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| Title: E | EFFECTS OF CHLORINATED BIOLOGICALLY |
| <u>.</u> | TABILIZED KRAFT MILL EFFLUENT ON THE |
| <u>v</u> | MORTALITY AND GROWTH OF COHO SALMON |
| Abstract | approved:Signature redacted for privacy |
| | / Gary L. Larson |

Ninety-six hour acute toxicity bioassays were employed to determine the tolerance of juvenile coho salmon to chlorinated stabilized kraft mill effluent (SKME). In addition the effect of chlorinated SKME on the growth of juvenile coho salmon in six and ten day experiments was studied.

Results of the acute toxicity tests indicated that the addition of as low as 1 mg/l of total residual chlorine to 100 percent SKME could cause acute toxicity in effluent concentrations as low as 18 percent. Since the non-chlorinated SKME used in these tests was not acutely toxic to the salmon, it appeared that chlorine or some chlorinated material formed in the chlorinated SKME was responsible for the cases of acute toxicity.

Effects of chlorinated SKME on the growth of the coho salmon were not clearly defined at the chlorine and SKME concentrations tested.

Effects of Chlorinated Biologically Stabilized Kraft Mill Effluent on the Mortality and Growth of Coho Salmon

by

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TABLE OF CONTENTS

| | Page |
|---|----------------|
| INTRODUCTION | 1 |
| METHODS AND MATERIALS | 4 |
| RESULTS Acute Toxicity Bioassays Growth Experiments | 10 10 15 |
| INTERPRETATIONS AND DISCUSSION | 23 |
| BIBLIOGRAPHY | 28 |
| APPENDICES | 32 |

LIST OF FIGURES

| Figure | | Page |
|-----------|---|------|
| 1, | (A) Relationship between percentage of mortality of coho salmon and percentage of SKME chlorinated with 1 mg/l of chlorine, and (B) relationship between average in-tank chlorine residual and percentage of SKME chlorinated with 1 mg/l of chlorine for the first 96-hr acute toxicity test. | 12 |
| 2. | Relationship between percentage of mortality of coho salmon and percentage of SKME chlorinated with 1.7 mg/l of chlorine for the second and third 96-hr acute toxicity tests. | 13 |
| 3. | Relationship between average in-tank chlorine residual and percentage of SKME chlorinated with 1.7 mg/l of chlorine for the second and third 96-hr acute toxicity test. | 14 |
| 4. | (A) Relationship between percentage of mortality of coho salmon and percentage of SKME chlorinated with 2.3 mg/l of chlorine, and (B) relationship between average in-tank chlorine residual and percentage of SKME chlorinated with 2.3 mg/l of chlorine for the fourth 96-hr acute toxicity test. | 16 |
| 5. | Relationships between growth rate and food consumption for coho salmon exposed to three concentrations of chlorinated SKME, three concentrations of non-chlorinated SKME, and a water control. Growth experiment conducted from September 5-11, 1975. | 18 |
| 6. | Relationships between growth rate and food consumption for coho salmon exposed to three concentrations of chlorinated SKME, three concentrations of non-chlorinated SKME, and a water control. Growth experiment conducted from September 19-29, 1975. | 20 |

| Figure | | Page |
|--------|---|------------|
| 7. | Relationships between growth rate and food consumption for coho salmon exposed to three concentrations of chlorinated SKME, three concentrations of non-chlorinated SKME, and a water control. Growth experiment conducted from October 8-18, 1975. | 21 |
| 8. | In-tank chlorine residual in 100% SKME chlorinated with 1.7 mg/l of chlorine as a function of the BOD of the 100% SKME for two 96-hr acute toxicity tests. | 2 6 |

LIST OF TABLES

| Table | | Page |
|-------|---|------|
| 1. | Concentrations between which the 96-hr TL ₅₀ 's occurred, chlorine added, mean dry weights of coho salmon and water quality data for each acute toxicity test. | 11 |
| 2. | Initial dry weights of coho salmon, ration sizes, amount of chlorine added and water quality data for each growth experiment. | 17 |

EFFECTS OF CHLORINATED BIOLOGICALLY STABILIZED KRAFT MILL EFFLUENT ON THE MORTALITY AND GROWTH OF COHO SALMON

INTRODUCTION

The kraft pulp and paper industry is one of the major industries in the Pacific Northwest. The large quantity of effluent discharged from the production of pulp and paper has led to concern about its toxicity and effects on the quality of receiving waters. Studies of the toxicity of kraft mill effluent show that undiluted primary treated effluent is usually acutely toxic to aquatic organisms such as chinook salmon (Tokar, 1968; Ellis, 1968), atlantic salmon and lobsters (Sprauge and McLeese, 1968), mayfly naiads (Van Horn, Anderson and Katz, 1949), stonefly naiads, caddisfly larvae and certain species of algae (DeWitt, 1963). Biological stabilization reduces the acute toxicity of primary kraft mill effluent (Borton, 1970; Seim, 1970). Such stabilization also reduces the sublethal effects of primary kraft mill effluent on salmon (Robinson-Wilson and Seim, 1976; Borton, 1975; Mower, 1974).

The Federal government has expressed concern over the presence of coliform bacteria in biologically stabilized kraft mill effluent (SKME) and about the possible contamination of receiving waters (Farrell et al., 1973). A study by Watkins (1973) on one

paper mill showed that the chlorination of biologically stabilized effluent was the most effective treatment tested for reducing the numbers of coliform bacteria to acceptable levels. The Environmental Protection Agency has considered the advisability of requiring all paper mills to chlorinate their SKME (Carter, 1975).

Secondary-treated domestic sewage is chlorinated to prevent contamination of receiving waters by bacteria in order to protect public health. Damage to aquatic organisms as a result of the discharge of chlorinated domestic sewage is a common occurrence and can often be attributed to the persistence of inorganic chloramines (Zillich, 1969a, b, and 1970). Tsai (1973) reported approximately 50 µg/l total residual chlorine five miles downstream from a chlorinated domestic wastewater outfall. Chloramines are formed when chlorine is added to water that contains ammonia, such as secondary-treated domestic sewage and certain other industrial effluents (Warren, 1971; Sawyer and McCarty, 1967).

Chloramines are acutely toxic to most species of aquatic life at concentrations less than 1 mg/l, the residual chlorine concentration usually maintained in domestic sewage effluents. Arthur and Eaten (1971) found reduced survival, in chlorinated water, of fathead minnow larvae at 108 μ g/l total chloramines and of the amphipod <u>Gammarus pseudolimnaeus</u> at 220 μ g/l total chloramines. The Michigan Department of Natural Resources (1971) found the

96-hr TL_{50} rainbow trout held in streams receiving chlorinated sewage, to be 22.8 μ g/l total residual chlorine. Lamperti (1975) found the 96-hr TL_{50} of juvenile coho ranged from 57 μ g/l to 82 μ g/l total chloramines with the 96-hr TL_{50} increasing with increasing fish size. Larson et al. (1976) found the 96-hr TL_{50} of juvenile brook trout in chlorinated water increased with increasing fish size.

