The Effect of Exposure to a Subacute Concentration of Parathion on the General Locomotor Behavior of the Goldfish

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Organophosphate compounds are a group of wide spectrum pesticides which enter the environment in great quantity (COPPAGE and MATTHEWS, 1974), especially since the ban of many of the ubiquitous chlorinated hydrocarbons. The organophosphates affect normal cholinergic neural transmission by inhibition of acetylcholinesterase (CORBETT, 1974). This effect has been well established in vivo as well as in isolated tissues of various animals, including fish, at both lethal and subacute concentrations (GUILBAULT et al., 1972; MAZUR and BODANSKY, 1946; WEISS, 1958, 1959).

The inhibition of acetylcholinesterase by exposure of the whole animal to dilute solutions of some organophosphates can be reversed by exposure to clean water in many fish. In contrast, in the case of parathion (O, O-diethyl O-4-nitrophenyl phosphorothioate), acetylcholinesterase activity continues to decrease even after interruption of treatment (WEISS, 1959, 1964).

In view of these persistent effects of parathion and of the crucial role of acetylcholinesterase in central nervous system function, which controls most behavioral responses, the behavioral toxicology of subacute parathion exposure in fish deserved investigation.

In establishing the approach to an investigation of the problem, an important consideration was the selection of a behavioral pattern suitable for quantitative analysis in "normal" and parathion-exposed fish. The measurement of fish swimming performance has been suggested and often claimed to be one of the best criteria for the determination of the subacute effects of pollutants (SPRAGUE, 1971). Because swimming performance may be expected to affect normal locomotor behavior and thus the orientation ability required in the location of an odor, mate, spawning ground, prey, or the evasion of a predator, exposure to a subacute concentration of a pollutant might result in serious biological damage. Surprisingly, there are few investigations on the deleterious effects of pollutants on fish locomotor behavior. DAVY et al. (1972) found that DDT impaired the locomotor pattern in the goldfish while BULL and McINERNEY (1974)

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demonstrated that coho salmon were unable to maintain position in a current after fenitrothion (Sumithion: 0, 0-dimethyl 0-(4-nitro-m-tolyl) phosphorothioate) exposure. More recently, it was shown that the locomotor orientation of the goldfish to a food "odor" source may be affected by parathion exposure (RAND et al., 1975).

In view of the importance of the locomotor behavior of fish and the effects of parathion, the aim of the present study was to ascertain the effect of exposure to a subacute concentration of parathion on the general locomotor behavior of the goldfish (*Carassius auratus*).

METHODS AND MATERIALS

Monitor Tank

Locomotor movements of a single fish were monitored in an acrylic, cylindrical tank (KLEEREKOPER, 1969). Hollow dividers oriented radially from the periphery, separate the tank into 16 compartments of equal dimensions, leaving an open area in the center. The entrance to each of the 16 compartments is guarded by a photoelectric gate and is triggered by the passage of the fish. The resulting electronic event and time of its occurrence are recorded by means of a logic interface by a paper tape punch located outside the experimental chamber.

The tank has a peripheral channel from which carbon-filtered water at 21.0 $\pm$ 0.5°C enters each compartment through a glass siphon. Water leaves the tank centrally through an overflow pipe. The laboratory water used throughout all phases of the experiments was of the same origin. In these experiments, the rate of flow through each siphon was 200 $\pm$ 5 ml/min.

Fish and Holding Conditions

Goldfish (25-30 cm) purchased from a commercial fish grower were kept in individual white fiberglass tanks and were fed Purina "trout chow" once each morning except when they were in the monitor tank. Water in these tanks was continuously re-circulated, aerated, and filtered. The temperature difference between holding and monitoring tanks never exceeded 2.0°C.

