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## Avoidance of Lime-Neutralized Iron Hydroxide Solutions by Coho Salmon in the Laboratory

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■ Laboratory avoidance studies indicated that coho salmon, raised in clean water, have shown a definite response to lime-neutralized iron hydroxide suspensions at the range of 4.25–6.45 mg Fe/l. The same species of fish, exposed for several months to different concentrations of the pollutant, has shown almost identical response as the fish raised in the control water.

Throughout the United States, there exist certain sections where the principal contributor to water pollution is acid drainage, most commonly originating from coal-mining activities and characterized by a high dissolved iron content. The passage of water through coal mines where iron pyrites (FeS<sub>2</sub>) have been exposed to the oxidizing action of air, water, and bacteria results in the production of acid and the release of soluble ferrous iron (1). When the pH is raised and oxidation takes place (e.g., in a receiving water body), most of the soluble ferrous iron is converted to insoluble ferric species.

The chemical processes in acid mine drainage treatment plants are based on the same principles (neutralization and oxidation) resulting in precipitation of metal compounds which are removed by sedimentation. Under normal treatment plant operation, ambient suspended iron concentrations are greatly reduced, but nevertheless, receiving waters may be furnished with low levels of ferric iron for indefinite periods, while somewhat higher levels of ferric iron enter intermittently when abnormal iron loadings occur.

To provide data useful in the regulation of iron discharges in neutral effluents, this bioassay was undertaken to ascertain the threshold concentration of solution containing suspended ferric hydroxide that would be detected and spontaneously avoided by coho salmon (*Oncorhynchus kisutch*, Walbaum).

### Methods and Materials

**Physical-Chemical System.** The diluent water was pumped from a well (18 m) by a 1/3-hp submersible pump to

a 378-l. Nalgene storage reservoir. This holding vessel was equipped with a 1-hp cooling unit, thermostatically controlled to maintain a water temperature of 10.0°C. The most recent diluent water analyses are summarized in Table I.

A modified proportional diluter, based on the design by Mount and Brungs (2) was used to deliver the pollutant. The apparatus simulates the neutralization phase of an acid mine drainage treatment plant. Thus, the basic operation of the dosing equipment entails (a) neutralization of a ferrous sulfate stock solution with lime slurry, and (b) oxidation of the resultant blue-green bivalent iron suspension to the red-brown trivalent iron precipitate, ferric hydroxide. Construction and function of the modified proportional diluter were described by Sykora et al. (3).

From the diluter each concentration of suspended iron was delivered to a 4-l. detention tank connected by a siphon tube to another 4-l. container. For avoidance experiments, the selected iron concentration was siphoned from

Table I. Dilution Water Characteristics

Characteristic	Mean	Std dev
Total iron, mg Fe/l. <sup>a</sup>	0.19	0.18
Total dissolved iron, µg Fe/l.	159.0	59.0
Total ferrous iron, µg Fe/l.	50.0	81.0
pH	7.76	0.16
Conductance, µmhos/cm	3236.0	135.0
Turbidity, JTU	5.3	0.9
Total acidity mg CaCO <sub>3</sub> /l.	2.49	1.31
Total alkalinity, mg CaCO <sub>3</sub> /l.	99.8	2.6
Total hardness EDTA, mg CaCO <sub>3</sub> /l.	159.0	10.6
Hardness Ca <sup>2+</sup> , mg CaCO <sub>3</sub> /l.	61.0	21.5
Total Ca <sup>2+</sup> , mg Ca <sup>2+</sup> /l.	24.4	8.6
SO <sub>4</sub> <sup>2-</sup> , mg SO <sub>4</sub> <sup>2-</sup> /l.	43.6	5.9
Total Mg <sup>2+</sup> , mg Mg <sup>2+</sup> /l.	5.66	...
Total Mn <sup>2+</sup> , mg Mn <sup>2+</sup> /l. <sup>a</sup>	0.03	...
O <sub>2</sub> , % saturation	92.6	3.0
Temperature, °C	10.0	0.6

<sup>a</sup> Most of these metals were removed by aeration and settling in the storage tank.

