

THE EFFECTS OF KRAFT MILL WASTE
EFFLUENTS ON KING AND SILVER
SALMON

by

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THE EFFECTS OF KRAFT MILL WASTE
EFFLUENTS ON KING AND SILVER SALMON

INTRODUCTION

The following is an account of the studies made on the effects of Kraft mill waste effluents on king salmon, Oncorhynchus tshawytscha (Walbaum)(Figure 2) and silver salmon, Oncorhynchus kisutch (Walbaum) (Figure 1) at Oregon State College in 1948-49. The importance of such an investigation as this becomes apparent upon consideration of the tremendous economic and recreational significance of Pacific salmon in this area. Due to the fact that salmon are anadromous, the presence of industrial wastes in rivers can constitute a barrier to either the adult fish on their spawning runs from the ocean, or to the young which are drifting back to sea. The immediate purpose of these experiments was to determine the critical and minimum lethal concentrations of the chemical substances involved on salmon. By critical concentration is meant the maximum concentration of a toxic substance that can be present in the water without injuring or killing any of the test organisms. The minimum lethal concentration is defined as the minimum concentration of a toxic substance in water that will destroy all of the test animals. In both cases, the concentrations are expressed as parts per million (p.p.m.). Throughout the investigations only the chemically pure forms of toxic substances known to occur in Kraft mill wastes were employed. Current work is being

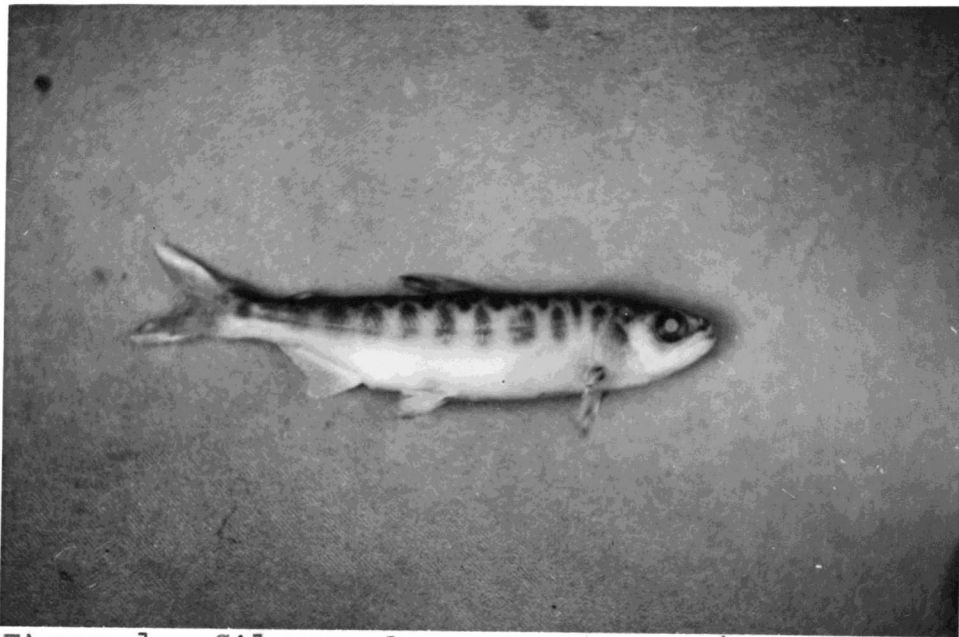


Figure 1. Silver salmon, O. kisutch (Walbaum)



Figure 2. King salmon, O. tshawytscha (Walbaum)

performed on king salmon using the waste products of the paper mills in the form in which it is discharged into streams. 3

The test animals, king and silver salmon were obtained in the wild form from pot-holes and streams. Pot-holes are depressions along river systems which become filled with water during flood stage (Figure 3). As flood waters subside, the pot-holes are disconnected from the river, thereby entrapping any remaining fish. Several attempts were made to employ hatchery fish as test specimens but were found to be unsatisfactory.

The experiments were performed at a laboratory located on the South Farm of Oregon State College (Figures 4 & 5), during the summer, fall and early winter of 1948-49. Procedures for conducting the toxicity tests were adapted from suggestions made by Dr. Willis Van Horn, Research Associate of the Institute of Paper Chemistry, and the methods as outlined by (Hart, Doudoroff and Greenbank, 1945) using warm-water game fishes. No effort was made to evaluate toxicity with respect to concentration and time, (Gersdorff, 1935).

The graphic illustrations that appear in this thesis are not intended to prove any definite point in the studies but rather to present graphically the relative effects of some of the toxic substances tested on king and silver salmon.



Figure 3. A pot-hole along the Willamette river. Three-hundred fingerling king salmon were taken from this source, which became completely dry a few days later.

Since the problem of pollution involves principally the wild forms of fish and to a much lesser degree, hatchery reared fish, the investigations to determine the effects of Kraft mill wastes on king and silver salmon were concerned mainly with the wild forms. In the course of events, it became expedient to resort to hatchery fish on several occasions. During the month of August, 1948, the supply of wild king salmon came to a temporary end. A local State hatchery provided a hundred small sized king salmon for continuing the work during the interim. To insure safe keeping, a sufficient amount of hatchery water was transported to the laboratory in which the fish were to be held. Overnight 50 percent of the fish died, and within 72 hours after leaving the hatchery only three or four fish were still alive. The hatchery reared fish appeared to be considerably smaller than the wild forms previously used. From a study of the scales of both types of salmon it was definitely established that both fish were of the same age class. Yet the hatchery reared fish measured an average of 7 centimeters in total length and 8 grams in weight, compared to 11.5 centimeters in total length and 13 grams in weight for the wild forms. The hatchery fish appeared extremely weak in their movements, and in proportion to the rest of the body their heads seemed large.

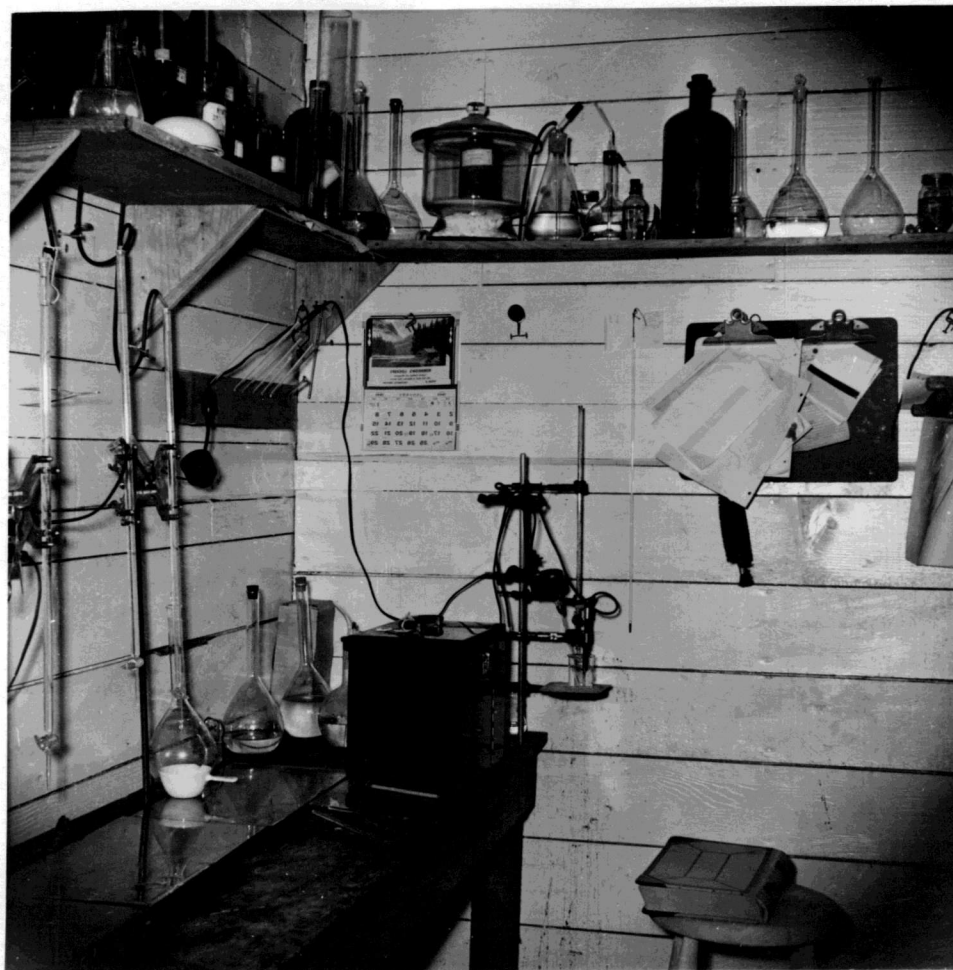


Figure 4. Section of the laboratory in which chemical solutions were prepared and standardized.

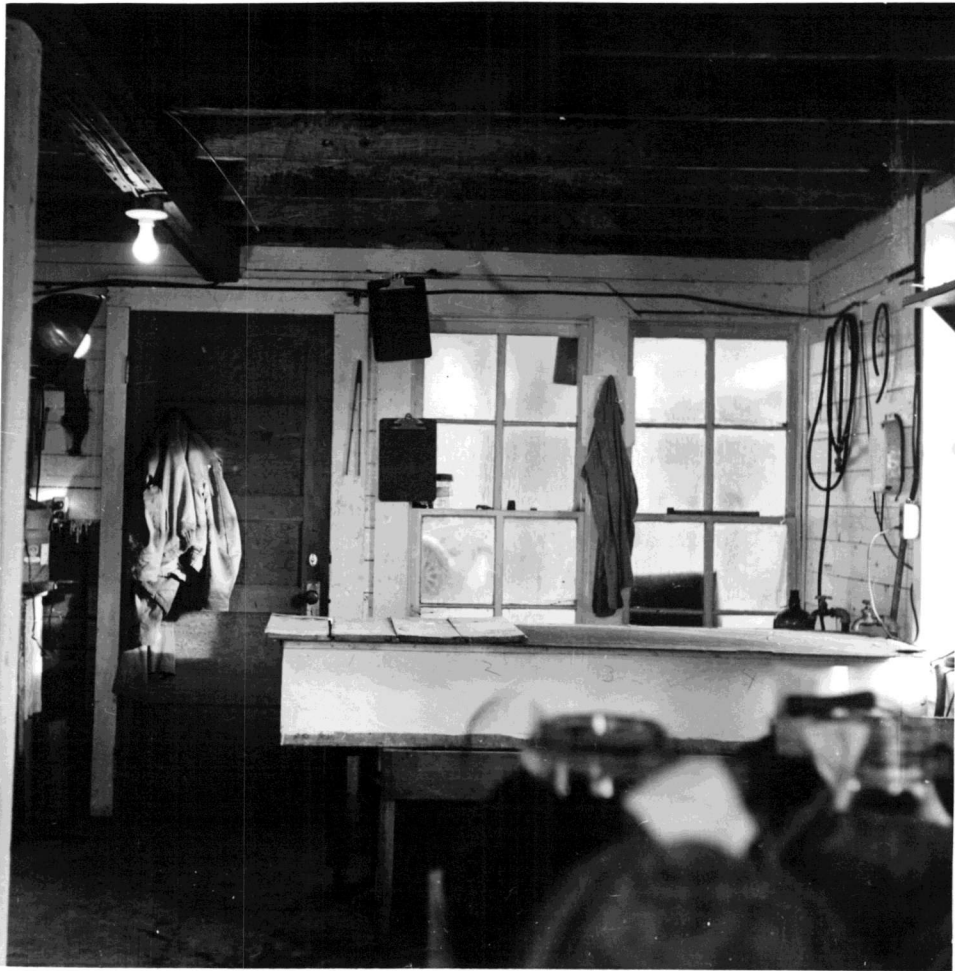


Figure 5. A view of the laboratory showing the type of tank used for water baths. Note the 19 liter glass jugs immersed in a water bath in the right foreground.

