FINAL REPORT

State: West Virginia

Project Number: F-18-R

Project Type: Research

Project Title: Research Studies on Chronic Effects of Acid Water on Aquatic Organisms.

Period Covered: July 1, 1975 to June 30, 1977

Job Objectives: Jobs I-4 and I-5. To determine the acute effects of pH and iron and pH and aluminum, and the effects of combinations of iron and aluminum at different pH levels on brook trout.

ABSTRACT

Acute bioassay tests were conducted on brook trout to determine median lethal levels, incipient lethal levels and toxicity of mixtures of iron and aluminum. Both the LC_{50} and ILL decrease with decreasing pH. The application factors for iron and aluminum were 0.16 mg/l and 0.07 mg/l, respectively. These values correspond to factors of 1/6 and 1/13 of the LC_{50}s.

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BACKGROUND

The effects of acid water and of several metals which enter the aquatic ecosystem as a result of mining activities have been intensively studied. The chronic effects of low pH has been described by Mount (1973) for fathead minnows and by Menendez (1976a) for brook trout. The effects of various metals are described in a review by McKim et al (1973).

Two metals of concern for which little data are available are iron and aluminum. Both metals are found in abundance and are associated with acid mine drainage and land disturbances associated with mining activities.

The reaction of these metals in natural waters and previous studies on toxicity have been previously discussed (Menendez 1975, 1976a, 1976b).

The study described here is an attempt to determine the acute effects of iron and aluminum at different pH levels and the effects of combinations of iron and aluminum at different pH levels.

PROCEDURES

The test apparatus and chemical analysis of the dilution water used in these tests have been previously described by Menendez (1976a and 1976b).

All bioassays were of the continuous-flow type and were conducted in accordance with methods established by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975).

Toxicant solution used in the iron exposure was prepared by dissolving reagent grade ferrous sulfate ($FeSO_4 \cdot 7H_2O$) into 20 l of dilution water acidified with 10 ml of concentrated HCL to prevent precipitation. The aluminum stock solution was prepared by dissolving the powder form of the metal in 15 ml HCL per l of stock solution.

Toxicant for the combined tests was prepared by mixing equal quantities of the two-stock solutions. Stock solutionswere introduced from a Mariotte bottle to the diluter and delivery rates were adjusted to achieve the desired concentration of the metal. A pH control unit with an upper and lower control level was used to achieve different pH levels in the tests. Since both stock solutions were highly acidic, it was also necessary to introduce a solution of 1 N. sodium hydroxide into the system to maintain pH values at the same level as the controls. This was accomplished using a delivery system identical to that used for the toxicants.

Chemical analyses for iron, aluminum, pH, and dissolved oxygen were made daily in all test tanks.

Domestic brook trout (Salvelinus fontinalis), of the Edray strains were used in all exposures. They averaged 105 mm in length and were approximately 6 months old. All fish were acclimated for a period of 14 days before testing. During the last 7 days they were held at the exact experimental conditions of temperature and pH. In all tests fish were exposed to five concentrations of the metal. Each duplicate tank contained 10 fish.

In addition to determining the median lethal concentration (LC_{50}) at 96 hours, the Median Lethal Time (LC_{50}) was also determined. This is a measure of the time to 50% mortality at a given concentration of metal (Sprague 1964). The resistance times were determined by inspections after suitable periods of exposure, which increased more or less logarithmically. Survival for 7 days was considered to represent indefinitely long survival, signifying no direct lethal effects of the metal.

The combined effects of iron and aluminum are expressed as a proportion or fraction of the incipient lethal level following the concept of Lloyd (1961) and as described by Sprague (1970). This method is based on the use of toxic units which may be expressed as a fraction or proportion of its lethal threshold concentration (measured in the same units). Hence, if this number is greater than 1.0, more than half of a group of fish will be killed by this poison. If less than 1.0, half the fish will not be killed. Thus, one toxic unit equals the incipient LC_{50}. The strength of any toxicant may be calculated as follows:

$$\text{Toxic units} = \frac{\text{Actual concentration in solution}}{\text{Lethal threshold concentration}}$$

For combinations of toxicants, the toxic units for each are then added and if the total units are 1.0 or larger the mixture is considered to be lethal.

FINDINGS

Iron Exposure. The results of all lethal tests for iron are shown in Figures 1 and 2. The median tolerance limit (LC_{50}) at 96 hours exposure was determined at three pH levels. These values were determined so that results of tests could be applied to that part of the West Virginia water quality standards relating to toxic substances. Through graphical interpolation, the LC_{50} values for iron were determined to be 2.3 mg/l, 3.3 mg/l, and 8.4 mg/l at pH 5.0, 6.0 and 7.0, respectively (Fig. 1).

In Figure 2 there is a sharp differentiation between lethal and non-lethal concentrations of iron, and the line relating the concentration to survival time breaks and runs parallel to the time axis. The point at which the line breaks and runs parallel to the time axis is the incipient lethal level (ILL). From Figure 2, the ILL for iron is very close to 0.22, 3.2 and 6.0 mg/l at pH values of 5.0, 6.0 and 7.0, respectively.