Parathion Solutions and Exposure

Fresh stock solutions of technical grade parathion (MONSANTO, 97%) in acetone (10 mg/ml), were prepared weekly and refrigerated. Dilutions were prepared from the stock solution. Fish were exposed individually to a stagnant, subacute parathion solution (0.33 ppm) in teflon-lined, 120 liter fiberglass tanks, at 21.0 ± 0.5°C, during 24 hours. Subacute concentrations were determined empirically using the criterion that fish survival be in excess of several months, following parathion exposure.

Experimental Procedures

Eighteen experiments were performed with nine goldfish. The locomotor behavior of each fish was monitored for eight hours before and after a 24-hour exposure to parathion. A single fish was transferred first from its holding tank to the monitor tank in a clear plastic bag. To allow for adjustment to minor temperature differences, the fish was allowed to remain in the bag, submerged in the monitor tank, for approximately 20 minutes prior to release. The locomotor movements were then recorded immediately after release. Because previous behavioral tests in this (DAVY et al., 1972) and other laboratories (AUBIN and JOHANSEN, 1969; HANSEN, 1969) failed to show an acetone effect, these controls were omitted in this study.

Data Treatment

The following locomotor parameters were analyzed: 1) total number of entries into compartments, 2) average duration of all pathways, and 3) orientation angles leaving a compartment, to the left (-180°) or the right (+180°).

To determine the effect of parathion treatment on the total number of entries and the average duration of pathways, a paired t-test and a Wilcoxon's signed rank test were used respectively.

To determine the consistency of the frequency distribution of orientation angles, for each fish before and after parathion exposure, a chi-square test with a 2 x n contingency table was used. Because this test might be biased as a result of variability in the fish's activity, only those orientation angles made by pathways in less than 10 seconds (time) were used in the analysis. This value was arrived at by inspection of the frequency distribution of all pathway durations.

The error rate for all sources of variation was fixed at a significance level of $\alpha = 0.05$(*).

RESULTS

The total number of entries decreased following parathion exposure (Table 1). Although the average duration of pathways increased after exposure in six of the nine fish (Fig. 1), the average of this value was not significantly different. Furthermore, in all nine fish the frequency distribution of orientation angles changed (*) following parathion exposure. Examples are illustrated in Figures 2 and 3.

TABLE 1
Total number of entries for each goldfish (n=9) before (Pre-PT) and after (PT) parathion exposure.

Fish	Total number of entries Pre-PT	PT
1	1651	517
2	807	1216
3	447	913
4	1364	773
5	753	455
6	1423	1382
7	1214	1256
8	1069	369
9	820	491

DISCUSSION

The data demonstrate that exposure to parathion resulted in a trend of overall decline in activity as measured by a decrease in total number of entries, and an increase in average duration of pathways. Although the parathion effect is confounded with the order of experimentation, past experience has shown that the effect of a second introduction alone into the tank has not by itself exhibited such an effect. It is of interest in this connection that during the 24-hour parathion exposure, fish were observed to remain listless on the bottom of the holding tank. Decreased activity as a result of exposure to organophosphate compounds has been documented elsewhere (BENKE and MURPHY, 1974; MAKI et al., 1973; PETERSON, 1974).

The results also demonstrate that "untreated" fish display an orientation angle distribution which is characterized by a preponderance of small angles (Figs. 2 and 3). After parathion exposure, in some of the fish, the above distribution was modified and distinguished by a shift in magnitude to larger angles (Figs. 2 and 3),

