SEAWATER CHALLENGE OF COHO SALMON SMOLTS EXPOSED TO ENDOTHALL

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ABSTRACT

A two-step procedure was used to determine if the contact herbicide endothall affected the ability of smolting coho salmon (*Oncorhynchus kisutch*) to survive in the marine environment. Thirty healthy smolts were first exposed to 5 mg/L endothall in freshwater for 96 hours, then challenged by seawater for 24 hours. Blood plasma sodium was measured as an indicator of the smolts' ability to osmoregulate. There was essentially no difference in plasma sodium concentrations of exposed fish (mean = 171 meq/L) compared to control fish (mean = 170 meq/L). These concentrations are consistent with those reported for fully smolted coho following a 24-hour seawater challenge.

INTRODUCTION

Endothall (chemical name 7-oxabicyclo [2,2,1]heptane-2,3-dicarboxylic acid) is a contact herbicide effective at controlling a variety of aquatic plant species (Westerdahl and Getsinger, 1988). It is produced as either a dipotassium salt (Aquathol K) or amine salt (Hydrothol 191), both of which are highly water soluble. Hydrothol 191 is toxic to fish and is not recommended for use where fishery resources are important (Pennwalt Corp.).

Since 1980, the Washington State Department of Ecology (Ecology) has permitted the use of endothall-containing herbicides (except Hydrothol 191) for the control of aquatic nuisance plants such as Eurasian watermilfoil (*Myriophyllum spicatum*). While endothall has relatively low acute toxicity to a variety of fish species (Bond *et al.*, 1960; Surber and Pickering, 1962), there are concerns that exposure to endothall or its formulated products may reduce the ability of salmon smolts to physiologically adapt for marine survival. Smolts are out-migrating juvenile fish that have undergone a physiological metamorphosis enabling them to excrete salt and retain water in a marine environment. The process of maintaining a proper salt-ion balance is called osmoregulation.

These concerns stem from a 1979 study in which 100% mortality was observed in coho salmon (*Oncorhynchus kisutch*) smolts challenged by seawater following a 5 mg/L endothall (commercial formulation not specified) exposure in freshwater for one hour (Bouck and Johnson, 1979). There were no mortalities in a second trial, and the inconsistency was never resolved. The researchers suggested that endothall may have interfered with the smolts' ability to properly osmoregulate.

Other investigators have shown that juvenile chinook salmon (*O. tshawytscha*) suffered high mortality when challenged by seawater following Aquathol K (3 mg/L endothall) exposure for 4 or 14 days (Ligouri *et al.*, 1984). The investigators also observed mild gill inflammation and hypothesized this as the cause of reduced osmoregulatory capacity. Coho smolts exposed to sublethal concentrations of heavy metals have also demonstrated osmoregulatory failure due to reduced activity of sodium-potassium-dependent ATPase, an enzyme important in maintaining salt balance in fish (Lorz *et al.*, 1978).

The purpose of this investigation was to measure the physiological response of coho smolts exposed to endothall. A two-step procedure was used: 1) fish were exposed to endothall in freshwater under static bioassay conditions for 96 hours, followed by 2) a 24-hour seawater challenge. Blood plasma sodium concentrations were then compared to values associated with normal marine adaptation for coho smolts challenged by seawater for 24 hours (170 meq/L; Clarke and Blackburn, 1977). Previous seawater challenge tests suggest that smolts with an extended osmoregulatory adjusting period have higher marine mortality due to factors such as slow growth and reduced swimming ability (Clarke and Blackburn, 1977; Clarke, 1982). Results may be used as a guide for Ecology's Water Quality Program in permitting the use of endothall in waters with anadromous salmonids.

METHODS AND MATERIALS

Static Endothall Exposure

The coho smolt bioassay/seawater challenge was conducted at the Ecology/EPA Manchester Environmental Laboratory in Manchester, Washington. Coho smolts averaging 27 g were obtained from the Minter Creek State Hatchery near Purdy, Washington, through the assistance of the Washington State Department of Fisheries during the second week of May 1992. Smolts were transported to Manchester by truck with appropriate precautions taken to reduce stress. Once at Manchester, dead or stressed fish were discarded. Unusual eye color, inactivity, nose and fin rot, and descaling were used as an indication of unhealthy fish. Healthy fish were held undisturbed in the bioassay laboratory for 7 days as an adjustment period prior to endothall exposure.

