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on

# "THE TOXICITY OF SELECTED SULFHYDRYL COLLECTORS TO RAINBOW TROUT"

by

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# INTRODUCTION

Since the discovery of the specificity of xanthate for sulfide minerals, use of this reagent as a collector has increased to the point where the metallurgical industry in this country alone uses approximately eight million pounds annually. In addition to xanthate, considerable quantities of other collectors, such as thiophosphate, thiocarbamate, carboxylate, sulfonate, and amine are also utilized. Billions of gallons of water are used in conjunction with these collectors, and although most flotation plants reclaim water used in processing, some water containing residual collector is lost to natural receiving waters. It is expected that these chemicals will exhibit toxic effects on aquatic life.

The toxic effects of some flotation collectors have already been demonstrated. For example, Laclerc and Devlaminck[1] have shown the limiting concentrations of sodium oleate, palmitate, and stearate to be 10-12 ppm in distilled water and in the case of sodium oleate, 900-1,000 ppm in hard water.

Schmid and Mann[2] have reported that a concentration of 5 ppm dodecylbenzenesulfonate is toxic to trout. These results are similar to those reported by Henderson, Pickering, and Cohen[3] for minnows.

Because of the quantity of collectors and water used in flotation processing, toxicity data are needed to determine the potential of these reagents for harming fish life. The objective of this investigation was to establish the 96-hour median lethal concentration (LC50-96) of selected collectors, namely ethylxanthate, isopropylxanthate, amylxanthate, diethyldithiophosphate, dibutyldithiophosphate, isopropylethylthiocarbamate, octylhydroxamate, decylhydroxamate, and tallow amine.

## EXPERIMENTAL MATERIALS AND PROCEDURE

Experiments were conducted with commercially available and purified collectors. Purification of the xanthates was effected by dissolving commercially available potassium xanthate in acetone and precipitating the salt with petroleum ether. The purified xanthate was filtered, washed with petroleum ether, dried under vacuum, and stored in a dessicator. The purity, based on potentiometric titration, was measured to be 99 percent.

Pure dithiophosphates were obtained by reaction of dithiophosphoric acid and the stiochiometric amount of potassium hydroxide dissolved in methyl alcohol. This solution was placed in a vacuum dessicator until the methyl alcohol and water formed by reaction were removed as vapor. The remaining material was dissolved in acetone and purified using the same process described for xanthate. Potentiometric titration revealed that this material was 99 percent in purity.

Pure isopropylethylthiocarbamate was prepared by vacuum distillation of Z-200 at 103°C and 10.6 mm mercury pressure. The distillate obtained before constant boiling temperature was reached was discarded as this product contained impurities of low molecular weight.

Octylhydroxamate was prepared by combining methyloctanoate with hydroxylamine hydrochloride dissolved previously in a solution of methanol and potassium hydroxide. Purification of potassium octylhydroxamate was effected by dissolution in methanol followed by recrystallization.

The experimental water used in the investigation was a naturally hard spring water and which is a source of water for a trout fish hatchery. The pH of the water was 8.6; other constituents are given in Table 1.

Fingerlings obtained from three different egg sources were used in the study. The fingerlings, which ranged in average weight from one to ten grams, were transported to the laboratory in polyethylene bags under an oxygen atmosphere. A 24-hour period was allowed to relieve the fish of the stress incurred during transportation.

After this time period, 20 fish were added to each of the test aquaria. The volume of water was adjusted to maintain a weight to volume ratio of two grams of wet fish per liter of solution. This ratio, suggested by Sprague[4] for static tests, is more than that required for consumption by trout which is 0.22 cc $O_2$/gm/hr at 14.7°C or 0.047 cc $O_2$/gm/hr at 11.6°C as found by Heilbrunn[5] and van Dam[6], respectively. Oxygen saturation was maintained in the control and test aquaria by bubbling air into the water contained in each of the aquaria. Oxygen content was monitored periodically to ensure that saturation was maintained.

Table 1. Composition of Experimental Water.

| Substance | ppm |
|---|---|
| Total Dissolved Solids | 338 |
| Volatile Dissolved Solids | 102 |
| Total Hardness | 348 |
| Carbonate Hardness | 203 |
| Non-Carbonate Hardness | 145 |
| Calcium | 83 |
| Magnesium | 34 |
| Sodium | 2 |
| Potassium | 1 |
| Iron | 0 |
| Manganese | 0.2 |
| Alkalinity (Methyl Orange) | 203 |
| Chlorides | 2.5 |
| Sulfate | 85 |
| Silica | 13 |
| Nitrates | 0.4 |
| Total Phosphate | 0.7 |
| pH | 8.6 |

Approximately one hour after the fish had been transferred to the test aquaria, a solution containing the collector was added. Water temperature was maintained at 12 ± 1°C, the temperature preferred by trout, unless temperature was the parameter under investigation.

Two of the collectors, xanthate and dithiophosphate, are capable of undergoing oxidation by dissolved oxygen. To establish whether oxidation of these collectors was occurring during the course of the bioassays, oxidation of these collectors was followed by titrating the solutions with silver nitrate and monitoring the silver ion activity with a silver/silver sulfide electrode. These experiments were conducted under the conditions of the bioassays but in the absence of fish.

## EXPERIMENTAL RESULTS

The initial experimental work involved establishing the extent of oxidation of xanthate and dithiophosphate by dissolved oxygen as a function of time at 12°C and pH 8.6. Oxygen saturation was maintained at 8 to 9 ppm by constant aeration. The measured concentrations of xanthate and dithiophosphate in solution are presented as a function of time in Tables 2 and 3. The values show clearly that essentially no collector oxidation or decomposition occurred during the course of the experiments.

Table 2. Solution Oxidation/Decomposition of Ethylxanthate (EX) and Amylxanthate (AX).

| Time (min) | EX (ppm) | AX (ppm) |
|---|---|---|
| 0 | 52.4 (initial) | 52.2 (initial) |
| 30 | 52.3 | 51.3 |
| 720 | 51.5 | 52.0 |
| 2880 | 52.6 | 51.3 |
| 5760 | 52.6 | 51.3 |

Table 3. Solution Oxidation/Decomposition of Diethyldithiophosphate (EDTP) and Disecondarybutyldithiophosphate (BDTP).

| Time (min) | EDTP (ppm) | BDTP (ppm) |
|---|---|---|
| 0 | 500 (initial) | 300 (initial) |
| 30 | 500 | 298 |
| 180 | 498 | 304 |
| 1440 | 496 | 300 |
| 4320 | 498 | 294 |

Bioassays were conducted first with purified potassium ethylxanthate. Median lethal concentrations for 96-hour exposures were established by plotting median survival times, LC50, as a function of the logarithm of the xanthate concentration. The data in Figure 1 show a well defined break between the concentrations which did not cause 50 percent mortality and those concentrations which caused 50 percent mortality in less than 96 hours and also show that source of eggs has no effect on sensitivity to xanthate. Those points with extending arrows at 96 hours indicate that LC50 was not attained in 96 hours.

It can also be noted that concentrations of less than 15 ppm potassium ethylxanthate did not cause 50 percent mortality in 96 hours, while concentrations greater than 20 ppm potassium ethylxanthate show decreasing median survival times as the concentration of xanthate is increased. Concentrations of potassium ethylxanthate greater than 25 ppm are seen to cause 50 percent mortality in approximately 20 hours. From the break in the bioassay data, the LC50-96 is seen to lie between 15 and 50 ppm potassium ethylxanthate.

Experiments were also run with commercially available potassium ethylxanthate, Z-3, the results of which are presented in Figure 2. As shown, LC50-96 was found to lie between 15 and 17 ppm.

Median survival times were established next for commercially available sodium ethylxanthate (Aeroxanthate 325 and Z-4) and sodium isopropylxanthate (Aeroxanthate 343 and Z-11). As can be noted in Figures 3, 4, 5, and 6, the median lethal concentration of Aeroxanthate 325 is 14 to 16 ppm, of Z-4 is 13 to 15 ppm, of Aeroxanthate 343 is 18 to 20 ppm, and of Z-11 is 18 to 20 ppm.

These results are considerably different from those reported by J. R. Hawley[7] on flathead minnows. Unfortunately, neither the experimental conditions nor the techniques employed were presented by Hawley so that a comparison with his data cannot be made.

Median survival times in the presence of various concentrations of purified potassium amylxanthate are plotted in Figure 7. As shown, 50 percent mortality was not caused by concentrations of less than 70 ppm in 96 hours. The LC50-96 is seen to lie in the range of 70-75 ppm potassium amylxanthate.

Experiments were also run with a potassium amylxanthate (Aeroxanthate 350) that is available commercially for industrial use. Median survival time is listed as a function of potassium amylxanthate concentration in Table 4. As can be noted, the LC50-96 lies between 70 and 80 ppm.

The role that temperature assumed in these systems was also investigated (Figure 8). Median lethal concentration of ethylxanthate was found to be the same at 8° and 12°C. Increasing the temperature to 16° and 20°C decreased this value from 15-20 ppm to 1.8-2.0 and 1.5-1.8 ppm, respectively.

Experiments were conducted next with purified and commercially available dithiophosphates. The median lethal concentration of purified potassium diethyldithiophosphate is 800-825 ppm at 12°C. See

Table 4. Median Survival Time for Various Concentrations of Commercial Potassium Amylxanthate (Aeroxanthate 350) at 12°C.

| Median Survival Time (hr) | Aeroxanthate 350 Concentration (ppm) |
|---|---|
| * | 50 |
| * | 70 |
| 69 | 80 |
| 61 | 90 |
| 50 | 100 |

*LC50 not attained

Figure 9. In the case of purified potassium disecondarybutyldithiophosphate, a value for LC50-96 was not established, since no deaths of trout fingerlings were observed at 1,000 ppm. Experiments with concentrations above this level were not conducted.

