Arch. Environ. Contam. Toxicol. 31, 232–238 (1996)

ARCHIVES OF
Environmental
Contamination
and Toxicology

Behavioral Effects of Waterborne Carbofuran in Goldfish

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Received: 18 November 1995/Revised: 28 February 1996

Abstract. The effects of concentration (1, 10, 100 μg/L) and duration (4, 8, 12 h) of exposure to carbofuran were assessed on the swimming activity, social interactions, and behavioral responses of goldfish to a flow (0.1 L/min) of water, with or without chironomids. Observations were also made on the behavioral responses of unexposed goldfish to a flow (0.1 L/min) of carbofuran-contaminated water. A 4-h exposure of goldfish 1 μg/L carbofuran produced a significant increase in sheltering, burst swimming, and nipping. Responses were enhanced at 100 μg/L. After a 12-h exposure, the behavioral effects of 1 μg/L carbofuran were less apparent. However, burst swimming at 10 μg/L, and sheltering, nipping and burst swimming at 100 μg/L, were still significantly increased after a 12-h exposure to carbofuran. Grouping was not consistently affected by exposure conditions. Chemical attraction to a filtrate of chironomids was significantly reduced after the 4-h exposure to 1 μg/L carbofuran. Decreased attraction to the food extract was less apparent after the 12-h exposure, except at 100 μg/L carbofuran. A significant decrease in attraction to a flow of uncontaminated water was also observed after a 4-h exposure to 10 and 100 μg/L carbofuran. Unexposed goldfish did not show avoidance reaction to a flow of carbofuran-contaminated water, even at a concentration (10 mg/L) exceeding the mean 96-h LC-50 in cyprinids (0.5–1 mg/L). However, at all concentrations tested (0.1, 1, 10 mg/L), goldfish quickly reacted to the introduction of the solution of carbofuran by increased burst swimming and nipping. These results are discussed in the light of the data concerning behavioral and neurotoxic effects of carbamate and organophosphorous insecticides in fish.

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate) is a broad spectrum systemic carbamate insecticide, commonly used in agricultural practice throughout the world. its relatively high hydrophilic and low adsorption to soils and sediments can result in concentrations up to 1 mg/L in runoff water from treated fields (Caro *et al.* 1973).

Carbofuran is very toxic to fish, with 96-h LC-50 generally below 1 mg/L (Trotter *et al.* 1991). Similar to other carbamate and organophosphate insecticides, the mode of action of carbofuran is based on inhibition of acetylcholinesterase activity at synaptic and neuromuscular junctions (Jash and Bhattacharaya 1983). In a freshwater teleost (*Channa punctatus*), alterations in the levels of neurotransmitters in the cerebral cortex have been recently shown in response to sublethal carbofuran concentrations (Gopal and Ram 1995). Such neurotoxic effects in brain area could adversely affect the behavior of fish on exposure to carbofuran.

The use of behavioral endpoints appears as a non invasive and sensitive method in fish toxicology. For the majority of pollutants tested, alterations in fish behavior have been observed in response to short-term exposures and sublethal concentrations (Giattina and Garton 1983; Atchison *et al.* 1987; Little and Finger 1990; Sandheinrich and Atchison 1990).

However, data on behavioral effects of carbamates in fish are scarce and mainly concern carbaryl (1-naphthyl methylcarbamate) (Hansen 1969; Hansen *et al.* 1972; Lunn *et al.* 1976; Peterson 1974; Little *et al.* 1990) and benthiocarb (S-4-chlorobenzyl diethylthiocarbamate) (Hidaka *et al.* 1984; Ishida *et al.* 1994). Recent observations in larval medaka (*Oryzias latipes*) showed that exposure to carbofuran caused an impairment of swimming performance (Heath *et al.* 1993).

In the present study, observations were made on the influence of concentration and duration of exposure to carbofuran on some behavioral activities in the goldfish, *Carassius auratus*.

Since behavioral alterations produced by exposure to toxic agents may be caused by adverse effects on chemosensory systems (Doving 1991; Klaprat *et al.* 1992), olfactometric tests were performed in order to assess the influence of carbofuran on behavioral responses to a food extract. Tests were also run to check whether goldfish can show an avoidance behavior in response to a flow of carbofuran-contaminated water.

Materials and Methods

Fish

Behavioral observations were run from August to October 1994, with 475 juvenile goldfish (total length 7–9 cm, weight 8–11 g), all from

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the same batch, extensively hatched and grown in an outdoor pond of 100 m^2. The fish were acclimated for one month, before being tested inside a greenhouse.

