three days of being transported to Manchester, apparently due to the presence of residual clorox. Survivors of the "bad" barrel were subsequently kept separate from fish in the other barrel, who suffered no mortality and showed no side effects from their relocation.

Fish were kept in Living Stream® (Frigid Unit, Inc., Toledo, Ohio) tanks for approximately four weeks as an adjustment period prior to herbicide exposure. During this adjustment period, and for the remainder of the experiment, fish were exposed to a photoperiod of 16 hours light: 8 hours dark. Smolts were fed Biomoist feed at approximately 2% of their body weight per day during the acclimation period. Feeding was stopped 48 hours prior to herbicide exposure and for the remainder of the experiment.

One week prior to testing, five fish were taken directly from the acclimation tanks and challenged with seawater for 24 hours to determine if these fish were indeed undergoing smoltification. The mean plasma sodium level from these fish was 165 meq/L indicating an ability to regulate their blood sodium levels characteristic of smolts (Clarke, 1982).

Hydrothol 191 Exposure

Four 190 L aquaria were used for the experiment: three test tanks and one control tank. Each tank contained 20 smolts in approximately 150 L fresh water. Average stocking density was 0.7 g/L. Prior to the introduction of fish, certified Hydrothol 191 (provided by ELF Atochem Technical Services, Bryan, Texas) was diluted in 2 L deionized water and mixed into the three test tanks to achieve nominal concentrations of 50, 100, and 200 µg/L (endothall acid equivalent). Duplicate water samples for endothall analysis were collected from each test tank at the beginning of the test, after 48 hours, and at 96 hours. Healthy chinook smolts were placed in the test and control chambers 30 minutes after herbicide introduction. Fish in the test tanks were exposed to Hydrothol 191 for 96 hours in freshwater. Controls were kept under identical conditions without herbicide exposure. A small stream of air bubbles was used in each tank to prevent dissolved oxygen depression.

Water and Hydrothol 191 were renewed after 48 hours by removing 80% of the tank volume and replacing it with new solution. Alkalinity, conductivity, hardness, and pH were measured at the beginning of the test, after 48 hours, and at 96 hours. Temperature and dissolved oxygen levels were measured every 24 hours. Fish were periodically observed to check for mortality and to assess abnormal behavior or changes in behavior. Dead fish were removed from the tanks immediately.

Seawater Challenge

After 96 hours of herbicide exposure, freshwater was replaced with seawater (Clam Bay in Puget Sound, Washington) over a period of approximately 30 minutes. Final salinity, measured by hand-held refractometer, was 26 parts per thousand.

Seawater challenge procedures followed those recommended by Clarke and Blackburn (1977). Following the 24-hour seawater exposure, fish were incapacitated with a blow to the head, blotted dry, weighed, measured, and tails were cut off with a clean scalpel for blood collection. Blood was collected by capillary action using ammonium heparinized micro-caraway tubes, then capped and centrifuged. Plasma aliquots of 5-20µL were pipetted and diluted in 50 mL deionized water for sodium analysis. Gill tissue was collected immediately following blood, placed in 2 mL conical centrifuge tubes with sucrose/EDTA/imidazole buffer (prepared by Wally Zaugg, Cook, Washington), and placed on dry ice for subsequent gill Na^+,K^+-ATPase assessment. Surgical gloves were worn by handlers of fish and blood.

Analytical Methods, QA/QC Procedures, and Data Quality

There were no problems associated with sample analysis or QA/QC procedures. Overall quality of the data was very good. See Appendix A for details.

Results

Complete results of water quality tests, endothall concentrations, plasma sodium levels, and gill ATPase activity are shown in Appendix B. A summary of these results is presented below.

Endothall Concentrations and Other Water Quality Measurements

Endothall concentrations were measured during the course of the experiment because Hydrothol 191, an organic salt of endothall, rapidly dissociates in water and therefore cannot be measured over time. There was no trend toward declining endothall concentrations during the 96-hour exposure period. This was somewhat expected because of similar results we obtained during a previous experiment (Serdar and Johnson, 1993). Overall, nominal and measured concentrations were in close agreement (Table 1). Measured concentrations were 2% greater

than nominal concentrations, on average. Other water quality measurements indicate that conditions were suitable for juvenile chinook salmon (Table 2).