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the second detention tank. The clean control water was pumped by a centrifugal pump from the cooling reservoir into an aerated 4-l. glass detention tank, siphoned into a second aerated detention tank, and delivered to the experimental trough.

**Avoidance Apparatus and Performance.** The avoidance trough employed similar design principles as those given by Jones and Sprague, and Shelford and Allee (4-6).

Constructed of pre-cut, single-strength (0.16 cm) glass, the rectangular trough measured 82 × 11 × 21 cm, with a total volume of 18,942 cm<sup>3</sup> (Figure 1). A depth of 14.5 cm was maintained in the trough by two outflow siphons, each equipped with a linear series of eight equally spaced holes along its side. Further, on each side of the trough were two additional overflow relief ports.

To establish a definite vertical gradient it was necessary to increase the clean water flow and decrease the hydraulic pressure of the clean water toward the middle. This was accomplished by a combination of holes cut into one side of the clean water inlet that directed the flow back against the trough's end, reducing the direct flow and giving an evenly distributed bulk flow in the middle. A similar piece of tubing with only one large square hole was placed on the polluted intake, with the flow directed toward the middle. This combination of inflow direction control gave a good vertical gradient. The avoidance system was in a separate laboratory room, and a black polyethylene canopy covered the trough to protect it from outside disturbances. Dissolved oxygen and temperature were measured in situ with a membrane type of oxygen electrode after each experiment. The hydrogen ion activity was determined by a pH meter in 300-ml samples taken from each side of the trough. For total iron determination a 50-ml sample was collected from each section and acidified with 2 ml of concentrated HCl. These samples were composited after each test, and analyzed with an atomic absorption spectrophotometer. Daylight hours were controlled according to the sunrise tables of Evansville, Ind., and the water temperature was maintained at 11 ± 1°C. Results from physical and chemical analysis indicate that pH variations were kept at a minimal level ranging from 7.63-7.90 in the clean

water and from 7.38-7.85 in the polluted section. Oxygen concentration was always high with saturation levels ranging from 86.7-93.5%.

**Biological System.** Coho salmon eggs were obtained from the Tionesta State Fish Hatchery, Tionesta, Pa. The ripe spawning coho salmon were netted in Walnut Creek, a tributary stream of Lake Erie. Their eggs were artificially fertilized in situ and taken to Tionesta, picked up, and brought to our Neville Island Research Laboratory. The eggs were incubated in fish breeders at 10.5-11°C, within 48 hr after fertilization. These eggs "eyed" 26 days after incubation, and hatched in 43-47 days, all eggs hatching by December 27, 1972.

Long-term sublethal bioassays to determine the effects of lime neutralized iron suspensions on avoidance of coho salmon exposed to different concentrations of ferric hydroxide were started immediately after the eggs hatched. Sac fry were randomly selected, placed in fish breeding cages in the six aerated 75-l. testing aquaria (5 concentrations and control). Each aquarium received 24 l./hr per concentration, with water temperature relatively constant at 11-13°C. These tests ended April 3, 1973. The fish were exposed to the following nominal concentrations of iron—0.75, 1.5, 3, 6, 12 mg Fe/l. and the control. During the long-term bioassay, fish tested in higher iron concentrations manifested a higher mortality rate than fish exposed to lower iron concentrations and control. Of all salmon tested in the highest iron concentration (12.0 mg Fe/l.), only five out of fifty survived. In other cases, fish mortality was very low, and many fish were available for subsequent avoidance testing.

The effect of lime neutralized iron hydroxide suspensions on growth of coho salmon are described and statistically evaluated by Smith (7). A short summary of the mean lengths for salmon before and after avoidance experiments is given in Table II.

**Test Procedure and Statistical Analysis.** Preliminary avoidance tests were performed using unmodified (control) water inflowing to both ends of the avoidance trough. Salmon were relatively active swimmers, moving back and forth in the trough eliciting common exploratory behavior. The fish were not influenced by flow nor bothered by the outflow siphons.