Median lethal concentrations of the commercially available forms of dithiophosphate are shown in Figures 10 and 11. Median lethal concentrations of commercially available sodium diethyldithiophosphate (Sodium Aerofloat) are 400-410 ppm and 310-330 ppm at 12°C and 16°C, respectively. The median lethal concentration of commercially available sodium disecondarybutyldithiophosphate (Aerofloat 238) is 600-625 ppm at 12°C.

The median lethal concentrations of purified and commercially available isopropylethylthiocarbamate (Z-200) were also established. As shown in Figures 12 and 13, these values are 40-45 ppm and 45-48 ppm respectively, at 12°C.

The toxic effects of two non-sulfhydryl collectors, hydroxamate and amine, were also investigated. The median lethal concentrations of purified potassium octylhydroxamate and potassium decylhydroxamate are 6-7 ppm and 10-12 ppm, respectively. See Figures 14 and 15.

As shown in Figure 16, the median lethal concentration of primary tallow amine is 2.0-2.5 ppm.

## DISCUSSION OF RESULTS

The conditions under which the bioassays were conducted were characteristic of the natural habitat of trout. Because of the similarity in pH, mineral content and temperature, the effects of acclimatization may be excluded from further consideration as the primary cause of death of the fish.

The accumulation of waste products, especially lactic acid, during transportation may be excluded from consideration as a holding period of 24 hours was used to allow ample time for the fish to compensate for the lactic acid.[8]

Significant oxidation or decomposition of the xanthate or dithiophosphate during the course of the experiment did not occur. As a result, the presence of these collectors can be assumed to be the primary cause of respiratory stress and death.

During the course of the experiments, it was noted that the presence of these reagents caused a change in the respiratory pattern of the fish. Upon addition of the reagents, an increase in breathing movements was noted which, in turn, caused an increase in the volume of collector-bearing solution to pass over the gill surfaces. Since the gill surfaces are probably the site of absorption of most poisons[9] and since mucus precipitates were abserved in the presence of amines only, xanthate, dithiophosphate, isopropylethylthiocarbamate and hydroxamate are assumed to affect the trout by way of the gills.

Although the determination of the toxic mechanism for the sulfhydryl collectors and hydroxamate was beyond the scope of this investigation, two possible mechanisms are: (1) absorption through the lamellae and reaction with hemoglobin, and (2) adsorption on the gill surfaces resulting in interference with waste removal and oxygen utilization.[10,11] Either of these mechanisms could account for the period of equilibrium loss and swimming difficulty if these symptoms were caused by the accumulation of urea and lactic acid. The accumulation of these waste products caused by the interference from collector with normal excretory processes, could reach lethal levels and kill the fish. The loss of equilibrium, decreased skeletal muscle control and the disruption of the central nervous system have been shown to be the result of lethal lactic acid concentrations.[11] However, the fact that more amylxanthate than ethylxanthate, that more dibutyldithiophosphate than diethyldithiophosphate and that more decylhydroxamate than octylhydroxamate are required to attain LC50 indicates that absorption of these collectors through the lamallae is involved. If adsorption on the gill surfaces were involved, less amylxanthate than ethylxanthate, for example, would be required, since greater adsorption of the longer-chain homologue would occur at equal concentration.

With regard to data established with purified and commercially available collectors, the fact that essentially the same values of LC50-96 were obtained for purified and commercially available ethyl and amylxanthate and isopropylethylthiocarbamate indicates that the commercially available samples were essentially 100 percent active and also that the samples did not contain any toxic impurities.

In the case of the dithiophosphates, however, considerable differences in values of LC50-96 were obtained between the pure and impure forms of the reagent. The fact that values of LC50-96 were considerably lower for the commercially available forms can probably be attributed to the hydrogen sulfide content of these samples. That is, hydrogen sulfide is a reaction product in the manufacture of dithiophosphates, accounting for its presence in the sample. Secondly, the distinctive odor of $H_2S$ was apparent when dithiophosphate was added to the test water. Thirdly, the lethal concentration of $H_2S$ is 1-3 ppm.[12]

Median lethal concentration of sodium ethylxanthate is lower than that for sodium isopropylxanthate or potassium ethylxanthate. This is expected in view of the differences in molecular weight between these collectors. That is, the molecular weight of sodium ethylxanthate is 144 gm/mole, that of sodium isopropylxanthate is 158 gm/mole, and that of potassium ethylxanthate is 160 gm/mole. If the median lethal concentration of potassium ethylxanthate is 15 to 17 ppm, the median lethal concentration of sodium ethylxanthate should be 15 to 17 ppm x 144/160 or 13 to 15 ppm, which is the value determined experimentally.

## CONCLUSIONS

The following conclusions can be drawn from this investigation with rainbow trout:

1. the median lethal concentration of Aeroxanthate 325 (sodium ethylxanthate) is 14-16 ppm at 12°C,
2. the median lethal concentration of Z-4 (sodium ethylxanthate) is 13-15 ppm at 12°C,
3. the median lethal concentration of Z-3 (potassium ethylxanthate) is 15-17 ppm at 12°C,
4. the median lethal concentration of pure potassium ethylxanthate is 15-20 ppm at 8°C and 12°C, 1.8-2.0 ppm at 16°C, and 1.5-1.8 ppm at 20°C,
5. the median lethal concentration of Aeroxanthate 343 (sodium isopropylxanthate) is 18-20 ppm at 12°C,
6. the median lethal concentration of Z-11 (sodium isopropylxanthate) is 18-20 ppm at 12°C,
7. the median lethal concentration of Aeroxanthate 350 (potassium amylxanthate is 70-80 ppm at 12°C,
8. the median lethal concentration of pure potassium amylxanthate is 70-75 ppm at 12°C,
9. the median lethal concentration of Sodium Aerofloat is 400-410 ppm at 12°C and 310-330 ppm at 16°C,
10. the median lethal concentration of pure potassium diethyldithiophosphate is 800-825 ppm at 12°C,
11. the median lethal concentration of Aerofloat 238 is 600-625 ppm at 12°C,
12. the median lethal concentration of pure potassium disecondarybutyldithiophosphate is greater than 1,000 ppm at 12°C,
13. the most toxic constituent of commercially available dithiophosphates is hydrogen sulfide,
14. the median lethal concentration of Z-200 (isopropylethylthiocarbamate) is 45-48 ppm at 12°C,
15. the median lethal concentration of pure isopropylethylthiocarbamate is 40-45 ppm at 12°C,

16. the median lethal concentration of pure potassium octylhydroxamate is 6-7 ppm at 12°C,

17. the median lethal concentration of pure potassium decylhydroxamate is 10-12 ppm at 12°C, and

18. the median lethal concentration of commercially available primary tallow amine is 2.0-2.5 ppm at 12°C.

## ACKNOWLEDGEMENTS

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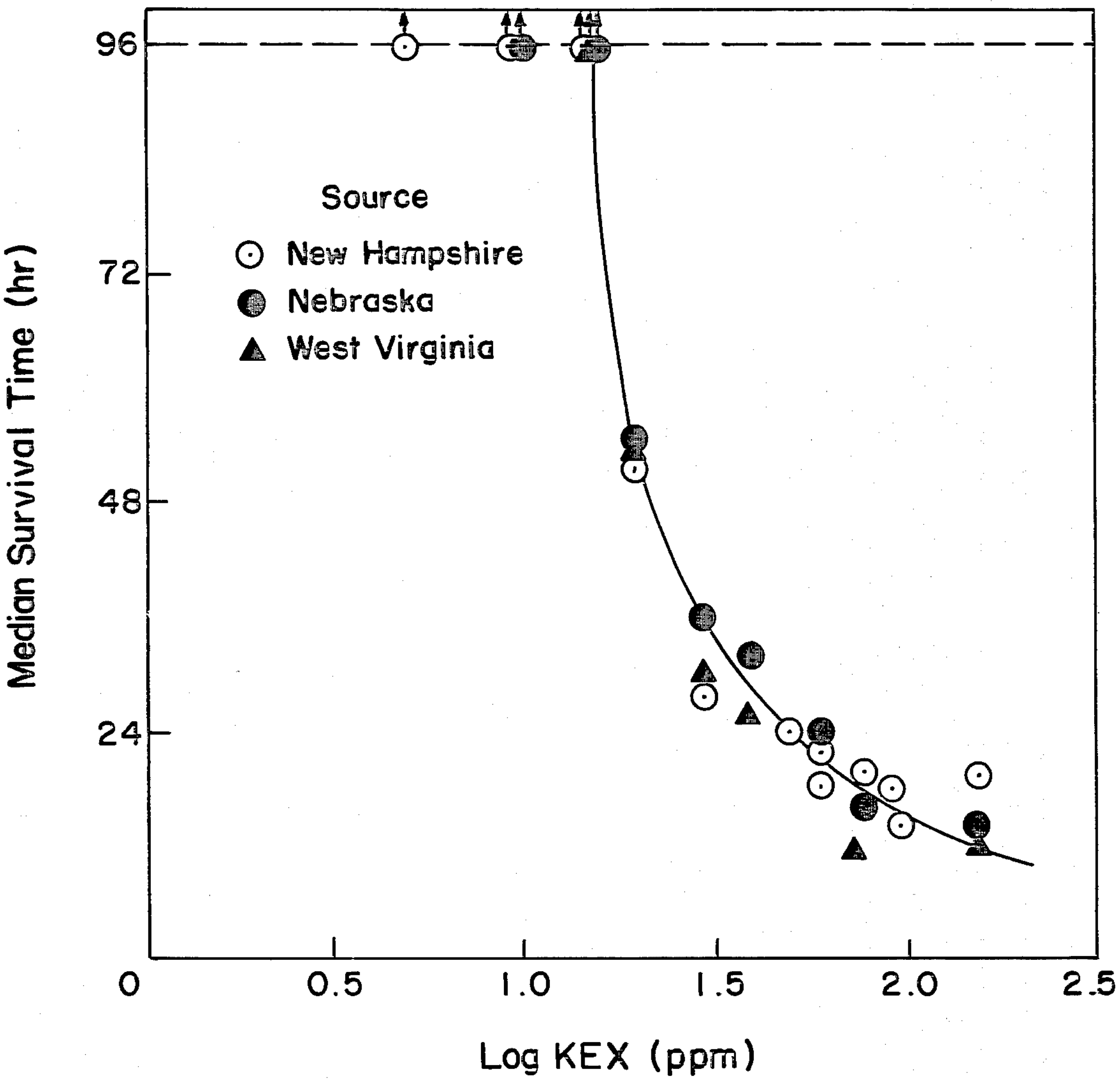

Figure 1. Median survival time as a function of the logarithm of purified potassium ethylxanthate concentration and egg source of fingerlings at 12°C.