Goldfish were kept at low densities (2.0–2.5 g. fish/L) in 4 thermoregulated 400-L polyvinyl chloride-lined fiberglass tanks (200 cm long × 50 cm wide × 40 cm deep), each supplied with charcoal-filtered recirculated and dechlorinated tap water flow-through (0.2 L/min) system. Water quality characteristics in both rearing and observation tanks throughout the experimental period ranged as follows: temperature 17–19°C; pH 7.5–7.8; dissolved oxygen 9–10.8 mg/L, total hardness 130–150 mg/L (as $CaCO_3$). All tanks received natural day light. Fish were fed until satiation twice daily, at 0800 h and 1700 h on frozen chironomids (Grebil Society, France) and carp food pellets (Aqualim, France).

Test Stimuli

Carbofuran (99.9% purity) was purchased from Cluzeau Info Labo (France). Carbofuran was diluted in acetone (0.2 mg/μL) before mixing in water.

In the part of the study concerning the behavioral effects of duration and concentration of exposure to carbofuran, the maximum acetone concentration in the 150-L test tanks was 0.5 μL/L. For the observations on the behavioral responses of unexposed goldfish to a flow of carbofuran-contaminated water in the test tanks, maximum amount of acetone used for dilution of carbofuran in the 1-L dripping test solution was 50 μL. In goldfish, Davy *et al.* (1972) failed to show a behavioral effect of acetone, at a concentration higher than that used in the present work for dilution of carbofuran. However, same amounts of acetone were added to controls to serve as a check on solvent effect. The chironomid extract was obtained by filtration, after 5 min maceration of 150 mg frozen chironomids in 1 L distilled water. All solutions were made prior to testing.

Test Apparatus

The fish were observed in two linear test tanks (Figure 1). Each device was identical in material and surface area to rearing tanks, but subdivided in 4 zones (45 × 50 cm) by lines drawn on the bottom. To minimize stress and wall-hugging tendency (Steele 1983), a shelter (shadow screen; 20 × 50 cm) was set above the center of each tank. Test tanks were filled with 150 L dechlorinated tap water. Both tanks were isolated with silent blocks and black plastic sheeting. Behaviors were observed at the side by means of a small opening (3 cm in diameter).

Observations on the behavioral effects of duration and concentration of exposure to carbofuran were made in contaminated static water. A drip (Perfusor Souplix R 30, Bruneau Laboratory, France) was used to test the behavioral responses of exposed and unexposed fish to a flow of a solution (vol 1 L, flow rate 0.1 L/min) of chironomids, uncontaminated water, or carbofuran-contaminated water. By the test using a dripping solution (vol 1 L, flow rate 0.1 L/min) of Methylene blue, the dye was ascertained to travel within 10 min from the inlet, below the surface of zone 1, to the other end (zone 4), without overflowing from the tank.

Responses Recorded

Within 10 min observation periods, the following behavioral activities of goldfish were recorded during 20 periods of 30 sec each:

Sheltering: fish remaining stationary under the shelter.
Grouping: 6 to 8 fish found in the same zone (1, 2, 3, 4, shelter).
Nipping: nips triggering avoidance reactions in the recipient.
Burst swimming: sudden spurt of nondirected movement. Burst swimming did not last more than a few seconds and was followed by immobilization of the fish.
Attraction: fish coming to a stop under the diffuser, in zone 1.

Number of fish sheltering and grouping were recorded every 30 sec. Other behavioral endpoints were counted within each 30-sec period.

Experimental Procedures

Observations were made on groups of 8 fish, not fed on the day of testing. Goldfish were introduced at 0800 h in the test tanks. Fish were tested only once and tanks were cleaned throughly between tests.

Within each experiment, the order in which treatments were applied in the test tanks was randomized. To eliminate observer bias, preparation of tests and behavior recordings were made by different persons, so that the observer (P. Saglio) was unaware of the experimental treatment he tested.

Procedures in the different experiments were as follows:

Effect of Carbofuran on Behavioral Activities

Water was contaminated with carbofuran 30 min before introduction of goldfish in the test tanks. Three concentrations tested were 1, 10, and 100 μg/L. For each concentration, observations were made after 4, 8, and 12 h of exposure to the static concentration. Behaviors were recorded for 10-min intervals.

Effect of Carbofuran on Behavioral Responses to a Food Extract

Immediately after the end of the previous recordings, 1 L of a chironomid extract was introduced (flow rate 0.1 L/min) in zone 1. Observations were made during the 10-min flow.

During each of the two consecutive observation periods, behaviors of exposed goldfish were compared to unexposed controls, 4, 8, or 12 h after their introduction in the test tank. Each combination concentration/duration of exposure was tested 3 times (4 replicates for the control).