Coho salmon raised in control water and coho salmon raised in five specific concentrations were tested individually in the avoidance trough. To reach the proper gradient, the pollutant was added to the experimental trough before the fish were tested. After transfer from the chronic testing aquaria or dilution water to the avoidance trough, each fish was given a 30-min acclimation period followed by a 10-min test period. Visit times to each side of the trough (verified by crossing the middle boundary) were measured by using two calibrated stop watches, and recorded in seconds. An

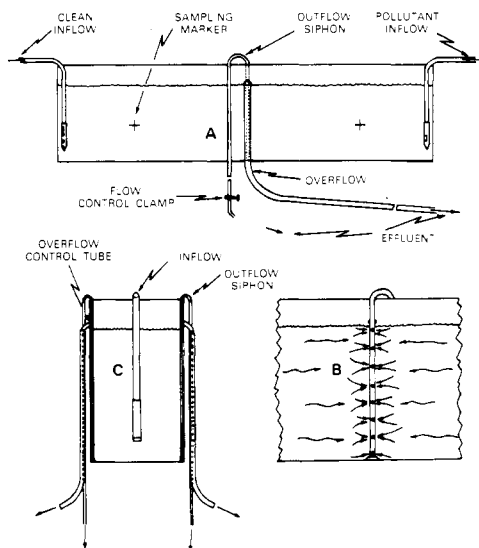


Figure 1. Avoidance trough  
A, side view; B, cut-away view; C, end view

Table II. Mean Length of Salmon

Nominal concns, total Fe, mg/l.	Initiation, cm	Termination, cm
Control	3.95	4.48
0.75	3.93	4.12
1.50	3.82	4.12
3.00	3.75	4.09
6.00	3.79	4.66
12.00	3.87	5.80

electric timer monitored the testing period and a buzzer sounded at the end of the 10-min experiment. Summation of the visit times for clean and polluted (modified) waters never exceeded  $\pm 4.0$  sec of the 600-sec test.

Only actively swimming fish were considered as exposing themselves to the modified and clean waters; therefore, any fish resting in one position for 95 sec was rejected and the test was initiated with another animal (5). The standard number of animals tested in each concentration was eleven, but more or less were tested depending on the number in the aquaria. The lowest concentration of metal was tested first, then the next concentration, and so forth. Each fish was used only for a single observation, tested, and returned to a marked chamber, thereby eliminating the possibility of retesting the same fish. After each test, while suspended iron was still being added, temperatures were recorded in both ends of the trough, and water samples were siphoned.

For each fish tested, the time spent in clean water as a percent of the total test time was calculated. A time response of 2–49% indicated preference for the modified water and a time response of 50% shows a neutral response. From 51–98% illustrates an avoidance of the polluted (modified) water.

Statistically significant avoidance reactions were tested by using the Kolmogorov-Smirnov, two-sample, one-tailed test (8). This is a nonparametric test of whether two populations have the same distribution—i.e., whether visits to the modified water were statistically different from visits to the clean water. However, each fish had to cross into the polluted section three times to be tested for significant avoidance, as suggested by Sprague and Drury (9).