Aluminum Exposure. The results of all lethal tests for aluminum are shown in Figures 3 and 4. The values presented were determined in the same manner as those for iron. The LC_{50} values for aluminum were 3.5 mg/l at pH 5.0 and 4.5 mg/l at pH 6.0. It was not possible to determine a value at pH 7.0 since mortality did not exceed 50% at any concentration within the 96 h. test period.

Figure 4 show the median mortality times for aluminum exposures. From these data the ILL was determined to be 3.3 mg/l, 3.9 mg/l and 7.9 mg/l at pH 5.0, 6.0 and 7.0, respectively.

Combined Toxicity of Iron and Aluminum. After determining the above values, any concentrations of iron or aluminum can then be expressed as a portion or fraction of the incipient lethal level (Lloyd 1961). The results plotted in this manner for brook trout are shown in Figures 5, 6 and 7. At a pH of 5.0, for any given fraction of the ILL, aluminum causes death more rapidly than iron (Fig. 5). At the higher pH levels the reverse is true (Fig. 6 and 7).

The combined action of the metals tested are also shown in Figures 5, 6 and 7. The LT_{50}'s are represented by adding the fraction of ILL for iron to the fraction of the ILL for aluminum. At 5.0 and 6.0 pH, fish died somewhat more rapidly than would be expected from the total fraction of the ILL. At these two pH levels the combined effects of the two metals are slightly less than additive. At pH 7.0 the combined toxicity falls somewhere between the TL_{50}'s for iron and aluminum singly.

DISCUSSION

The incipient lethal levels for iron and aluminum do not differ greatly from the LC_{50} values. The one exception to this is the ILL value for iron at pH 5.0 which was considerably less than the LC_{50} value. No comparisons can be made between results obtained and other work since there is a lack of literature dealing with toxicity of these two metals.

A comparison with data from Menendez (1976b) shows that the ILL is higher for both metals than the safe values he gives (1.37 mg/l for iron and 0.58 mg/l for aluminum). A comparison of the ILL with adult survival in chronic exposures shows close agreement for aluminum while the ILL for iron is greater. These data indicate that mortality rates of brook trout exposed to aluminum occur more rapidly and then cease, while those for iron occur over a longer time interval.

The results of the long-term effects of iron and aluminum on survival, growth, and reproduction establish the "no-effect level" or maximum acceptable toxicant concentration (MATC, as described by Mount and Stephens 1967) for brook trout at a pH of 7.0 in the water used for testing. Therefore, the application factor for iron (MATC/96h LC_{50}) based on a 96 h LC_{50} of 8.4 mg/l and a no effect level of 1.37 mg/l is 0.16 mg/l. The application factor for aluminum based on a ILL of 7.9 mg/l and a no effect level of 0.58 mg/l is 0.07 mg/l. These values correspond to a factor of 1/6 of the 96 h LC_{50} for iron and 1/13 of the 96 h LC_{50} for aluminum

Mixtures of iron and aluminum reduce survival times at pH levels of 5.0 and 6.0. This phenomenon has also been reported for copper and zinc by Lloyd (1961) and Sprague (1964). Possibly some effects of pH are responsible for adding to the toxicity of the mixture at these two pH levels. Based on the data, it appears that when mixtures of these two metals exceed 1 toxic unit, a 50% mortality can be expected within a 96 h period.

SUMMARY

Median tolerance levels (LC_{50} at 96 h exposure) for iron were 2.3, 3.3 and 8.4 mg/l at pH 5.0, 6.0 and 7.0, respectively.

Incipient lethal levels for the same pH levels were 0.22, 3.2 and 6.0 mg/l.

Median tolerance levels at 96 h exposure for aluminum were 3.5 and 4.5 mg/l at pH 5.0 and 6.0.

Incipient lethal levels were 3.0, 3.9 and 7.9 mg/l at pH 5.0, 6.0 and 7.0.

The application factors for iron and aluminum are 0.16 mg/l and 0.07 mg/l respectively. These values correspond to a factor of 1/6 to 1/13 of the LC_{50}'s.

The data show that when mixtures of these two metals exceeds 1 toxic unit, 50% mortality can be expected within 96 h.

RECOMMENDATIONS

It is recommended that additional testing be performed to more fully understand the combined effects of mixtures of these two metals. These tests should consist of chronic exposures.

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LITERATURE CITED

Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates and amphibians. National Environmental Research Center. EPA. Corvallis, Oregon. 61 pp.

Lloyd, R. 1961. The toxicity of zinc sulphate to rainbow trout. Ann. Appl. Biol., 48(1):84-94.

McKim, J. E., G. M. Christensen, J. H. Tucker, D. A. Benoit, and M. J. Lewis. 1973. Effects of pollution on freshwater fish. Jour. WPCF. 45(6):1370-1407.