Effect of Carbofuran on Behavioral Responses to the Flow of Water

Tests were carried out to differentiate the chemical attraction produced by the presence of food in the flowing test solution from the orientated movements to the flow (rheotaxis). Four hours after introduction of goldfish in the test tanks, attraction responses of controls and carbofuran-exposed fish to the filtrate of chironomids were compared to responses to an effluent of dechlorinated tap water, at same flow rate (0.1 L/min). The effects of concentrations of exposure to carbofuran (1, 10, 100 μg/L) on responses to the water flow were tested 3 times (4 replicates for the control).

Behavioral Responses of Unexposed Fish to a Flow of Carbofuran-Contaminated Water

Goldfish were introduced in uncontaminated water and left for 4 h to acclimate. Behaviors were observed at 1200 h during three consecutive periods lasting 10 min each. During the first period, water was uncon-

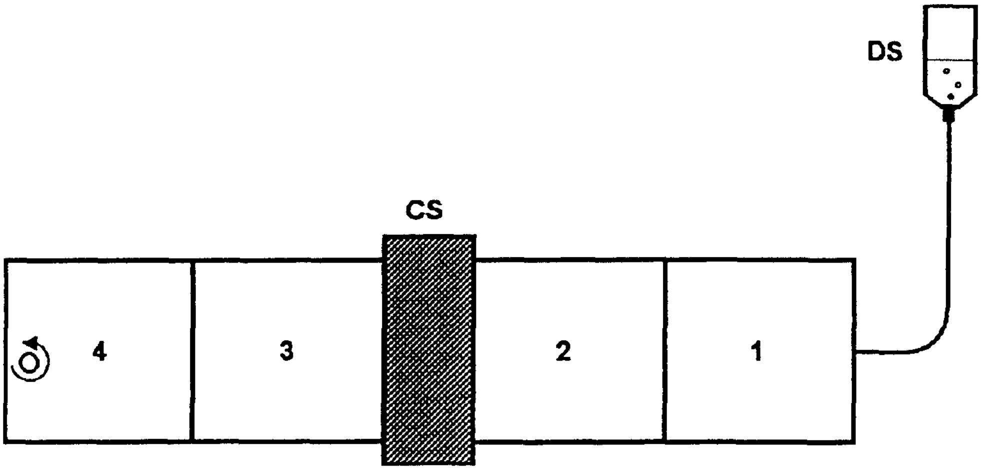

Fig. 1. Experimental device. Verticl view of a test tank. Dripping solutions were filtrate of chironomids, uncontaminated water, or carbofuran-contaiminated water. Solutions were introduced (flow rate: 0.1 L/min) below the surface, in zone 1. Test tanks were isolated with silent-blocks and black plastic sheeting. Behaviors were observed at the side by means of a small (3 cm in diameter) opening. Observations were made on swimming activity, social interactions, and attraction to the diffuser. All behavioral recordings were performed during 10-min periods on groups of 8 fish. CS: central shelter; DS: dripping solution; 1, 2, 3, 4: observation zones

Table 1. Influence of duration and concentration of exposure to carbofuran on behavioral activities in goldfish

Duration and concentration of exposure	Behavioral activities[a]			
	Sheltering	Grouping	Nipping	Burst swimming
4 Hours				
0 (Control)	6.2	14.2	0.2	0
1 μg/L	20.6 S	12.3 NS	6.6 S	8 S
10 μg/L	23.3 S[b]	8.3 S	3 S	12.3 S
100 μg/L	127.3 S	19 NS	7.3 S	24.6 S[b]
8 Hours				
0 (Control)	6.2[b]	11	0	0
1 μg/L	10.3 NS[b]	15 NS	0 NS	6 S
10 μg/L	18 NS[b]	15.3 NS	1 NS	14 S
100 μg/L	101 S[b]	19.3 NS	4.3 S	24 S
12 Hours				
0 (Control)	5.5[b]	12.7	0.2	0
1 μg/L	9 NS[b]	15.3 NS	1.3 NS[b]	2 NS
10 μg/L	11.3 NS[b]	10.6 NS	1.3 NS[b]	8.6 S
100 μg/L	98.6 S[b]	14.6 NS	4.6 S	15 S

[a] Behaviors were recorded on groups of 8 fish. Values are means of 3 replicates (4 in controls). Significance (S) is indicated for behaviors differing ($p \leq 0.05$) from unexposed controls. NS: nonsignificant difference

[b] Indicates significant heterogeneity ($p \leq 0.05$) among replicates

taminated. During the second period, 1 L of a solution of carbofuran was introduced (flow rate 0.1 L/min) above zone 1. Observations were made during the third period, after the flow of the test solution ceased. Three concentrations were tested: 0.1, 1, 10 mg/L carbofuran. These values represent the concentrations at the level of the dripping solution, which were then diluted in the test tanks. Tests of dripping solutions of Methylene blue showed that the dye did not overflow. However, precise carbofuran concentrations in the different zones of the test tank, during and after the flow, were not measured. Behavioral activities were compared to controls receiving a flow of dechlorinated tap water. Each concentration was tested 3 times (4 replicates for the control).