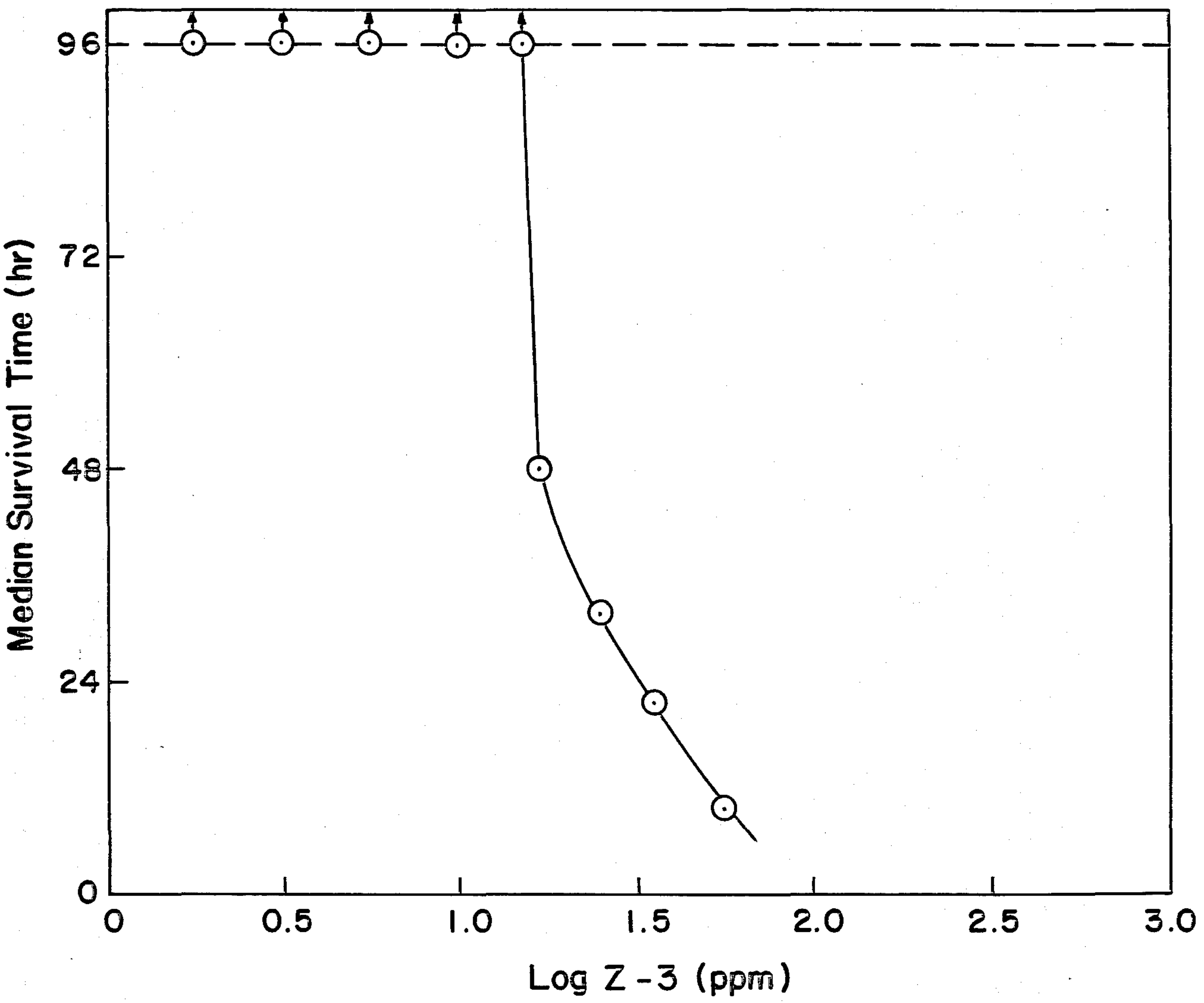

Figure 2. Median survival time as a function of the logarithm of Z-3 (potassium ethylxanthate) concentration at 12°C.

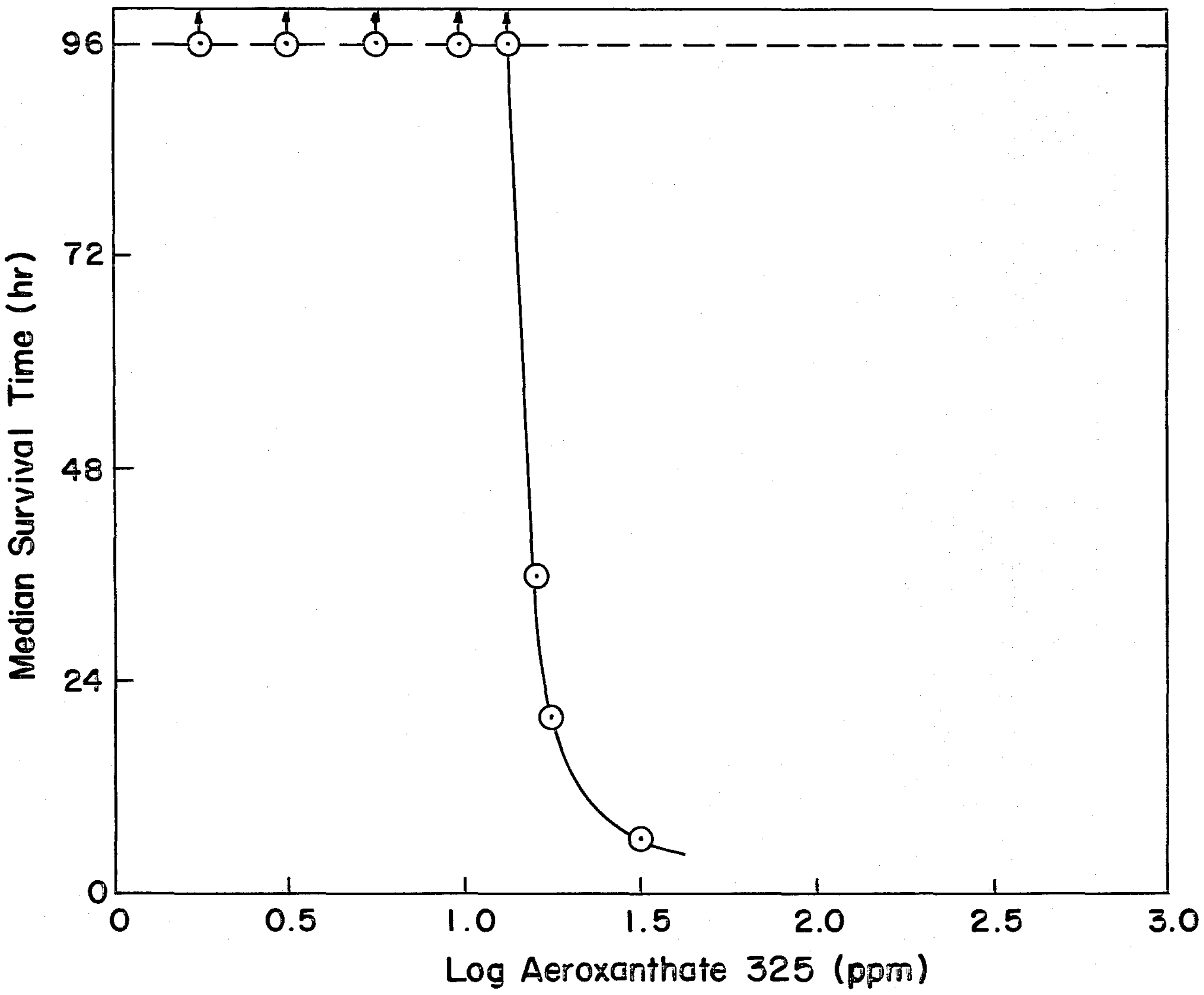

Figure 3. Median survival time as a function of the logarithm of Aeroxanthate 325 (sodium ethylxanthate) concentration at 12°C.