Menendez, R. 1975. Conduct acute and chronic bioassay tests using brook trout with iron and aluminum at various pH levels. D-J Project F-18-R-3. W. Va. Dep. Nat. Resour., 1 p. mimeo.

_____. 1976a. Chronic effects of reduced pH on brook trout (Salvelinus fontinalis). J. Fish. Res. Bd. Can., 33(1):118-123.

_____. 1976b. Chronic effects of iron and aluminum on brook trout. Final Report, D-J Project F-18-R. W. Va. Dep. Nat. Resour. 16 p. mimeo.

Mount, D. I. 1973. Chronic effects of low pH on fathead minnow survival, growth and reproduction. Water Resour., 1:21-29.

_____ and C. E. Stephen. 1967. A method for establishing acceptable toxicant limits for fish, malathion and the butoxyethanol ester of 2, 4-D. Trans. Am. Fish. Soc. 96(2):185-193.

Sprague, J. B. 1964. Lethal concentrations of copper and zinc for young Alantic salmon. J. Fish. Res. Bd. Can., 21(1):17-26.

_____. 1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Resour. 4:3-32.

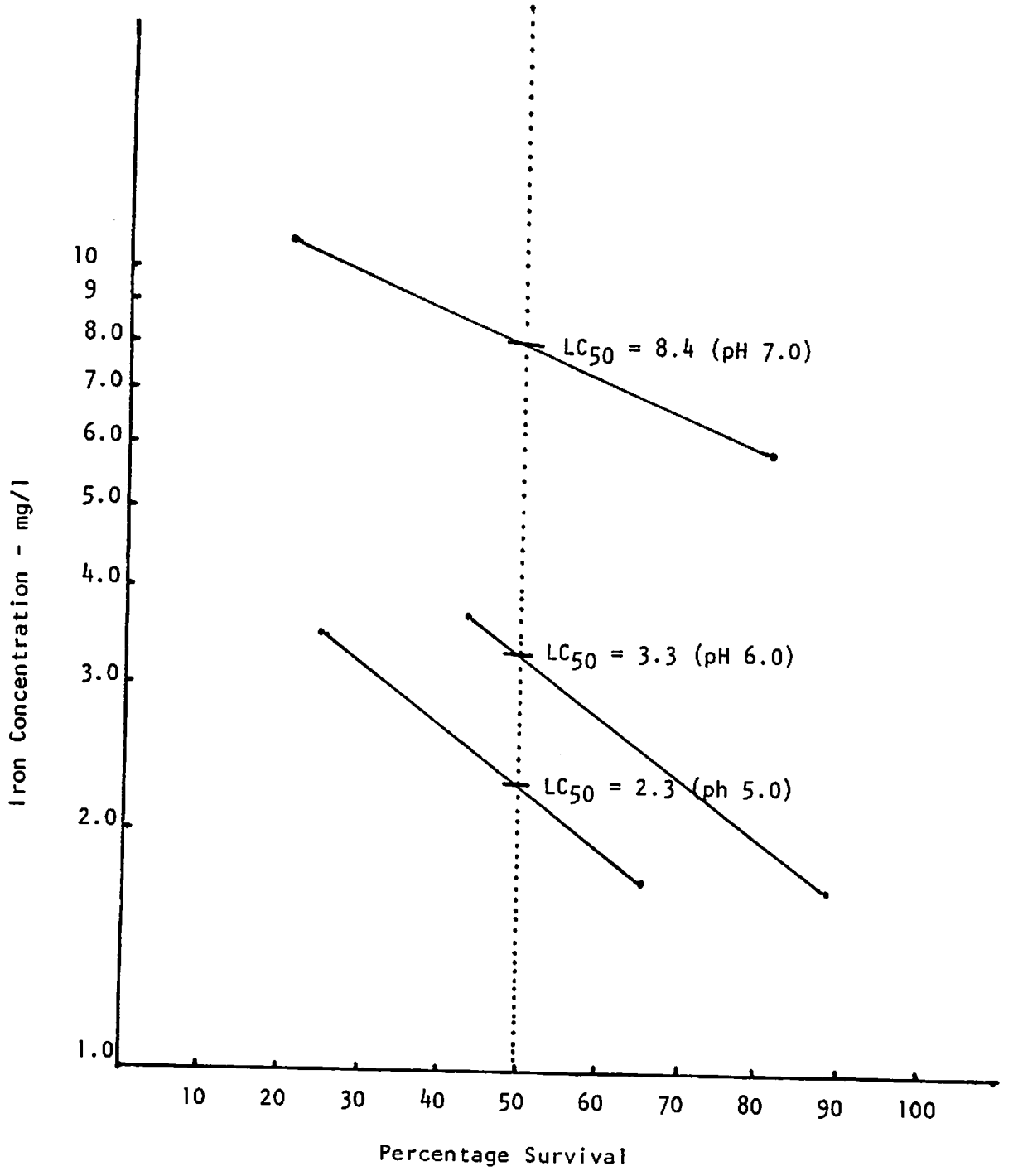

Figure 1. Median tolerance limits (LC_{50}) for iron at different pH levels.

