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Fathead Minnow Optomotor Response as a Behavioral Endpoint in Aquatic Toxicity Testing

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Fish behavior tests have long been recognized as good indicators of sublethal levels of toxicants in water (Kleerekoper 1976; Marcucella and Abramson 1978; Rand 1985). Endpoints being developed for use in controlled laboratory studies include swimming behavior, chemoreception, spontaneous locomotor activity, ventilatory behavior, and preference-avoidance. Here we report an evaluation of an optomotor response test in fathead minnows (Pimephales promelas) as a behavioral toxicity assay for the laboratory. The goal of these experiments was to determine whether optomotor response measurements might be suitable for use with these fish, either in effluent biomonitoring programs or for establishing maximum allowable contaminant levels (e.g., MATCs) for specific pollutants.

Optomotor response has been defined as movement of an animal in the direction of moving reference points within its field of vision (Scherer and Harrison 1979). Optomotor response is a component of rheotaxis, defined as the reactions a fish displays in a current of water, responding to the visual, tactile and inertial stimuli resulting from the displacement of the fish relative to the position of natural landmarks along the stream bed and to water flowing over its body (Harden Jones 1963; Arnold 1974). The optomotor response can be elicited in the laboratory by moving the background past the fish, and Scherer and Harrison (1979) proposed using this technique to determine impaired visual-orientational functions. Dodson and Mayfield (1979a,b) examined optomotor response to sublethal concentrations of diquat, simazine (fenitrothion) and 2,4-D butoxyethanol ester in rainbow trout, concluding then that it was a sensitive bioassay for sublethal effects of biocides. The main objectives of our experiments were to determine whether consistent optomotor responses could be observed with the fathead minnow, a widely-used standard test species, and to compare relative sensitivity of this test with swimming capacity, one of the more commonly used fish behavior tests.

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MATERIALS AND METHODS

Juvenile fathead minnows (Pimephales promelas) were obtained from a commercial fish supplier (Chico Game Fish Farm, Chico, CA) and were acclimated in tap water purified by carbon filtration for a minimum of two weeks prior to testing. This water was also used for toxicant dilution water. Water was aerated to at least 60% oxygen saturation, water temperature was 22 +/- 1°C, photoperiod was 12:12, and fish were fed Tetramin (TetraWerke, Melle, Germany) daily, with the last feeding 24 hours before exposures began.

Fish were exposed to toxicants or toxicant dilution water under static conditions for 24 hours. Diquat and fenitrothion were selected for these studies because both are currently used in substantial quantities, levels toxic to fish were known, and although both are biocides they have different mechanisms of action. Fenitrothion (CAS# 112-14-5, 99% pure) was obtained from Crescent Chemical Co., Haupauge, NY. A commercial preparation of diquat (CAS# 85-00-7, 35% formulation) was provided by the Ortho Division of Chevron Chemical, Fresno, CA. The remaining inert ingredients were proprietary and were not analyzed, nor were test concentrations, because the primary interest was whether fish responded consistently to the optomotor stimulus with any given test substance.

Three independent 24-hr exposure and paired optomotor/swim capacity experiments were conducted for each compound using three different batches of fish. Static 24-hr LC50s for each compound were determined with each batch by the method of Weil (1952). Sublethal concentrations used to evaluate behavioral endpoints in each batch of fish were expressed as percentages of the LC50 to facilitate comparison among different shipments of fish.

An optomotor response test apparatus (Figure 1) was constructed based on designs described by Harden Jones (1963) and Dodson and Young (1977). When the black and white striped cylindrical screen revolved slowly around the outside of the aquarium, the fish normally responded by swimming in the same general direction as the moving screen. When the direction of screen rotation was reversed, the fish also reversed direction.

After exposures, individual fish were transferred to the optomotor aquarium containing fresh dilution water. Each fish was allowed to acclimate to the test conditions for four minutes while the screen revolved slowly. Each fish was then evaluated using a standard 8-min test protocol. First the screen was rotated slowly (approximately 2 rpm) in alternating directions during six 1-min periods to test response latency (RL). This was defined as time elapsed from the second the screen began moving in a given direction after the acclimation period to when the fish began swimming in the direction of