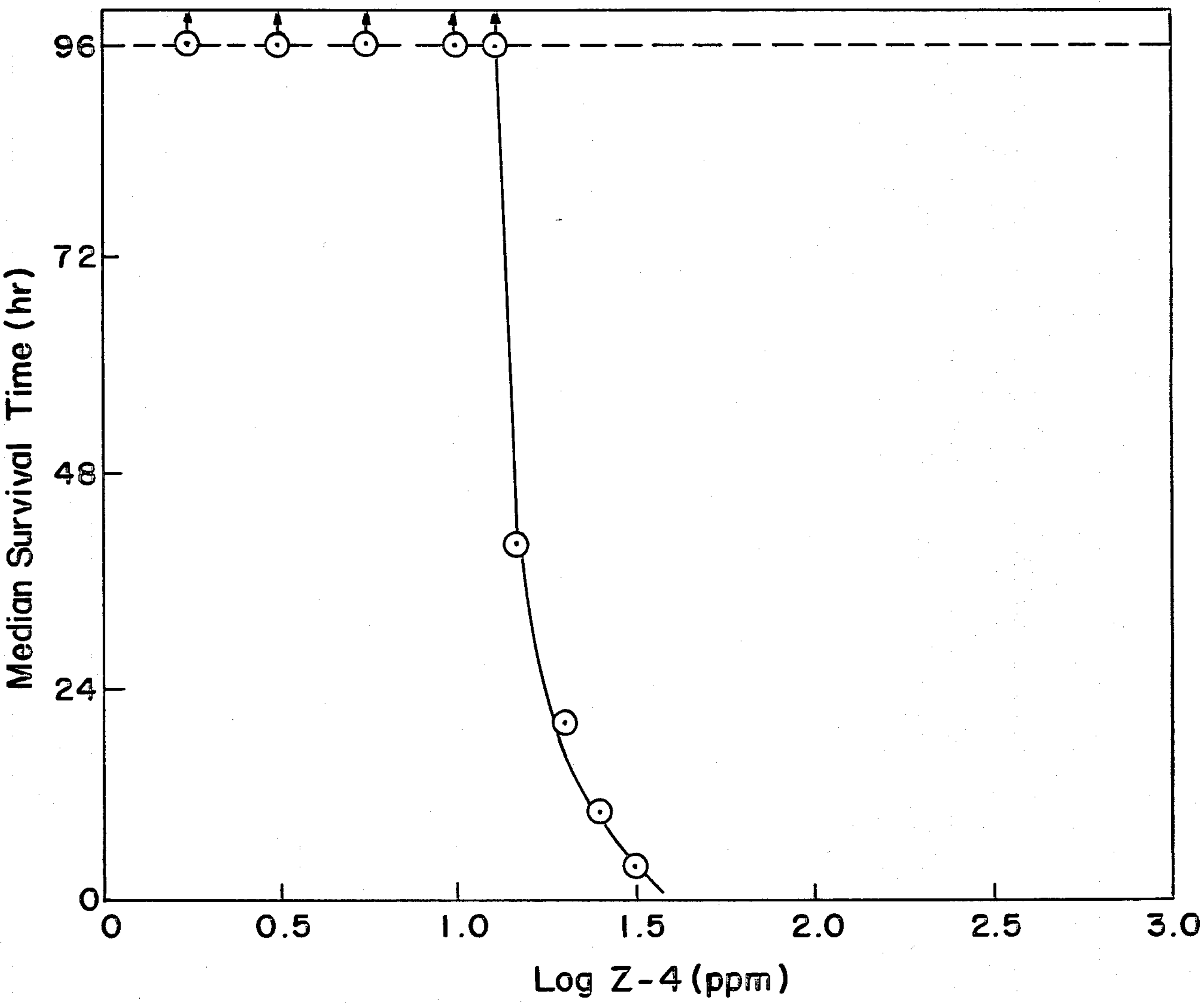

Figure 4. Median survival time as a function of the logarithm of Z-4 (sodium ethylxanthate) concentration at 12°C.

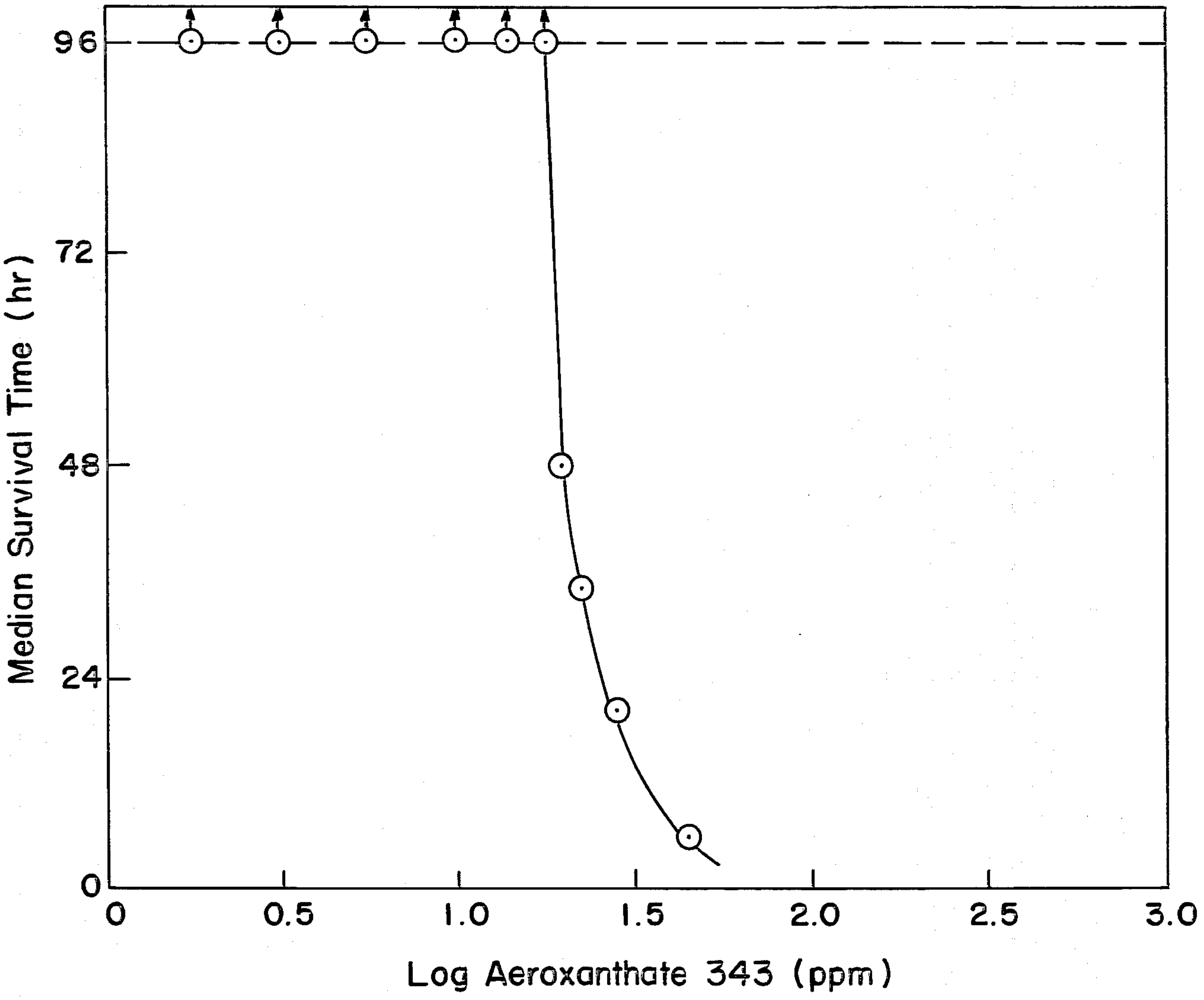

Figure 5. Median survival time as a function of the logarithm of Aeroxanthate 343 (sodium isopropylxanthate) concentration at 12°C.

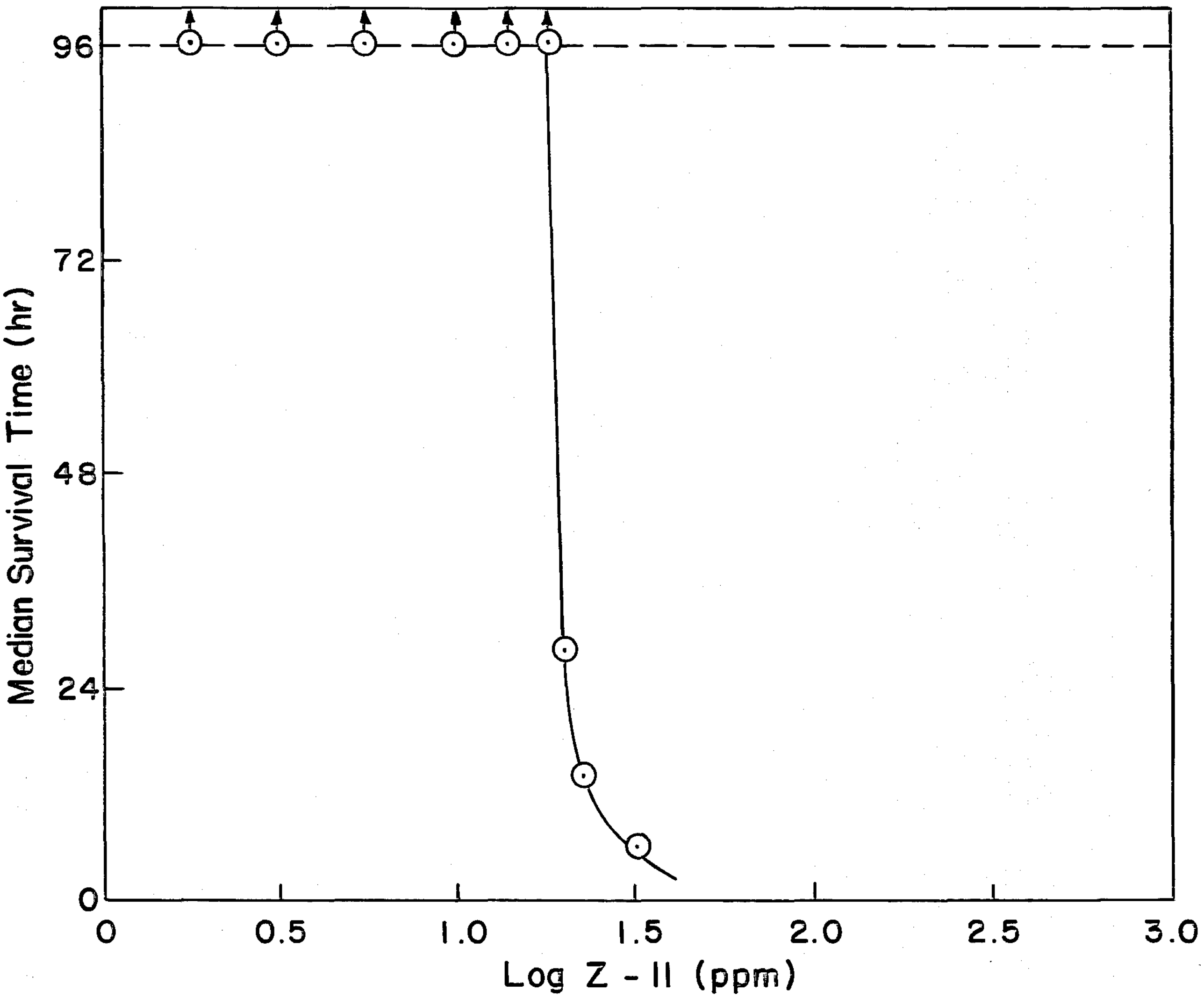

Figure 6. Median survival time as a function of the logarithm of Z-11 (sodium isopropylxanthate) concentration at 12°C.