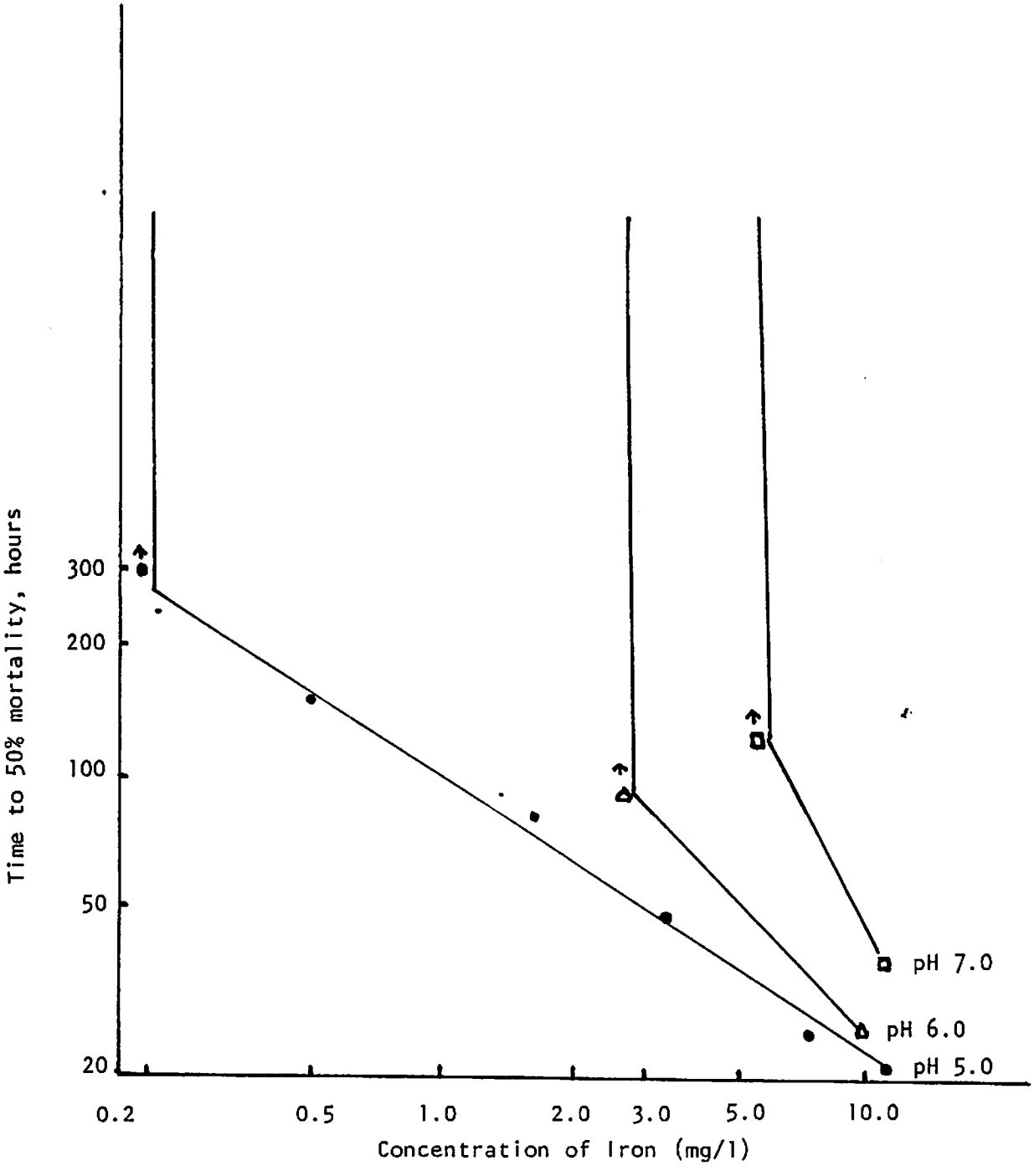

Figure 2. Median mortality times of brook trout exposed to iron. The straight lines fitted to the points break and run parallel to the time axis at the incipient lethal level.