Fish Survival

Table 3 shows survival of fish in each tank. The greatest mortality occurred in the tank containing 200 µg/L Hydrothol 191, but only after fish were challenged with seawater. An EPA probit analysis program (version 1.4) was used to calculate EC values (Appendix C). The LC_{50} was calculated to be 240 µg/L, with lower and upper 95% confidence limits of 170 and 880 µg/L.

Table 1. Endothall Concentrations (mean ± SD in µg/L; n=8).

	Nominal Endothall Concentrations	Measured Endothall Concentrations
Tank050	50	55 ± 10
Tank100	100	108 ± 14
Tank200	200	174 ± 33
Control	__	4.7 U

U=Undetected at detection limit shown

Table 2. Water Quality Data (mean and ranges).

	Temp. (C)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L as $CaCO_3$)	Conductivity (µmhos/cm)	Hardness (mg/L as $CaCO_3$)	pH (s.u.)
Tank050	13.5 (12.4-14.2)	6.9 (5.4-7.8)	37 (37)	120 (120)	56 (46-61)	7.13 (7.04-7.21)
Tank100	13.5 (12.6-14.1)	7.3 (6.3-7.8)	40 (32-45)	120 (110-120)	71 (55-79)	6.94 (6.74-7.22)
Tank200	13.4 (12.4-14.1)	7.7 (7.0-8.1)	38 (32-45)	120 (110-120)	67 (59-79)	6.84 (6.64-7.46)
Control	13.5 (12.6-14.1)	7.4 (6.8-7.7)	35 (22-45)	140 (110-210)	64 (55-79)	6.82 (6.58-7.21)

Table 3. Fish Survival (cumulative %).

	Freshwater				Seawater
	Day 1	Day 2	Day 3	Day 4	Day 5
Tank050	100	100	100	95	95
Tank100	100	100	100	95	95
Tank200	100	100	100	100	55
Control	100	100	100	100	100

Plasma Sodium Concentrations and Gill ATPase Activity

Condition factors, sodium levels, and gill ATPase activities in fish demonstrated little difference among groups (Table 4). No statistically significant differences were found among groups for any of the variables tested (one-way ANOVA, $p<0.05$).

Table 4. Fish Condition Factors, Plasma Sodium Concentrations, and Gill ATPase Activity

	Survival (%)	Condition Factor ($100w/l^3$)	Plasma Sodium (meq/L)	Gill ATPase (μmoles P_i/ mg protein/hr)
Tank050	95	0.89 ± 0.05	156.4 ± 24.5	11.3 ± 3.2
Tank100	95	0.85 ± 0.05	166.0 ± 22.0	11.4 ± 1.7
Tank200	55	0.85 ± 0.08	173.0 ± 16.1	9.5 ± 2.6
Control	100	0.86 ± 0.07	170.0 ± 30.8	12.0 ± 2.2

Gill ATPase activity appears to be inversely related to Hydrothol concentrations and plasma sodium levels. This enzyme is involved in excretion of sodium and other monovalent ions, and lower ATPase activity would be expected to result in higher sodium levels. However, control fish, which showed the highest ATPase activity, did not have lower sodium levels than herbicide-exposed fish, except for those exposed to 200 μg/L Hydrothol 191.

Discussion and Conclusions

Results of this study indicate that Hydrothol 191 does not cause a decrease in the osmoregulatory capacity of chinook smolts. Plasma sodium concentrations in all groups of fish, except those exposed to 200 µg/L of the herbicide, were consistent with normal seawater adaptation for chinook smolts (≤170 meq/L; Clarke and Blackburn, 1977). Smolts exposed to 200 µg/L Hydrothol 191 had slightly higher sodium levels (173.0 meq/L), but the difference from normal levels was not significant (student's *t*, $p<0.025$).

High mortality (45%) in the group of fish exposed to 200 µg/L Hydrothol 191 represents the most significant finding in this study. Although the fish died only after having been challenged with seawater, reduced osmoregulatory capacity does not appear to have been the cause. These fish were observed congregating at the surface of the water on the fourth day of herbicide exposure which suggests they were suffering respiratory distress, possibly caused by gill irritation as a result of the Hydrothol 191. This condition may have been exacerbated by subsequent exposure to seawater.