The potential toxicity of chlorinated SKME could be a serious problem to aquatic organisms. Little is known about its toxicity, however. The objectives of this research were to determine the acute toxicity and a sublethal effect (i.e., growth) of chlorinated SKME as compared to non-chlorinated SKME, on juvenile coho salmon.

METHODS AND MATERIALS

Coho salmon (Oncorhynchus kisutch) was chosen for study because it is an important sport and commercial species present in many Oregon waters receiving kraft pulping wastes. The coho were obtained from the Fall Creek Salmon Hatchery (Oregon Department of Fish and Wildlife).

The effluent used in this study came from a kraft liner board pulp and paper mill producing about 890 tons per day (tpd), consisting of 590 tpd unbleached kraft pulp, 200 tpd neutral sulfite, semichemical pulp, and 100 tpd waste paper clippings. Digestion chemicals and turpene were recovered in the mill. Primary treatment of the effluent occurred in sedimentation ponds having a total retention time of 24 hours. Secondary treatment of the effluent was provided in biological stabilization basins, having a retention time of about 7 days. The effluent was then subjected to an additional 10 days of biologican treatment in a small stabilization basin operated by personnel of the National Council for Air and Stream Improvement (NCASI) (Seim, 1975). This additional treatment was provided to produce an effluent of high and relatively consistent quality, one that might be characteristic of stabilized kraft process effluents produced by this mill in the future. The stabilized kraft mill effluent was then pumped directly into the laboratory effluent

headboxes. SKME pH values ranged from about 7.5 to 8.5 and the five day biological oxygen demand (BOD) ranged from about 6.0 mg/l to 22 mg/l. The effluent temperature ranged from about 17 C to 25 C. SKME samples were analyzed for BOD and resinacids by personnel of the NCASI.

Dilution water from the Willamette River was pumped through a sand filter before entering a cooling reservoir. It was then pumped through an ultraviolet light purifier to destroy pathogens, before entering the dilution water headboxes. The pH values of the water ranged from approximately 7.6 to 8.2 and the temperature ranged from about 15 C to 19 C.

Chlorine was introduced into the SKME in combination with ammonia. Studies done on a neutral sulfite, semi-chemical pulp and paper mill effluent showed that the combination of chlorine and ammonia was more effective in disinfection of stabilized pulp effluent and more economical than the addition of chlorine alone (Farrell et al., 1973). Complete coliform destruction was achieved by the addition of 40 mg/l of ammonia and 20 mg/l of chlorine. Without the ammonia, the amount of chlorine necessary to achieve 100 percent coliform destruction was 40 mg/l.

Sodium hypochlorite and ammonium chloride, kept in separate

Mariotte bottles, entered a mixing box at equal rates, allowing

partial chloramine formation. The molar ratio of chlorine to

ammonia was 0.7:1.0 and retention time in the box was about 90 minutes. From the mixing box, the toxicant (averaging approximately 71 percent inorganic chloramines and 29 percent free residual chlorine) dripped into the 100 percent SKME entering the dilutor. Concentrations of residual chlorine were measured with a Wallace and Tiernan Amperometric Titrator as free chlorine (chlorine gas, hypochlorite ion and hypochlorous acid), dichloramine (NHCl2), monochloramine (NH,Cl), and total residual chlorine (sum of the free and chloramine residuals). Residual chlorine concentrations were measured just before the toxicant was added to the full strength (100 percent) SKME entering the dilutor (chlorine added), as well as in the solution of the aquaria. The in-tank residual samples were taken from the aquaria solutions directly under the inflows, and measured immediately. Chlorine concentrations in these samples were used as an index of the total residual chlorine to which the coho were exposed. Chlorine concentrations in each aquarium were averaged for the duration of each acute toxicity test and reported as in-tank total residual chlorine. Concentrations were measured twice each day throughout each test. Although in-tank chlorine residuals were measurable in the acute studies, none were measurable in the growth studies due to the low concentrations of chlorine applied.

Constant flow dilution systems (Chadwick et al., 1972) were

used to deliver the desired proportions of SKME and water to 15 gallon (56.8 l) glass aquaria within which standpipe drains maintained the liquid volume in each aquarium at 45 liters. Separate dilution systems were used for the chlorinated effluent and for the non-chlorinated effluent plus water controls. The flow rate into each aquarium was 330 ml/min.

Fish used in the 96-hr acute toxicity tests were acclimated to the dilution water for three days without food and then sorted to nearly uniform size in groups of ten and placed in the aquaria. At the end of each test the fish were removed from the aquaria, dried for four days at 70°C and then weighed to obtain the dry weight of each group. Concentrations of 18, 32, 56, and 100 percent chlorinated SKME by volume were maintained during acute tests 1, 2, and 3. As no in-tank chlorine residuals could be measured in the 18 percent concentration during these three tests, this concentration was replaced by a 75 percent concentration in the fourth acute test. Each concentration of chlorinated effluent was replicated once. Flows were measured daily and adjusted when needed. Fish held in 100 percent non-chlorinated SKME and in water were used as controls.

Two graphs were constructed for each acute toxicity test to illustrate the relationships between percent mortality and percent effluent, and percent effluent and the average in-tank chlorine

residual for each aquarium. From the plot of percent mortality and percent effluent, the percent effluent resulting in 50 percent mortality could be estimated for each test. The residual chlorine concentration present in that percent effluent could be estimated from the graph of in-tank chlorine residual and percent effluent for the same test. The chlorine residual and the percent effluent at which 50 percent mortality occurred in each 96 hour test was called the 96-hr TL₅₀ for that test. Fish used in the growth studies were acclimated in the dilution water one week prior to testing. The fish were fed Tubifex for five days, and then starved for two days. Each group of fish was then weighed and placed into an appropriate aquarium. In each experiment a subsample of fish was dried at 70°C for 4 days and then weighed to estimate the initial dry weight of the fish groups used in the experiment.

Concentrations of 0 (control), 5, 10, and 20 percent by volume of chlorinated and non-chlorinated SKME were maintained during the growth studies. Fish in each concentration were fed daily one of three ration sizes of <u>Tubifex</u> worms. The rations were fed for six days in growth study 1 and ten days in growth studies 2 and 3. At the end of each test, food was withheld for one day prior to the removal of the fish from the aquaria. Each group of fish was dried for four days at 70°C and weighed to determine the final dry weight. Periodically subsamples of <u>Tubifex</u> were dried at 70°C for

four days and weighed during each growth study to provide estimates of the percent dry weight of <u>Tubifex</u> fed the fish. Fish growth rates were calculated by dividing total change in milligrams dry weight, by the mean dry weight in grams and by time in days. Food consumption rate was calculated by dividing total dry weight in milligrams of food consumed by the mean dry weight of fish in grams and by time in days (Warren, 1971). Gross efficiency of food utilization was calculated by dividing growth rate by the consumption rate.