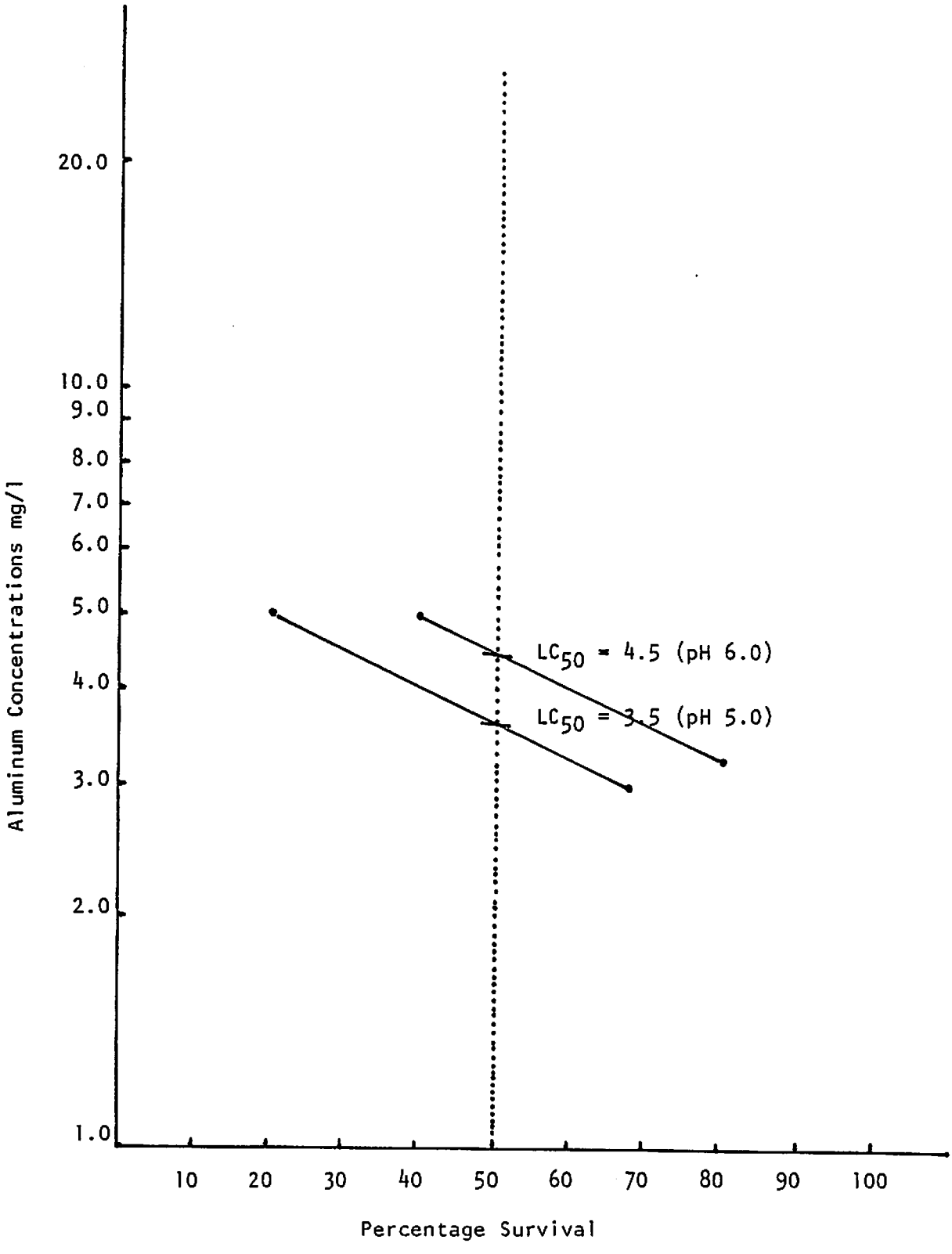

Figure 3. Median tolerance limits (LC_{50}) for aluminum at different pH levels.