The LC_{50} value calculated from results of this study (240 µg/L) is low compared to those for other species. The Final Supplemental Environmental Impact Statement for Ecology's Aquatic Plants Management Program (Ecology, 1992) lists Hydrothol 191 LC_{50}s for rainbow trout ranging from 700 to 1,300 µg/L for a 48-hour static exposure and 560 to 1,200 µg/L for a 96-hour static exposure. Other species are equally or more sensitive under static tests for 48 hours (bluegill - 800 to 1,180 µg/L; golden shiner - 350 µg/L) or 96 hours (golden shiner - 350 µg/L). Comparability of the LC_{50} to those found in other studies is limited because 1) there are no data on lethal concentrations of Hydrothol 191 based on seawater challenge tests, and 2) only three concentrations of Hydrothol 191 were tested. Mortality numbers from the 96-hour herbicide exposure in this study do not lend themselves to a precise estimate of the LC_{50}.

Finally, the gill ATPase activity measured in all four groups of fish leaves some question about the level of smolt development. Zaugg (1982) observed activities for parred (pre-smolt) salmonids ranging from 5 to 10 µmoles P_i/mg protein/hr and smolt activities from 12 to 65 µmoles P_i/mg protein/hr. Hatchery fall chinook often have gill ATPase activities in excess of 20 µmoles P_i/mg protein/hr in late spring (W. Zaugg, written communication). This is in apparent contradiction to salinity tolerance these fish exhibited which would indicate a high level of smolt development, and the silvery, streamlined appearance of the fish, a less definitive indicator of smoltification (Folmar and Dickhoff, 1980; Wedemeyer *et al.*, 1980).

Acknowledgements

The proposal for this study was reviewed by Larry Goldstein, Cliff Kirchmer, Steve Saunders (Ecology), Joe Solga (Elf Atochem), and Vince Piccirillo (NPC, Inc.). Bob Foster and Dennis Popochock of the Washington Department of Fish & Wildlife provided the chinook smolts. Myrna McIntosh and Jim Ross from the Manchester Lab conducted the sodium analysis. Dick Huntamer, also from the Manchester Lab, did the endothall analysis. Gill ATPase assays were conducted by Wally Zaugg. Joan LeTourneau formatted the report. We thank all of these people. We are also grateful to Elf Atochem who provided the funding for this study.

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APPENDIX A

Analytical Methods, QA/QC Procedures, and Data Quality

ANALYTICAL METHODS

Table A-1. Methods Used for Sodium, Hydrothol 191, and Gill Na^+,K^+-ATPase Analysis.

Parameter	Method	Practical Quantitation Limit (PQL)	Method/ Reference	Laboratory
Sodium	Acidification, ICAP	10 µg/L (=.00043 meq/L)	EPA 200.7	Manchester ERL
Endothall	Solid phase extraction, GC/MS	5 µg/L	EPA 548.1	Manchester ERL
Gill Na^+,K^+-ATPase	Differential measurement of ATP hydrolysis	N/A	Zaugg, 1982	W. Zaugg, Cook, WA

N/A=Not Available

QA/QC PROCEDURES AND DATA QUALITY

The QA/QC procedures used in this study follow those outlined in *Effects of Hydrothol on Smolting Salmon - Quality Assurance Project Plan* (Serdar, 1995).

Sampling

The bioassay conditions were those used by Ecology for static acute fish toxicity tests (Ecology, 1981). Test chambers were constructed of glass and silicone and were pre-cleaned using the following procedure: Soaked and hand-scrubbed in hot water for 20 minutes, followed with tap water rinse, acetone rinse, 15 minute soak in 10% hydrochloric acid, and 10 rinses in tap water. Dechlorinated municipal water (Manchester, Washington) was used in the test chambers. Air used for aeration was free of oil and fumes.

All necessary steps were taken to reduce sample contamination. Care was taken to reduce cross-contamination while spiking test tanks and collecting samples for endothall analysis. No endothall was detected (PQL = 4.7 µg/L) in one pair of duplicate samples collected from the control tank.