Percent mortality and dry weight of coho salmon, in-tank chlorine residuals and water quality for each concentration of SKME and controls for each acute toxicity test are presented in Appendices I through IV. Fish weights, growth rates, food consumption rates and gross efficiency of food utilization for growth for coho salmon in chlorinated and nonchlorinated SKME and water controls for each growth experiment are presented in Appendices V through VII.

RESULTS

Acute Toxicity Bioassays

No fish died in the water controls or the nonchlorinated 100 percent SKME controls in any of the 96-hr acute toxicity bioassays. Recent studies by Robinson-Wilson and Seim (1976) have also demonstrated that SKME from the same mill is rarely acutely toxic to coho salmon.

In test 1, the 96-hr TL₅₀ occurred between SKME concentrations of 18 and 32 percent (Figure 1a). No in-tank chlorine residuals were detectable at these effluent concentrations (Figure 1b). The amount of total residual chlorine applied to the SKME before dilution was 1 mg/1. The mean dry weight of the fish was 0.14 grams (Table 1). The average temperature of the 100 percent SKME was 22°C. The average BOD of the 100 percent SKME was 17.6 mg/1 before chlorination.

In test 2, the 96-hr TL_{50} occurred between SKME concentrations of 56 and 100 percent and between in-tank chlorine residual concentrations of 3 and 14 μ g/l (Figures 2 and 3). The amount of total residual chlorine applied to the SKME before dilution was 1.7 mg/l. The mean dry weight of the fish was 0.7 grams (Table 1). The average temperature of the 100 percent SKME was 23.6 °C. The

Table 1. Concentrations between which the 96-hr TL₅₀'s occurred, chlorine added, mean dry weights of coho salmon and water quality data for each acute toxicity test.

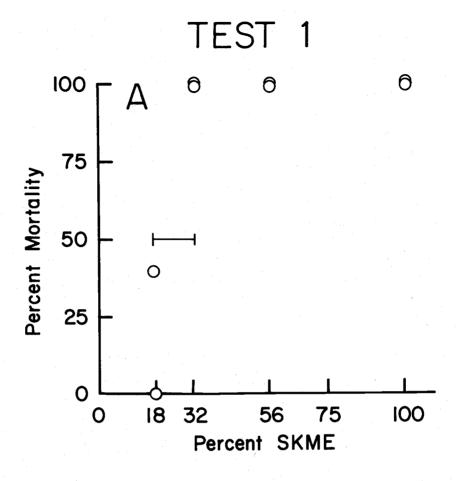
| Test | Date | Mean dry wt per fish (g) | Chlorine added (mg/l) | Concentrations between which the 96-hr TL ₅₀ occurred | | Mean temp. 100% | Temp. range 100% | Mean | Mean ¹ / | <u>2</u> / Mean | |
|------|---------|--------------------------------------|-----------------------------|--|-------------------------------|-----------------------|------------------------------|------------------------|---------------------|--------------------|---------------------------|
| | | | | SKME conc. | In-tank chlorine $(\mu g/l)$ | SKME (°C) | SKME (°C) | temp. water (°C) | BOD (mg/l) | D. O. (mg/l) | Mean ³ / pH |
| 1 | 7/17/75 | 0. 14 | 1.0 | 32-18 | 0 | 22. 0 | 23.0-21.5 | 19.0 | 17.6 | 7.33 | 8. 13 |
| 2 | 7/22/75 | 0. 7 2 | 1.7 | 100-56 | 18-3 | 23.6 | 25.0-20.0 | 19.0 | 15.0 | 8.79 | 7.99 |
| 3 | 7/30/75 | 0.60 | 1.7 | ud 4/ | > 2 6 | 20. 5 | 22 . 0 - 19. 0 | 16.9 | 9.0 | 7.74 | 7.95 |
| 4 | 8/6/75 | 1. 10 | 2.3 | 100-56 | 20-4 | 17.8 | 18.4-17.2 | 17.7 | 17.0 | 8.60 | 8. 01 |

 $[\]frac{1}{2}$ Measured in 100% non-chlorinated SKME.

^{2/}For all aquaria.

 $[\]frac{3}{2}$ For all aquaria.

 $[\]frac{4}{}$ Unable to determine.



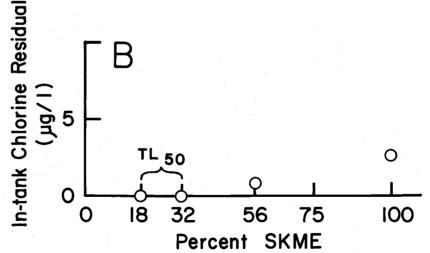


Figure 1. (A) Relationship between percentage of mortality of coho salmon and percentage of SKME chlorinated with 1 mg/l of chlorine (—denotes SKME concentrations between which TL₅₀ occurred), and (B) relationship between average in-tank chlorine residual and percentage of SKME chlorinated with 1 mg/l of chlorine (—denotes chlorine concentrations between which TL₅₀ occurred) for the first 96-hr acute toxicity test.

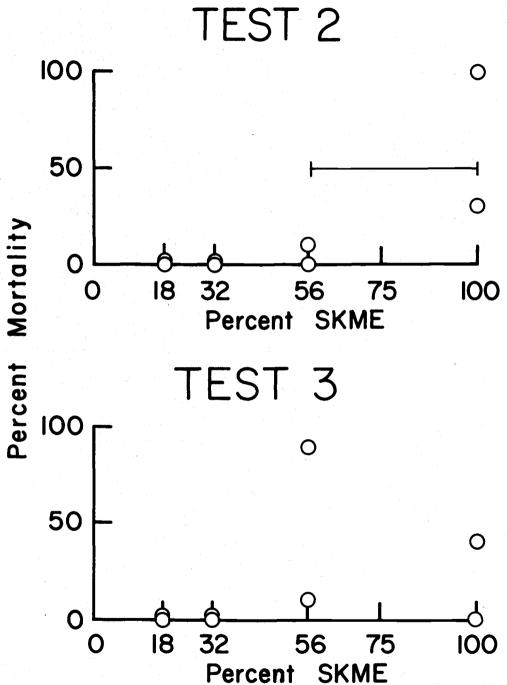
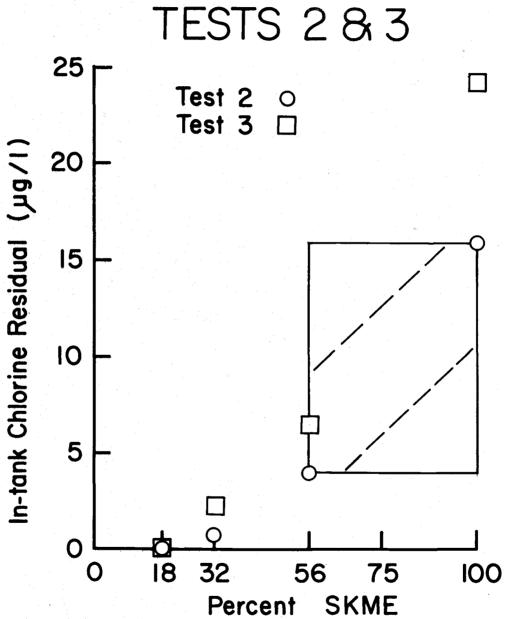


Figure 2. Relationship between percentage of mortality of coho salmon and percentage of SKME chlorinated with 1.7 mg/l of chlorine for the second and third 96-hr acute toxicity tests (—denotes SKME concentrations between which TL50 occurred in test 2).