Four 190 L glass aquaria were used for the experiment; two test and two control. Fifteen healthy smolts were randomly assigned to each aquarium. Stocking density was approximately 2.7 g/L. The bioassay conditions were those specified by Ecology for static acute fish toxicity tests (Washington State Department of Ecology, 1981). Water

temperatures were maintained at 12-14°C during the course of the test. All materials and equipment were specially constructed and cleaned to avoid introduction of potentially interfering chemicals. Aquaria were situated in one corner of the laboratory to reduce outside visual disturbances. Dechlorinated municipal water (Manchester, Washington) was used in the test chambers, and air used for aeration was free of oil and fumes.

Prior to the introduction of fish, technical-grade endothall (ChemService, Westchester, Pennsylvania) was diluted and mixed into the test aquaria to achieve a nominal concentration of 5 mg/L endothall (free acid equivalent), which is the maximum application rate recommended by the manufacturer. Duplicate water samples were collected from the test tanks for endothall analysis at the beginning and end of the freshwater exposure period. Healthy coho smolts were placed in the test and control chambers 30 minutes after herbicide introduction.

Fish in the test tanks were exposed to endothall for 96 hours in freshwater. Fish in control aquaria were kept under identical conditions without endothall exposure. Temperature, pH, and dissolved oxygen levels were monitored throughout the course of the experiment. Alkalinity and conductivity were measured at the beginning and end of the freshwater exposure period. Fish were not fed during the course of the experiment.

Seawater Challenge

After 96 hours, freshwater was replaced with seawater (Clam Bay in Puget Sound, Washington) over a period of about 30 minutes. Final salinity, measured by hand-held refractometer, was 28-30 parts per thousand.

Seawater challenge procedures were similar to those recommended by Clarke and Blackburn (1977). Following a 24-hour seawater challenge, fish were anesthetized with MS-222, 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 with critocaps® (Oxford Labware, St. Louis, Missouri) and centrifuged. Plasma aliquots of 20 μL were pipetted and diluted in 50 mL deionized water for sodium analysis. Surgical gloves were worn by handlers of fish and blood.

Chemical Analysis and Data Quality

Overall, quality of the analytical data was very good. Sodium concentrations were determined at the Manchester Laboratory using inductively coupled plasma (ICP) methodology. Precision was excellent (average relative percent difference = 3.4%) for 18 samples analyzed in duplicate. Trace sodium was detected in the method blank (deionized water in place of plasma), but at about 2% of the concentrations in plasma.

Endothall analysis was conducted at A & S Environmental Laboratory in Reading, Pennsylvania. Gas chromatography (EPA method 548) was employed at detection limits of

0.1 mg/L. No endothall was detected in either control aquarium. Average precision for four pair of duplicate samples from the test aquaria was ± 9%. A single matrix spike analysis had 90% recovery, well within control limits, and endothall was not detected in the procedural laboratory blank.

RESULTS

Water Quality

Water quality of test and control aquaria remained within a range appropriate for the health of coho smolts (Table 1).

Table 1. Range of water quality measurements during the 96-hr freshwater endothall exposure.

	Parameter					
Aquarium	Temp. (C)	pH	Dissolved Oxygen (mg/L)	Conduct-ivity (μmhos/ cm)	Alkal-inity (mg/L $CaCO_3$)	Hardness (mg/L $CaCO_3$)
Test 1	12.8-13.7	7.4-7.5	5.1-9.6	260	75	65
Test 2	12.9-13.9	7.4-8.0	6.0-9.5	260-270	75-85	63-65
Control 1	12.8-13.7	7.5	6.6-9.2	260	75	66
Control 2	12.9-13.9	7.5-8.3	4.2*-9.5	260-270	75-85	62-68

*Dissolved oxygen dipped briefly below 5 mg/L when aeration was lost in this tank.

Except for dissolved oxygen, none of the water quality parameters varied widely within or among the four aquaria. Temperature of the water was 1-2°C higher than anticipated but well within tolerance limits of coho smolts.

Tanks were aerated after dissolved oxygen levels began to drop during the first 6 to 8 hours of the experiment. Aeration was lost briefly in control aquarium 2 twice during the experiment and dissolved oxygen dipped below 5 mg/L. However, fish did not appear to be affected by the oxygen depression.