To assess possible sodium contamination during sample collection and/or analytical procedures,

one blank was submitted for each 10 plasma sodium samples. These blanks were submitted in order (*i.e.*, as the 11th, 22nd, etc. sample) to ensure that each blank was representative if samples were split into two or more analytical batches. Blanks were prepared in a manner identical to plasma samples, using deionized water (< 5 μmhos/cm) in place of blood. Of eight blanks submitted, six had undetectable concentrations of sodium (PQL = 10 μg/L). The remaining two blanks had sodium concentrations of 15 and 27 μg/L, less than 2% of the average plasma sample concentrations. Laboratory blanks (n=2) had no measurable sodium, indicating that the sodium contamination was introduced during sample collection.

Duplicate samples were collected to assess total precision. For sodium analysis, 9 pairs of duplicate samples were analyzed. The relative standard deviation (RSD, standard deviation/mean) was 13.4%, somewhat higher than expected. The RSD for 12 duplicate analyses of endothall was 6.0% and RSD for seven duplicate ATPase analyses was 20.4%.

Laboratory

Analysis of sodium and endothall adhered to quality criteria for EPA Methods 200.7 and 548.1, respectively. This included requirements for holding times, instrument calibration, procedural blanks, spiked sample analysis, precision data, and laboratory control sample analysis. Review of the sodium and endothall analyses were done by Manchester ERL staff and reported in the attached Quality Assurance Memoranda.

STATE OF WASHINGTON
DEPARTMENT OF ECOLOGY
MANCHESTER ENVIRONMENTAL LABORATORY
7411 Beach Drive East • Port Orchard, Washington 98366-8204 • (206) 871-8860 • SCAN 871-8860

June 22, 1995

TO: Dave Serdar, Project Officer

FROM: Myrna McIntosh, Metals Chemist

SUBJECT: Metals Quality Assurance memo for the Smolts in Hydrothol Project
Sample Numbers: 95238500 - 95238585

QUALITY ASSURANCE SUMMARY

Data quality for this project is excellent. No significant quality assurance issues are noted.

SAMPLE INFORMATION

The samples from the Smolts in Hydrothol project were received by the Manchester Laboratory on 6/12/95 in good condition.

HOLDING TIMES

All analyses were performed within the USEPA Contract Laboratory Program (CLP) holding times for metals analysis (28 days for mercury, 180 days for all other metals).

INSTRUMENT CALIBRATION

Instrument calibration was performed before each analytical run and checked by initial calibration verification standards and blanks. Continuing calibration standards and blanks were analyzed at a frequency of 10% during the run and again at the end of the analytical run. All initial and continuing calibration verification standards were within the relevant USEPA (CLP) control limits. AA calibration gave a correlation coefficient (r) of 0.995 or greater, also meeting CLP calibration requirements.

PROCEDURAL BLANKS

Since this project was not digested, the intial calibration blanks were reported as the procedure blanks.

SPIKED SAMPLE ANALYSES

Spiked and duplicate spiked sample analyses were performed on this data set. All spike recoveries are within the CLP acceptance limits of +/- 25%.

PRECISION DATA

The results of the spiked and duplicate spiked samples are used to evaluate precision on this sample set. The Relative Percent Difference (RPD) for all analytes is within the 20% CLP acceptance window for duplicate analysis.

LABORATORY CONTROL SAMPLE (LCS) ANALYSES

LCS analyses are within the windows established for each parameter. The initial calibration verification was reported as the run LCS.

Please call Bill Kammin at SCAN 360-871-8801 to further discuss this project.

MMM:mmm

MANCHESTER ENVIRONMENTAL LABORATORY

7411 Beach Drive E , Port Orchard Washington 98366

CASE NARRATIVE

June 28, 1995

Subject: Smolts in Hydrothol

Samples: 95 - 238600 to -238625

Case No. 1915-95

Officer: Dave Serdar

By: Dickey D. Huntamer
Organics Analysis Unit

ENDOTHALL

ANALYTICAL METHODS:

The samples were prepared and analyzed using EPA Method 548.1.

HOLDING TIMES:

All sample and extraction holding times were within the recommended limits.

BLANKS:

No Endothall was detected in the laboratory blanks.

SURROGATES:

There are currently no surrogate compounds available at this time for the Endothall analysis.

MATRIX SPIKE AND MATRIX SPIKE DUPLICATE:

Matrix spike recoveries ranged from 36.5% to 73% and the relative Percent Differences (RPD) ranged from 51% to 67%.