Relationship between average in-tank chlorine residual and percentage of SKME chlorinated with 1.7 mg/l of chlorine for the second and third 96-hr acute toxicity tests (denotes area within chich TL₅₀ occurred for test 2).

average BOD of the 100 percent SKME was 15.0 mg/l before chlorination.

In test 3, insufficient deaths occurred to estimate the 96-hr TL₅₀ (Figure 2). The maximum in-tank chlorine residual, 26 µg/l, occurred in the aquaria receiving 100 percent chlorinated SKME (Table 1 and Appendix III). The amount of total residual chlorine applied to the SKME before dilution was 1.7 mg/l. The mean dry weight of the fish was 0.6 grams.

In test 4, the 96-hr TL $_{50}$ occurred between SKME concentrations of 56 and 100 percent and between in-tank chlorine residual concentrations of 4 and 20 μ g/l (Figure 4a and 4b). The amount of total residual chlorine applied to the SKME before dilution was 2.3 mg/l. The mean dry weight of the fish was 1.1 grams (Table 1).

Growth Experiments

The relationships between the growth rates and the food consumption rates for the coho salmon in the first growth study are shown in Figure 5. The amount of total residual chlorine applied to the SKME before dilution was 0.768 mg/l (Table 2). The mean initial dry weight of the fish was 2.72 grams. There were 5 fish in each aquaria. The ration sizes were 2.8, 6.5, and 9.3 percent of the mean dry body weight of the fish. The fish in 10 percent chlorinated and nonchlorinated SKME did not consume all of the

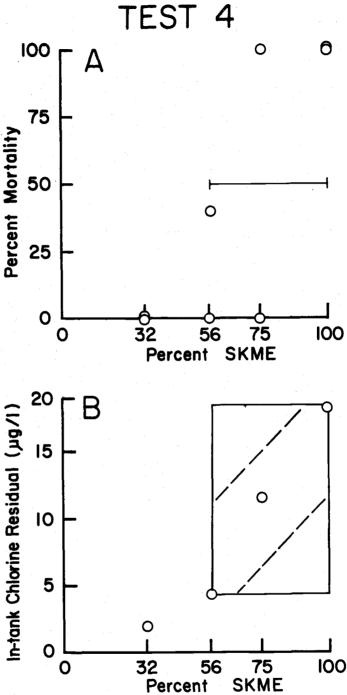


Figure 4. (A) Relationship between percentage of mortality of coho salmon and percentage of SKME chlorinated with 2.3 mg/l of chlorine (I denotes SKME concentrations between which TL₅₀ occurred), and (B) relationship between average in-tank chlorine residual and percentage of SKME chlorinated with 2.3 mg/l of chlorine for the fourth 96-hr toxicity test (denotes area within which TL₅₀ occurred).

Table 2. Initial dry weights of coho salmon, ration sizes, amount of chlorine added and water quality data for each growth experiment.

| Test | Date | Mean estimated initial dry weight per fish (g) | Chlorine added (mg/l) | Ration 1/ sizes (%) | Number of fish per aquarium | Mean temp. (°C) | <u>2</u> / Mean BOD (mg/l) | Mean 3/ D.O. (mg/1) | Mean 4/ | Mean ⁵ / Isopimaric acid conc. (mg/1) | |
|------|---------------------|--|-----------------------------|---------------------|--------------------------------------|-----------------------|-------------------------------------|---------------------------|---------|--|--|
| 1 | 9/5-11/75 | 2.72 | 0.768 | 2.8 6.5 9.3 | 5 | 15.8 | 12.0 | 8.4 | 7.74 | 0, 32 | |
| 2 | 9/19 -2 9/75 | 1. 11 | 0.441 | 2.4 4.8 7.2 | 6 | 15.6 | 7.0 | 7.7 | 7.78 | 0. 2 6 | |
| 3 | 10/8-18/75 | 0.28 | 0.609 | 2.7 5.5 8.2 | 8 | 15.7 | 12.5 | 6/ | | 0.45 | |

^{1/}As percent dry body weight.

^{2/}Measured in 100% nonchlorinated SKME.

 $[\]frac{3}{\text{For all aquaria.}}$

 $[\]frac{4}{}$ For all aquaria.

^{5/}Measured in 100% nonchlorinated SKME.

 $[\]frac{6}{Not}$ measured.

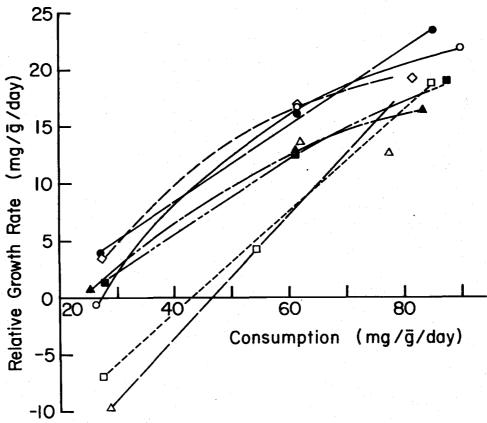
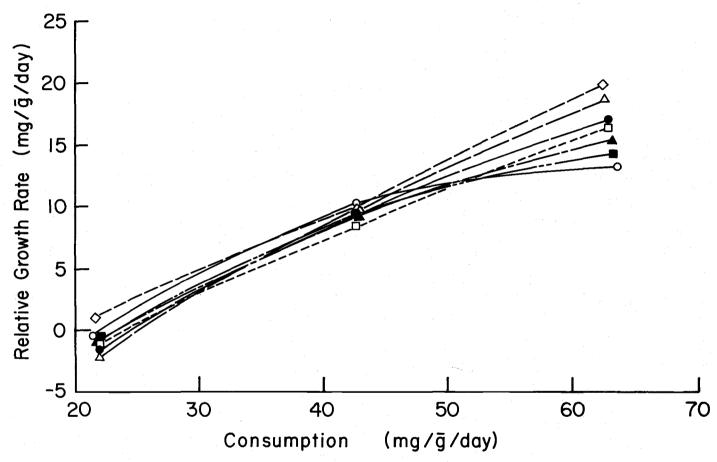


Figure 5. Relationships between growth rate and food consumption for coho salmon exposed to three concentrations of chlorinated SKME (5%,): 10%, : 20%, ...,), three concentrations of non-chlorinated SKME (5%, : 10%, : 20%, ...,), and a water control (: - :). Growth experiment conducted from September 5-11, 1975.