Data Analysis

Homogeneity among trials within treatments was analyzed, using a chi-square test of homogeneity. All results were transformed, using arc sin $\sqrt{X}$, to stabilize the variance and to approach more closely a normal distribution (Sokal and Rohlf 1969). The mean values for each treated group of fish were compared with that of the control group using the Student's t-test. For each comparison, significant differences from controls were identified at $p \leq 0.05$. A regression analysis (Sherrer 1984) was used to test for relationships between concentration or duration of exposure and behavioral endpoints.

Results

Homogeneity Among Trials

A good homogeneity was found among replicates for the behavior of control groups. Exposure to carbofuran resulted in an increased heterogeneity in behavior, particularly in sheltering and swimming orientation (attraction) to the diffusor.

Effect of Exposure on Behavioral Activities

Comparison with control fish pointed out significant effects of duration and concentration of exposure to carbofuran on sheltering, nipping and burst swimming (Table 1). Increase in these behaviors was found after the 4-h exposure, at the lowest concentration (1 μg/L) tested. Responses were enhanced at 100 μg/L. The effects of 1 μg/L carbofuran on behavioral activities were less apparent after 12 h of exposure. However, burst swimming at 10 μg/L, and sheltering, nipping, and burst swimming at 100 μg/L, were still significantly increased after a 12-h exposure to carbofuran. Grouping was not consistently affected by exposure conditions. The regression analysis showed a significant ($p \leq 0.05$) relation between the concentration of exposure to carbofuran and the observed changes in

Table 2. Influence of duration and concentration of exposure to carbofuran on behavioral responses to a filtrate of chronomids in goldfish

Duration and concentration of exposure	Behavioral responses[a]				
	Sheltering	Grouping	Nipping	Burst swimming	Attraction
4 hours					
0 (Control)	1.2	18.7	0	0	187.5
1 μg/L	14.3 S[b]	17.3 NS	5 NS[b]	2.6 S	115.6 S[b]
10 μg/L	16 S[b]	13.6 S	5.3 S	2 NS[b]	68 S
100 μg/L	106 S	14.6 NS	9 S	25.5 S	30 S
8 Hours					
0 (Control)	2.2	17.7	0	0.6	181.5
1 μg/L	10 NS[b]	18 NS	1 NS	4.6 S	132 S
10 μg/L	12 NS[b]	16.6 NS	0.6 NS	3.3 NS[b]	127 NS[b]
100 μg/L	74.3 S[b]	17 NS	1.6 NS	15 S	66 S[b]
12 Hours					
0 (Control)	2.2	18.7	0	0.2	200
1 μg/L	3.6 NS	17.3 NS	0 NS	0 NS	173.3 NS[b]
10 μg/L	5.3 NS[b]	16.3 NS	0 NS	2.6 NS[b]	152.6 NS[b]
100 μg/L	50.6 S[b]	18.3 NS	0.3 NS	7 S[b]	97.6 S[b]

[a] Behaviors were recorded on groups of 8 fish. Values are means of 3 replicates (4 in controls). Significance (S) is indicated for behaviors differing ($p \leq 0.05$) from unexposed controls. NS: nonsignificant difference

[b] Indicates significant heterogeneity ($p \leq 0.05$) among replicates

Table 3. Influence of a 4-h exposure to different carbofuran concentrations on attraction responses to chironomid-free water and solutions of chironomids in goldfish

Carbofuran concentration	Attraction responses to chironomid-free water	Attraction responses to solutions of chironomids
0 (control)	30.6	187.5
1 μg/L	34 NS[a]	115.6 S[b]
10 μg/L	11.6 S	68 S
100 μg/L	4.3 S[b]	30 S

[a] Significance (S) is indicated for responses differing ($p \leq 0.05$) from unexposed control. Observations were made on groups of 8 fish. Values are means of 3 replicates (4 in controls). NS: nonsignificant difference

[b] Indicates significant ($p \leq 0.05$) heterogeneity among replicates

sheltering ($R^2 = 0.67$), grouping ($R^2 = 0.17$), nipping ($R^2 = 0.33$), and burst swimming ($R^2 = 0.35$).