Considerable difficulty in collecting and holding of test fish was encountered during the initial period of the project. Repeated efforts invariably resulted in a very high degree of mortality. The problem presented a three-fold aspect; proper collecting technique, transportation and holding at the laboratory. By careful analysis and continued improvements of methods employed, satisfactory results were eventually realized.

The collecting equipment included one-quarter inch mesh seines of varying length. The seine most frequently used was 50 feet long and 5 feet wide. Glass jugs of 19 liter capacity with metal caps excellently served the purpose of carrying fish from the point of capture to the laboratory. Jugs of this size could be handled quite easily, and were often carried as far as a half-mile to the truck (Figure 7). The number of fish placed in each jug varied with the size of the fish, the temperature of the water and the distance to be transported. King salmon for example, were obtained from within a 15 mile radius of the laboratory, hence the time factor in transporting was negligible, and 15 fish averaging 12 centimeters in total length were held in each jug very successfully even though the temperature of the water reached 19° C. at times. All of the glass jugs used both in collecting and in testing were covered with burlap sacks



Figure 6. King salmon of the same year class taken from two different sources, showing variation in size.

(Figure 7). This practice was followed as a precautionary¹⁰ measure to prevent any unnecessary excitement of the fish during transport from its native habitat to the laboratory.

Before actual seining operations began, a suitable landing site would be chosen. To be a suitable landing site, the bank should slope gradually and evenly, and should have a fairly smooth bottom in order to prevent the bottom of the seine from tipping up and permitting some of the fish to escape (Figure 8). Having selected the landing site, the next step was to fill the jugs with cool water reasonably free of algae or other suspended materials. It is believed that considerable injury to the gill membranes of the fish were incurred due to the presence of any excessive amount of algae in the water while transporting to the laboratory. The specific seining technique would, of course, depend on the species or type of fish desired and the nature of the water in which they were found (Figure 9). King salmon, for example, found in pot-holes along the Willamette river were present in large numbers in a fairly concentrated area and could be readily obtained by wading out to some point where the water was cool and then seining towards the shore. Upon reaching the landing site, the seine would be brought out of the water at either end, leaving only a center pocket in the water. The fish would be concentrated here and were quickly transferred to the jugs by means of a small dip net. Prompt and efficient handling of the test specimens



Figure 7. Test fish were carried in these 19 liter cylindrical glass jugs from the point of capture to the laboratory.

plus a rapid journey to the laboratory would insure a high degree of fish survival.

Silver salmon were all obtained from Horn Creek, a tributary of the Nestucca river approximately 75 miles from the laboratory. Although the silver salmon tended to be dispersed along the creek, they were found to occur in greater numbers in the larger pools. Whenever possible, a pool would be seined against the current towards the landing site. In situations where suitable landing sites were not available, various landing techniques were improvised to best meet the existing conditions. Although the success ratio was not as high as for king salmon, 50 silver salmon were averaged per hour of seining effort. The practice was to work up stream from the truck, and placing 15 fish averaging 11.5 centimeters in total length in each jug. As each jug was filled with its compliment of fish it was left behind, immersed in shallow water in order to keep the water temperature down in the container. When a designated quota of fish was obtained, the jugs were quickly carried back to the truck, where a final exchange of water was made to replenish the dissolved oxygen supply. Although the distance to the laboratory was 75 miles, no further changes in water were made enroute. This was possible because the work with silver salmon took place in the fall of the year when temperatures were considerably cooler.



Figure 8. A gradually sloped bank that provides an excellent landing-site for quick and efficient transfer of captured fish to a transporting jug.

Wild king salmon were obtained during the period of June to September from the many pot-holes found along the Willamette river. Hahn's Pond, situated about eight miles south of Corvallis, Oregon, was the closest source and yielded approximately a thousand fish for use at the laboratory before it eventually dried up. The most distant point from which king salmon were taken was at Irish Bend, about fifteen miles south of Corvallis. The number of young migrant king salmon lost annually in pot-holes during flood stages of the Willamette river must reach tremendous figures. It was noted that salmon obtained from different pot-holes, differed in size, color and susceptibility to some of the toxic substances tested throughout the experiments (Figure 6). In a few pot-holes, the fish were somewhat stunted in size due presumably to overpopulation and food scarcity, in other ponds, salmon would be found in fewer numbers but unusually larger in size. The physico-chemical environment in which fish live might exert a profound influence on the ability of fish to resist certain toxic substances to which they were later subjected.

All of the silver salmon used in the toxicity tests were from Horn Creek, a tributary of the Nestucca River, approximately 75 miles north of Corvallis. The young silver salmon were found most abundantly in pools about one mile up the creek from its mouth.



Figure 9. Seining the quiet eddies and pools along a stream would insure a greater success ratio in the capture of silver salmon.

At the laboratory the fish were quickly transferred¹⁶ to a large coffee urn of approximately 250 gallon capacity (Figure 10). These coffee urns were constructed of a stainless variety of steel, a comparatively unreactive metal. They were purchased as government surplus property, and as holding tanks for fish proved to be very satisfactory. As many as 150 fish averaging 12 centimeters in total length were held successfully in each urn for indefinite periods of time provided that the water was aerated occasionally

The fish at the laboratory were kept in ordinary city water, which by chemical analysis was found to be very similar to the native waters from which both the king and silver salmon were taken. The chemical analysis referred to above was a cursory one, including the hydrogen-ion concentration (pH), dissolved oxygen, acidity and alkalinity. The ultimate criterion for the suitability of the city water for holding test animals at the laboratory was the ability of the fish to survive indefinitely in good shape. Fish were fed once daily during the holding period, and food consisted of freshly ground crayfish or beef liver. Aquatic insects were also fed whenever available. Care was taken to see that the fish were adequately fed, for failure to do so would invariably result in fish nipping each others fins, in some instances causing serious injury. The silver salmon were especially pugnacious and would frequently harass one another. It may be as a



Figure 10. Holding tanks. King and silver salmon were held in these coffee urns prior to testing.

point of interest that although both species of salmon ¹⁸ were of wild origin, they readily became tame and would eagerly await their food at regular feeding periods, even nipping the fingers of the person providing them with the food.

During the summer months the residual chlorine content of the city water was practically nil, and the water in the holding tanks could safely be changed once daily. Later in the fall and early winter the chlorine content in the water increased, necessitating continued aeration of the water until such a time that fish could be safely introduced, hence the water was not changed as often.

Both king and silver salmon exhibited a great deal of tolerance to their own metabolic wastes. Two king salmon 12 centimeters long were kept 45 days in 15 liters of tap water in a 19 liter glass jug, before they finally succumbed. Throughout the entire period the water remained unchanged, no aeration other than atmospheric exchange took place, and the fish were not fed. Starvation undoubtedly caused the death of the fish. Former students held silver salmon under somewhat similar conditions for approximately 35 days. The only other factor connected with the holding of salmon that required constant care was the temperature of the water in the holding tanks during the summer months. When the temperature reached 20° C. the king salmon would begin to show distress; a determination of dissolved oxygen indicated a paucity of this substance, being as low

as 3.8 p.p.m. on several occasions. It is thought that due to the crowded conditions in the holding tanks the temperature of the water was a limiting factor as far as the dissolved oxygen supply was concerned. During the colder part of the winter the temperature of the water would drop to 3° C. without any apparent ill effects on the fish.

WATER SUPPLY

The laboratory where the bioassays with salmon were performed had as its source of supply, Corvallis City water. A number of analyses made, indicated the suitability of this water for testing purposes. The residual chlorine content of the water as determined by the ortho-toluidene colorimetric method was found to be less than 0.1 p.p.m. The alkalinity of the water expressed as methyl orange alkalinity in parts per million calcium carbonate ranged from 30 to 65. Total acidity as parts per million calcium carbonate using phenolphthalein indicator was less than 1. The carbon dioxide tension of the water was satisfactory for supporting fish fauna as determined by differences in the pH values of aerated and non-aerated samples according to (Hart, Doudoroff and Greenbank, 1945). The dissolved oxygen content of the water varied with temperature and season, ranging from 9 p.p.m. in summer months to 12 p.p.m. in the winter.

The acidity, alkalinity and dissolved oxygen were determined as indicated by the American Public Health Association and the American Water Works Association (1946). The pH of the city water was found to be approximately 7.5 during the summer and early fall and 7.0 in the late fall and early winter. The presence of nitrites, sulfites and other substances present in water were evaluated only to the extent that they influenced the dissolved oxygen

calculations.

The ultimate criterion for the suitability of the water for test purposes was the ability of the salmon to survive in excellent condition for indefinite periods of time.

Test Solutions

All of the test solutions were prepared from pure grade chemicals and distilled water, and were made up as normal solutions or fractions thereof. Since only an ordinary torsion balance was used in weighing out chemical substances, the prepared solutions were all standardized with suitable reagents and indicators. The normality of any chemical solution could readily be determined by the following formula:

$$\frac{\text{Volume 1}}{\text{Volume 2}} = \frac{\text{Normality 2}}{\text{Normality 1}}$$

The calculations of the concentrations (as p.p.m.) to be used in any given test were based upon the normality of a given substance, since a part per million is defined as one milligram of solute per liter of solution. Thus a 0.2 N solution of sodium hydroxide would contain 8 grams or 8000 milligrams per liter of solution, or 8000 p.p.m. The formula for diluting a solution of a given normality to a given concentration expressed as p.p.m. would be as follows:

$$\frac{\text{p.p.m. of stock solution}}{\text{p.p.m. desired}} = \frac{\text{c.c. of test solution desired}}{\text{c.c. of stock solution}}$$

Standardization of basic substances such as sodium hydroxide and sodium carbonate were made by titrating against a 0.2 N solution of sulfuric acid using methyl orange as an indicator. Sodium sulfate was not standardized but was accurately weighed and prepared. Sodium thiosulfate was standardized against potassium bichromate as described by (Willard and Furman, 1940). Sodium sulfide, sodium sulfhydrate, hydrogen sulfide and methyl mercaptan were standardized against 0.1 N silver nitrate potentiometrically in a basic media using blue point and silver-silver sulfide electrodes, (Van Horn et al, 1946).