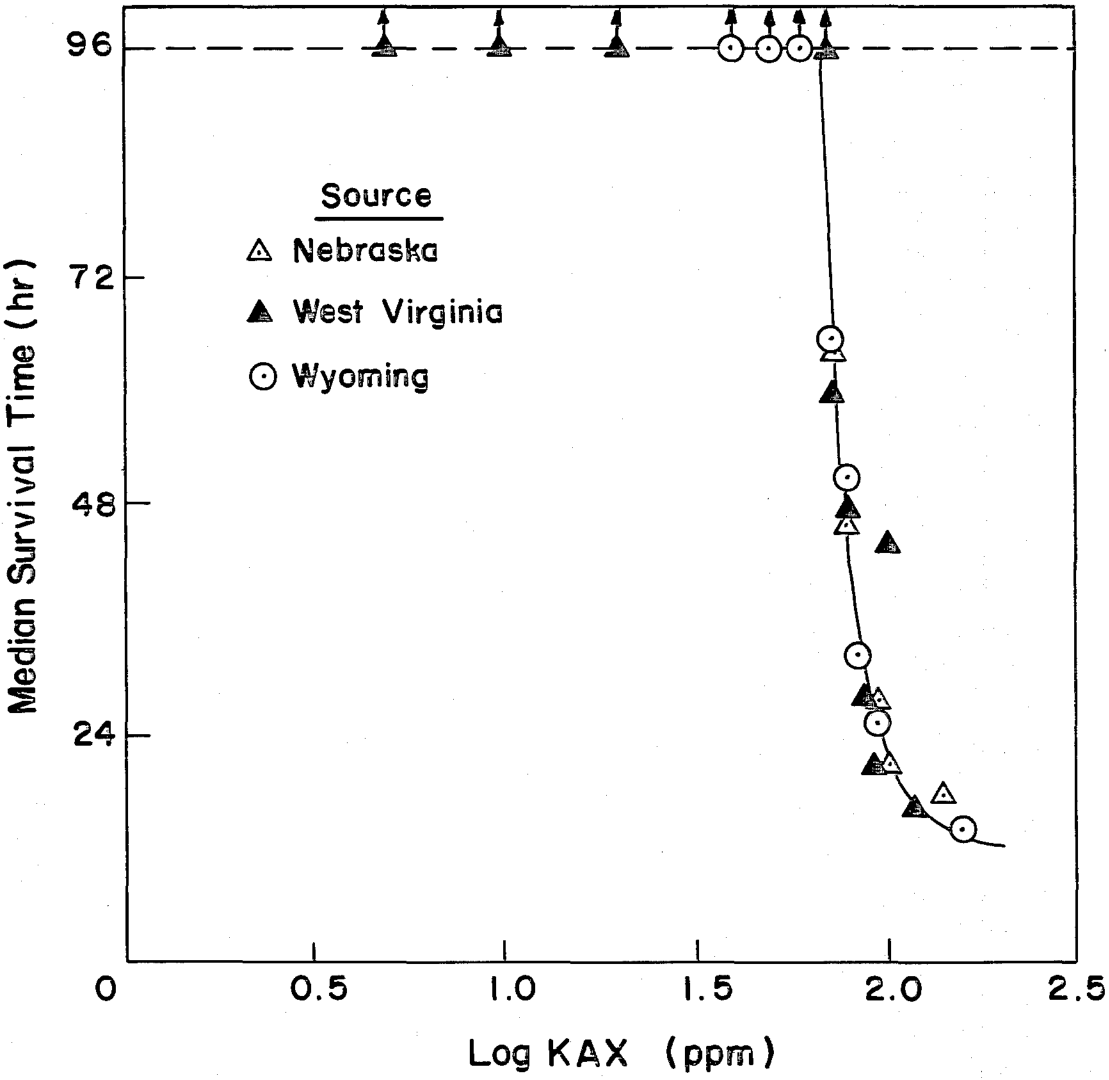

Figure 7. Median survival time as a function of the logarthm of purified potassium amylxanthate concentration and egg source of fingerlings at 12°C.

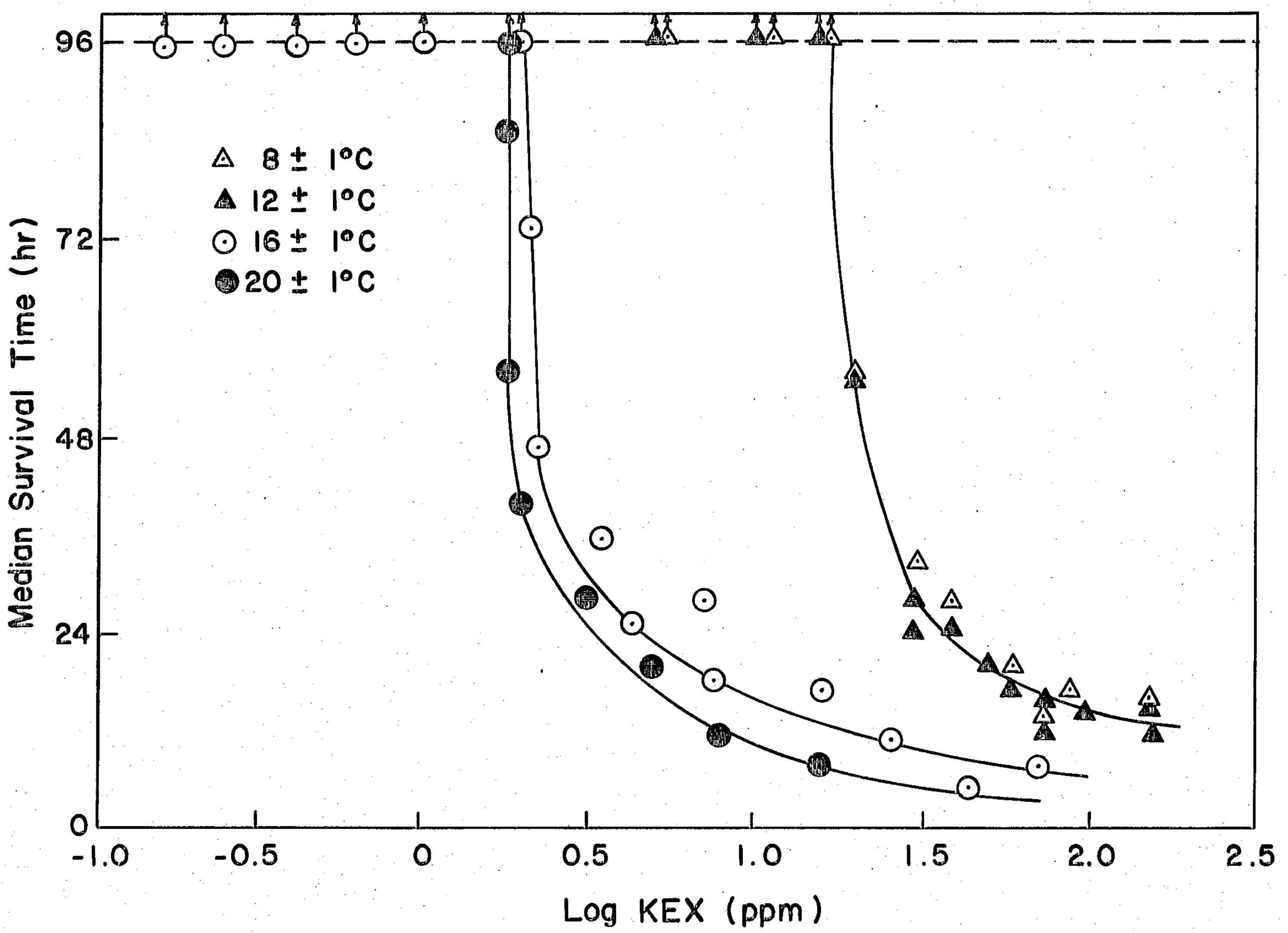

Figure 8. Median survival time as a function of the logarithm of purified potassium ethylxanthate concentration at various temperatures.