Smolt Survival and Blood Sodium Concentrations

There were no mortalities in either control or test aquaria during the static freshwater exposure and seawater challenge. All fish appeared to be healthy except for one of the

control fish which had tail rot. Blood was collected from all sixty fish for plasma sodium analysis.

Mean plasma sodium concentrations of exposed and control fish are summarized in Table 2. Mean sodium concentrations did not differ substantially between exposed and control fish. Plasma sodium concentrations of 170 meq/L are considered normal in healthy coho smolts following a 24-hour seawater challenge (Clarke and Blackburn, 1977; Clarke, 1982).

Table 2. Plasma sodium concentrations of smolts exposed to endothall and control fish. Values are means ± standard deviation.

Aquarium	n	Nominal Endothall Conc. (mg/L)	Plasma Sodium Conc. (meq/L)	Weight (g)	Total Length (mm)
Test 1	15	5.0	173 ± 6	27 ± 6	155 ± 9
Test 2	15	5.0	169 ± 8	26 ± 6	152 ± 12
Control 1	15	0	170 ± 9	26 ± 6	152 ± 10
Control 2	15	0	171 ± 6	28 ± 7	156 ± 11

Persistence of Endothall in Test Aquaria

To assess the degradation or other loss of endothall during the 96-hour static exposure period, concentrations were measured in the aquaria at the beginning and end of the test. Results are shown in Table 3. There was no net loss of endothall in the aquaria during the exposure period, and endothall was not detected in water samples from a control aquarium.

Table 3. Endothall concentrations at the beginning and end of the 96-hr static exposure period. Values are means (n=2) except for the control aquarium (n=1).

	Endothall Concentration (mg/L)	
Aquarium	Beginning	Ending
Test 1	4.4	4.4
Test 2	5.0	5.2
Control 1	U(0.1)	U(0.1)

U=Undetected at detection limits shown in parentheses

DISCUSSION AND CONCLUSIONS

Results of this investigation indicate that coho smolts do not suffer a decreased ability to regulate their blood sodium concentrations when exposed to maximum endothall levels recommended for herbicide application. Plasma sodium concentrations in exposed and control fish were well below the 200 meq/L level where high mortality has been observed shortly after transfer to seawater (Clarke and Blackburn, 1977). These results appear to contradict those of previous studies reporting increased mortality in endothall-exposed fish challenged with seawater (Bouck and Johnson, 1979; Ligouri *et al.*, 1984). Although these investigators used death as an experimental endpoint, they concluded that inability to physiologically adjust to saltwater caused mortality.

One explanation is that "inert" ingredients present in the commercial formula(s) (Aquathol K in the 1984 study) may have caused mortality. Inert ingredients and product formulations are proprietary corporate information, and are generally not revealed. In addition, sublethal toxicity testing is not usually required for product testing and registration. Therefore, the possibility exists that inert ingredients, alone or in combination with endothall, elicit a physiological response, possibly caused by gill irritation, which results in poor marine survival. Lack of information about the inert ingredients may have led to oversight in reaching conclusions about their sublethal toxicity.

Another explanation may be that coho are less sensitive than chinook as used by Ligouri *et al.* (1984). Unfortunately, there are no available data comparing toxicity or sublethal effects of endothall on each species. However, juvenile chinook have been shown to be more sensitive than coho in acute toxicity tests using a variety of organic compounds (National Academy of Sciences and National Academy of Engineering, 1973) and are more easily stressed by changes in their environment (Kurt Fresh, personal communication). A third possibility is that juvenile chinook salmon tested by Liouri *et al.* (1984) were not fully smolted. This is supported by the 20% mortality of control fish after 24 hours in seawater, suggesting that exposed fish were predisposed to sensitivity in seawater, a condition perhaps exacerbated by the herbicide.

RECOMMENDATIONS

The bioassay and seawater challenge should be repeated using Aquathol K if concerns linger about its effects on the osmoregulatory capacity of coho smolts. Other aquatic herbicides may also be tested if they are used in the vicinity of smolting salmon. In future seawater challenge tests, measurement of ATPase and other biochemical parameters should be considered to provide a more sensitive indication of physiological response to herbicides.

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