Chemical Determinations Made on Test Solutions

Determinations of the solutions employed in biological assays included; the dissolved oxygen content, pH values, alkalinity and temperature. The gaseous oxygen content of water was calculated according to the Winkler method as outlined by the American Public Health Association and the American Water Works Association (1946). Tests were made to evaluate the presence of nitrites and sulfites in the water to the extent of interfering with the dissolved oxygen readings as directed by (Ellis et al, 1946), and were found to be no import. The hydrogen-ion concentrations (pH) were obtained by the use of a LaMotte Colorimeter. The total acidity of the solutions were generally so small as to be of no great significance and hence were disregarded. The methyl orange alkalinity was

expressed as calcium carbonate (p.p m.) and was determined as indicated before.

LABORATORY TECHNIQUES

Water Baths and Aquaria

Several metal tanks of various sizes served as water baths for maintaining a constant temperature in the aquaria. The depth of water in these tanks was adjusted to the depth of water within the aquaria. Regulation of the temperature in the summer months was accomplished by keeping a constant flow of tap water through the tanks. As indicated by a maximum, minimum thermometer a temperature variance of $\pm 2^{\circ} \text{C.}$, existed over a 24 hour period. During colder weather the problem of maintaining constant temperature became more difficult. Lacking adequate heat regulating devices, an attempt was made to control the water bath at a warmer temperature than that existing atmospherically by means of several heating elements of varying wattage. The best control for constancy of temperature that could be obtained under these circumstances was a $\pm 3^{\circ} \text{C.}$, variance over a 24 hour period.

During the early stages of the tests, rectangular aquaria were used of the 20 liter type. However, these aquaria were proved unsatisfactory due to their fundamental construction. Pressure from the water in the tanks would off-set the pressure normally exerted by the water within the aquaria, and the glass sections of the aquaria would become loose enabling chemicals to diffuse outward into the water baths.

Cylindrical glass jugs of 19 liter capacity were found to be far more satisfactory (Figure 7). The number of fish capable of being held in a jug of this size varied, of course, with the size of the fish. This point will be discussed in a later section. The mouths of the jugs were sufficiently wide to permit their washing out and cleaning after each test. Some other advantages of the cylindrical jugs are to be found in their cheaper cost, greater ease in handling and less liability of injury to the fish. Certain of the toxic substances employed in the studies possessed such irritating qualities as to cause the fish to dart and dash wildly. In rectangular aquaria the fish would dash against the sides producing injury which often proved fatal. In the cylindrical jugs the fish would merely swim in circles. Often the test fish would survive the irritating effects of the toxic substances to which they were subjected provided that they were prevented from injuring themselves during the interim. As previously mentioned all of the jugs were covered with burlap sacks.

Temperature

Temperature has a pronounced effect, not only on chemical reactions but also on the metabolism of fish. In order to remove as many of the variables as possible, an attempt was made to keep the temperature at which the toxicity tests were run constant. The assays with king salmon during the summer months were conducted at 17.5° C.

$\pm 2^{\circ} \text{C.}$, while with silver salmon during the fall and early winter the temperature average was $15^{\circ} \text{C.} \pm 3^{\circ} \text{C.}$ That temperature influences the rate at which toxic substances affect fish seems quite definite, but just what effect, if any, temperature has on the ultimate lethal concentrations is as yet not too definite. For example, at 15°C. , a concentration of 3.0 p.p.m. sodium sulfide proved 100 percent lethal to all silver salmon tested within 24 hours, but at 6°C. , in the same concentration of sodium sulfide 50 percent mortality would occur within 48 hours and 80 percent mortality after two weeks. This last result would indicate that lower temperatures not only effect the rates at which toxic substances kill fish, but that the ultimate lethal concentration may be lowered. The effects of temperature per se, on fish in the absence of pollutants is significant in itself. It is known that sudden changes in temperature can seriously injure fish. In one incident a coastal cutthroat trout, Salmo Clarkii clarkii (Richardson), apparently in excellent condition was removed from water at a temperature of 1.5°C. , and accidentally dropped into a water bath which was 17°C. Within five minutes the fish exhibited great distress and was quickly replaced to the water from which it was taken where it succumbed a few hours later.

Since tap water was used both in the holding tanks and in the test jugs, little trouble was encountered in transferring fish from the one source to the other. Moving the fish however from the spacious areas of a holding tank to the more confined spaces of a 19 liter glass jug caused them to become somewhat excited and sufficient time was permitted them to become acclimated before innoculating the test water with toxic chemicals. Usually several hours sufficed for this purpose. During the warmer months the temperature of the water in the holding tanks coincided closely to the temperature of the water baths in which the aquaria were held. However, during the colder periods it was necessary to acclimate the fish by gradually increasing the temperature. This was accomplished by mixing the bath water with colder water thus lowering the temperature of the bath. The fish were then placed in jugs having water the same temperature as the water in the holding tanks. The test jugs and the bath water were then gradually heated to the desired temperature. The entire process took place over a period of 24 hours during which time the temperature increase ranged from 9 to 12° C.

Several trials were made to determine the effects of introducing toxic chemicals at different times into the test jugs, but since no significant difference was found, chemicals were usually introduced several hours after the

tests were set up. Fish selected for test purposes were ²⁵ not fed 24 hours prior to testing.

Selection of Fish for Test Purposes

Fish were selected for uniformity of size, age and place of origin or source, whenever possible. The size limits of king salmon used throughout the tests ranged from 9 to 12 centimeters in total length and 9 to 14 grams in weight. The silver salmon averaged slightly smaller than the king salmon ranging in total length from 7.5 to 11 centimeters and 7 to 12.5 grams in weight. A number of experiments were made to ascertain the reactions of king salmon differing in size to given concentrations of pollutants. Results indicated that a size differential with a species of the same age class does not exist within limits. The smallest fish employed in the above tests measured 8 centimeters, in length while the largest reached a length of 15 centimeters. However, current work with coastal cut-throat trout suggests a definite size differential to toxic materials. This later size difference may be due to difference in age classes. Age factor in king and silver salmon may exert considerable influence on the effects which some of the pollutants have on these fish. Due to part of their life being spent in the ocean they must possess some sort of salt compensating mechanism which would enable them to survive in high salt concentrations. According to (Keys, 1932), salmon, Salmo salar, possess cells

of a secretory type in the gills which he has termed as ²⁹
"chloride secreting cells". On the basis of one specimen
he found these chloride secreting cells to be non-function-
al in the young of salmon. However, the findings in this
series of experiments would seem to indicate that the salt
regulating devices do develop in the young of salmon and
at varying rates within an age class. Sodium sulfate and
sodium thiosulfate produced such varied effects on king
and silver salmon both inter and intraspecifically that
such a contention seems probable. The identification and
the exact function of this salt regulating mechanism for
the present remains problematical, but that its rate of
development varies within the groups mentioned above may
be postulated by studying Tables 1 to 4. It will be noted
for example, that the minimum lethal concentration of so-
dium thiosulfate for king salmon is 75.0 p.p.m., while for
silver salmon it is 15,000 p.p.m. Likewise the range be-
tween the minimum lethal and the critical concentrations
of sodium thiosulfate for silver salmon is tremendous.

Fish taken from different sources were found to react
to a given pollutant in varying degrees. This was especi-
ally apparent in the king salmon which were obtained from
more numerous sources than were the silver salmon. King
salmon taken from one pot-hole all succumbed in a sodium
hydroxide solution of 10.0 p.p.m. concentration, while
others taken from another pot-hole required a 50.0 p.p.m.
concentration of sodium hydroxide to effect a 100 percent

Table 1. - The effects of sodium sulfate on king salmon, tests carried out in 10 liters of solution at 17° C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO ₃ | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|-----|--|-----|
| | | | Start | End | Start | End | Start | End |
| 20,000 | 6 | 100 | 8.7 | 5.0 | 7.5 | 7.4 | 70 | 78 |
| 15,000 | 7 | 100 | 8.5 | 5.0 | 7.5 | 7.3 | 70 | 75 |
| 12,500 | 28 | 100 | 9.0 | 5.2 | 7.5 | 7.3 | 70 | 80 |
| 11,000 | 10 | 20 | 10.0 | 5.6 | 7.5 | 7.5 | 60 | 65 |
| 10,000 | 10 | 100 | 6.8 | 6.0 | 7.5 | 7.4 | 70 | 75 |
| 8,500 | 16 | 0 | 9.3 | 5.6 | 7.5 | 7.4 | 70 | 84 |
| 750 | 16 | 0 | 8.5 | 6.0 | 7.5 | 7.4 | 60 | 75 |
| 500 | 10 | 10 | 9.0 | 6.0 | 7.5 | 7.4 | 60 | 65 |
| 375 | 8 | 0 | 8.6 | 5.4 | 7.3 | 7.4 | 50 | 75 |
| 300 | 6 | 0 | 9.2 | | 7.2 | | 60 | |
| 200 | 6 | 0 | 9.0 | | 7.2 | | 60 | |
| 100 | 4 | 0 | 8.6 | | 7.3 | | 55 | |
| 75 | 4 | 0 | 9.0 | | 7.3 | | 55 | |

Table 2. - The effects of sodium sulfate on silver salmon, tests conducted in 11 liters of solution at 16° C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO ₃ | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|-----|---|-----|
| | | | Start | End | Start | End | Start | End |
| 20,000 | 4 | 100 | 11.0 | 6.4 | 7.4 | 7.5 | 50 | 50 |
| 17,500 | 8 | 100 | 11.2 | 6.0 | 7.5 | 7.5 | 50 | 55 |
| 16,500 | 12 | 100 | 11.0 | 6.6 | 7.5 | 7.5 | 55 | 55 |
| 15,000 | 14 | 83 | 10.6 | 5.4 | 7.6 | 7.5 | 45 | 50 |
| 13,500 | 10 | 20 | 10.4 | 5.8 | 7.6 | 7.5 | 50 | 50 |
| 12,500 | 6 | 66 | 11.0 | 5.6 | 7.5 | 7.5 | 55 | 50 |
| 10,000 | 10 | 0 | 11.2 | 6.2 | 7.5 | 7.4 | 50 | 55 |
| 8,500 | 4 | 0 | 11.4 | 6.8 | 7.6 | 7.4 | 45 | 50 |

Table 3. - The effects of sodium thiosulfate on king salmon, tests conducted in 10 liters of solution at 18° C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO ₃ | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|-----|---|-----|
| | | | Start | End | Start | End | Start | End |
| 200 | 8 | 100 | 9.0 | | 7.5 | 7.2 | 80 | 70 |
| 125 | 8 | 100 | 9.2 | | 7.4 | 7.4 | 70 | 60 |
| 75 | 18 | 95 | 6.3 | | 7.4 | 6.8 | 70 | 60 |
| 68 | 16 | 50 | 6.8 | | 7.5 | 7.0 | 55 | 55 |
| 60 | 12 | 10 | 7.2 | | 7.3 | 7.0 | 65 | 60 |
| 30 | 8 | 0 | 7.0 | | 7.3 | 7.0 | | |