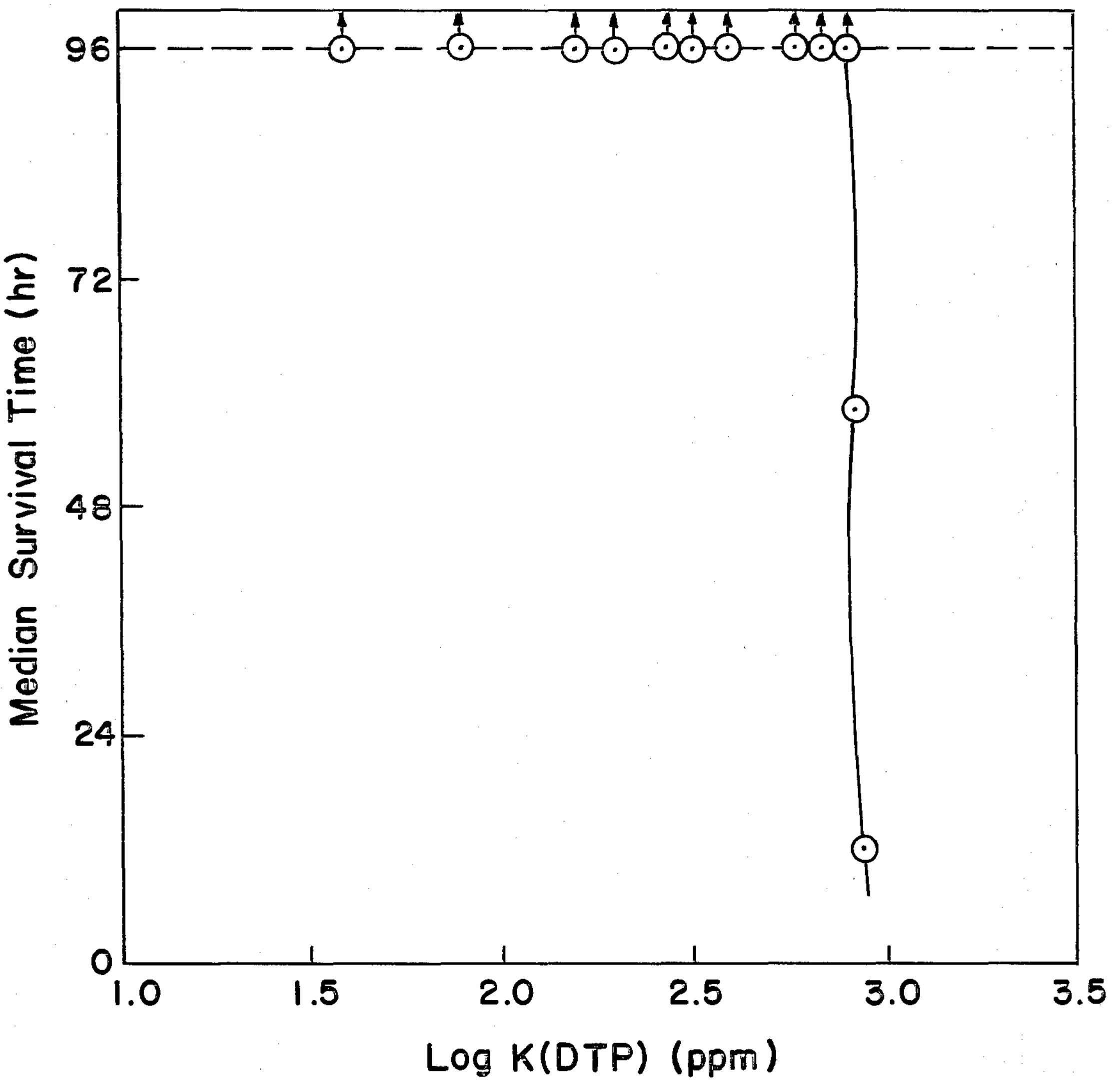

Figure 9. Median survival time as a function of the logarithm of purified potassium diethyldithiophosphate concentration at 12°C.

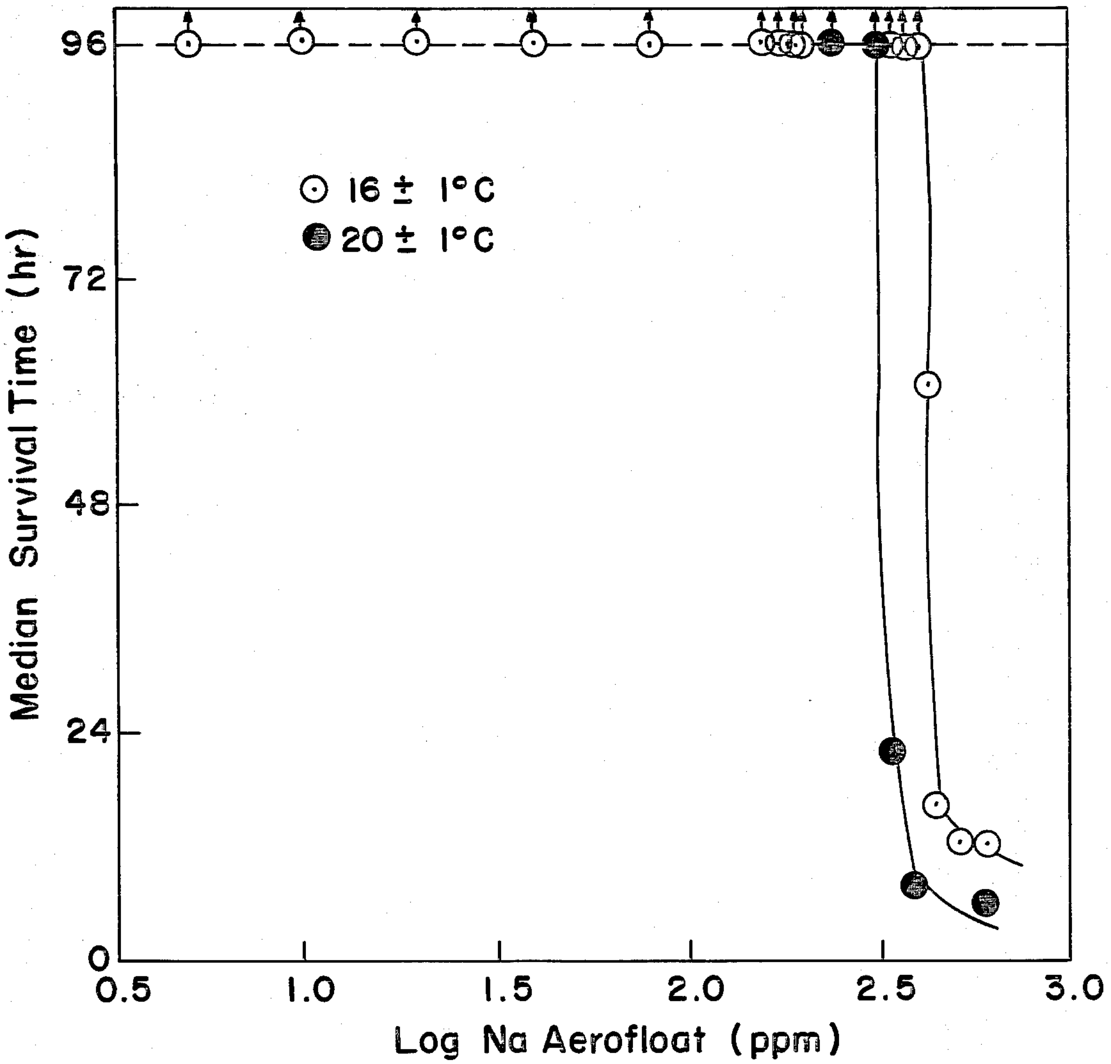

Figure 10. Median survival time as a function of the logarithm of Sodium Aerofloat concentration at two temperatures.

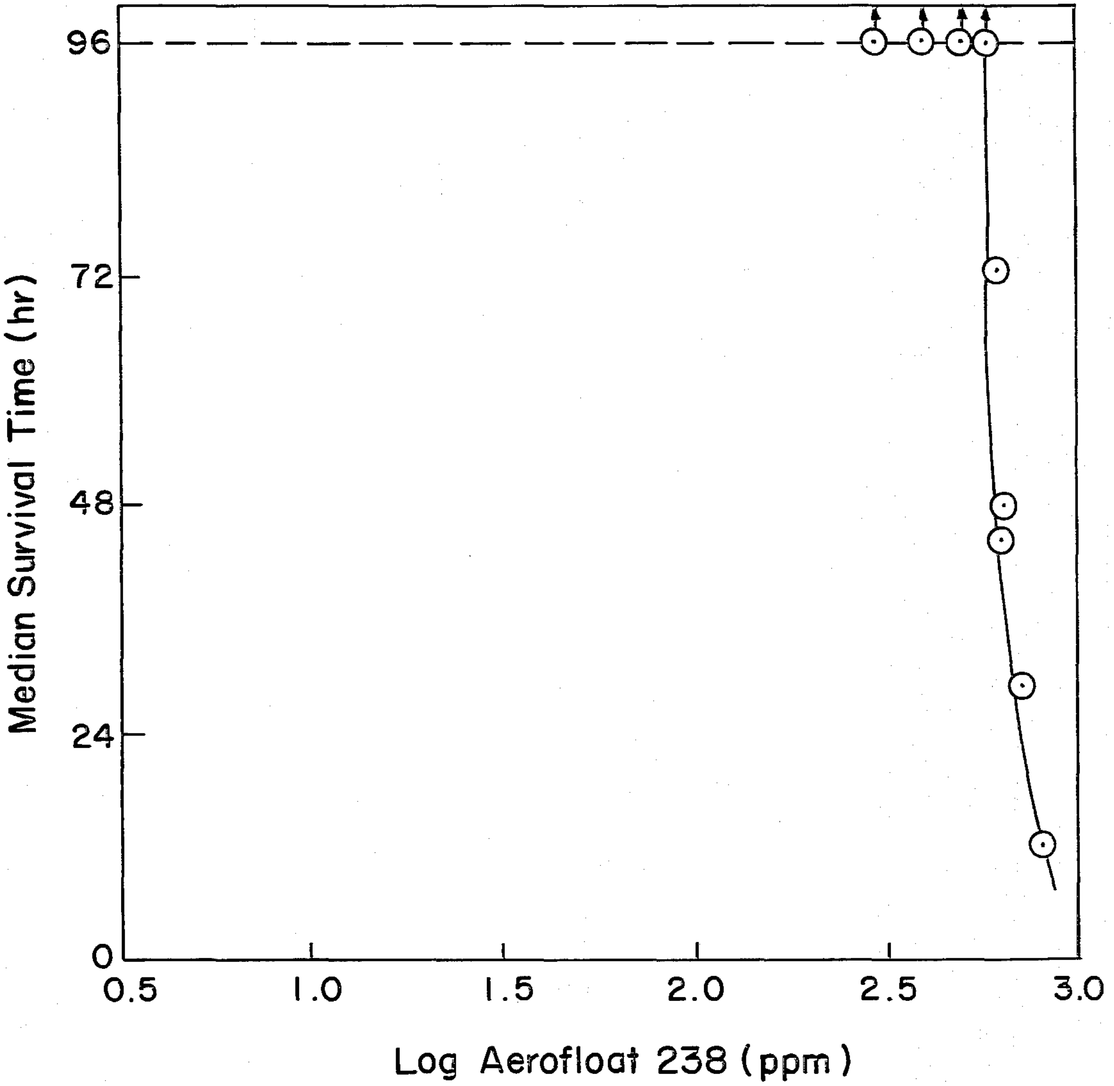

Figure 11. Median survival time as a function of the logarithm of Aerofloat 238 concentration at 12°C.