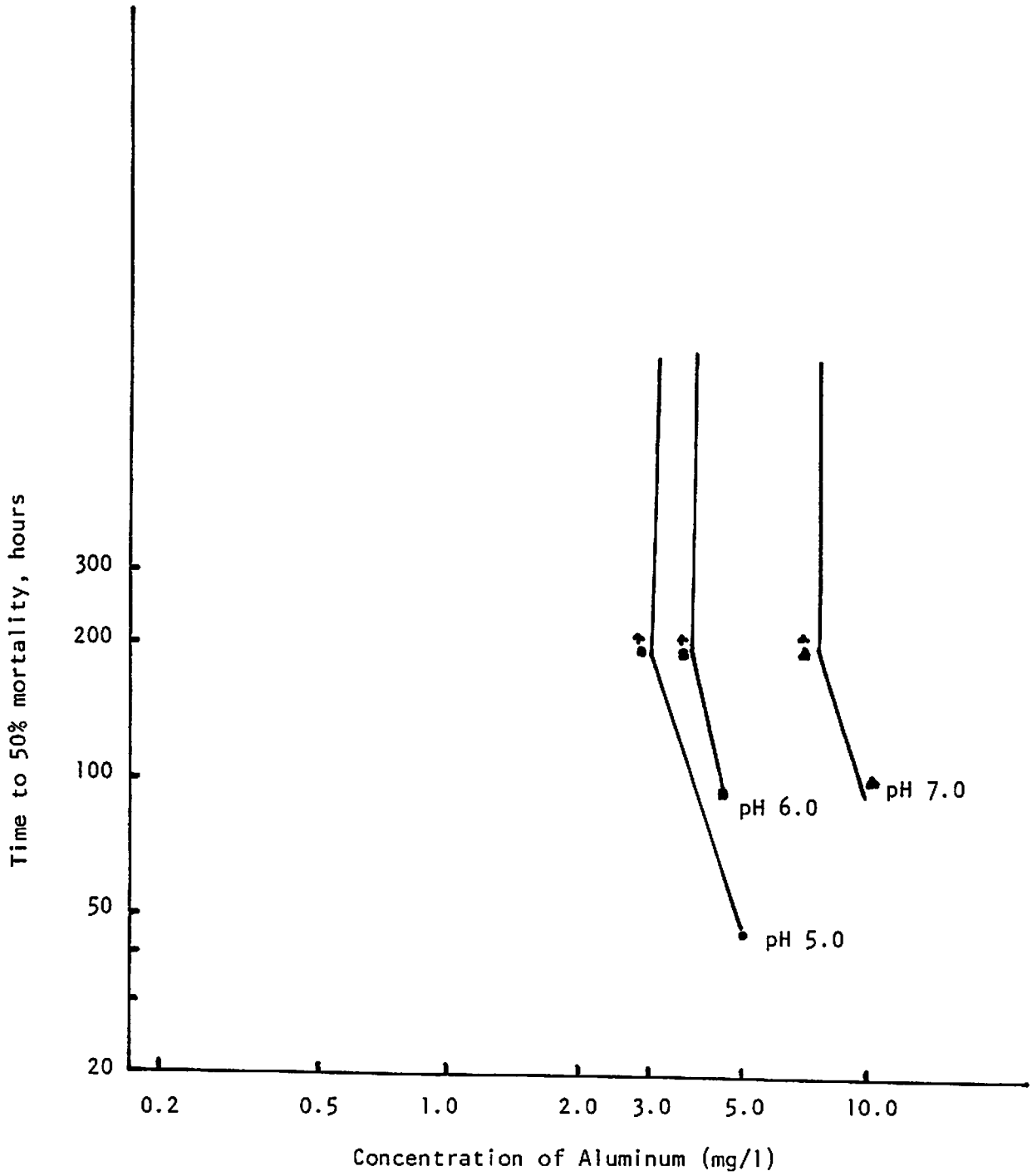

Figure 4. Median mortality times of brook trout exposed to iron. The straight lines fitted to the points break and run parallel to the time axis at the incipient lethal level.