Table 4. - The effects of sodium thiosulfate on silver salmon, tests conducted in 11 liters of solution at 15° C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO ₃ | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|-----|---|-----|
| | | | Start | End | Start | End | Start | End |
| 16,000 | 8 | 100 | 11.4 | | 7.4 | 7.5 | 35 | 35 |
| 15,000 | 10 | 100 | 10.2 | | 7.4 | 7.5 | 38 | 38 |
| 13,500 | 10 | 80 | 9.6 | | 7.0 | 7.2 | 35 | 35 |
| 12,500 | 8 | 50 | 10.2 | | 7.2 | 7.3 | 30 | 35 |
| 10,000 | 6 | 50 | 12.4 | | 7.5 | 7.3 | 30 | 35 |
| 5,000 | 6 | 50 | 11.2 | | 7.3 | | 35 | |
| 2,500 | 4 | 25 | 11.2 | | 7.4 | | 40 | |
| 750 | 6 | 0 | 10.8 | | 7.5 | | 30 | |
| 500 | 4 | 100 | 10.6 | | 7.3 | | 35 | |
| 300 | 4 | 0 | 11.0 | | 7.5 | | 30 | |
| 250 | 4 | 50 | 11.2 | | 7.6 | | 30 | |
| 175 | 4 | 0 | 11.6 | | 7.4 | | 30 | |
| 150 | 2 | 100 | 10.8 | | 7.5 | | 35 | |
| 135 | 6 | 33 | 10.8 | | 7.3 | 7.4 | 40 | |
| 120 | 6 | 83 | 11.2 | | 7.5 | 7.4 | 30 | |
| 100 | 4 | 25 | 11.0 | | 7.4 | 7.3 | 30 | |
| 75 | 4 | 25 | 10.8 | | 7.3 | 7.3 | 35 | |
| 50 | 4 | 0 | 11.0 | | | | 35 | |
| 30 | 8 | 0 | 12.0 | | 7.2 | 7.3 | 35 | 35 |

kill. The inference from these results would appear to be that fish living under differing physico-chemical conditions would have greater or lesser susceptibility to sundry pollutants depending on the nature of their environment and the pollutant tested. Fortunately the king and silver salmon were found in sufficient numbers so as to be obtained from a minimum of sources.

Preliminary tests to determine the approximate minimum lethal and critical concentrations were undertaken as a measure to conserve fish. With king salmon this procedure involved a considerable amount of exploratory work since data from similar investigations on the effects of chemically pure components of Kraft mill waste effluents on king and silver salmon were not to be found in available literature. (Ericksen, 1940) and (Nightingale, 1928) conducted biological studies to ascertain the effects which various dilutions of sulfite liquors had on king salmon, but since the pollutants discharged in the sulfite process are not the same as those discharged in the sulfate (Kraft) process either qualitatively or quantitatively, their results could not be applied to the present investigation. (Van Horn, et al, 1946) includes a summary of the minimum lethal concentrations of toxic substances found in Kraft pulp mill waste liquors using local species of minnows as test fish, which served as an approximate guide for the studies on salmon. A comparison of the effects of some of the substances tested on the minnows of Van Horn and on king and silver salmon is discussed in the conclusions.

The results found with king salmon served as an index in subsequent work with silver salmon. The steps taken in setting up the screening tests were similar to those taken in the final trials, with the exception that fewer fish

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were used. Only two fish were employed for control purposes. Control fish and their significance together with procedures in conducting toxicity tests will be discussed in a later section.

The purpose of making these biological assays was to determine the minimum lethal and critical concentrations of some of the toxic components known to be present in Kraft mill waste liquors on king and silver salmon.

The following chemicals found in Kraft mill wastes were tested with salmon; hydrogen sulfide (Tables 5 and 6), methyl mercaptan (Tables 7 and 8), sodium sulfide (Tables 9 and 10), sodium sulfhydrate (Tables 11 and 12), sodium hydroxide (Tables 13 and 14), sodium carbonate (Tables 15 and 16), sodium thiosulfate (Tables 3 and 4), and sodium sulfate (Tables 1 and 2). The minimum lethal concentration is defined as the minimum amount of a toxic substance present in the water which will kill 100 percent of the test organisms. The critical concentration is the maximum amount of a toxic substance which may be present in the water without injuring or killing any of the test organisms. The concentrations are expressed in parts per million (p.p.m.).

The duration for any given test was 120 hours. It is thought that test organisms capable of survival for a period of 120 hours would live indefinitely at that particular concentration of pollutant. Actually in most cases a 72 hour test time was sufficient to give conclusive results. Because some of the more volatile toxic substances were so easily lost from a solution, a 24 hour test period

Table 5 (a). - The effects of hydrogen sulfide on king salmon, tests conducted in 10 liters of solution at 15° C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO ₃ | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|-----|---|-----|
| | | | Start | End | Start | End | Start | End |
| 1.5 | 10 | 100 | 10.8 | 9.8 | 7.5 | 7.5 | 60 | 60 |
| 1.0 | 12 | 100 | 10.2 | 10 | 7.4 | 7.4 | 55 | 58 |
| .75 | 8 | 75 | 11.2 | 9.4 | 7.5 | 7.4 | 50 | 53 |
| .5 | 6 | 50 | 11.2 | 9.6 | 7.5 | 7.5 | 50 | 50 |

Table 5 (b). - The effects of hydrogen sulfide on king salmon, tests conducted in 1 liter of solution at 15°C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO ₃ | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|-----|---|-----|
| | | | Start | End | Start | End | Start | End |
| 2.0 | 10 | 100 | 8.8 | | 7.4 | 7.4 | 60 | 63 |
| 1.0 | 10 | 50 | 10.0 | 8.0 | 7.5 | 7.4 | 58 | 55 |
| 0.5 | 4 | 0 | 10.0 | 8.0 | 7.4 | 7.5 | 60 | 60 |

Table 6. - The effects of hydrogen sulfide on silver salmon, tests conducted in 10 liters of solution at 7 to 9° C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO_3 | |
|----------------------------|------------------------|---------------------------|-------------------------------|------|-------|-----|---|-----|
| | | | Start | End | Start | End | Start | End |
| 1.5 | 10 | 100 | 10.5 | 10.0 | 7.0 | 7.0 | 55 | 60 |
| 1.25 | 28 | 100 | 11.0 | 11.0 | 7.1 | 7.2 | 55 | 57 |
| 1.0 | 20 | 80 | 11.0 | 10.5 | 7.0 | 7.1 | 55 | 60 |
| 0.75 | 15 | 0 | 10.5 | 9.4 | 7.1 | 7.2 | 52 | 54 |

Table 7 (a). - The effects of methyl mercaptan on king salmon, tests conducted in 10 liters of solution at 17.5°C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO ₃ | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|-----|---|-----|
| | | | Start | End | Start | End | Start | End |
| 2.5 | 6 | 100 | 6.4 | 7.0 | 7.4 | 7.5 | 55 | 70 |
| 1.9 | 10 | 100 | 6.6 | | 7.4 | | 60 | |
| 1.5 | 14 | 100 | 9.0 | 9.0 | 7.5 | 7.5 | 65 | 65 |
| 1.0 | 20 | 100 | 9.0 | 8.6 | 7.5 | 7.4 | 60 | 60 |
| 0.75 | 12 | 83.3 | 9.0 | 8.0 | 7.5 | 7.3 | 63 | 70 |
| 0.5 | 8 | 12 | 9.0 | 9.0 | 7.5 | 7.2 | 60 | 65 |

Table 7 (b). - The effects of methyl mercaptan on king salmon, tests conducted in 1 liter of solution at 17.5°C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO ₃ | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|-----|---|-----|
| | | | Start | End | Start | End | Start | End |
| 5.0 | 4 | 100 | 7.0 | | 7.5 | | 50 | |
| 2.5 | 8 | 100 | 7.0 | | 7.5 | | 60 | |
| 1.0 | 10 | 10 | 8.0 | | 7.4 | | 55 | |
| 0.5 | 4 | 0 | 8.2 | | 7.5 | | 63 | |

Table 8. - The effects of methyl mercaptan on silver salmon, tests conducted in 10 liters of solution at 7 to 9°C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO_3 | |
|----------------------------|------------------------|---------------------------|-------------------------------|------|-------|-----|---|-----|
| | | | Start | End | Start | End | Start | End |
| 2.0 | 8 | 100 | 10.5 | 10.1 | 7.4 | 7.4 | 55 | 60 |
| 1.75 | 20 | 100 | 10.0 | 9.7 | 7.3 | 7.3 | 53 | 58 |
| 1.5 | 16 | 50 | 10.0 | 9.8 | 7.5 | 7.5 | 60 | 60 |
| 1.25 | 12 | 0 | 11.0 | 10.0 | 7.0 | 7.2 | 60 | 60 |
| 1.0 | 10 | 33 | 11.0 | 9.0 | 7.2 | 7.3 | 60 | 60 |
| 0.75 | 10 | 0 | 11.0 | 9.0 | 7.0 | 7.2 | 55 | 60 |

Table 9. - The effects of sodium sulfide on king salmon, tests conducted in 10 liters of solution at 17.8°C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO_3 | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|-----|---|-----|
| | | | Start | End | Start | End | Start | End |
| 5.0 | 6 | 100 | 8.5 | | | | | |
| 3.5 | 12 | 100 | 8.5 | 7.9 | 7.9 | 7.1 | 60 | 70 |
| 3.0 | 10 | 80 | 8.7 | 7.9 | 7.9 | | 55 | |
| 2.0 | 8 | 10 | | | | | | |
| 1.0 | 6 | 0 | 8.7 | | | | | |

Table 10. - The effects of sodium sulfide on silver salmon, tests conducted in 11 liters of solution at 16°C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO ₃ | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|-----|---|-----|
| | | | Start | End | Start | End | Start | End |
| 5.0 | 6 | 100 | 10.5 | 7.8 | 7.6 | 7.5 | 50 | 55 |
| 3.5 | 12 | 100 | 11.2 | 8.0 | 7.6 | 7.5 | 50 | 50 |
| 3.0 | 16 | 100 | 11.4 | 6.2 | 7.7 | 7.6 | 45 | 40 |
| 2.75 | 12 | 75 | 12.0 | 5.8 | 7.6 | 7.5 | 45 | 40 |
| 2.5 | 6 | 50 | 11.0 | 6.2 | 7.6 | 7.5 | 40 | 35 |
| 2.0 | 6 | 50 | 10.4 | 5.6 | 7.6 | 7.4 | 40 | 35 |
| 1.5 | 8 | 12 | 10.2 | 5.6 | 7.6 | 7.4 | 35 | 35 |
| 1.0 | 10 | 0 | 10.6 | 6.4 | 7.6 | 7.4 | 40 | 35 |
| 0.75 | 8 | 0 | 11.0 | 5.8 | 7.5 | 7.3 | 40 | 35 |