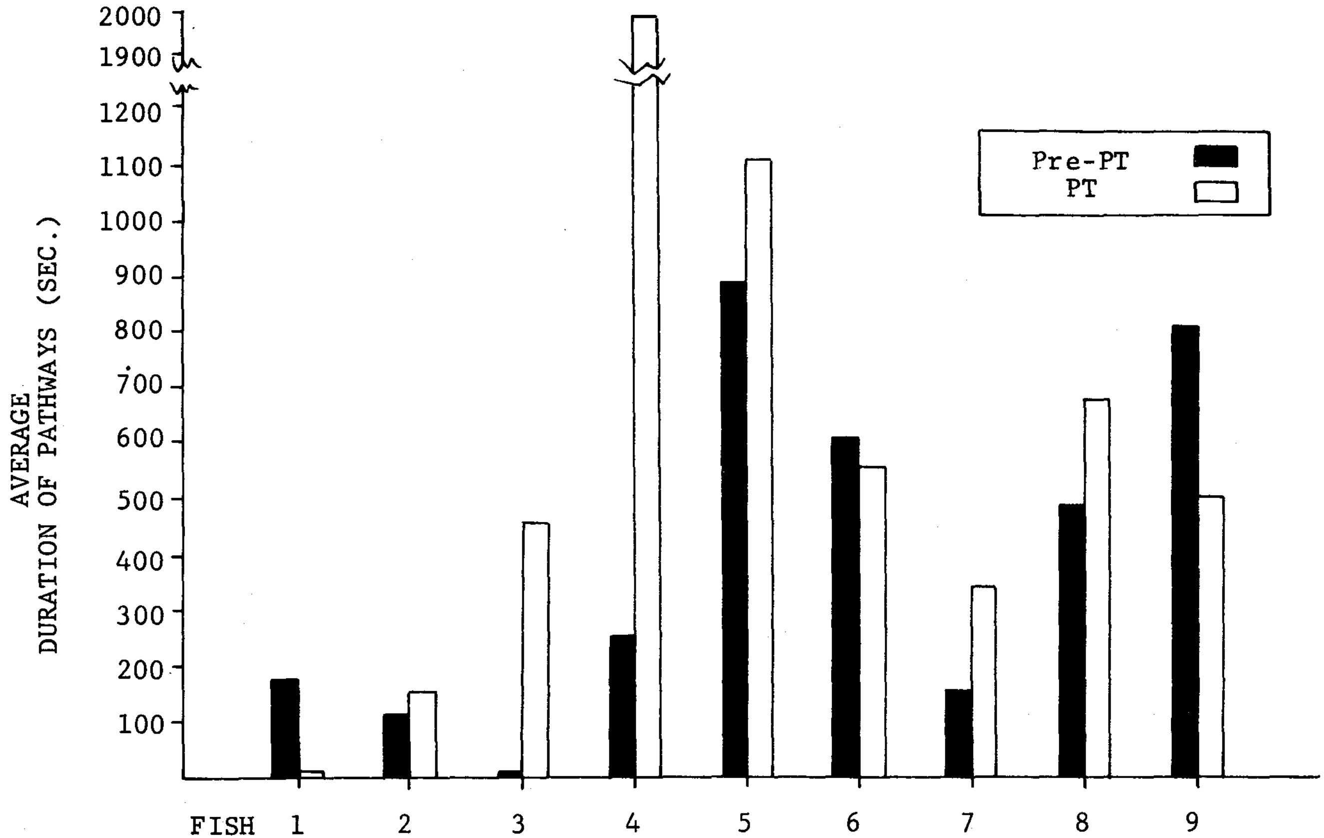

Figure 1. Average duration of pathways of pre-parathion treated (Pre-PT) and parathion treated (PT) goldfish (n=9).