6.5 percent ration offered (Appendix V). The fish in the water control, 5 percent nonchlorinated SKME, and 10 and 20 percent chlorinated SKME did not consume all of the 9.3 percent ration offered. One fish died the first day of the test in one of the 20 percent chlorinated SKME aquaria offered the 9.3 percent ration. The average BOD of the 100 percent SKME was 12 mg/l before chlorination.

The relationships between the growth rates and the food consumption rates for the coho salmon in the second growth study are shown in Figure 6. The amount of total residual chlorine applied to the SKME before dilution was 0.441 mg/l (Table 2). The mean initial dry weight of the fish was 1.11 grams. There were 6 fish in each aquaria. The ration sizes were 2.4, 4.8, and 7.2 percent of the mean dry body weight of the fish. All of the food offered was consumed in each aquarium. The average BOD of the 100 percent SKME was 7 mg/l before chlorination.

The relationships between the growth rates and the food consumption rates for the coho salmon in the third growth study are shown in Figure 7. The amount of total residual chlorine applied to the SKME before dilution was 0.609 mg/l (Table 2). The mean initial dry weight of the fish was 0.28 grams. There were 8 fish in each aquaria. The ration sizes were 2.7, 5.5, and 8.2 percent



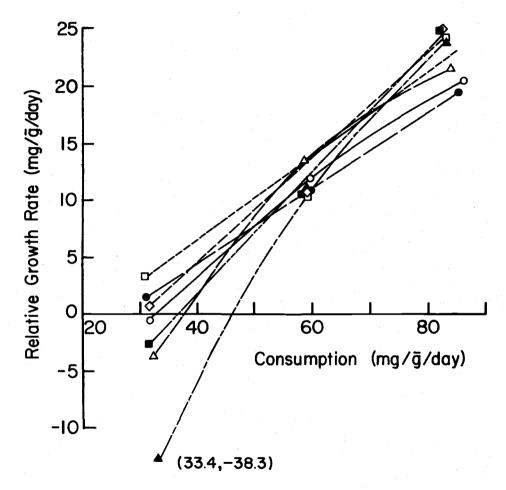


Figure 7. Relationships between growth rate and food consumption for coho salmon exposed to three concentrations of chlorinated SKME (5%, 0 ; 10%, 1 - - -; 20%, 1 - - 1), three concentrations of non-chlorinated SKME (5%, 0 - - ; 10%, 1 - - - ; 20%, 1 - - 1), and a water control (- - -). Growth experiment conducted from October 8-18, 1975.

of the mean dry body weight of the fish. All of the food offered was consumed in each aquarium. The average BOD of the 100 percent SKME was 12.5 mg/l before chlorination.

INTERPRETATIONS AND DISCUSSION

In the present study, the results of the acute toxicity tests on juvenile coho salmon with chlorinated SKME indicated that the addition of chlorine concentrations as low as 1 mg/l to 100 percent SKME could cause acute toxicity in effluent concentrations as low as 18 percent. Since the nonchlorinated SKME used in these tests was not acutely toxic to the salmon, it would appear that chlorine or some chlorinated material formed in the chlorinated SKME was responsible for the cases of acute toxicity.

The effects of chlorinated SKME on the growth of coho salmon were not clearly defined at the chlorine and SKME concentrations tested. In the first growth test, 20 percent chlorinated SKME appeared to increase the maintenance ration and reduce growth rates of the coho at all ration sizes as compared to the other test groups. There were no obvious effects of chlorinated SKME on growth in test two as compared to fish exposed to nonchlorinated SKME and the water controls. In the third growth test, 20 percent chlorinated SKME appeared to improve the growth rate at the low ration, as compared to the 20 percent nonchlorinated SKME concentration. The effects on the growth of coho salmon exposed to concentrations of less than 20 percent chlorinated SKME were not clearly demonstrated in the three growth experiments.

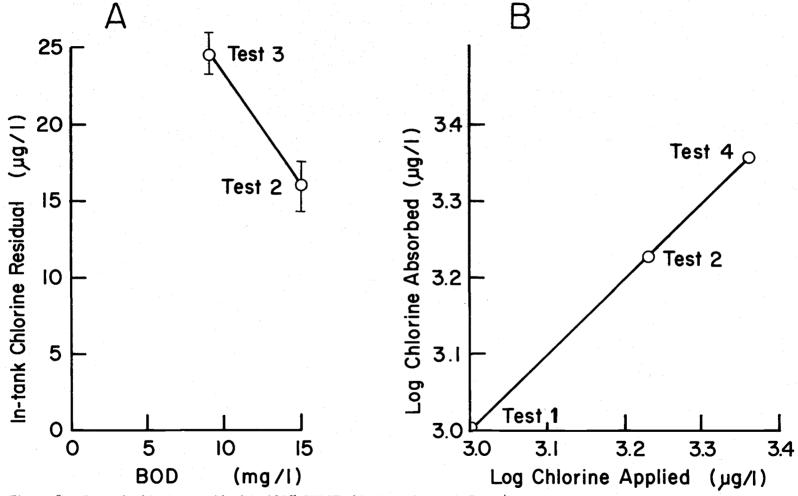
The toxicity of chlorinated pulp and paper mill effluents probably varies with a number of factors. The chemical and other characteristics of the effluent and the amount of treatment it receives before chlorination affects its toxicity. The amount of chlorine that can react with the effluent depends on the concentrations of the oxidizable and chlorine absorbing compounds present (Gilcreas et al, 1951). The chlorine demand of most pulp and paper mill effluents is high, approximately 20 to 60 mg/l. even after secondary treatment (Weston, 1973). BOD is an indicator of chlorine demand (Enslow, 1932). BOD levels indicate the amount of oxygen needed to oxidize biochemically the remaining organic substrate. Generally, the higher the BOD the greater the amount of degradable organics present. These organics also exert a chlorine demand; the more organics present, the higher the concentration of chlorine that can be tied-up in unmeasured organochlorine compounds and the smaller the concentration of measured residual chlorine, such as inorganic chloramines with the amperometric titration method. In acute toxicity tests 2 and 3, the SKME was chlorinated with the same amount of chlorine and the mean fish size was approximately the same. Test 3 had higher in-tank residual concentrations than test 2 (Appendicies II and III). The higher BOD of test 2 (40 percent higher than the BOD in test 3) indicates that more chlorine was in combined forms, probably with the organic compounds, than in

test 3 (Figure 8).