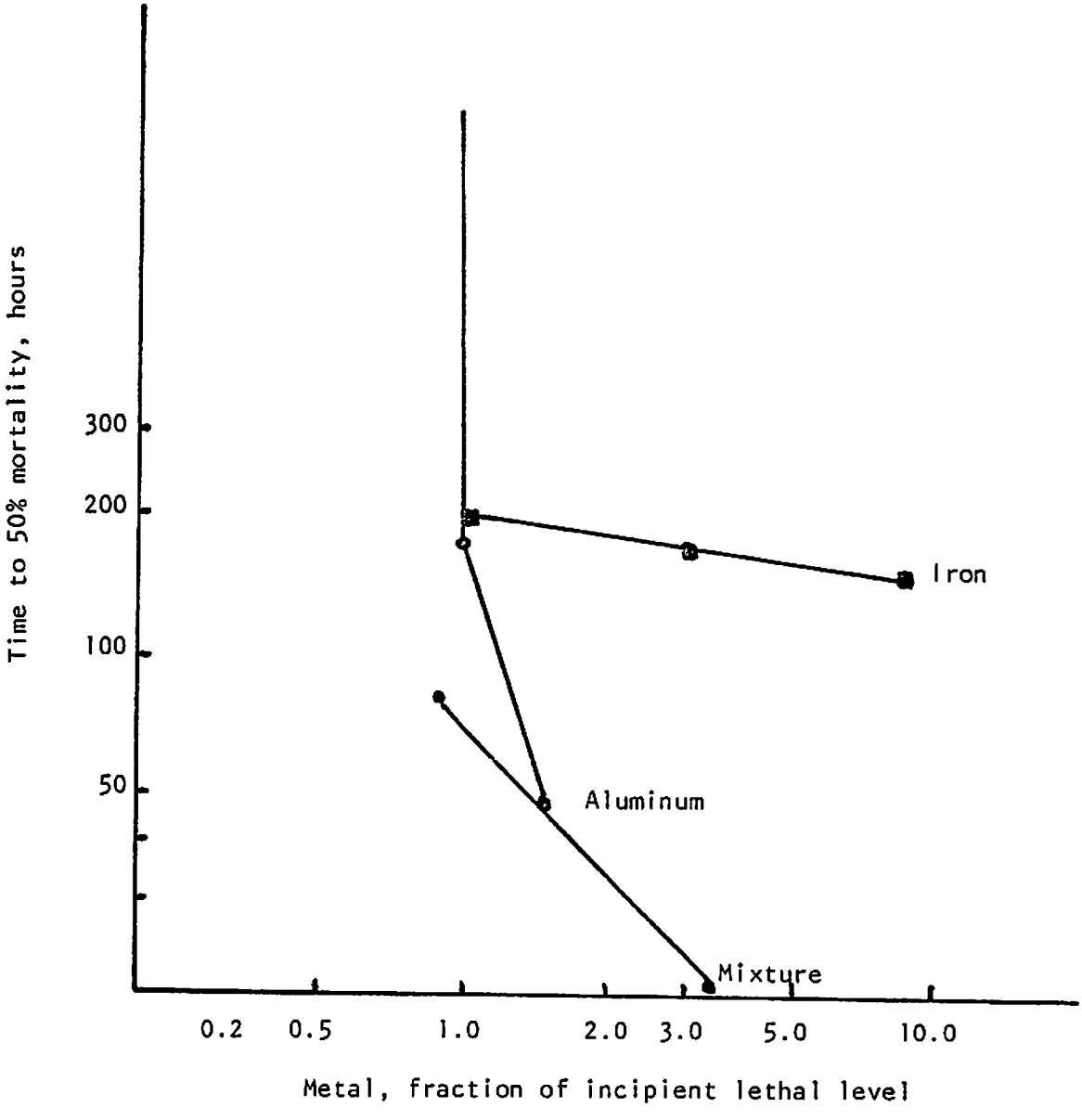

Figure 5. Comparison of median mortality times for brook trout exposed to solutions of iron, aluminum and mixtures at pH 5.0 concentrations are expressed as fractions of the ILL.