Table 11. - The effects of sodium sulphhydrate on king salmon, tests carried out in 10 liters of solution at 16°C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO ₃ | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|-----|---|-----|
| | | | Start | End | Start | End | Start | End |
| 10.0 | 6 | 100 | 8.5 | 6.2 | 7.5 | | 60 | |
| 5.0 | 8 | 100 | 9.0 | 5.8 | 7.3 | | 50 | |
| 4.0 | 10 | 100 | 9.0 | 6.0 | 7.7 | 7.7 | 60 | 60 |
| 3.6 | 10 | 100 | 9.3 | 5.4 | 7.6 | 7.5 | 60 | 60 |
| 3.3 | 16 | 100 | 8.7 | 6.0 | 7.7 | 7.6 | 60 | 60 |
| 3.0 | 10 | 50 | 8.5 | 5.8 | 7.3 | 7.4 | 50 | 60 |
| 2.0 | 6 | 10 | 9.0 | 6.4 | 7.4 | | 60 | |

Table 12. - The effects of sodium sulphhydrate on silver salmon, tests carried out in 10 liters of solution at 16°C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO ₃ | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|-----|---|-----|
| | | | Start | End | Start | End | Start | End |
| 5.0 | 6 | 100 | 10.0 | 7.4 | 7.6 | 7.5 | 50 | 55 |
| 3.5 | 12 | 100 | 10.8 | 6.2 | 7.5 | 7.5 | 50 | 50 |
| 3.25 | 10 | 50 | 11.0 | 6.4 | 7.4 | 7.5 | 45 | 40 |
| 3.0 | 6 | 17 | 11.2 | 5.8 | 7.5 | 7.4 | 50 | 55 |
| 2.0 | 6 | 0 | 11.0 | 5.6 | 7.6 | 7.5 | 45 | 45 |
| 1.0 | 6 | 17 | 11.4 | 6.2 | 7.5 | 7.5 | 45 | 50 |
| 0.5 | 12 | 0 | 10.2 | 6.4 | 7.4 | 7.5 | 45 | 40 |

Table 13. - The effects of sodium hydroxide on king salmon, tests conducted in 10 liters of solution at 14°C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO_3 | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|-----|---|-----|
| | | | Start | End | Start | End | Start | End |
| 50.0 | 20 | 100 | 10.8 | 9.2 | 10.5 | 9.5 | 130 | 130 |
| 42.0 | 15 | 87 | 9.0 | 8.8 | 10.5 | 9.7 | 110 | 115 |
| 35.0 | 10 | 20 | 10.2 | | 9.8 | | 115 | |
| 25.0 | 6 | 0 | 9.0 | 9.0 | 9.5 | 7.5 | 90 | 140 |
| 20.0 | 4 | 0 | 9.2 | 8.8 | 9.3 | 7.5 | 80 | 120 |

Table 14. - The effects of sodium hydroxide on silver salmon, tests conducted in 11 liters of solution at 16°C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO_3 | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|------|---|-----|
| | | | Start | End | Start | End | Start | End |
| 75.0 | 4 | 100 | 11.5 | 8.0 | 11.5 | 10.0 | 230 | 130 |
| 50.0 | 4 | 100 | 11.5 | 8.3 | 10.5 | 9.7 | 110 | 100 |
| 35.0 | 6 | 100 | 12.0 | 7.4 | 10.0 | 9.5 | 80 | 80 |
| 30.0 | 8 | 100 | 10.6 | | 9.7 | | 70 | |
| 25.0 | 8 | 100 | 10.6 | 9.0 | 9.3 | 9.0 | 60 | 65 |
| 20.0 | 12 | 100 | 10.4 | 7.2 | 9.2 | 9.1 | 60 | 65 |
| 15.0 | 6 | 33 | 11.0 | 5.2 | 10.0 | 7.3 | 60 | 60 |
| 10.0 | 10 | 0 | 11.0 | 5.6 | 9.7 | 7.5 | 60 | 60 |

Table 15. - The effects of sodium carbonate on king salmon, tests carried out in 10 liters of solution at 17.8°C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO_3 | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|------|---|-----|
| | | | Start | End | Start | End | Start | End |
| 90.0 | 8 | 100 | 9.0 | | 9.6 | | 140 | |
| 75.0 | 8 | 100 | 9.0 | | 9.3 | | 120 | |
| 68.0 | 16 | 100 | 10.0 | | 9.5 | 7.3 | 124 | 75 |
| 60.0 | 10 | 20 | 9.0 | | 9.3 | | 100 | |
| 25.0 | 8 | 0 | 9.0 | | 8.5 | 7.25 | | |
| 10.0 | 8 | 0 | 9.2 | | 8.0 | 7.1 | | |

Table 16. - The effects of sodium carbonate on silver salmon, tests conducted in 11 liters of solution at 14°C.

| Concentration in p.p.m. | Number of test fish | Mortality in 120 hours | Dissolved oxygen in p.p.m. | | pH | | Total alkalinity as p.p.m. CaCO ₃ | |
|----------------------------|------------------------|---------------------------|-------------------------------|-----|-------|-----|---|-----|
| | | | Start | End | Start | End | Start | End |
| 100.0 | 6 | 100 | 11.0 | 9.4 | 9.3 | 7.7 | 240 | 140 |
| 85.0 | 6 | 100 | 11.0 | 9.0 | 10.0 | 9.1 | 115 | 115 |
| 75.0 | 14 | 100 | 11.2 | 6.4 | 9.7 | 9.0 | 120 | 110 |
| 68.0 | 8 | 87 | 10.4 | 5.6 | 9.6 | 8.5 | 100 | 110 |
| 60.0 | 6 | 100 | 10.2 | 5.2 | 9.3 | 7.7 | 160 | 130 |
| 50.0 | 7 | 42 | 11.4 | 5.4 | 9.5 | 7.5 | 90 | 90 |
| 45.0 | 10 | 0 | 11.0 | 6.0 | 9.4 | 8.4 | 80 | 80 |
| 40.0 | 8 | 0 | 11.2 | 5.6 | | | | |

proved to be adequate in most of these cases.

After the preliminary surveys had established the approximate lethal and critical concentrations, a more extensive study was made in order to obtain more accurate results. A total of at least ten fish were considered necessary to give conclusive evidence for establishing the minimum lethal and critical concentrations of a pollutant. The entire lot of ten fish were not necessarily run in any one test, but were often conducted in consecutive tests in groups of three or four fish.

Control fish were subjected to exactly the same conditions as were the test animals with the exception that no pollutant was added to the water. They were selected in such a way as to represent a cross section of all of the test specimens employed especially in regards to size and vigor. The number used in any test varied with the number of test fish. Generally four control fish were used, rarely less. If more than one control fish succumbed during the test, the entire experiment was discarded.

The 19 liter capacity test jugs were thoroughly washed before and after each experiment to remove any trace of compounds previously used. The test water was then added at a quantity somewhat less than ultimately desired. Thus, for king salmon, after nine liters of water had been placed into a jug, two fish were introduced. The calculated volume of toxic chemical was then made up to one liter of solution and this placed into the jug, bringing the total volume to 10 liters. After the experiment had been set up, the pH value, the alkalinity as p.p.m. CaCO_3 , the dissolved oxygen content and the temperature were ascertained and recorded. To avoid possible confusion, all of the test jugs were tagged with the following information; species of fish, the chemical employed and the concentration. Routine tests were made during the bioassay to determine any changes in the pH value, dissolved oxygen content and the temperature. Test fish that succumbed during the course of an experiment were removed from the jugs in order to prevent any decomposition products from affecting the remaining specimens. Upon completion of a test, any fish still surviving were placed into a separate holding tank where an effort was made to restore their original vigor. These fish were later released into nearby streams.

The volume of water used in the test solutions was computed so as to assure an adequacy of dissolved oxygen for consumption by the test fish. By empirical methods it was found that two fish averaging 10 centimeters in total length and 12 grams in weight could be safely held in 10 liters of solution for an indefinite period at 15° C., without aerating the water. The lowest concentration of dissolved oxygen which will support a varied fauna of warm-water fishes in fresh water streams is 5.0 p.p.m. (Ellis, 1946). Therefore it would be reasonable to expect that certain fishes of colder waters (trout and salmon) would have a higher dissolved oxygen minimum under certain conditions than have many other fishes (Welch, 1935). A standard of 5.0 p.p.m. dissolved oxygen was adopted for the toxicity tests, although the content would occasionally drop to 3.5 p.p.m. Even at this lower oxygen concentration the fish would often survive though showing signs of distress. In general however, experiments which at their termination indicated a dissolved oxygen content of less than 5.0 p.p.m. were considered unreliable, since it is believed that fish weakened by a lack of oxygen are more susceptible to destruction by toxic materials than they would be if that essential element were present in a sufficient amount.

The bioassays with king salmon were carried out in 10 liters of solution while in most of the tests with silver salmon 11 liters of solution were used. The change from 10 to 11 liters of solution evolved during the course of time as an expedient in setting up the experiments. It was noted however, that when the threshold at which the minimum lethal concentration occurred had been reached, a difference in the volume of the solution at the same concentration would produce varied effects on the test fish. For example, 50 percent of the king salmon tested in 1 liter of solution containing 1 p.p.m. hydrogen sulfide would survive, but in 10 liters of solution containing 1 p.p.m. hydrogen sulfide all of the salmon would die. In similar tests, king salmon would have a 90 percent survival in 1 liter solution of 1 p.p.m. methyl mercaptan, but in a 10 liter solution of methyl mercaptan at 1 p.p.m. a 100 percent kill would be effected. The data for these experiments are contained in Tables 5 and 6.

From the foregoing it may be assumed that although the concentration in both volumes remained the same, the overall number of toxic ions in the larger volume were present in greater numbers to the effect that the organisms failed to successfully overcome them. In the smaller volume the extent of the toxic ions present were sufficiently few to enable defensive mechanisms within the fish to satisfactor-

ily combat their inimical effects. It will be observed that in the above results highly toxic materials were involved. No attempts were made to evaluate the volume differences of solutions containing less toxic compounds.

RECOVERY OF TEST FISH SUBJECTED TO POLLUTANTS

The successful recovery of a fish subjected to sundry pollutants was found to depend largely on the vigor of the individual fish, the nature of the toxic substance involved, the concentration of same and the time during which the test specimen is exposed. Highly toxic compounds would kill fish in a matter of minutes or a few hours, whereas the slower acting compounds would not produce appreciable effects until a few days had elapsed.

Attempts were made to compare the ability of fish to recover from exposure to highly poisonous and to slow acting pollutants. As a temporary standard, fish were subjected to the toxic materials to a point where they assumed a more or less permanent "belly-up" position, whence they were immediately removed and placed into fresh water. Attention is drawn to the fact that one of the first symptoms of serious distress in a fish is the loss of equilibrium. The complete loss of equilibrium does not generally occur at once, since it is generally preceded by a period of excitement characterized by an increase of respiration and by wild movements with an occasional loss of balance that becomes more and more pronounced. Having been placed into fresh water the fish would continue to float "belly-up", but its respiration would become stronger and more regular if it was capable of recovery. This would be followed by an increase in bodily activity and

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subsequent recovery of equilibrium. A rectangular tank similar to those used as water baths, served for recovery purposes. A rubber hose was attached at one end of the tank through which fresh water was supplied, and at the other end of the tank the excess water was permitted to flow through a valve. Although obstacles were planted from one end of the tank to the other, recovering fish would slowly swim around each obstacle to the source of water and maintain its position there until fully revived.

