The Effects of Lapsed Time Since Feeding Upon the Toxicity of Zinc to Fish

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Most fish bioassays advise a period of no feeding before exposing the fish to potential toxicants (e.g. British Ministry of Agriculture, Fisheries, and Food [KEIJ et al., 1969]; the 1971 edition of Standard Methods; the 1971 Annual Book of ASTM Standards; and the Ohio River Valley Water Sanitation Commission [ORSANCO], [SMITH et al., 1973]). SPRAGUE (1972) recommends not feeding fish for at least a full day before testing. However, the justification for the lapsed time from the last feeding until the beginning of exposure seems to be intuitive rather than scientific. Since emergency tests are often necessary and waiting for digestive tracts to empty not always desirable, it seemed worthwhile to determine the effects of different lapsed times between feeding and testing. This research was undertaken to determine how the mortality of the goldfish used in the ORSANCO tests varies when feeding is done at different times prior to their being placed in a toxicant (Zn).

Materials and Methods

The recommendations in the ORSANCO 24 hour bioassay (SMITH et al., 1973) were followed so that the results could be related to an existing "standard" bioassay. A specific strain of goldfish is recommended for the ORSANCO tests and obtainable from hatcheries in Indiana and Missouri. These were shipped air express to Pellston, Michigan. Fish were acclimated for at least a week in Douglas Lake (Michigan) water. The day after arrival antibiotics were added to the holding tanks as recommended; the fish were kept in this solution two days. There were about 1.4 liters of water per fish. The temperature was 22°C (±1°).

All experimental runs began between 0600 and 0700. Pelletized fish food was added in excess to the holding tanks and feeding was allowed to proceed 25 minutes (15 minutes in the first run). At the end of this period, any remaining food was removed from the tanks. Ten fish were then immediately transferred into two test containers (20 liter glass jugs filled with 16 liters of Douglas Lake water), one with zinc (100 mg/l as $ZnSO_4$) and one without.

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These fish were put into the test containers at 1, 2, 3, 4, 6, 12, 24, 48, and 72 hours after feeding. These times were carried out in a series of experimental runs. All fish were observed every 15 minutes after their first two hours of exposure to zinc. Dead fish were removed and their survival time recorded. In the few cases (13 out of 420 fish) where a run was terminated before the last fish died, the time of death for the last fish was arbitrarily determined to be the next 15-minute period after termination. This was undoubtedly an underestimate in some cases but the fish were nearly dead in all cases and in these special cases extreme circumstances required termination of the tests. The test containers were aerated to prevent any flocculent material from settling out. Physical parameters including temperature, pH, hardness, and dissolved oxygen were measured at the beginning and end of the experiment. Because of the very low mortality in the control fish during the first three runs, only one control group was used in subsequent runs. The last run had more controls because it extended over three days.

Preliminary analysis of the results indicated non-homogeneous variances in four of the seven runs. Thus, non-parametric statistical methods were used to examine the relation between survival time of the fish and the time after feeding the fish was placed in a toxicant. Analysis of variance was done by the Kruskol-Wallis test while correlation coefficients were determined by the Spearman test.

Results

Chemical and physical determinations for both experimental and control tanks are summarized in Table I. As might be expected, the zinc solution had a lower pH and higher hardness than the control. Dissolved oxygen concentration showed little variation and was always well over 5 p.p.m.

TABLE I

Summary of Water Quality Data

	Control	Zinc
Temperature (°C)	22.2 ± 0.49*	22.2 ± 0.49
Dissolved Oxygen (mg/l)	7.1 ± 0.13	7.0 ± 0.0
pH	8.5 ± 0.02	7.3 ± 0.06
Hardness (mg/l as $CaCO_3$)	127.0 ± 4.5	188.0 ± 7.9

*Mean ± S.D.; n = 13

TABLE II. Analysis of the Relation Between Survival Time and Elapsed Time from Feeding to Start of Exposure to Zinc.

Experimental Run	Fish Exposed at These Times After Feeding (Hr.)	Number of Controls (Number Died)	Kruskal - Wallis Analysis of Variance df	Kruskal - Wallis Analysis of Variance Significance (.05 level)	Spearman Correlation Coefficient
1	0, 1, 3, 4, 6	50 (0)	4	+	+0.500**
2	0, 1, 2, 4, 6	50 (0)	4	-	+0.344*
3	0, 1, 2, 4, 6, 12, 24	50 (2)	6	+	-0.135
4	0, 1, 2, 4, 6, 12, 24	10 (0)	6	-	+0.233*
5	0, 1, 2, 4, 6, 12, 24	10 (0)	6	-	+0.127
6	0, 1, 2, 4, 6, 12, 24	10 (0)	6	-	+0.107
7	0, 24, 48, 72	40 (0)	3	+	+0.544**

* Significant at .05 level
** Significant at .01 level

The results from each run are reported in Table II. The Kruskol-Wallis analysis of variance showed that, in three of the seven runs, the mean survival times were significantly different for several of the time intervals between feeding and exposure to zinc. Positive correlation between these two variables were found in six of the seven runs; in four runs the correlation was significant, including two of the three runs showing a significant difference in the means by the Kruskol-Wallis test.