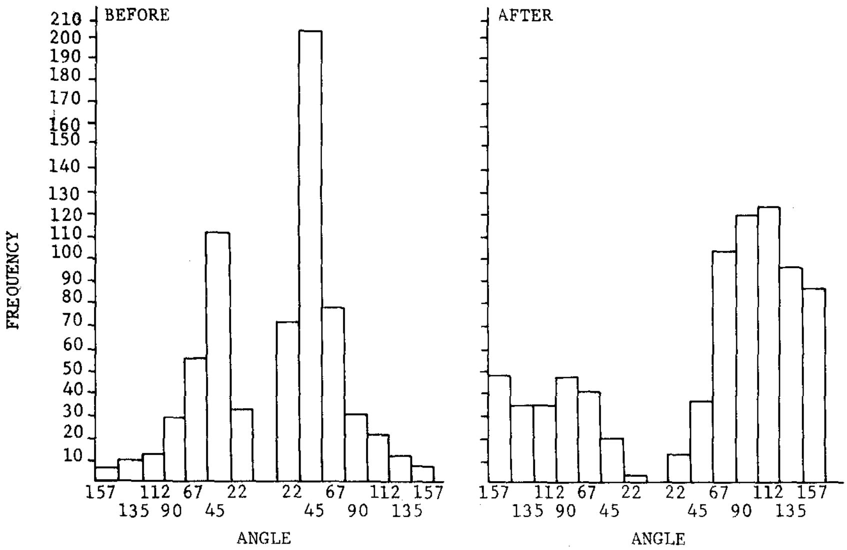

Figure 2. Frequency distribution of orientation angles from all compartments before and after parathion exposure for fish number two.

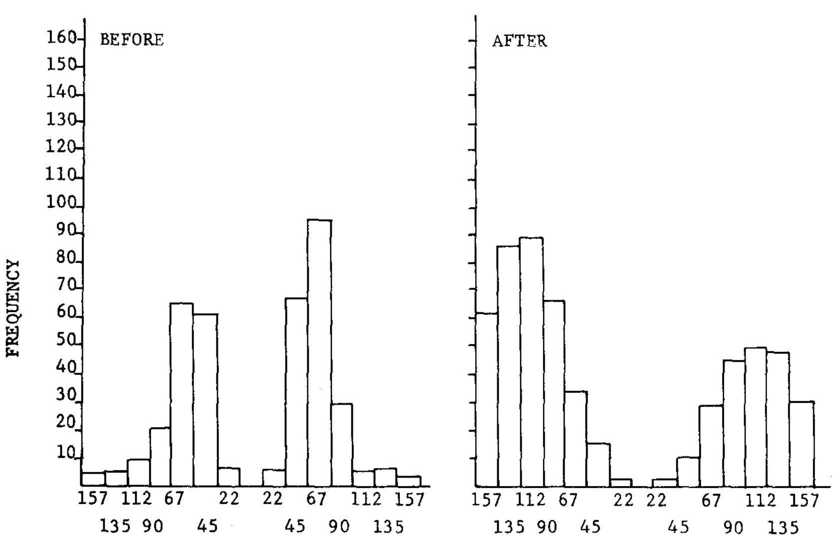

Figure 3. Frequency distribution of orientation angles from all compartments before and after parathion exposure for fish number three.

whereas the remainder of fish did not show such a trend. This suggests that parathion exposure may affect motor control mechanisms. This has not been demonstrated but it has been shown that exposure to subacute concentrations of parathion induce irreversible tonic contractures beyond tetany in skeletal muscle of crab (Carcinus maenas) (BRADBURY, 1973a, 1973b).

In earlier work (WESTLAKE and KLEEREKOPER, 1970), it was shown that a "memory" or retention process was involved in the turning behavior of the goldfish. This behavior may be assumed, at least in part, to be under central nervous system control. More recently, it was found (DAVY et al., 1972) that the above behavior in the goldfish was significantly affected by DDT exposure and as a result, it was postulated that DDT impaired the retention mechanism involved in this behavior.

Because orientation angles are a function of turning behavior, and thus may also be assumed to be under the control of a neural "retention" mechanism, it is evident from the results that parathion exposure may affect this mechanism. It is suggested that if this is so, a large number of other behaviors involving locomotion (localization of a mate, defense against predators, orientation, etc.) may also be affected.

This and other behavioral toxicity studies have indicated that tests (LD_{50}, LC_{50}, TL_m) which assess the acute effects of a pollutant, using death as an endpoint, have serious biological limitations. Because of water dilution, organisms usually experience pollutant concentrations far below lethal levels which may modify a "normal" behavioral response not directly leading to death but which nonetheless may threaten the organism's survival. Therefore, it is important that research be continued in behavioral toxicology because behavioral observations not only lend themselves to direct interpretation concerning water quality, but they also provide a relatively rapid means of bioassay.

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