Temperature can affect the toxicity of chlorinated effluents. Increasing temperatures can lower the ability of organisms to withstand toxicant stress, and can decrease the TL₅₀ determined for short exposure periods (Warren, 1971). The effect of increasing temperature increasing the sensitivity of fish to chlorine toxicity was shown in the study by Thatcher et al. (1976). The 96-hr TL₅₀ of brook trout in chlorinated river water was approximately 0.153 mg/l in 7 to 15°C water and 0.105 mg/l in 20°C water. Eren and Langer (1973) found that Tilapia were more sensitive to chlorinated water at high temperatures than at low temperatures. The high SKME temperatures in the first two acute toxicity tests probably lowered the resistance of the coho to the toxicant.

The pH of the chlorinated SKME may affect its toxicity. Lamperti (1975) found an increase in the pH of chlorinated water from 7.5 to 8.1 decreased the 96-hr TL_{50} from about 71 μ g/l to about 61 μ g/l for juvenile coho. Watkins (1973) found that at high secondary paper mill effluent pH values, chlorine residuals persist longer than at low pH values. The relatively high pH of the SKME used in the present study may have increased the toxicity of chlorine and the chlorine residuals to the coho salmon.

It is not known if the amounts of chlorine used in the present study, that were found to be acutely toxic in some cases were



sufficient to reduce coliform numbers to acceptable Federal standards. Watkins (1973) found that chlorine concentrations in secondary paper mill effluent that reduced the numbers of coliform bacteria to acceptable levels were toxic to salmon. He found, however, that when the effluent was aged for two hours after chlorination, the acute toxicity was reduced. Adequate retention of a chlorinated pulp and paper mill effluent before discharge, as well as sufficient dilution in receiving waters, probably would reduce the acute toxicity of the effluent to fish. Whether aged and diluted chlorinated pulp and paper mill effluents would induce sublethal effects in the fish exposed for prolonged periods of time, as well as induce other effects on the productivity of aquatic systems, requires further study.

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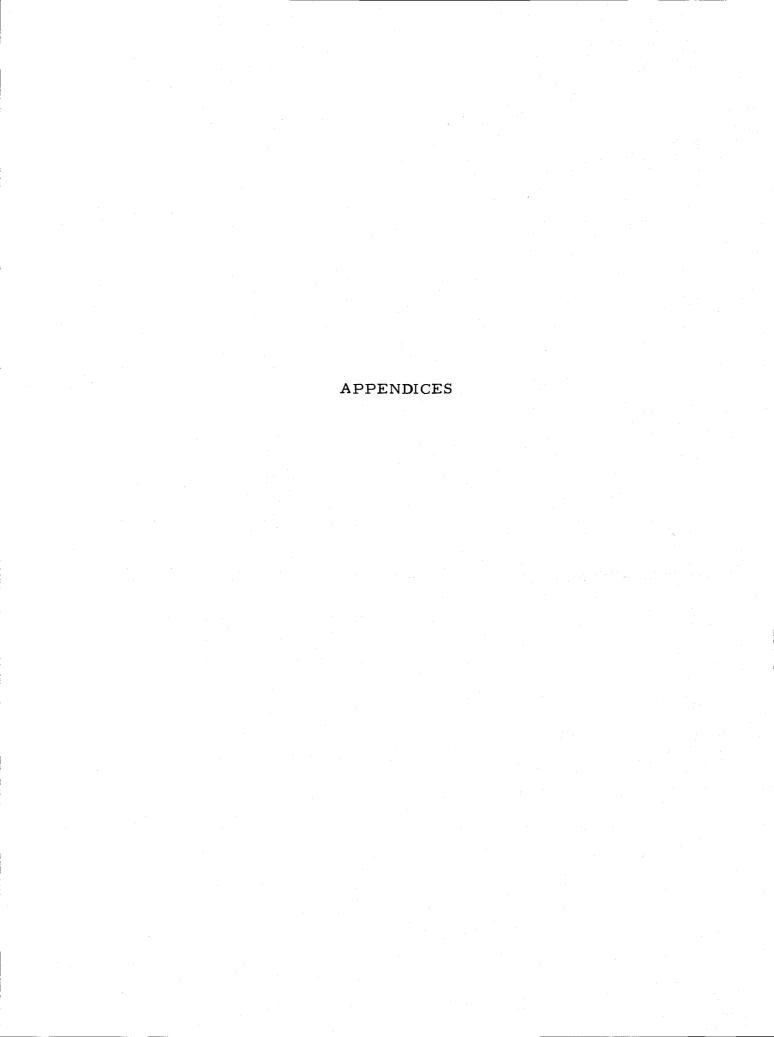
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Appendix I. Percent mortality and dry weight of coho salmon, in-tank chlorine residuals and water quality for each concentration of SKME and controls in the first acute toxicity test (July 17-21, 1975).

| SK. | rinated ME ntration %) | In-tank chlorine residual (µg/l) | Dry weight (g) | Mortality (%) | Mean temp. (°C) | Mean D.O. (mg/l) | Mean pH |
|---------------|---------------------------------|---|----------------------|---------------|-----------------------|------------------------|------------|
| 100 | A ¹ / | 5.3 | . 117 | 100 | 22 | 2/ | 8. 19 |
| | В | 0 | . 091 | 100 | 22 | 6.6 | 8.10 |
| 56 | A | 1.8 | . 122 | 100 | 20 | | 8.16 |
| | В | 0 | . 134 | 100 | 20.5 | 7.3 | 8.07 |
| 32 | A | 0 | . 119 | 100 | 21.5 | | 8.22 |
| | В | 0 | . 130 | 100 | 20 | 8.0 | 8.01 |
| 18 | Α | 0 | . 199 | 0 | 20 | | 8.21 |
| | В | 0 | . 171 | μΟ | 20 | 7.8 | 8.10 |
| 100% nonch | lorinated | | | | | | |
| | control | 0 | .201 | 0 | 22 | 6.1 | 8.05 |
| Water | control | 0 | .163 | 0 | 19 | 8.2 | 8.16 |

^{1/}A and B refer to the two replicate aquaria at each chlorinated SKME concentration.

^{2/--} not determined.

Appendix II. Percent mortality and dry weight of coho salmon, in-tank chlorine residuals and water quality for each concentration of SKME and controls in the second acute toxicity test (July 22-26, 1975).

| SKN | ntration | In-tank chlorine residual (µg/l) | Dry weight (g) | Mortality (%) | Mean temp. | Mean D.O. (mg/1) | Mean pH |
|-------|----------------------|---|----------------------|---------------|---------------|------------------------|------------|
| 100 | A ^{1/} | 17.7 | .604 | 100 | 21 | 8.1 | 8.03 |
| | В | 14.3 | . 881 | 30 | 23 | 8.9 | 8.02 |
| 56 | A | 5.1 | .741 | 10 | 21 | 8.5 | 8.17 |
| | В | 3.0 | .681 | . 0 | 21. | 8.9 | 7.88 |
| 32 | A | 1.1 | .750 | 0 | 20. | 8.5 | 8.06 |
| | В | . 3 | .740 | 0 | 20 | 8.9 | 7.98 |
| 18 | A | 0 | .671 | 0 | 19 | 8.7 | 7.71 |
| | В | 0 | .679 | 0 | 20 | 9.6 | 7.83 |
| 100% | 1 4 - 4 | | | | | | |
| | lorinated control | 0 | .840 | 0 | 24 | 8.3 | 8.00 |
| Water | control | 0 | .640 | 0 | 19 | 9.4 | 7.87 |

 $[\]frac{1}{2}$ A and B refer to the two replicate aquaria at each chlorinated SKME concentration.