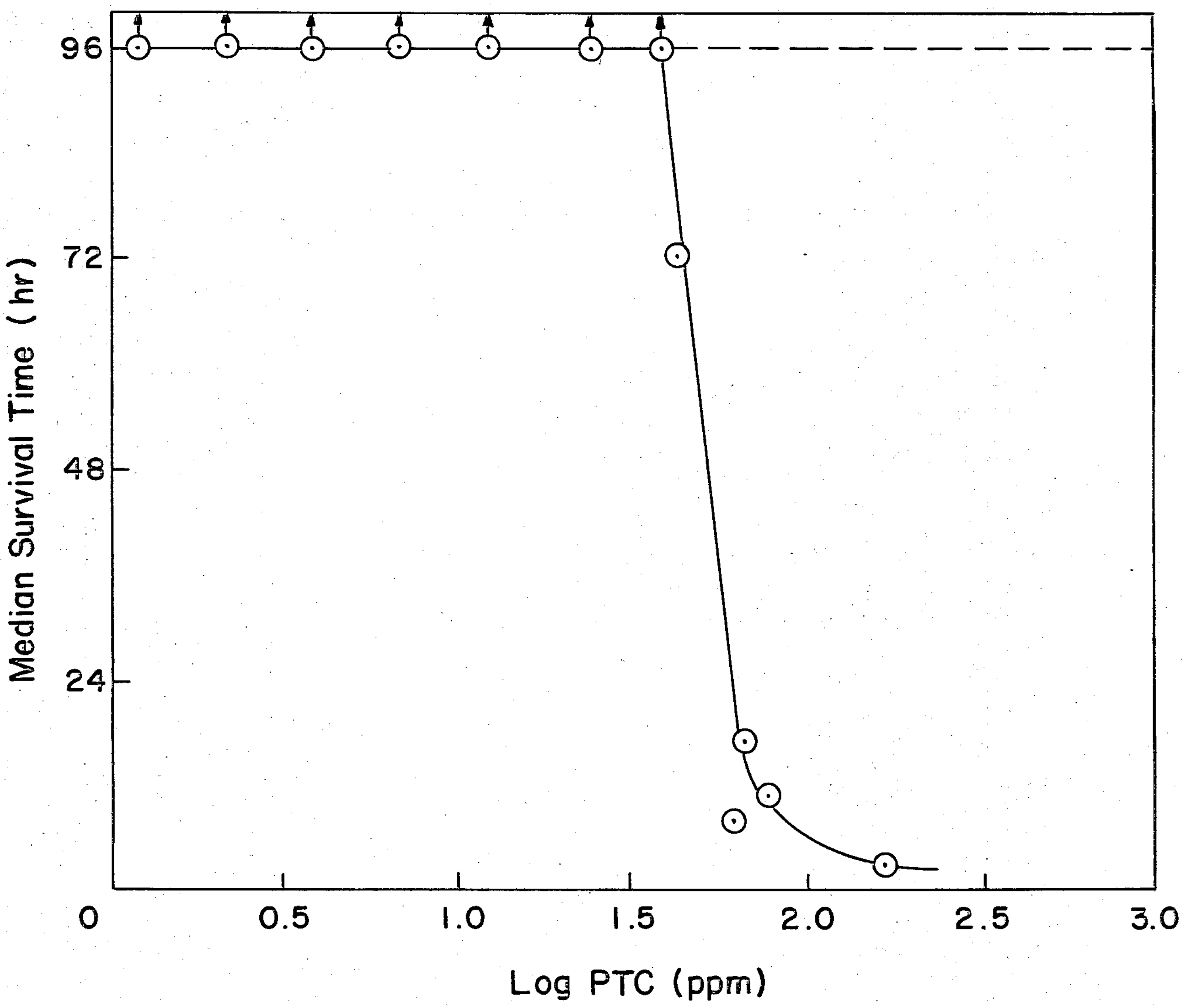

Figure 12. Median survival time as a function of the logarithm of purified isopropylethylthiocarbamate concentration at 12°C.

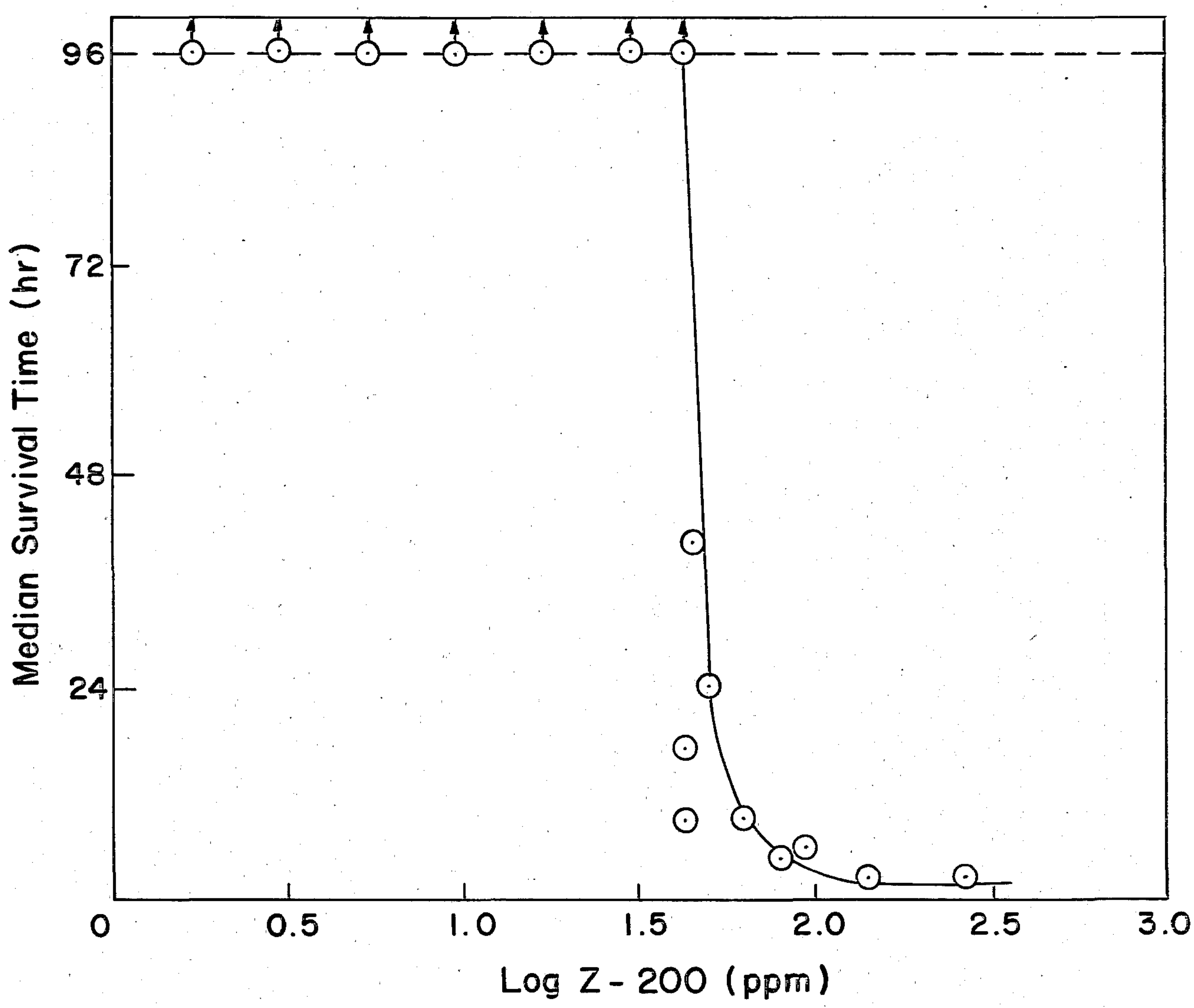

Figure 13. Median survival time as a function of the logarithm of commercially available isopropylethylthiocarbamate (Z-200) concentration at 12°C.