The results of these studies indicated that a high percentage of salmon were capable of recovering from the effects of such highly lethal compounds as hydrogen sulfide and methyl mercaptan, provided they were removed immediately upon reaching a permanent "belly-up" position. Slower acting pollutants such as sodium hydroxide, sodium carbonate, sodium sulfate and sodium thiosulfate probably had accumulative effects on the salmon because very few of the fish recovered having once assumed a permanent "belly-up" position in solutions of these materials.

Figure 11. - Relative effects of hydrogen sulfide on
king and silver salmon.
Solid line = king salmon. Dotted line
= silver salmon.

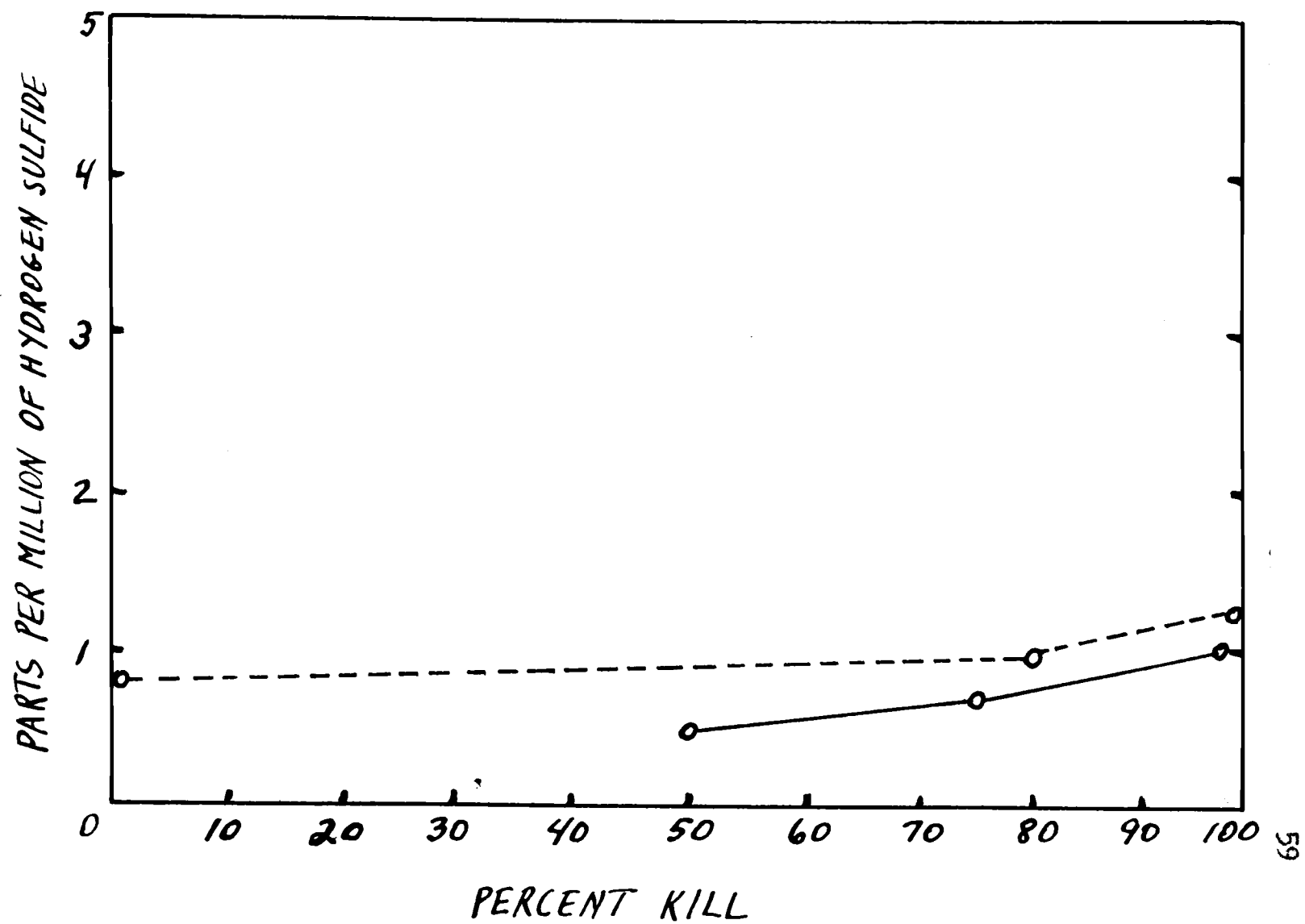


Figure 12. - Relative effects of methyl mercaptan on
king and silver salmon.
Solid line = king salmon.
Dotted line = silver salmon.

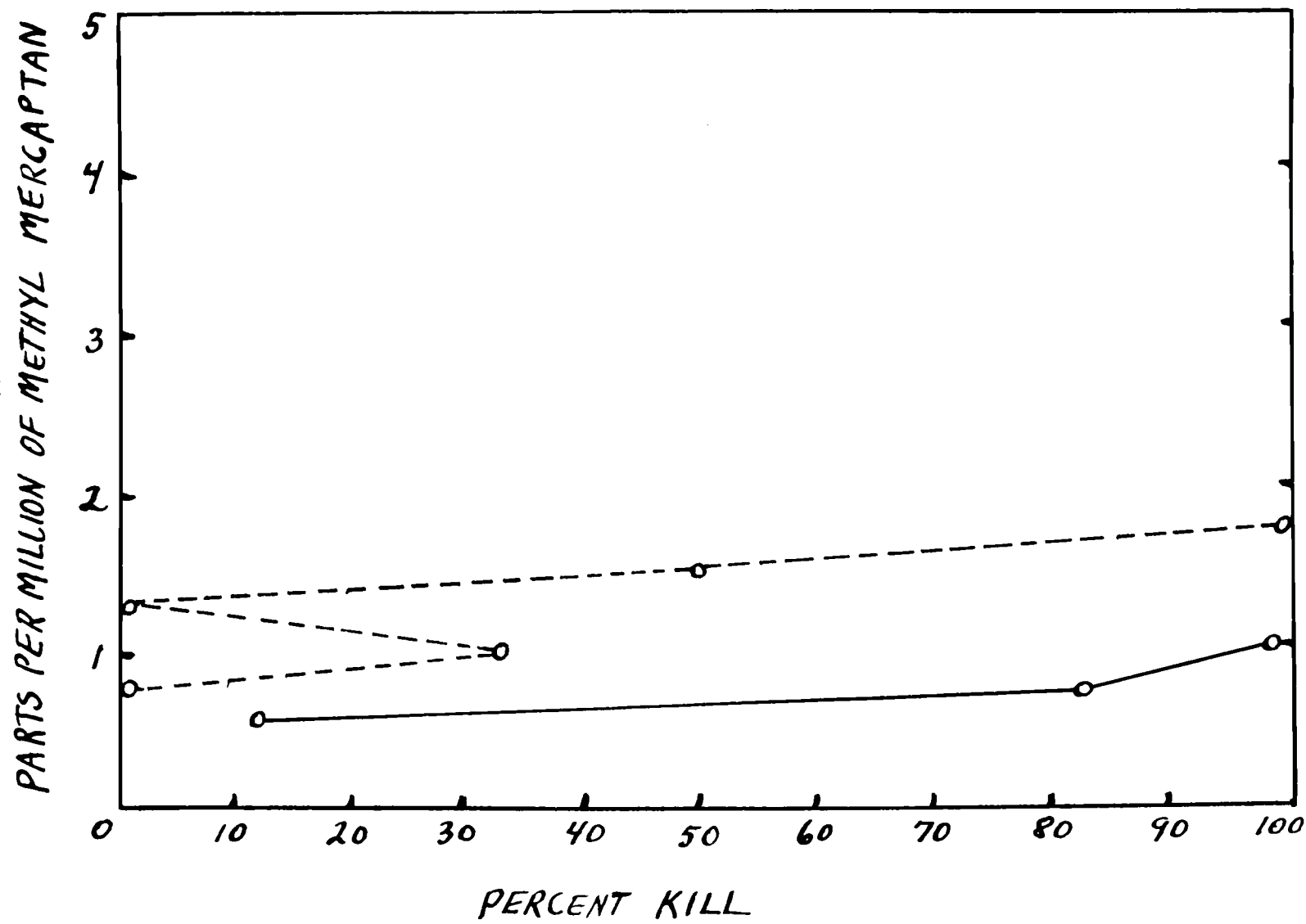


Figure 13. - Relative effects of sodium sulfide on
king and silver salmon.
Solid line = king salmon.
Dotted line = silver salmon.

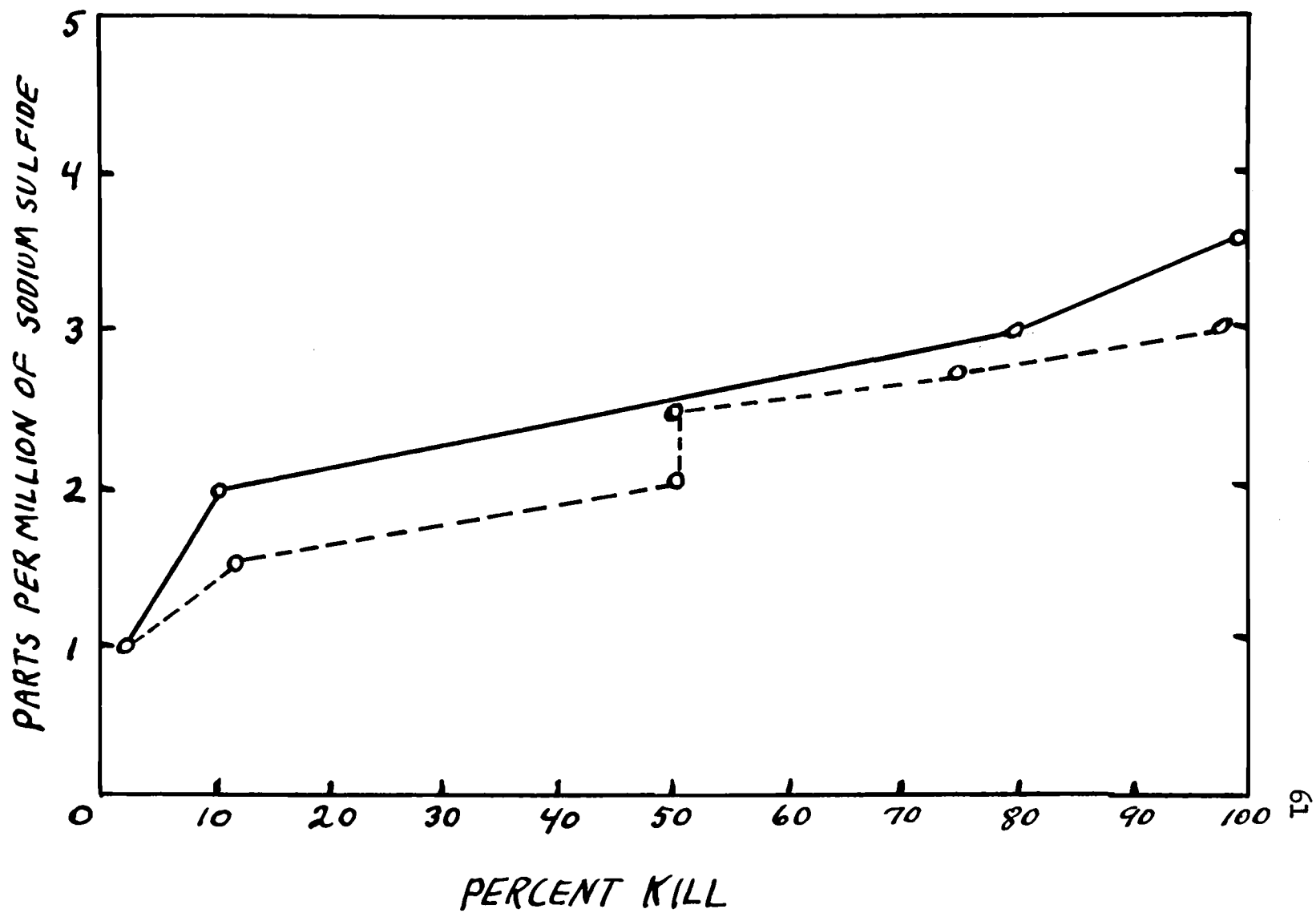


Figure 14. - Relative effects of sodium sulfhydryte
on king and silver salmon.
Solid line = king salmon.
Dotted line = silver salmon.