Effect of Exposure on Behavioral Responses to a Food Extract

During olfactometric stimulation by the filtrate of chironomids, the behaviors of carbofuran-exposed fish differed from those of controls (Table 2). In fish exposed for 4 h to 1 μg/L, sheltering and burst swimming were increased during the flow of the filtrate, while attraction was decreased. At 10 μg/L and 100 μg/L, nipping significantly increased during stimulation by the food extract. The effects of carbofuran on behavioral responses to the filtrate were less apparent after the 12-h exposure, except at the highest exposure concentration where differences in sheltering, burst swimming and attraction were still significant. The regression analysis showed significant effects ($p \leq 0.05$) of duration of exposure on nipping ($R^2 = 0.30$) and attraction ($R^2 = 0.15$) and of concentration of exposure on sheltering ($R^2 = 0.56$), nipping ($R^2 = 0.30$), burst swimming ($R^2 = 0.50$), and attraction ($R^2 = 0.52$).

Effect of Exposure on Behavioral Responses to a Flow of Water

In all groups of fish exposed for 4 h to carbofuran, attraction produced by the solution of chironomids was significantly higher than the slight attraction observed in response to a flow of water (Table 3). However, comparison to unexposed controls showed that exposure to the concentrations of 10 and 100 μg/L significantly decreased attraction to the water effluent.

Behavioral Responses of Unexposed Fish to a Flow of Carbofuran-Contaminated Water

Some of the observed behaviors of goldfish were not affected by the flow of solutions of carbofuran in the test tank (Table 4). This is the case for grouping. Similarly, the slight attraction observed in controls in response to a drip of pure water was not significantly reduced by the concentrations of carbofuran in the flow, thus suggesting that goldfish did not avoid a flow of carbofuran-contaminated water. Burst swimming reactions were observed in response to all concentrations tested, during and after the stimulation. Number of nips were also significantly increased after introduction of the solutions in the tank.

Discussion

This study showed that short-term exposures of goldfish to low carbofuran concentrations affected swimming pattern, social interactions and orientation to a food extract or to an uncontaminated water source. Goldfish showed little reaction to a flow of carbofuran-contaminated water, with no avoidance at concentrations (10 mg/L) that exceeded the mean 96-h LC-50 (0.5–1 mg/L) in cyprinids (Trotter *et al.* 1991).

Carbofuran concentrations of up to 25 μg/L may occur in surface water (Matthiessen *et al.* 1995). Significant behavioral

Table 4. Behavioral activities of goldfish before, during and after the introduction of carbofuran contaminated water, at three concentrations

Concent mg/L	Sheltering			Grouping			Nipping			Burst swimming			Attraction		
	Before	During	After	Before	During	After	Before	During	After	Before	During	After	Before	During	After
0	16[b]	10.3	14.6	8	11	7.6[b]	0.3	0.3	0	0	0	0	0.3	30.6	2.6
0.1	12.6 NS	13 NS[b]	9.3 NS	8.6 NS	15.3 NS	8 NS	2.3 NS	7.6 S	9.6 S	0.3 NS	9 S[b]	4 S	0 NS	29.3 NS[b]	5.3 NS[b]
1	28.6 NS[b]	19.3 NS[b]	18.3 NS[b]	14.3 NS	15.6 NS	12.6 NS	0.6 NS	4.3 S	4.3 S	0.3 NS	7 S	10.6 S	0 NS	21.6 NS[b]	3 NS[b]
10	14 NS	32.3 S	32 NS[b]	15 NS	12.6 NS[b]	10.6 NS[b]	0.3 NS	1.3 NS	8 S[b]	0.6 NS	11.3 S[b]	18.6 S[b]	0 NS	12.3 NS[b]	3 NS

[a] Behaviors were recorded on groups of 8 fish. Within each 10-min observation period, behavioral activities are compared to controls receiving a solution of pure water. Values are means of 3 replicates (4 in controls). Significance (S) is indicated for behaviors differing ($p \leq 0.05$) from controls. NS: nonsignificant difference

[b] Indicates significant ($p \leq 0.05$) heterogeneity among replicates

changes were observed here in goldfish exposed to concentrations as low as 1 μg/L.

Most studies on behavioral effects of organophosphate and carbamate insecticides in fish concern responses to long-term exposures (24 h and more), but some authors have reported changes in swimming activity during the first hours of exposure to sublethal concentrations of fenitrothion (*O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate) (Bull and Mc Inerney 1974) and dichlorvos (2,2-dichlorovinyl dimethyl phosphate) (Ghosh 1986). Our observations also showed significant behavioral alterations after 4 h of exposure to carbofuran. Early-occuring behavioral effects may be related to the fact that, in fish brain, exposure to carbofuran may induce a quick decrease in levels of acetylcholinesterase (AChE) activity. Incubation of brain tissue of an Indian siluroid fish (*Heteroprieustes fossilis*) with carbofuran (320 μg/L) caused a 50% reduction in AChE activity after 40 min (Sur and Ghose 1978). However, behaviors of goldfish did not differ significantly from those of controls after 12-h exposure to 1 μg/L, while compensation was almost complete in fish exposed to 10 μg/L, but only partial at 100 μg/L. Such a compensation process might be linked to a partial repletion of AChE levels. In mammals, almost complete recovery of brain AChE levels after acute intoxication to carbofuran may occur within 24 h (Gupta and Kadel 1989; Gupta 1994).