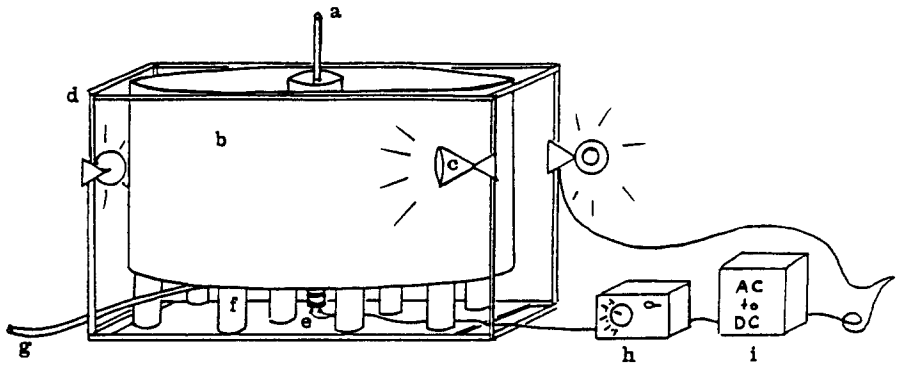

Figure 1. Optomotor response test apparatus, showing (a) motor shaft, (b) 60-cm diameter donut-shaped aquarium and screen (vertical stripes on screen not shown), (c) illumination sources; (d) frame, (e) motor, (f) platform, (g) drain tube, (h) rheostat control box, (i) and power transformer.

screen rotation, and was averaged for the six periods. During the final 2-min period the screen revolved in one direction. Total time spent swimming in the same direction as screen rotation (T+) was measured during this period, as well as the number of complete circles made by the fish around the center core of the aquarium (REVs). Exposed and control fish were tested in random order. The optomotor trials were videotaped and scored blind after all fish had been tested.

Swim capacity was tested in a 92-cm x 2.5-cm diameter (OD) tunnel fitted at the downstream end with a ball valve to control water flow. Each fish was challenged with fresh dilution water immediately after completing the optomotor trial to enable direct comparison of the two behavior tests. Flow rate started at approximately 3 cm/sec and was increased every 30 sec in step-wise increments until the fish was carried out the end of the tunnel by the flow. The standard length of each fish was used to convert terminal flow rates to fish lengths per second to account for any differences in fish size.

One-way analysis of variance (ANOVA) was used to determine significant differences among exposure and control group means. Duncan's multiple range test, reported as shortest significant range, was used to determine which of the group means were significantly different from each other. Tests showing a p-value less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The 24-hr LC50s determined in two or more separate tests for the fish batch used in experiments described here were 76.5 +/- 21.0 ppm for diquat and 7.46 ppm (both tests) for fenitrothion. No mortality was observed at the concentrations used for behavior testing.

Table 1 shows typical results of optomotor tests for diquat, with swim speed test results also shown for comparison. Response latency time (RL) was significantly reduced from that of controls ($p<0.05$) at all exposure concentrations. Mean time spent moving in the same direction as screen rotation (T+) was increased over control times ($p<0.01$). Complete revolutions made by treated fish around the aquarium in the direction of

TABLE 1. Optomotor response and swimming capacity of fathead minnows exposed to diquat. Data shown are means (S.D.) of 10 fish per concentration.

	Toxicant Concentration Expressed as Percentage of LC50			
	Control	12%	23%	46%
Optomotor Response Measures[1]				
RL	19 (16)	7* (5)	6* (5)	9* (9)
T+	36 (28)	82** (24)	107** (13)	76** (37)
REVs	0.7 (1.0)	2.1 (2.1)	3.6** (1.3)	2.5 (2.0)
Maximum Swimming Performance (fish lengths per second)				
	11.4 (2.1)	5.8** (1.4)	3.6** (0.9)	3.2** (1.2)

[1]RL=response latency (sec), T+=time spent swimming in positive direction during 2-min test period (sec), REVs=total revolutions in positive direction during 2-min period. Treatment mean significantly different from control: *, $p<0.05$ and **, $p<0.01$ according to Duncan's multiple range test.

screen rotation (REVs) were also increased over those observed in control fish, with statistical significance seen only at 23% of the diquat LC50 ($p<0.01$). With diquat, a biphasic trend was seen in all of the optomotor response measures. Judging from all experiments with this compound, some of which tested other diquat concentrations, RL decreased while T+ and REVs increased with exposures up to approximately 35% of the LC50, at which point all three measures apparently began to return to control levels. Statistical differences from controls were seen with two of the measures (RL and T+) at exposures as low as 12% of the LC50.

Typical optomotor response and swim capacity after fenitrothion exposure are shown in Table 2. Like diquat, fenitrothion decreased response latency time (RL), but increased both number of revolutions made in the direction

TABLE 2. Optomotor response and swimming capacity of fathead minnows exposed to fenitrothion. Data shown are means (S.D.) of 10 fish per concentration.

	Toxicant Concentration Expressed as Percentage of LC50		
	Control	35%	71%
Optomotor Response Measures[1]			
RL	19 (16)	9 (9)	4* (2)
T+	36 (28)	63 (35)	87** (26)
REVs	0.7 (1.0)	3.3* (3.1)	1.5 (1.4)
Maximum Swimming Performance (fish lengths per second)			
	11.4 (1.1)	8.7* (2.4)	7.3** (3.1)

[1]RL=response latency (sec), T+=time spent swimming in positive direction during 2-min test period (sec), REVs=total revolutions in positive direction during 2-min period. Treatment mean significantly different from control: *, $p<0.05$ and **, $p<0.01$ according to Duncan's multiple range test.

of the screen (REVs) and time spent swimming in the direction of screen rotation (T+) at the concentrations tested. Unlike diquat, fenitrothion RL and T+ measures show no biphasic tendency over this concentration range. This may be either a reflection of differing mechanisms of action of the two compounds or levels of exposure. Statistical differences from controls were seen in RL at 35% of the fenitrothion LC50, and in T+ and REVs only at 71%.