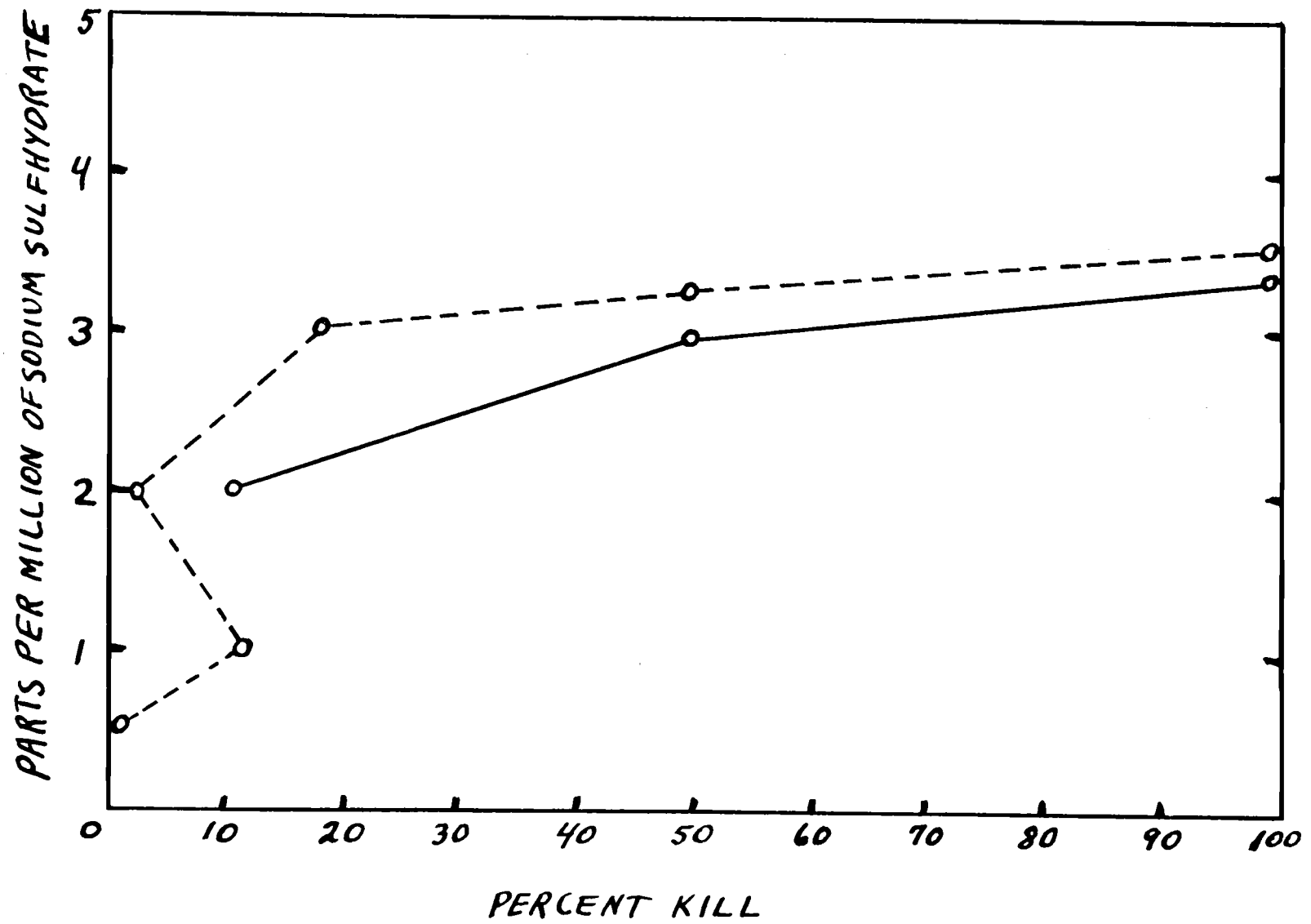


Figure 15. - Relative effects of sodium hydroxide
on king and silver salmon.
Solid line = king salmon.
Dotted line = silver salmon.

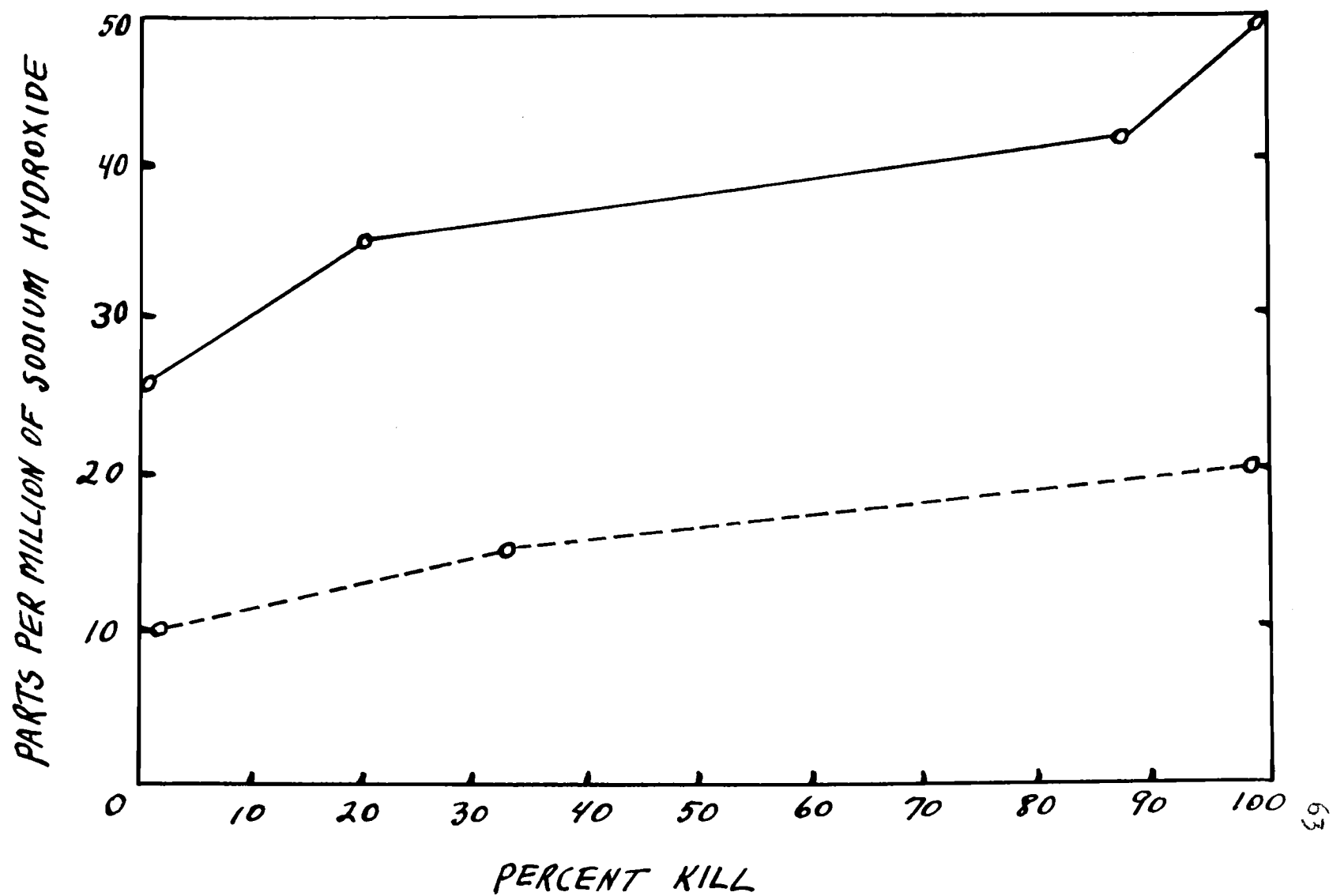


Figure 16. - Relative effects of sodium carbonate on
king and silver salmon.
Solid line = king salmon.
Dotted line = silver salmon.