This eventuality remains questionable in fish, where it has been showed that weeks can elapse before brain AChE return to normal levels after a 48-h exposure to high (300 to 462 μg/L) carbofuran concentrations (Jash and Bhattacharaya 1983). A lack of correspondence between behavioral alterations and inhibition of AChE activity has been also reported for organophosphorous insecticides known for their delayed neurotoxic effects (Post and Leasure 1974; Heath 1987; Heath *et al.* 1993).

The quick compensation observed here upon exposure to low concentrations of carbofuran cannot either be explained by half-life of carbofuran in water. Owing to characteristics of water used in the present study, half-life of carbofuran might exceed 10 days (NRCC 1979; Chapman and Cole 1982). However, discrete changes in carbofuran concentrations in the test tanks within the 12-h exposure were not analyzed.

The short-term compensation in behavioral alterations might rather be related to some efficient process of carbofuran detoxication, possibly activated by induced decrease in AChE and other neurotransmitters in the fish brain (Gopal and Ram 1995). Apart from possible interspecific differences in sensitivity, such process might account for the fact that Heath *et al.* (1993) did not observe any significant changes in swimming activity of larval medaka after 4 days of their exposure to 110 μg/L carbofuran. In the same way, Henry and Atchison (1984) observed that changes in the behaviors of bluegill (*Lepomis macrochirus*) exposed for 96 h to sublethal concentrations of methyl parathion (*O,O*-dimethyl *O*-4-nitrophenyl phosphorothioate) mainly occurred within the first 24 h. Further investigations are thus needed concerning the relations between neurophysiological and behavioral responses to carbamates and organophosphorous insecticides in fish.

Locomotor of fish is subjected to high intraspecific variability (Rand 1977; Hose and Stoffel 1980; Hidaka and Tatsukawa 1986; Little and Finger 1990; de Peyster and Long 1993; Heath *et al.* 1993). In the present work, sheltering and swimming orientation showd increased heterogeneity following exposure to carbofuran.

Heterogeneity in swimming behavior within exposed groups might be related to carbofuran-induced changes in social interactions (increased aggressiveness). Conversely, social interactions have been found to affect the behavioral responses to toxic compounds (Sparks *et al.* 1972; Henry and Atchison 1984, 1986; Hidaka and Tatsukawa 1986). This study showed that grouping was not consistently affected by carbofuran exposure, but it is likely that the effects would have been different using a social group of fish of different number, size, or life history. In goldfish, schooling behavior can be a sensitive indicator of their physiological state (Weis and Weis 1972, 1974), and the influence of such basic social factors on the behavioral responses to toxicants needs further investigations.

Burst swimming and aggressive interactions (nipping) significantly increased upon exposure of goldfish to carbofuran. Virtually absent in controls, both behaviors can be considered as indicative of stressfull or conflicting situations. Burst swimming is used by fish to avoid predators (Fuiman 1986). In goldfish, either burst swimming and nipping can be produced by the chemical perception of alarm pheromone (Saglio and le Martret 1982). Burst swimming is an alarm behavior of social importance since it can be rapidly transmitted by visual contacts among individuals. The present observations showed that burst swimming reactions were followed by sheltering of the group of fish. An increase in the number of burst swimming had been noticed in bluegill exposed to 320 μg/L methyl parathion (Henry and Atchison 1984). They also observed an increase in the number of nips in social grooups of bluegill after a 24-h exposure to methyl parathion.

Behavior primarily depends on functional integrity of sensory systems. In fish, chemical sense regulates numerous behavioral processes (Hara 1992) and effects of aquatic contaminants on chemonsensory organs may be responsible for alterations in various aspects of feeding, social and reproductive behaviors (Klaprat *et al.* 1992). Olfactory and gustatory fish receptors are in direct and permanent contact with the environment and thus highly accessible to toxic agents. After a 2-h exposure of a siluroid fish *(Heteropneustes fossilis)* to methyl parathion (200 μg/L), Chakraborty *et al.* (1989) found a higher percentage of depletion of AChE in the olfactory organ than in the brain. In all groups of goldfish treated with carbofuran, chemical attraction produced by a filtrate of chironomids was significantly decreased after a 4-h exposure. Exposure also decreased attraction of goldfishto a flow of uncontaminated water. Then, both chemotactic and rheotaxic components of attraction response to food extract appeared affected by short-term exposure to carbofuran. In the goldfish, Rand *et al.* (1975) similarly found that a 24-h exposure to 330 μg/L parathion (*O*,*O*-diethyl *O*-4-nitrophenyl phosphorothioate) affected not only the swimming orientation to food odor and flow individually, but also the interaction between these two environmental stimuli. However, all carbofuran-treated groups significantly prefered the solution of chironomids over pure water, thus suggesting that food chemical perception remained—at least partly—intact.

