Avoidance of Fenitrothion by Goldfish (*Carassius auratus*)

by E. SCHERER
Department of the Environment
Fisheries and Marine Service, Freshwater Institute
Winnipeg, Man., Canada, R3T 2N6

ABSTRACT

Goldfish (*Carassius auratus*) avoided water containing the pesticide fenitrothion. The response was graded over the tested concentration range of 5 orders of magnitude, with a threshold around 10 µg/l, equivalent to 3.5×10^{-8} M.

INTRODUCTION

It is of importance to know whether fish avoid an environmental toxicant, and at what concentration, for two reasons: (1) detection and avoidance at a sublethal level will provide a chance to escape lethal exposure; (2) at the same time, the altered spatial distribution of the species may severely affect an ecosystem, with an immediate impact on commercial and recreational fisheries.

Fenitrothion (0,0-dimethyl 0-4-nitro-*m*-tolyl phosphorothioate) has been applied on a large scale in Canada since 1968 to control the spruce budworm. This pesticide is marketed worldwide at the present time (Sumithion(R), Folithion(R)). In acute bioassays, it seems less toxic to fish than several other popular pesticides (WILDISH *et al.* 1971); data on avoidance are lacking.

The goldfish is easily handled in the laboratory and has been used in other bioassays. Its sensitivity as an organism for sublethal assays, however, has not been adequately explored.

MATERIALS AND METHODS

Ten goldfish, obtained from a local dealer and ranging in length from 9-11 cm, were acclimated to 15°C for 4 weeks in sodium thiosulfate - dechlorinated Winnipeg tap water. They were held together in one tank and fed once daily. No mortalities occurred, nor were any signs of impairment seen as long as the tests lasted or for 3 months thereafter. The fish were tested one at a time, usually proceeding from lower to higher concentrations. In each test, the specimen was given a choice between pure water in one half of a counter-current chamber, and one fixed concentration of the pesticide in the other half, and movements of the fish were recorded continuously, as described by

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SCHERER and NOWAK (1973). Control recordings with pure water in both halves indicated no bias such as could have been caused by unsymmetries of lighting, flow rates, temperature or visual marks; times accumulated to the left and the right of the tank midline during 55 control runs differed by less than 1%.

Movement of fish towards or across a chemical boundary is a prerequisite to this type of test. In practice it was found that certain individuals were not as motile as others. Consequently we adopted the rule of replacing a fish that did not cross the tank midline at least 5 times during a 10 minute period prior to the introduction of the toxicant. While this procedure effectively eliminated the original intention to use randomly selected fish, it insured that the subsequent test was imposed on a reasonably active specimen.

Ten minutes, measured in full seconds, were assigned to each test. Three hundred seconds spent by the fish in both halves of the chamber would signify a perfectly neutral reaction. This situation can be expressed as the time spent in unmodified water in percent of total time (e.g. SPRAGUE 1964), any increase over 50% being considered as increasing avoidance. I chose to convert those percentage figures into "degrees of avoidance", in order to arrive at a handier, immediately comprehensible notation for the relative intensity or completeness of the response. The conversion was done in the following manner. Fifty percent time accumulated in the pure water side of the chamber equals "zero" on the degree of avoidance scale. Ten arithmetic divisions over the range 50% to 100% will convert 55% to 10 degrees, 50% to 20 degrees of avoidance, and so on; symbolically:

degree of avoidance = 2 x (% time in pure water - 50)

For values below 50%, a degree of preference - scale can be used just as easily.

Mean, median, standard error of the mean of degrees of avoidance at each concentration, and a regression analysis were computed.

Fenitrothion (97% purity) was solubilized in dechlorinated tap water by adding Atlox(R) (Atlas Chemical Co., Bradford/Ont.) emulsifier at 15% of fenitrothion weight, and subsequent sonication. Comparable solutions containing Atlox alone were also prepared. All solutions were made daily, and appropriate dilutions were delivered to the test tank by a precision peristaltic pump. Flow velocities in the test chamber ranged from 6 to 8 mm/sec. In all experiments, the peristaltic pump added less than 0.07 mm/sec to the test side. Oxygen was kept at saturation level through aeration; pH ranged from 7.6 to 8.0, without significant changes by fenitrothion and/or Atlox.

RESULTS

A total of 102 tests was carried out at 11 different concentrations from 0.01 mg/l to 100 mg/l fenitrothion; 8 tests each at 1, 2, 5 and 10 mg/l; 10 tests each at all other levels.