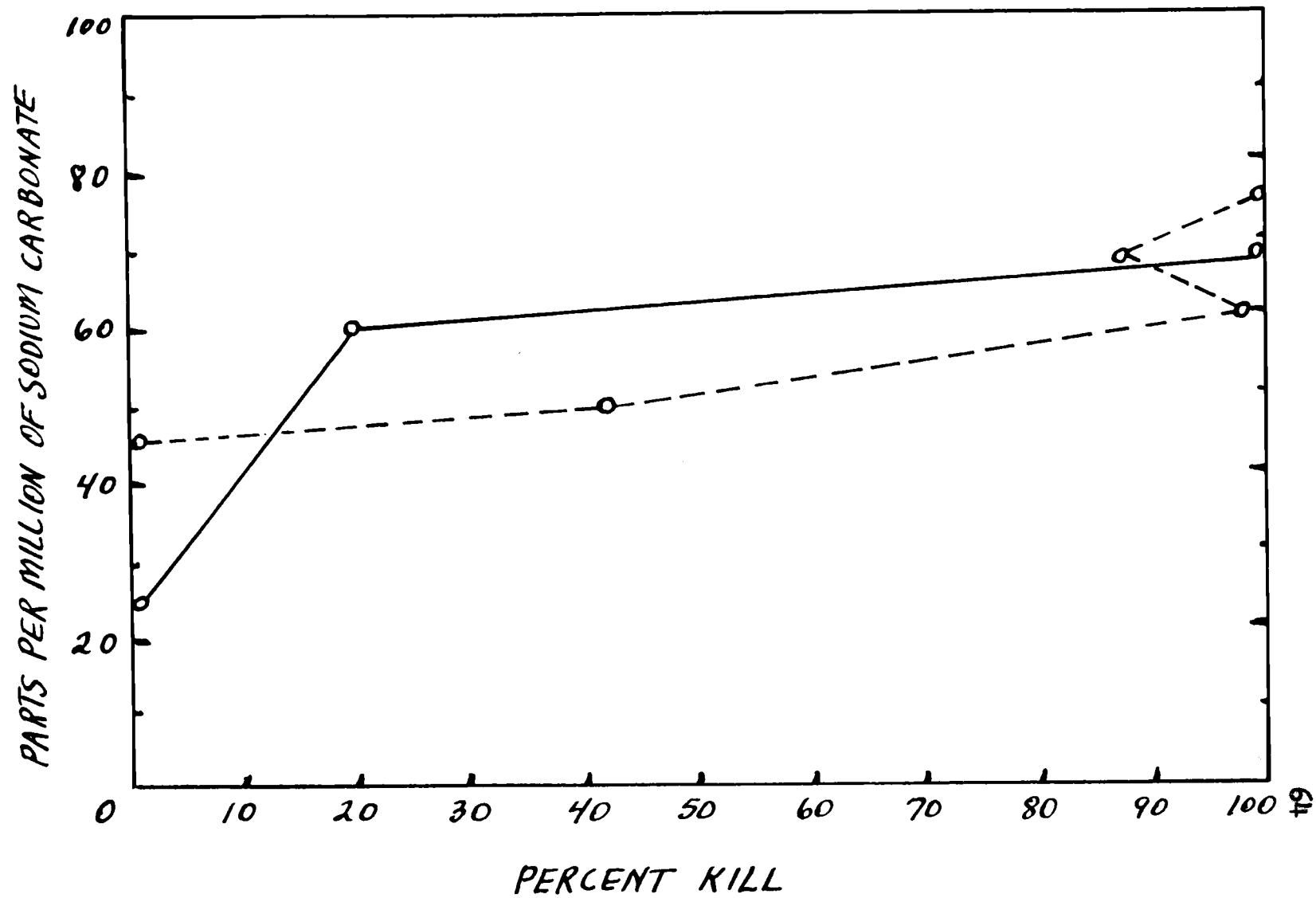
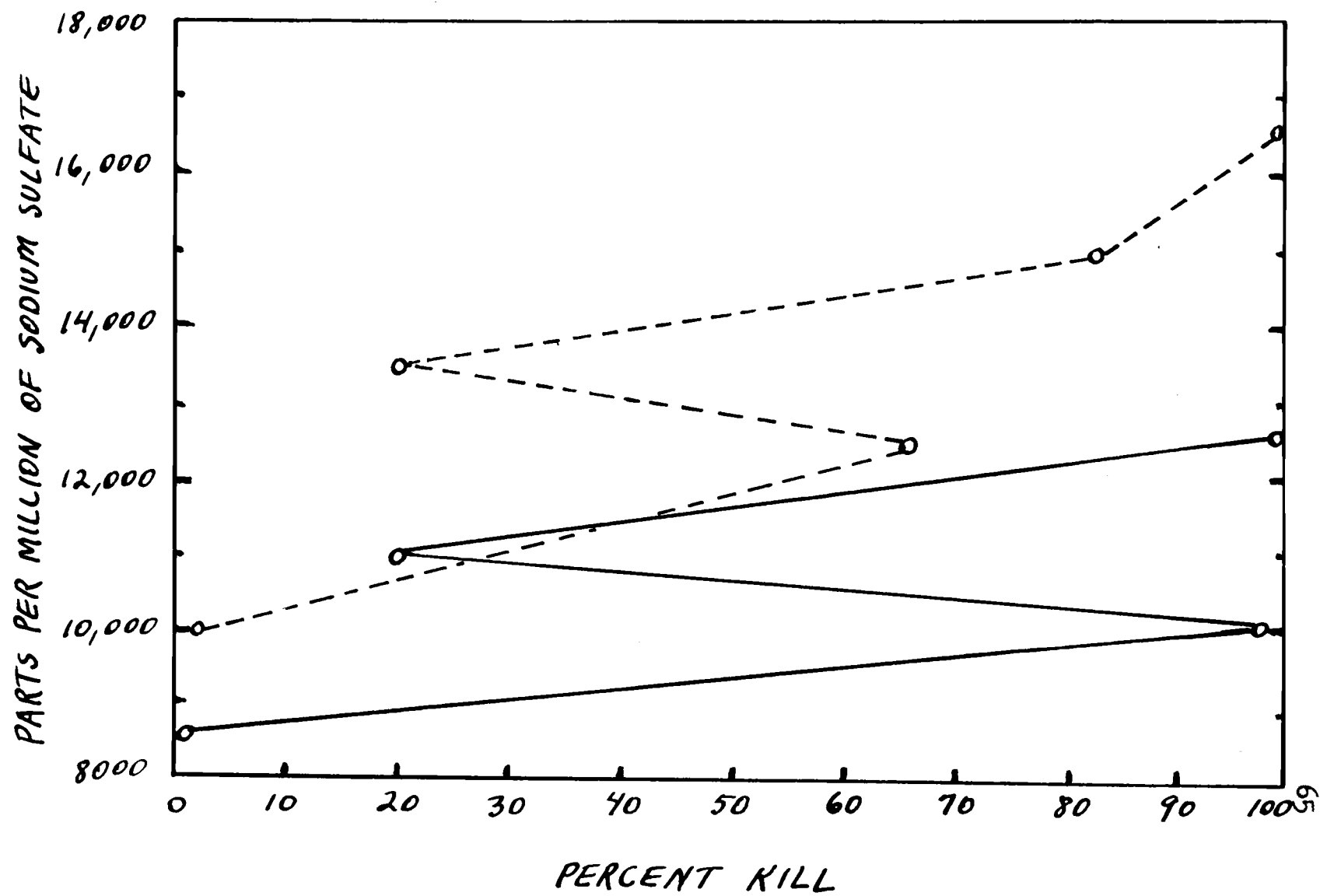


Figure 17. - Relative effects of sodium sulfate on
king and silver salmon.
Solid line = king salmon.
Dotted line = silver salmon.



For the most part, the chemical compounds known to occur in Kraft mill waste liquors have proved to be highly injurious to king and silver salmon while a few are tolerated to such an extent as to be of relatively little import (Table 17). In general, hydrogen sulfide, methyl mercaptan, sodium sulfide and sodium sulfhydrate are the most noxious, followed by sodium hydroxide, sodium carbonate and sodium thiosulfate. Sodium sulfate is tolerated in very high concentrations by young salmon.

Hydrogen sulfide (Figure 11) was very toxic to king and silver salmon, the minimum lethal concentration being 1.0 p.p.m. for the king and 1.25 p.p.m. for the silver. The critical concentration for king salmon was not determined; for silver salmon it was 0.75 p.p.m. The minimum lethal concentration for the minnows tested by Van Horn is 1.0 p.p.m.

Methyl mercaptan (Figure 12) was also very toxic to king and silver salmon, the minimum lethal concentration being 1.0 p.p.m. for the king and 1.75 p.p.m. for the silver salmon. The critical concentration for king salmon was not determined, but for silver salmon it is 0.75 p.p.m. The minimum lethal concentration for the minnows tested by Van Horn is 0.5 p.p.m.

Sodium sulfide (Figure 13) although lethal in very small concentrations was slower acting than hydrogen

Table 17. - A resume of the minimum lethal and critical concentrations of chemical substances known to be present in Kraft mill waste liquors on king and silver salmon.

| Chemical substance | King salmon | | Silver salmon | |
|--------------------|-------------------------------------|-------------------------------|-------------------------------------|-------------------------------|
| | Minimum lethal concentration p.p.m. | Critical concentration p.p.m. | Minimum lethal concentration p.p.m. | Critical concentration p.p.m. |
| Hydrogen Sulfide | 1.0 | | 1.25 | 0.75 |
| Methyl Mercaptan | 1.0 | | 1.75 | 0.75 |
| Sodium Sulfide | 3.5 | 1.0 | 3.0 | 1.0 |
| Sodium Sulphydrate | 3.3 | | 3.5 | 0.5 |
| Sodium Hydroxide | 50.0 | 25.0 | 20.0 | 10.0 |
| Sodium Carbonate | 68.0 | 25.0 | 75.0 | 45.0 |
| Sodium Thiosulfate | 75.0 | 30.0 | 15,000.0 | 30.0 |
| Sodium Sulfate | 12,500.0 | 8,500.0 | 16,500.0 | 10,000.0 |

sulfide and methyl mercaptan. The minimum lethal concentration for king salmon is 3.5 p.p.m. and for silver salmon 3.0 p.p.m. The critical concentration for both the king and silver salmon is 1.0 p.p.m. The minimum lethal concentration which Van Horn found for minnows was 3.0 p.p.m.

Sodium sulfhydrate (Figure 14) proved to be lethal at lower concentrations (0.5 p.p.m.) for the minnows tested by Van Horn than for the salmon. The minimum lethal concentration for king salmon is 3.3 p.p.m. and for silver salmon 3.5 p.p.m. The critical concentration for king salmon was not determined; for silver salmon it is 0.5 p.p.m.

Sodium hydroxide (Figure 15) proved to be tolerated in much smaller concentrations by both salmon than that tolerated by the minnows of Van Horn. The minimum lethal concentration for king salmon is 50.0 p.p.m. and for silver salmon is 20.0 p.p.m. The critical concentration for king salmon is 25.0 p.p.m. and for silver salmon 10.0 p.p.m. The minimum lethal concentration for the minnows tested by Van Horn is 100.0 p.p.m.

Sodium carbonate (Figure 16) at a concentration of 250.0 p.p.m. killed 100 percent of the fresh water minnows tested by Van Horn. The king and silver salmon were far more susceptible to this compound than the minnows. The minimum lethal concentration for king salmon was

68.0 p.p.m. and for silver salmon 75.0 p.p.m. The critical⁶⁹ concentration for king salmon is 25.0 p.p.m. and for silver salmon 45.0 p.p.m.

Sodium thiosulfate killed king salmon at much lower concentrations than silver salmon. The range between the critical and minimum lethal concentration for silver salmon is very great. The minimum lethal concentration for king salmon is 75.0 p.p.m. and for silver salmon it is 15,000.0 p.p.m. The critical concentration for both king and silver salmon is 30.0 p.p.m.

Sodium sulfate (Figure 17) was tolerated in very high concentrations by both species of salmon. The minimum lethal concentration for king salmon is 12,500.0 p.p.m. and for silver salmon, 16,500.0 p.p.m. The minimum lethal concentration for the fresh-water minnows tested by Van Horn is 100.0 p.p.m.

With the exception of sodium thiosulfate, both king and silver salmon appear to show generic relationship in their abilities to resist the pollutants tested.

Differences in the volume of solutions of hydrogen sulfide and methyl mercaptan at given concentrations produced varied effects on king and silver salmon. The significance and possible application of this fact to future pollution studies should be seriously considered.

King salmon taken from dissimilar sources reacted differently to sodium hydroxide. The minimum lethal concentration for salmon taken from ompot-hole is 10.0 p.p.m.

while for salmon taken from another hole it is 50.0 p.p.m.

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