In Figure 1, the data from all the runs was pooled to give one mean survival time for each of the times after feeding at which the fish were exposed to zinc. The brackets are plus or minus one standard deviation.

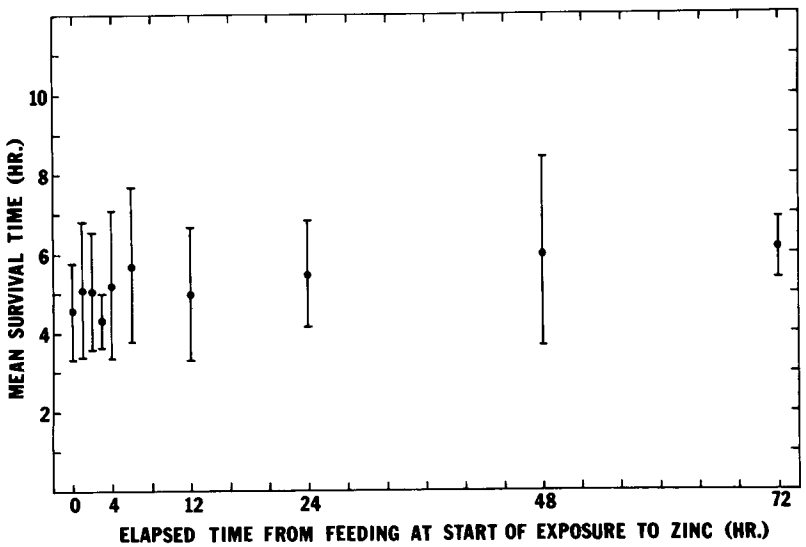

Figure 1. Mean survival time vs. elapsed time from feeding at start of exposure to zinc.

Discussion

The lack of a significant difference in mean survival times in four of the seven runs and the generally low correlation coefficients indicate that if there is any effect of lapsed time since feeding on the toxicity of zinc to goldfish, it is small relative to the individual variations in survival time among the fish. In six of the seven runs there was a positive but very low correlation between survival time and time from feeding, including the two runs which had significant results in both the Kruskol-Wallis and Spearman tests. Thus there may be a slight trend towards increased survival time as the time between feeding and exposure to zinc increases. This conclusion is further illustrated

by Figure 1. There seems to be a slight increase in mean survival time between 0 and 72 hours after feeding but the size and overlaps of the standard deviation makes a definite conclusion impossible.

This experiment indicates that, at least with goldfish and $ZnSO_4$, there is little need for the standard bioassay procedure of not feeding test fish for 24 or 48 hours prior to their exposure to a toxicant. Certainly, fish in a natural environment will not be under any such feeding restriction when they encounter a pollutant. Thus, further knowledge on the relation of feeding to the toxicity of a material involving other fish and toxicants would be valuable to those who must use the results of standard laboratory bioassays to set standards for the concentration of pollutants in natural bodies of water. However, for crude acute bioassays, such as the ORSANCO method, lapsed time from feeding to test is not likely to markedly affect the results.

SUMMARY

This study was undertaken to determine the effects of elapsed time from feeding to exposure upon the tolerance of goldfish (Carassius auratus L.) to zinc. Methods followed those in the 24 hour static bioassay of the Ohio River Valley Water Sanitation Commission (SMITH et al., 1973). Fish were fed for 25 minutes and at specific times after feeding (0, 1, 2, 4, 6, 12, 24, 72 hours) groups of 10 fish were placed into 18 liters of water containing a lethal concentration (100 mg/l) of zinc as $ZnSO_4$. Time until death was recorded for individual fish. A total of 420 fish (excluding controls) were exposed to zinc in the course of seven experimental runs. Statistical analysis revealed a slight but non-significant increase in survival time as the interval between feeding and exposure to zinc increased. These variations in survival time do not appear to be of sufficient magnitude to support the standard acute bioassay requirement that fish not be fed for 24 or 48 hours prior to their exposure to a toxicant.

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REFERENCES

ANONYMOUS: Annual Book of ASTM Standards. Part 23. Easton, Md: American Society for Testing and Materials. 1971.

ANONYMOUS: Standard Methods for the Examination of Water and Wastewater. 13 ed. New York: American Public Health Assoc. 1971.

KEIJ, A., et al. (technical committee). Fish toxicity tests. British Ministry of Housing and Local Government. London: Her Majesty's Stationary Office. 1969.

SMITH, L.L., et al. ORSANCO 24-Hour Bioassay. Cincinnati, Ohio: Ohio River Valley Water Sanitation Commission. 1973.

SPRAGUE, J.B. The ABC's of pollutant bioassay using fish. American Society for Testing and Materials. Special Publ. No. 528:6-30 (1973).