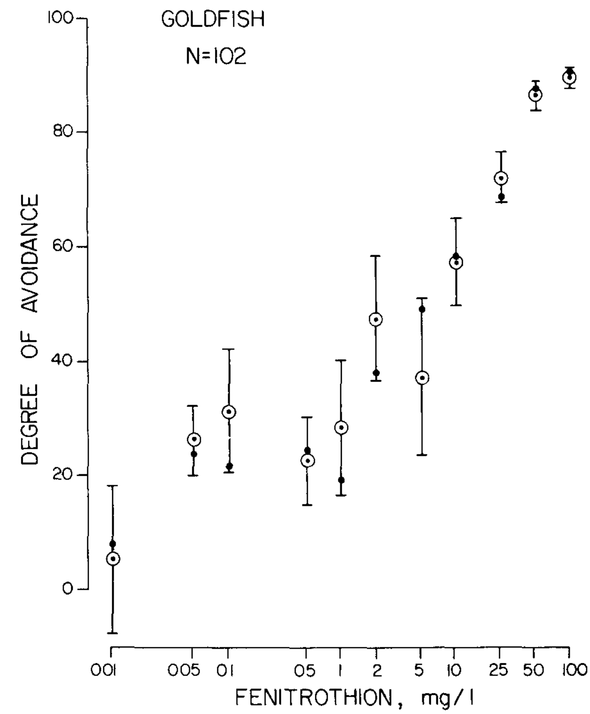

Fig. 1. Avoidance of fenitrothion by goldfish
⊙ = response mean
• = response median
I = standard error of mean

Fig. 1 shows the relation between degrees of avoidance and pesticide concentration. All response medians fall within one standard error of their respective means. The coefficient of variation reaches its largest value (V = 38.8%) at 0.01 mg/l. At this concentration, 3 out of 10 tests yielded no avoidance (i.e., less than 5 minutes was spent in the pure-water half of the tank), and 3 others resulted in less than 20 degrees of avoidance each. Therefore, 0.01 mg/l = 10 μg/l is considered to represent the threshold area for avoidance.

The distribution of means and medians over the range of concentrations suggests a sigmoid curve with a rectilinear central portion. A regression analysis supports this assumption:

TABLE 1

Regression of avoidance on fenitrothion concentration

	0.01 - 100 mg/l	0.5 - 25 mg/l
Equation for rectilinear regression	$y = 19.6 \log x + 41.1$	$y = 27.0 \log x + 29.9$
Test on regression coefficient	$p < 0.01$	$p < 0.01$
Test on rectilinearity	$F = 1.67$	$F = 0.75$

Atlox alone was assayed in 25 tests, using concentrations identical with those used for emulsifying the various aliquots of fenitrothion. Avoidance was found in 15 of these tests (not significant by chi-square, $p > 0.05$). The response mean of all Atlox tests amounted to 18 degrees of avoidance; no significant regression on concentration was found ($0.05 < p < 0.06$). This suggests: Atlox did not contribute to the increase of avoidance with higher pesticide concentration, but it appears to have slightly augmented the avoidance response over the whole range.

Finally some observations under dark conditions were carried out at 25 to 100 mg/l fenitrothion, with the aid of an infrared viewing device ($\lambda > 750$ nm). The reason for this was the slightly milky to opaque appearance of the test water at these high concentrations, suggesting the possibility that the avoidance response was elicited visually rather than through chemoreceptors. This hypothesis, however, found no support in our observations; avoidance in the dark reached the same degrees as in light.

DISCUSSION

It is common practice to derive a statement on avoidance from proportions of time accumulated in water with and without the substance in question. This measure is integrative and appears suitable as a first approach for assessing to what extent a species may suffer exposure to a toxicant, or may disappear from a certain area. Analysis of constituent behavioural elements (e.g., directed and random movements) is underway.

How repeated testing of a specimen changed its response to the pesticide could not be clearly derived from the present series.

It was observed, however, that some individuals were consistently more sensitive than others, and motility and sensitivity seemed to be positively correlated.

An avoidance threshold of 10 μg/l, even though a conservative estimate according to Fig. 1, appeared to be well below an incipient lethal level. To verify this, the same fish were, after conclusion of avoidance tests, exposed to 100 μg/l for 10 days under continuous-flow conditions. No death resulted; the only noticeable effect, if any, was a slight decrease in locomotor activity.

Ten μg/l is more than 2 orders of magnitude below the reported ILL for Atlantic salmon (WILDISH *et al.* o.c.), and 1 order below a concentration reducing the response to electric shock (HATFIELD and JOHANSEN 1972) and causing a decline in territorial behaviour (SYMONS 1973). The latter author also observed rejection of this pesticide by Atlantic salmon in the form of regurgitation of fenitrothion-stuffed mealworms.

Behavioural tests may sometimes ascertain lower thresholds than physiological techniques, because the response results from an intact, integrated functional system. The molarity equivalent to 10 μg/l, at the given chemical purity, is 3.5×10^{-8}. This value ranks lower than some gustatory and olfactory thresholds determined by various other techniques for a number of test substances and species of fish (cf. HARA 1971).

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