Almost complete compensation was observed in attraction response to the food extract, after 12 h of expsoure to 1 and 10 μg/L. In fish, olfaction is mainly responsible for chemical attraction to food (Jones 1992). Short-term compensation in chemoattraction might be related to the presence of high levels of biotransformation enzymes in the fish olfactory organ, possibly involved in detoxication process (Brittebo *et al.* 1986; Monod *et al.* 1994).

Investigations are thus needed on the relationship between biotransformation enzymatic activities in the olfactory organ and effects of toxicants on olfactory perception and orientation in fish.

Quickly detecting and avoiding an adverse chemical is of survival value for an organism. However, there are a number of pesticides which do not stimulate either attraction or avoidance by fish at sublethal concentrations (Beitinger and Freeman 1983). Avoidance response to organophosphorous and carbamate insecticides may be induced by olfaction in fish (Hidaka and Tatsukawa 1989; Ishida *et al.* 1994), but the concentration-response relationship greatly depends on the compound and species tested. For example, mosquitofish *(Gambusia affinis)* avoids malathion (diethyl {dimethoxyphosphinothioylthio} succinate) at the concentration of 50 μg/L, but not at 5 mg/L, while sheepshead minnow *(Cyprinodon variegatus)* did not avoid the same compound at the concentrations of 100 μg/L, 1 mg/L, and 10 mg/L (Hansen 1969; Hansen *et al.* 1972).

The observations showed that the slight attraction observed in controls in response to a drip of pure water was not significantly reduced by carbofuran in the flow. Our observations showed that goldfish did not avoid a flow of carbofuran-contaminated water, even at high (10 mg/L) concentration. However, the onset of the contaminated flow induced immediate burst swimming responses, at all carbofuran concentrations tested.

In summary, the results of this study indicate that a short-term exposure to low concentrations of carbofuran may produce severe behavioral alterations in fish. Swimming impairment, enhanced intraspecific aggressiveness and decreased responses to chemical food stimuli caused by sublethal carbofuran exposure may indirectly affect survival of fish in the aquatic environment. Non-avoidance reactions to an acutely contaminated area can also be fatal to the fish. Owing to their sensitivity and ecological relevance, the behavior endpoints selected here are easy accessible indicators of toxicity which might be worthwhile to consider in environmental risk assessment.

Acknowledgments. We thank Gilles Monod and Laurent Lagadic for their helpful comments on the manuscript.

References

Atchison GJ, Henry MG, Sandheinrich MB (1987) Effects of metals on fish behavior: A review. Environ Biol Fishes 18:11–25

Beitinger TL, Freeman L (1983) Behavioral avoidance and selection responses of fishes to chemicals. Res Rev 90:35–55

Brittebo EB, Darnerud PO, Larsson J, Svanberg O, Brandt I (1986) O-dealkylation of phenacetin in the olfactory rosette in rainbow trout (Salmo gairdneri). Acta Pharmacol Toxicol 58:259–264

Bull CJ, McInerney JE (1974) Behavior of juvenile coho salmon *(On corhynchus kisutch)* exposed to sumithion (fenitrothion), an organophosphate insecticide. J Fish Res Bd Can 31:1867–1872

Caro JH, Freeman HP, Glotefty DE, Turner NC, Edwards WM (1973) Dissipation of soil-incorporated carbofuran in the field. J Agric Food Chem 21:1010–1015

Chakraborty PS, Mallik A, Dingal DK, Banerjee S (1989) Effect of methyl parathion on brain and olfactory organ acetylcholinesterase activity of the fish *Heteropneustes fossilis.* Environ & Ecol 7:310–314

Chapman RA, Cole CM (1982) Observations on the influence of water and soil pH on the persistence of insecticides. J Environ Sci Health 17B:487–504

Davy FB, Kleerekoper H, Gensler P (1972) Effects of exposure to sublethal DDT on the locomotor behavior of the goldfish *(Carassius auratus).* J Fish Res Bd Can 29:1333–1336

Fuiman LA (1986) Burst-swimming performance of larval zebra danios and the effects of diel temperature fluctuations. Trans Am Fish Soc 115:143–148

Ghosh TK (1986) Nuvan induced physiological, biochemical and behavioral changes in *Barbus stigma.* Poll Res 5:63–68