Appendix III. Percent mortality and dry weight of coho salmon, in-tank chlorine residuals and water quality for each concentration of SKME and controls in the third acute toxicity test (July 30 - August 3, 1975).

| Chlorinated SKME concentration (%) | | In-tank chlorine residual (µg/l) | Dry weight (g) | Mortality (%) | Mean temp. | Mean D.O. (mg/l) | Mean pH |
|------------------------------------|----------------------|---|----------------------|------------------|---------------|------------------------|------------|
| 100 | A-1/ | 23.3 | .559 | 40 | 21 | 7.4 | 8.06 |
| | В | 26 | .553 | 0 | 19 | 7.3 | 8.04 |
| 56 | Α | 5.4 | .618 | 10 | 19 | 7.7 | 8.02 |
| | В | 7.5 | . 413 | 90 | 18 | 7.5 | 8.02 |
| 32 | Α | 2.0 | .660 | 0 | 18 | 8.1 | 7.97 |
| | В | 2.5 | .671 | 0 | 18 | 7. 8 | 7.98 |
| 18 | Α | 0 | .715 | 0 | 17 . | 8.1 | 7.86 |
| | В | 0 | .605 | 0 | 17. | 7.7 | 7.84 |
| 100% | 1 | | | | | | |
| | lorinated control | 0 | .632 | 0 | 21, | 7.4 | 8.04 |
| Water | control | 0 | .611 | 0 | 17 | 8.3 | 7.77 |

 $[\]frac{1}{2}$ A and B refer to the two replicate aquaria at each chlorinated SKME concentration.

Appendix IV. Percent mortality and dry weight of coho salmon, in-tank chlorine residuals and water quality for each concentration of SKME and controls in the fourth acute toxicity test (August 6-10, 1975).

| SKN concen | inated ME atration %) | In-tank chlorine residual (µg/l) | Dry weight (g) | Mortality (%) | Mean temp. (°C) | Mean D.O. (mg/l) | Mean pH |
|---------------|--------------------------------|---|----------------------|------------------|-----------------------|------------------------|------------|
| 100 | A-1/ | 18,7 | , 108 | 100 | 17.6 | 8.7 | 8.05 |
| | В | 19.9 | .107 | 100 | 17.7 | 8.1 | 8.03 |
| 75 | A | 11.0 | . 110 | 0 | 17.3 | 8.7 | 7.96 |
| | В | 12.1 | . 112 | 100 | 17.7 | 8.4 | 8.04 |
| 56 | Α | 4.6 | . 109 | 40 | 17.1 | 8.7 | 8.07 |
| | В | 3.8 | . 109 | 0 | 17.7 | 8.2 | 8.00 |
| 32 | A | 1.8 | , 119 | 0 | 17.2 | 8.9 | 7.99 |
| | В | 2.3 | . 112 | 0 | 17.6 | 8.6 | 8.11 |
| 100% | | | | | | | |
| | lorinated control | 0 | . 113 | 0 | 17.8 | 8.5 | 8.05 |
| Water | control | 0 | . 112 | 0 | 17.7 | 8.7 | 7.89 |

A and B refer to the two replicate aquaria at each chlorinated SKME concentration.

Appendix V. Fish weights, growth rates, food consumption rates and efficiency of food utilization for growth for coho salmon in chlorinated and non-chlorinated SKME and water controls in the first growth experiment (September 5-11, 1975).

| | <u>1/</u> | <u>2</u> / | | Relative | Food | Food | |
|---------------|----------------|------------|-------------|------------------|------------|-------------|------------|
| SKME | Chlorine | Initial | Final | growth | ration | consumption | <u>3</u> |
| concentration | added | dry wt. | dry wt. | rate | offered | rate | Efficiency |
| (%) | | (g) | (g) | (mg/g/day) | (mg/g/day) | (mg/g/day) | (%) |
| | | | Lo | w Ration (2.8%) | | | |
| 0 (control) | - | 13.53 | 13.82 | 3.53 | 27.53 | 27.53 | 12.82 |
| 5 | - | 13.36 | 13.71 | 4.00 | 27.50 | 27.50 | 14.50 |
| 5 | + | 13.59 | 13.55 | -0.49 | 26.65 | 26.65 | - 1.84 |
| 10 | - | 13.52 | 13.64 | 1.47 | 27.74 | 27.74 | 5.30 |
| 10 | + | 14.35 | 14.29 | -6.90 | 27.38 | 27.38 | -19.9 |
| 20 | - | 14.09 | 14.15 | 0.71 | 25.61 | 25.61 | 2.77 |
| 20 | + | 13.22 | 12.47 | -9.73 | 28.53 | 28.53 | -34.10 |
| | | | Medi | um Ration (6.5%) | | | |
| 0 (control) | · - | 13.94 | 15.43 | 16.91 | 61.49 | 61.49 | 27.50 |
| 5 | - | 13.91 | 15.32 | 16.08 | 61.44 | 61.44 | 26.17 |
| 5 | + | 13.02 | 14.38 | 16.54 | 61.68 | 61.68 | 26.82 |
| 10 | - | 14.09 | 15.19 | 12.52 | 62.45 | 61.03 | 20.51 |
| 10 | + | 13.04 | 13.37 | 4.17 | 63.91 | 54.38 | 7.67 |
| 20 | - | 13.38 | 14.44 | 12.70 | 61.12 | 61.12 | 20.78 |
| 20 | + | 13.39 | 14.54 | 13.72 | 62.15 | 62.15 | 22.08 |
| | | | <u>Hi</u> g | gh Ration (9.3%) | | | |
| 0 (control) | - - | 13.13 | 14.72 | 19.16 | 87.36 | 81.30 | 23.67 |
| 5 | - | 12.96 | 14.93 | 23.43 | 86.07 | 84.95 | 27.58 |
| 5 | + | 13.38 | 15.26 | 21.87 | 89.50 | 89.50 | 24.44 |
| 10 | - | 13.68 | 15.32 | 18.85 | 87.36 | 87.36 | 21.57 |
| 10 | + | 13.67 | 15.31 | 19.06 | 95.86 | 84.26 | 22.60 |

Appendix V. Continued

| SKME concentration (%) | 1/ Chlorine added | <u>2</u> / Initial dry wt. (g) | Final dry wt. (g) | Relative growth rate (mg/g/day) | Food ration offered (mg/g/day) | Food consumption rate (mg/g/day) | <u>3</u> / Efficiency (%) |
|------------------------------|-------------------------|---|-------------------------|--|---|---|---------------------------------|
| 20 | - | 14.43 | 15.92 | 16.36 | 83.56 | 83.56 | 19.58 |
| 20 | + | $11.16\frac{4}{}$ | 12.05 | 12.78 | 89.66 | 77.38 | 16.52 |

 $[\]frac{1}{2}$ Chlorinated SKME indicated by +.