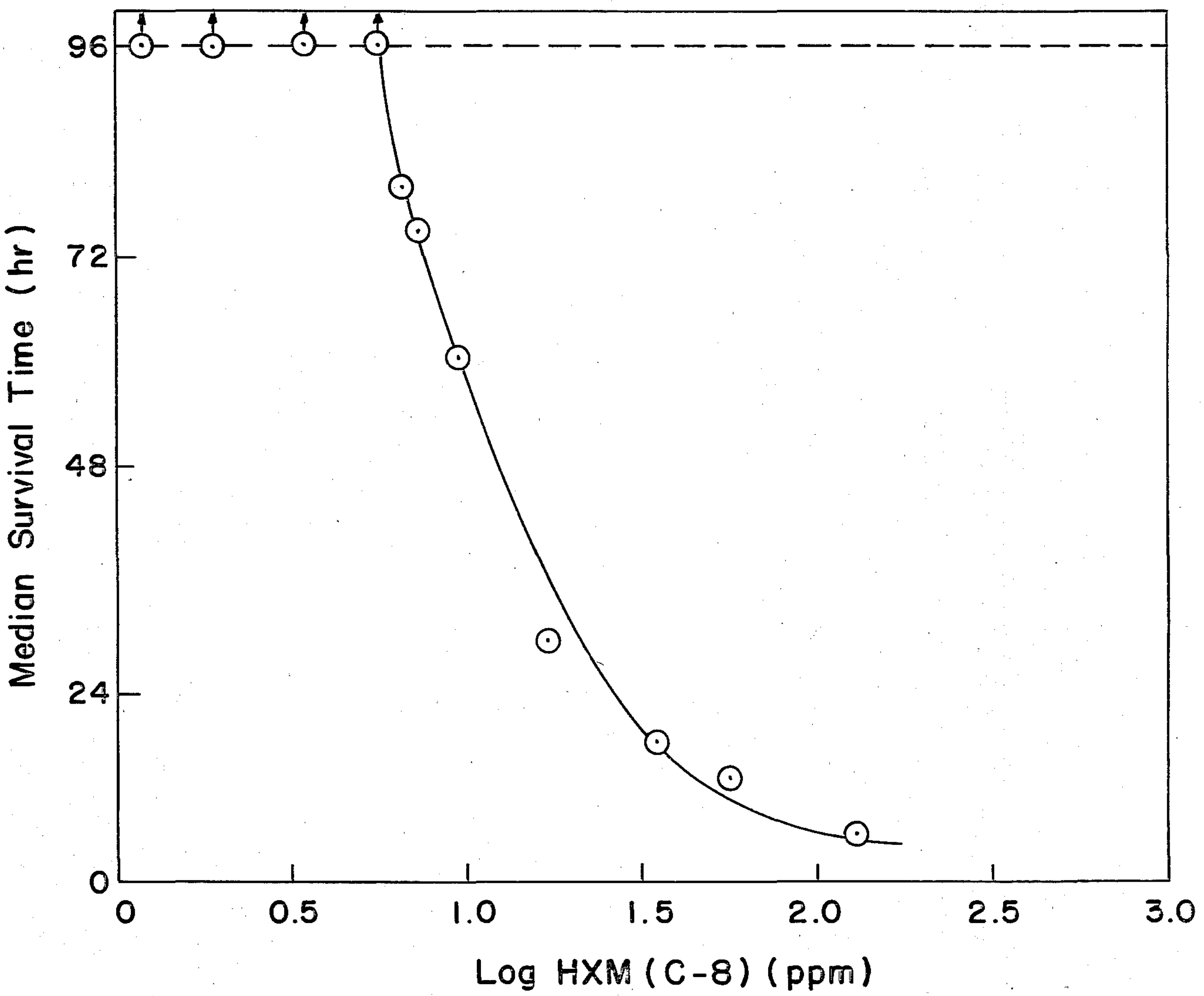

Figure 14. Median survival time as a function of the logarithm of purified potassium octylhydroxamate at 12°C.

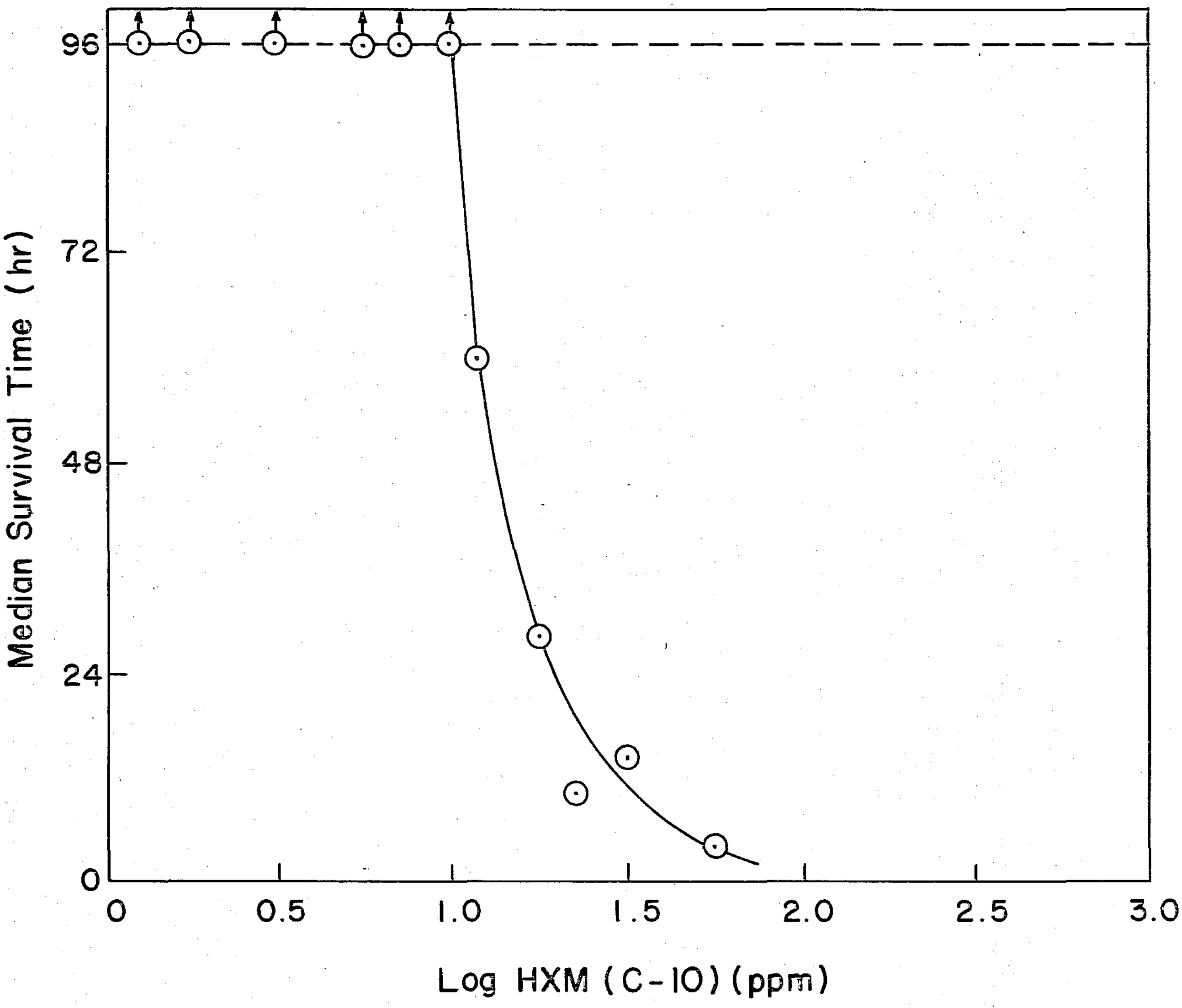

Figure 15. Median survival time as a function of the logarithm of purified potassium decylhydroxamate at 12°C.

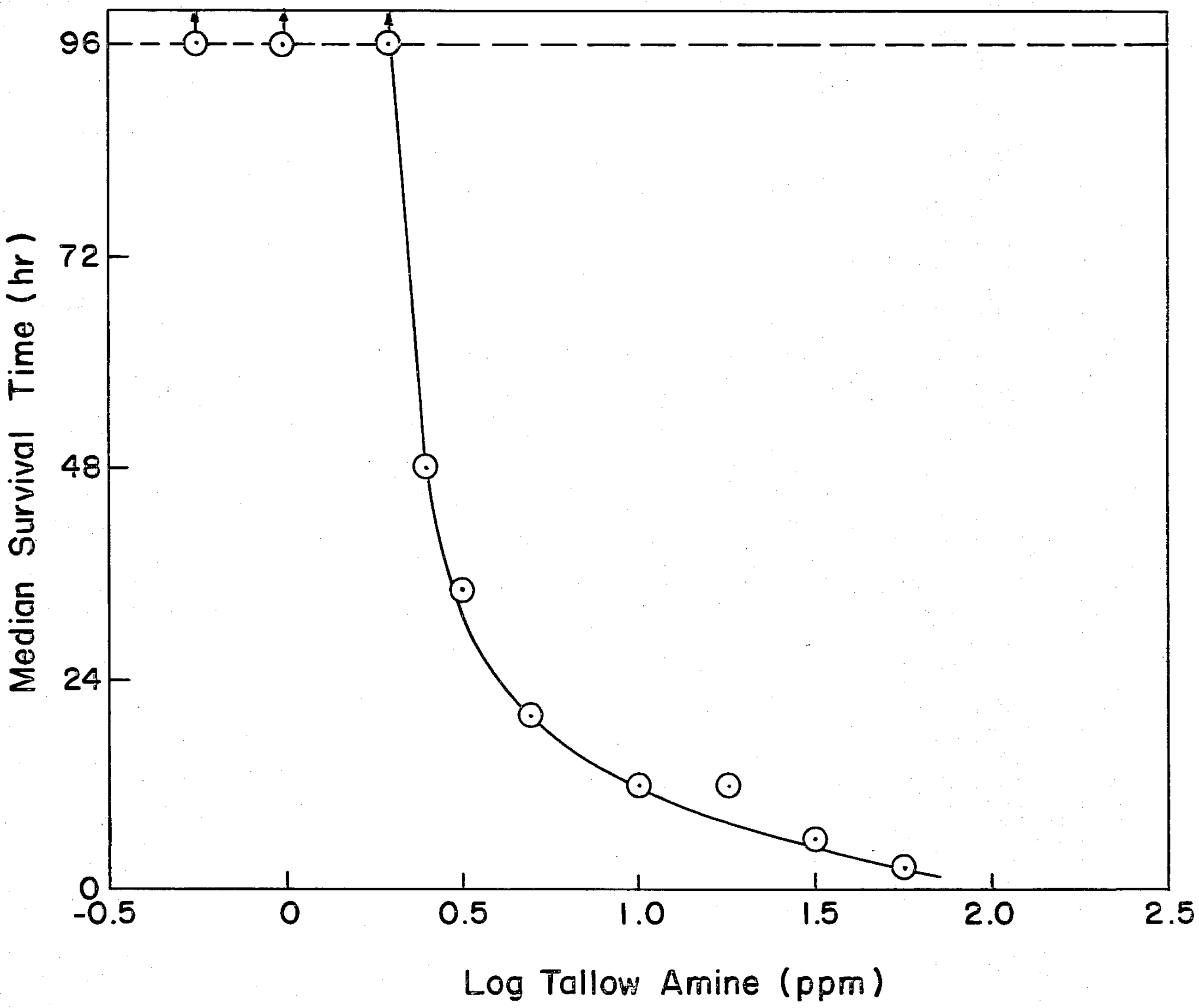

Figure 16. Median survival time as a function of the logarithm of primary tallow amine concentration at 12°C.