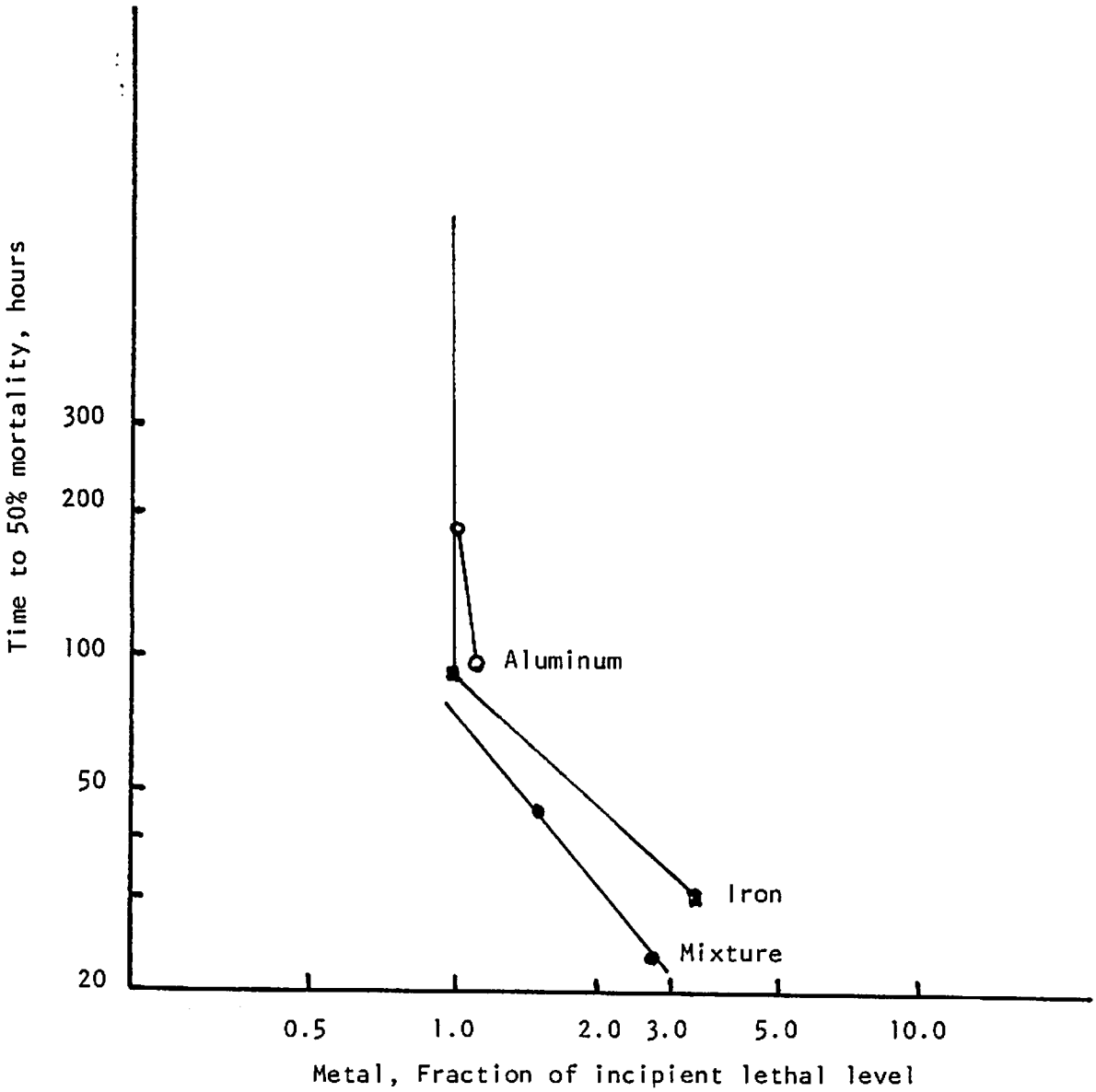

Figure 6. Comparison of median mortality times for brook trout exposed to solutions of iron, aluminum and mixtures at pH 6.0 concentrations are expressed as fractions of the ILL.

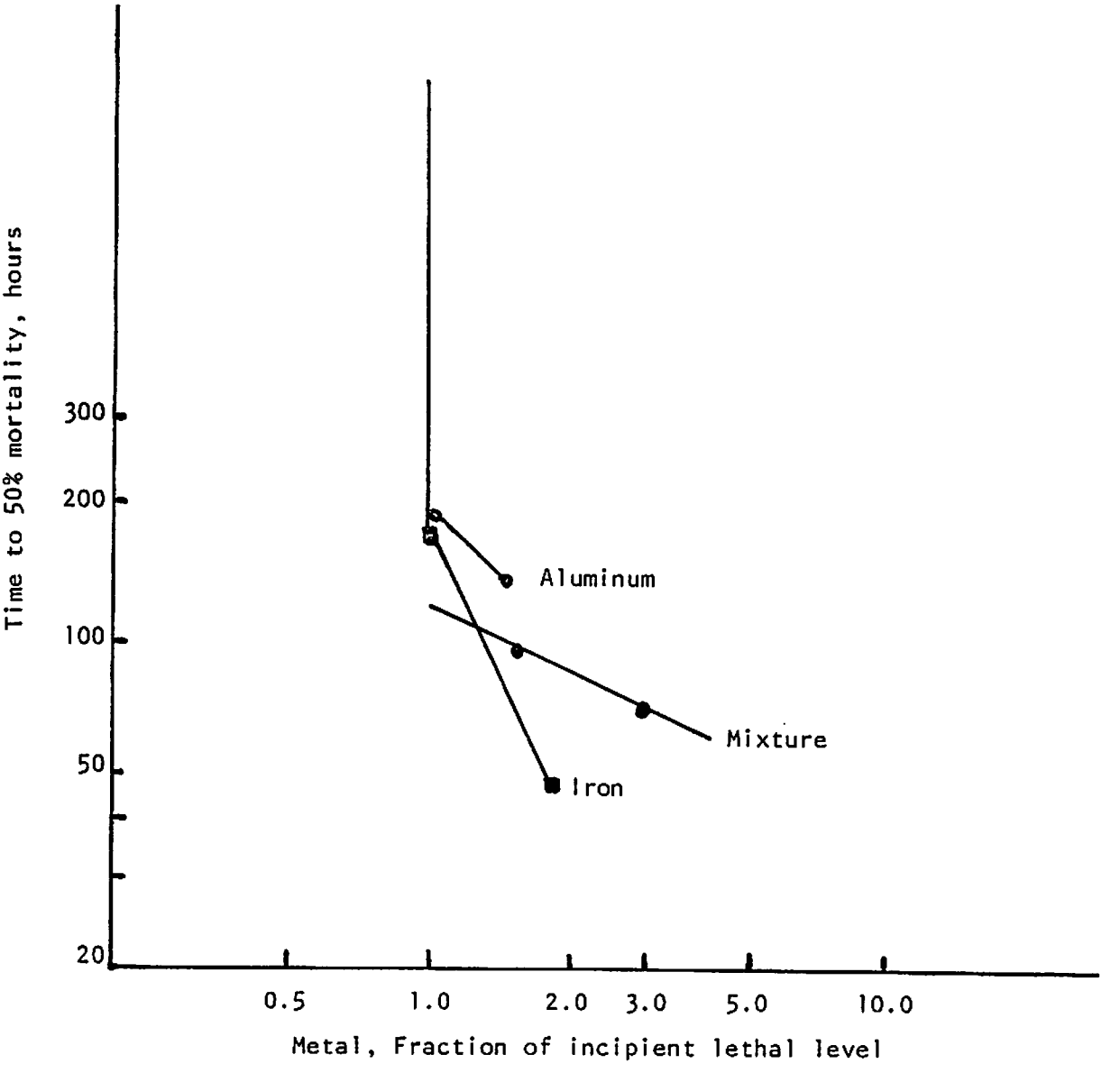

Figure 7. Comparison of median mortality times for brook trout exposed to solutions of iron, aluminum and mixtures at pH 7.0 concentrations are expressed as fractions of the ILL.