In contrast to the heightened optomotor response, average swimming capacity was consistently reduced in fish exposed to all toxicant concentrations tested relative to controls (Tables 1 and 2). The lowest toxicant concentrations at which statistically significant effects were seen in swimming performance were the same as those at which effects were observed in the optomotor response: 12% of the diquat LC50 and 35% of the fenitrothion LC50. However, the coefficients of variation within a single concentration were typically much lower for the swim speed test, ranging from 19% to 42%, than those seen with the optomotor response measurements, which ranged from 12% to 143%. T+ showed the lowest coefficients of variation of all optomotor measures. It is also apparent in Tables 1 and 2 that interindividual variability in optomotor responses within an exposure group was quite large in control fish and decreased as the effects of these toxicants became more noticeable.

Prerequisites for a useful aquatic bioassay are reproducibility, clearly defined quantifiable endpoints, reasonable sensitivity, and ecological relevance. Optomotor responses of fathead minnows exposed to diquat and fenitrothion were surprisingly reproducible; that is, responses were similar between fish lots when concentrations were expressed relative to the LC50 for that cohort. However, interindividual variability within a treatment group was often much higher than that seen in the swim speed test. The lower interindividual variability in swimming capacity tests contributed to consistently higher statistical significance in these tests than that seen with optomotor tests.

Videotaping the optomotor trials both eliminated observer bias during scoring and also facilitated evaluation of three different quantifiable endpoints. A computerized scoring system using a three-dimensional array of photobeam detectors could also be developed into the test system, similar to the activity box used by mammalian toxicologists. Time to response (RL) was the simplest measurement to score manually, but time spent swimming in the positive direction (T+) most consistently discriminated exposed from control fish, partly because individual variation in a treatment group was lowest for this measure.

With regard to sensitivity, the optomotor test was comparable to the swimming performance test in its ability to detect the lowest level of these

two toxicants causing effects at levels of $p<0.05$. Although swimming behavior in general is enjoying increasing acceptance as a measure of sublethal toxicity, the capacity to swim against a flowing current is not considered to be among the most highly sensitive measures of effect (Little and Finger 1990). Optomotor responses were sometimes enhanced with these test substances, and some optomotor responses appeared to be biphasic within the concentrations tested. Although swim capacity was consistently reduced with these compounds at the concentrations tested, biphasic swim speed responses have also been reported with a number of chemicals (Little and Finger, 1990).

Enhanced optomotor responses in fish exposed to these pollutants were not expected and differ from the diminished optomotor responses of rainbow trout exposed to these same toxicants as reported by Dodson and Mayfield (1979a,b). Although adapted from earlier work, the method we used to quantify results also appears to discriminate more readily between exposed and unexposed fish. Alternatively, the contradictory results may reflect interspecies differences in response. Enhanced, biphasic or hormetic effects have been documented in many plant and animal systems (Calabrese et al. 1987). McKim et al. (1987) observed that overreaction to stimuli is one characteristic of a behavior syndrome seen with exposure to cholinesterase inhibitors, like fenitrothion, as well as with other agents. It seems reasonable to suggest that an abnormally enhanced response could represent an overreaction to the boldly striped screen stimulus in cases where o ther more subtle cues used by a normal fish to orient itself are masked by effects of the toxicant. Enhanced responses to diquat in this study could also have been due to other ingredients in the formulation. Other pollutants would not necessarily produce enhanced optomotor responses. Furthermore, enhanced optomotor responses may be as deleterious to an organism overall as diminished ones.

Establishing the ecological significance of aquatic behavior tests requires extensive laboratory and field validation well beyond the scope of this initial evaluation. Clearly, however, the rheotropic response involves integration of numerous visual, tactile and kinetic cues and responses (Smith, 1984) used by fish to feed, mate, avoid predators and otherwise function in a stream or in a school of cohorts. Optomotor response is more apparent in pelagic, schooling or stream dwelling fish than in bottom dwellers (Harden Jones 1963). Possessing these qualities, fathead minnows seem to be a suitable species to use for further work with the optomotor test, bearing in mind that they may predict the response of only certain species. Large interindividual variability in response may limit the value of this test as a routine toxicity bioassay. Even so, this laboratory optomotor test could be a useful research tool for investigating the impact of toxicants and other environmental stressors on those species dependent upon optomotor skills for survival.

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