ANALYTICAL COMMENTS:

No analytical problems were encountered in the analysis. The data is acceptable for use as qualified.

APPENDIX B

Complete Results of Water Quality Tests, Endothall Concentrations, Plama Sodium Levels, and Gill ATPase Activity

Table B-1. Endothall Concentrations and Water Quality During the 96-Hour Bioassay

Parameter:	Endothall (ug/L)*				Temperature (C)				Dissolved Oxygen (mg/L)			
Tank No.:	050	100	200	CTL	050	100	200	CTL	050	100	200	CTL
Pretest	44 ± 2	95 ± 1	152 ± 1	N/A	14.2	14.1	13.7	13.9	N/A	N/A	N/A	N/A
					@ 24 hrs: 13.8	13.8	13.8	13.7	@ 24 hrs: 7.8	7.5	8.0	7.7
48 Hours (original water)	50 ± 4	97 ± 1	139 ± 1	N/A	13.1	13.1	12.9	12.9	5.4	6.3	7.7	7.3
48 Hours (renewed water)	61 ± 0.4	118 ± 6	202 ± 4	N/A	12.4	12.6	12.4	12.6	6.5	7.1	7.0	6.8
					@ 72 hrs: 14.0	14.1	14.1	14.1	@ 72 hrs: 7.8	7.8	8.1	7.7
96 Hours	65 ± 11	120 ± 10	201 ± 2	4.7 U	13.6	13.5	13.8	13.9	7.1	7.6	7.6	7.7

Parameter:	Alkalinity (mg/L CaCO3)				Conductivity (umhos/cm)				Hardness (mg/L CaCO3)			
Tank No.:	050	100	200	CTL	050	100	200	CTL	050	100	200	CTL
Pretest	37	37	45	22	120	120	110	110	55	55	79	79
48 Hours (original water)	37	45	45	37	120	120	120	120	46	79	60	61
48 Hours (renewed water)	37	32	32	45	120	120	110	120	61	79	59	60
96 Hours	37	45	32	37	120	110	120	210	60	70	70	55

Parameter:	pH (s.u.)			
Tank No.:	050	100	200	CTL
Pretest	7.20	7.06	6.75	7.15
48 Hours (original water)	7.10	6.89	7.46	6.58
48 Hours (renewed water)	7.21	7.22	6.86	7.21
96 Hours	7.04	6.74	6.64	6.68

*mean ± range of duplicate analysis
N/A=Not Analyzed
U=Undetected at detection limit shown

Table B-2. Fish Lengths, Weights, Plasma Sodium Concentrations, and Gill ATPase Activity Following the Seawater Challenge Test.

	Fork Length (mm)	Weight (g)	Condition Factor (100w/l3)	Plasma Sodium (meq/L)	Gill ATPase (µmoles Pi/ mg protein/hr)
Tank005	85	5.55	0.90	149.0	11.8
"	85	5.34	0.87	164.2	10.1
"	79	4.47	0.91	156.6	12.1
"	83	5.39	0.94	134.4	9.3
"	89	6.39	0.91	165.3	12.0
"	79	4.47	0.91	165.3	12.8
"	84	4.87	0.82	138.5	10.1
"	90	7.25	0.99	155.5	14.2
"	83	5.38	0.94	118.5	11.1
"	80	4.60	0.90	106.4	18.0
"	78	4.23	0.89	147.3	10.3
"	89	6.62	0.94	166.4	14.4
"	78	3.97	0.84	182.7	10.3
"	85	5.34	0.87	140.3	17.5
"	80	4.44	0.87	195.7	4.8
"	91	6.70	0.89	145.7	9.4
"	77	4.05	0.89	192.5	8.5
"	85	5.02	0.82	150.1	8.1
"	77	3.63	0.80	196.8	9.9
"	76	5.64	1.28	died	- -
Tank010	80	4.00	0.78	152.5	13.6
"	79	4.03	0.82	182.7	8.2
"	87	5.28	0.80	146.8	13.1
"	93	7.19	0.89	155.5	13.6
"	78	4.21	0.89	135.1	10.2
"	89	5.75	0.82	172.9	12.2
"	85	5.79	0.94	131.6	9.9
"	85	5.42	0.88	213.1	14.0
"	88	5.99	0.88	182.7	11.8
"	77	3.56	0.78	159.9	11.0
"	81	4.74	0.89	183.8	11.7
"	92	7.37	0.95	157.5	11.8
"	77	3.88	0.85	153.5	12.1
"	87	5.04	0.77	169.6	12.8
"	85	5.18	0.84	150.8	11.1
"	85	5.01	0.82	184.9	11.0
"	83	4.86	0.85	154.4	9.9
"	82	4.53	0.82	207.7	8.5
"	86	5.18	0.81	158.2	10.3
"	88	6.83	1.00	died	- -