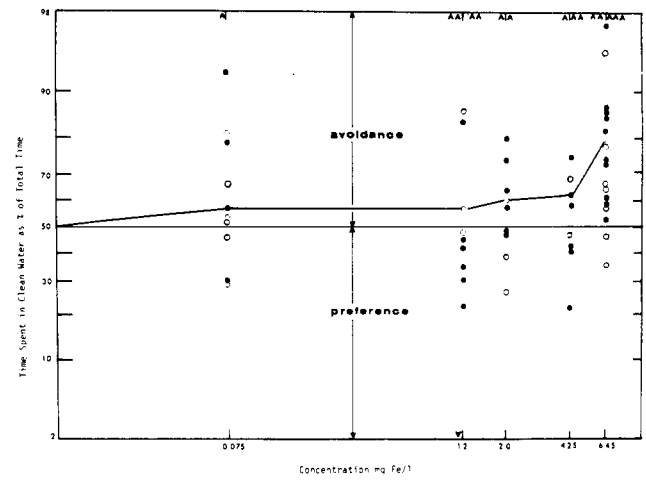
To find out whether salmon raised in control and salmon raised in specific concentrations varied significantly, a *t*-test was used. The difference between the mean percentage of total test time spent in clean water for each group (control and experimental) was tested at the 0.05 significance level. Before employing the *t*-test, the variances of the two groups were tested for homoscedasticity (homogeneity of variances) by Hartley's *F*-max test at the 0.05 significance level. This test relies on the statistic which is the variance ratio of the largest to the smallest of several sample variances (10).

### Results and Discussion

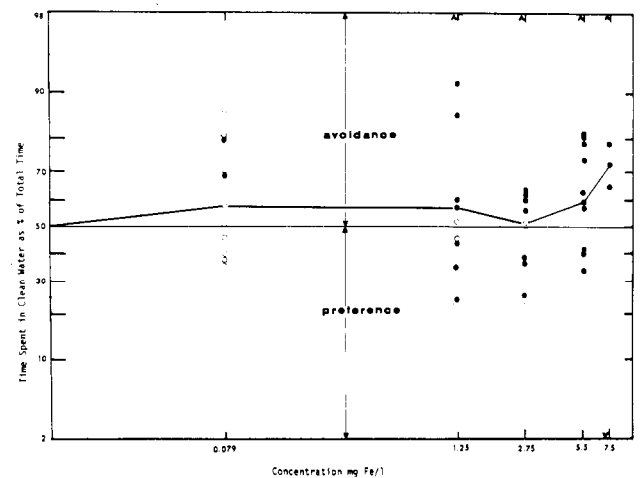
Salmon raised in control and salmon raised in nominal concentrations of lime-neutralized iron solutions elicited similar avoidance responses. Percents of time spent by coho salmon in clean water are presented graphically in Figures 2 and 3, with percents on the probability ordinate and measured concentrations on the logarithmic abscissa. Circles represent each fish, with statistically significant fish determined by the Kolmogorov-Smirnov test represented as the solid black circles. The median percents at each concentration are connected on the graphs.

At the lowest experimental (measured) concentration (0.075 mg Fe/l.), coho salmon raised in control have shown nearly neutral response, with the median time of 57.5% spent in the clean water. Almost the same reaction was observed in the next solution containing 1.20 mg Fe/l. At each remaining concentration, fish spent successively more time in clean water indicating a definite avoidance reaction at the range of 4.25–6.45 mg Fe/l.

Coho salmon, long-term tested in specific solutions of suspended iron hydroxide and exposed to the same or similar concentrations of the metal during avoidance experiments, show almost identical response as the fish raised in the control water. The measured test concentrations for the two salmon groups (control and experimental) were reason-



**Figure 2.** Avoidance responses as percent times spent in "clean" water to salmon raised in control  
Solid points = statistically significant choice of one fish; open points = nonsignificant response; line = median response; arrows = response more or less than 98% or 2% respectively



**Figure 3.** Avoidance responses as percent times spent in "clean" water to salmon raised in specific concentration of lime-neutralized ferric hydroxide

ably close. Hartley's *F*-max test indicated that the two group variances were homoscedastic. The *t*-tests showed that the two salmon groups means were not significantly different at each concentration. This suggests that salmon, exposed to specific nominal concentrations of iron since hatching, detected and avoided almost identical concentrations of iron hydroxide suspensions in the same pattern as salmon from control water.