 $[\]underline{2}'$ Initial and final dry weights for each group of 5 fish.

 $[\]frac{3}{2}$ (grow + comsumption) x 100

 $[\]frac{4}{4}$ 4 fish (1 died the first day of the test).

Appendix VI. Fish weights, growth rates, food consumption rates and efficiency of food utilization for growth for coho salmon in chlorinated and non-chlorinated SKME and water controls in the second growth experiment (September 19-29, 1975).

| SKME concentration (%) | 1/ Chlorine added | 2/ Initial dry wt. (g) | Final dry wt. (g) | Relative growth rate (mg/g/day) | Food consumption rate (mg/g/day) | 3/ Efficiency (%) |
|------------------------------|-------------------------|---------------------------------|-------------------------|--|---|-------------------------|
| | | Lov | w Ration (| 2.4%) | | |
| 0 (control) | _ | 6.79 | 6.86 | 1.03 | 23.72 | 4.34 |
| 5 | - , | 6.37 | 6.27 | -1.58 | 24.05 | -6.57 |
| 5 | + | 7.12 | 7.09 | -0.42 | 23.38 | -1.80 |
| 10 | - | 5.97 | 5.92 | -0.67 | 24.38 | -2.75 |
| 10 | + | 6.60 | 6.53 | -1.07 | 24.05 | -4.45 |
| 20 | - | 6.46 | 6.41 | -0.93 | 23.91 | -3.89 |
| 20 | + | 6.52 | 6.38 | -2.17 | 24.19 | -8.97 |
| | | Med | ium Ratio | <u>n (4.8%</u>) | | |
| 0 (control) | _ | 6.65 | 7.37 | 10.27 | 45.65 | 22.5 |
| 5 | · - | 6.26 | 6.91 | 9.71 | 45.68 | 21.26 |
| . 5 | + | 7.10 | 7.88 | 10.41 | 45.53 | 22.68 |
| 10 | - | 6.52 | 7.16 | 9.36 | 45.61 | 20.52 |
| 10 | + | 6.84 | 7.45 | 8.54 | 45.73 | 18.67 |
| 20 | - | 6.75 | 7.41 | 9.32 | 45.76 | 20.37 |
| 20 | + | 6.71 | 7.36 | 9.09 | 45.74 | 19.87 |
| | | <u>Hig</u> | h Ration (| 7.2%) | | ÷ |
| 0 (control) | _ | 6.63 | 8.09 | 19.84 | 64.67 | 30.69 |
| 5 | - | 6.46 | 7.67 | 17.13 | 65.63 | 26.1 |
| 5 | + | 6.60 | 7.55 | 13.43 | 66.95 | 20.06 |
| 10 | - | 7.06 | 8.14 | 14.33 | 66.62 | 21.51 |
| 10 | + | 6.71 | 7.92 | 16.54 | 65.85 | 25.12 |
| 20 | _ | 6.56 | 7.66 | 15.47 | 66.39 | 23.30 |
| 20 | + | 7.21 | 8.72 | 18.93 | 64.91 | 29.16 |

 $[\]frac{1}{}$ Chlorinated SKME indicated by +.

Initial and final dry weights for each group of 6 fish.

 $[\]frac{3}{}$ (growth + consumption) x 100.

Appendix VII. Fish weights, growth rates, food consumption rates and efficiency of food utilization for growth for coho salmon in chlorinated and non-chlorinated SKME and water controls in the third growth experiment (October 8-18, 1975).

| | <u>1</u> / | <u>2</u> / | | Relative | Food | |
|---------------|--------------|------------|--------------|------------|-------------|------------|
| SKME | Chlorine | Initial | Final | growth | consumption | <u>3</u> / |
| concentration | added | dry wt. | dry wt. | rate | rate | Efficiency |
| (%) | | (g) | (g) | (mg/g/day) | (mg/g/day) | (%) |
| | | L | ow Ration (| 2.7%) | | |
| 0 (control) | - | 2.5 | 2.52 | .80 | 31.47 | 2.54 |
| 5 | _ | 2.65 | 2.69 | 1.50 | 31.05 | 4.83 |
| 5 | + | 2.38 | 2.37 | - 0.42 | 31.62 | - 1.33 |
| 10 | - | 2.34 | 2.28 | - 2.60 | 31.69 | - 8.20 |
| 10 | + | 2.42 | 2.5 | 3,25 | 30.53 | 10.65 |
| 20 | - | 2.58 | 2.27 | -12.78 | 33.36 | -38.3 |
| 20 | + | 2.31 | 2.22 | - 3.75 | 32.28 | -11.16 |
| | | Mi | ddle Ration | (5.5%) | | |
| 0 (control) | - | 2.43 | 2.71 | 10.89 | 59.26 | 18.38 |
| 5 | - | 2.42 | 2.70 | 10.90 | 59.40 | 18,35 |
| 5 | + | 2.28 | 2.57 | 11.96 | 59.38 | 20.14 |
| 10 | - | 2.31 | 2.57 | 10.66 | 58.63 | 18.18 |
| 10 | + | 2.28 | 2.53 | 10.39 | 59.0 | 17.61 |
| 20 | - | 2.47 | 2.76 | 11.09 | 58.89 | 18.83 |
| 20 | + . | 2.42 | 2.77 | 13.49 | 58.57 | 23.03 |
| | | <u>H</u> | igh Ration (| 8.2%) | | |
| 0 (control) | - | 2, 35 | 3.02 | 24.95 | 82.68 | 30.18 |
| 5 | - | 2.23 | 2.71 | 19.43 | 84.99 | 22.86 |
| 5 | + | 2.52 | 3.09 | 20.32 | 85.92 | 23.65 |
| 10 | - | 2.18 | 2.80 | 24.90 | 81.93 | 30.39 |
| 10 | + | 2.36 | 3.01 | 24.21 | 83.05 | 29.15 |
| 20 | - | 2.47 | 3.14 | 23.89 | 83.07 | 28.76 |
| 20 | + | 2.23 | 2.77 | 21.60 | 84.0 | 25.71 |

 $[\]underline{1}/$ Chlorinated SKME indicated by +.

Initial and final dry weights for each group of 8 fish.

 $[\]frac{3}{}$ (growth --- consumption) x 100.