Table B-2. (Cont'd)

	Fork Length (mm)	Weight (g)	Condition Factor (100w/l3)	Plasma Sodium (meq/L)	Gill ATPase (µmoles Pi/ mg protein/hr)
Tank020	76	3.57	0.81	187.0	10.8
"	86	5.67	0.89	152.4	11.0
"	77	3.77	0.83	192.5	10.7
"	84	5.41	0.91	153.3	3.1
"	85	4.25	0.69	203.3	9.1
"	84	4.92	0.83	168.6	6.5
"	88	6.82	1.00	169.6	13.1
"	75	3.56	0.84	178.3	10.5
"	79	3.97	0.81	169.6	9.9
"	83	4.78	0.84	158.8	10.1
"	78	4.33	0.91	169.6	9.5
"	74	3.26	0.80	died	- -
"	82	5.21	0.94	died	- -
"	80	4.03	0.79	died	- -
"	79	4.47	0.91	died	- -
"	79	3.96	0.80	died	- -
"	77	4.97	1.09	died	- -
"	75	4.66	1.10	died	- -
"	84	5.44	0.92	died	- -
"	78	4.26	0.90	died	- -
Control	88	6.30	0.92	193.6	9.7
"	90	6.92	0.95	170.7	12.2
"	82	4.60	0.83	199.0	11.8
"	87	6.24	0.95	187.0	13.9
"	88	5.81	0.85	197.9	13.9
"	86	4.81	0.76	139.2	14.5
"	91	7.66	1.02	158.0	15.2
"	83	4.45	0.78	120.5	10.8
"	87	5.68	0.86	199.0	10.2
"	80	4.21	0.82	204.4	10.0
"	81	4.67	0.88	145.5	9.6
"	83	4.64	0.81	226.2	8.5
"	65	2.03	0.74	127.0	8.9
"	84	4.59	0.77	139.2	12.2
"	76	3.51	0.80	198.6	12.2
"	81	4.50	0.85	119.6	11.2
"	82	5.02	0.91	168.3	16.6
"	81	4.60	0.87	169.6	14.6
"	89	6.63	0.94	164.2	12.5
"	80	4.19	0.82	172.5	10.8

APPENDIX C

Probit Analysis

EPA PROBIT ANALYSIS PROGRAM
USED FOR CALCULATING EC VALUES
Version 1.4

PROBIT ANALYSIS FOR HYDROTHOL BIOASSAY

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
0.0500	20	1	0.0500	0.0500	0.0206
0.1000	20	1	0.0500	0.0500	0.1270
0.2000	20	9	0.4500	0.4500	0.4051

Chi - Square Heterogeneity = 2.092

Mu = -0.618639
Sigma = 0.334330

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	6.850385	0.913779	(5.059378, 8.641392)
Slope	2.991056	1.025514	(0.981049, 5.001063)

Theoretical Spontaneous Response Rate = 0.0000

PROBIT ANALYSIS FOR HYDROTHOL BIOASSAY

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence	Upper Limits
EC 1.00	0.0401	0.0031	0.0696
EC 5.00	0.0678	0.0148	0.0992
EC10.00	0.0897	0.0329	0.1239
EC15.00	0.1084	0.0541	0.1497
EC50.00	0.2406	0.1696	0.8766
EC85.00	0.5344	0.2920	9.3411
EC90.00	0.6454	0.3290	16.5040
EC95.00	0.8537	0.3917	38.4457
EC99.00	1.4425	0.5407	188.6191

PROBIT ANALYSIS FOR HYDROTHOL BIOASSAY

PLOT OF ADJUSTED PROBITS AND PREDICTED REGRESSION LINE