As evidenced from the percent time graphs, tests with salmon at the lower concentrations showed almost neutral responses. At the higher concentrations, there was discretely noticeable turbidity in the modified waters. This may have been a key factor affecting the choices of the salmon for the "clean", less turbid waters. The fact that salmon from control and from specific concentrations elicited similar avoidance patterns seemed to indicate that the iron was not directly amenable to adverse chemoreception. However, the physical nature of the water—i.e., turbidity, may have been inimical to instinctive food-gathering behavior or visual orientation. The above sensory cues probably also hold true for brook trout. Sykora et al. (3) have shown that

juvenile brook trout, after exposure to 50 mg/l. of lime-neutralized iron suspensions were not directly (toxicologically) affected, but the attrition rate seemed dependent on adverse physical conditions.

In applying the results of the present study to nature, it must be remembered that the chemical characteristics of a particular water body have a major influence in determining which iron species predominate. This experiment simulated an aquatic environment with neutral pH and a high oxygen content, especially familiar to shallow, fast-flowing streams where aeration is maximized. Under these conditions, ferric hydroxide floc formation would most likely eliminate food sources and would cause fish to avoid this stretch of water. In contrast, a slightly acidic water with low oxygen content often contains large quantities of dissolved  $Fe^{2+}$ . Fish present in this environment would be exposed to the insidious deposition of iron on their gills and on the perivitelline membrane covering their eggs.

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## Evaluating Environmental Impacts of Stack Gas Desulfurization Processes

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■ The environmental impacts of the five most advanced and promising stack gas desulfurization processes, namely, limestone slurry scrubbing, lime slurry scrubbing, magnesia slurry scrubbing—regeneration to  $H_2SO_4$ , sodium solution— $SO_2$  reduction to sulfur, and catalytic oxidation have been examined. In these desulfurization processes, various kinds of pollutants/wastes are formed; the lime slurry scrubbing generates the greatest quantity while the catalytic oxidation the least. From this information, an attempt was made to assess the external costs of these control processes which could serve as an additional criterion for selecting control processes. The limestone slurry scrubbing process is the best choice when operating cost is the sole criterion. However, given the marketability of the resultant sulfuric acid, the catalytic oxidation process emerges as the best process when external costs are added to the operating cost.

In controlling pollution of all kinds, two apparently important facts are often neglected: Removal of a particular pollutant from a particular source and location may merely result in transforming that substance into different forms of pollutants in different locations; and, as first suggested by Leontief (1), the activity of pollution control itself requires inputs of various kinds, and the production of those inputs in turn may generate additional pollutants.

In some cases, although it is possible to recycle part of the residuals from consumption and production activities into useful products, economic considerations and physical limitations often lead to conversion of one form of pollutant into another form. In our ecological system, most pol-

lutants can, in principle, be removed from water and air streams by use of appropriate equipments, chemicals, and utilities, but what is left must be disposed of in solid form.

As previously asserted by Ayres and Kneese (2), Kohn (3), and Russel (4), the interrelations among the various waste streams call for an integrated approach to the environmental control, rather than handling pollution control as separate and isolated problems of disposing gas, liquid, and solid wastes. As an example, this study attempts to incorporate environmental interrelations in evaluating five specific air pollution control alternatives.

#### Five Stack Gas Desulfurization Processes to Be Evaluated

As coal becomes more and more important as a power plant fuel in this time of energy shortage, and the combustion of high-sulfur content Eastern coal would impair air quality, efforts have been made to devise efficient processes to control  $SO_2$  emission. Besides desulfurization before combustion, stack gas desulfurization is considered the most promising method that could also be applicable to all other fossil fuels. In this method, the sulfur-bearing fossil fuels are burned and the resulting pollutants, such as  $SO_2$  and particulates in the stack gases, are removed by physical and chemical means. Of the many stack gas desulfurization processes being developed, the following five processes are most promising: Limestone slurry, lime slurry, magnesia slurry—regeneration to sulfuric acid, sodium solution scrubbing— $SO_2$  reduction to sulfur, and catalytic oxidation—80% sulfuric acid.

In their recent study, McGlarmery and Torstrick (5) have compared in detail the control costs of these five processes designed for 90%  $SO_2$  removal from 500-MW new coal-fired power plants burning fuels containing 3.5% sul-