Giattina JD, Garton RR (1983) A review of the preference-avoidance responses of fishes to aquatic contaminants. Res Rev 87:43–90

Gopal K, Ram M (1995) Alteration in the neurotransmitter levels in the brain of the freshwater snakehead fish *(Channa punctatus)* exposed to carbofuran. Ecotoxicology 4:1–4

Gupta RG (1994) Carbofuran toxicity. J Toxicol Environ Health 43: 383–418

Gupta RG, Kadel WL (1989) Concerted role of carboxylesterases in the potentiation of carbofuran toxicity by iso-OMPA pretreatment. J Toxicol Environ Health 26:447–457

Hansen DJ (1969) Avoidance of pesticides by untrained sheepshead minnows. Trans Am Fish Soc 3:426–429

Hansen DJ, Matthews E, Nall SL, Dumas DP (1972) Avoidance of pesticides by untrained mosquitofish, *Gambusia affinis.* Bull Env Contam Toxicol 8:46–51

Hara TJ (ed) (1992) Fish chemoreception. Fish and fisheries series 6. Champan & Hall, London

Heath AG (1987) Water pollution and fish physiology. CRC Press, Boca Raton, FL

Heath AG, Cech Jr JJ, Zinkl JG, Steele MD (1993) Sublethal effects of three pesticides on japanese medaka. Arch Environ Contam Toxicol 25:485–491

Henry MG, Atchison GJ (1984) Behavioral effects of methylparathion on social groups of bluegill *(Lepomis macrochirus).* Environ Toxicol Chem 3:399–408

———, ——— (1986) Behavioral changes in social groups of bluegills exposed to copper. Trans Am Fish Soc 115:590–595

Hidaka H, Tatsukawa R (1986) Variations by sex and body size in the avoidance tests of sodium linear laurylbenzene sulfonate and fenitrothion using a fish, medaka *Oryzias latipes*. Bull Jap Soc Sci Fish 52:1753–1757

———, ——— (1989) Avoidance by olfaction in a fish, medaka *(Oryzias latipes)*, to aquatic contaminants. Environ Pollut 56: 299–309

Hidaka H, Hattanda M, Tatsukawa R (1984) Avoidance of pesticides with medakas (*Oryzias latipes*). J Agric Chem Soc Jap 58:145–151

Hose JE, Stoffel RJ (1980) Avoidance response of juvenile *Chromis punctipinnis* to chlorinated seawater. Bull Environ Contam Toxicol 25:929

Ishida Y, Yoshikawa H, Kobayashi H (1994) Avoidance of pesticides by olfaction in carp. In: Kurihara K, Suzuki N, Ogawa H (eds) Olfaction and taste XI. Springler-Verlag, Tokyo, 765 pp

Jash NB, Bhattacharaya S (1983) Delayed toxicity of carbofuran in fresh water teleost *Channa punctatus*. Indian J Exp Biol 17: 693–697

Jones KA (1992) Food search behaviour in fish and the use of chemical lures in commerical and sports fishing. In: Hara TJ (ed) Fish chemoreception. Fish and Fisheries series 6. Chapman and Hall, London 288 pp

Klaprat DA, Evans RE, Hara TJ (1992) Environmental contaminants and chemoreception in fishes. In: Hara TJ (ed) Fish chemoreception. Fish and fisheries series 6. Chapman & Hall, London, 321 pp

Little EE, Finger SE (1990) Swimming behavior as an indicator of sublethal toxicity in fish. Environ Toxicol Chem 9:13–19

Little EE, Archeski RD, Flerov BA, Kozlovskay VI (1990) Behavioral indicators of sublethal toxicity in rainbow trout. Arch Environ Contam Toxicol 19:380–385

Lunn CL, Toews DP, Pree DJ (1976) Effects of three pesticides on respiration, coughing, and heart rates of rainbow trout *(Salmo gairdneri* Richardson). Can J Zool 54:214–219

Mattiessen P, Sheahan D, Harrison R, Kirby M, Rycroft R, Turnbull A, Volkner C, Williams R (1995) Use of a *Gammarus pulex* bioassay to measure the effects of transient carbofuran runoff from farmland. Ecotoxicol Environ Safety 30:111–119

Monod G, Saucier D, Perdu-Durand E, Diallo M, Cravedi JP, Astic L (1994) Biotransformation enzyme activities in the olfactory organ of rainbow trout (*Oncorhynchus mykiss*). Immunocytochemical localization of cytochrome P4501A1 and its induction by β-naphtoflavone. Fish Physiol Biochem 13:433–444

NRCC (1979) Carbofuran: criteria for interpreting the effects of its use on environmental quality. Ottawa: National Research Council of Canada, Associate Committee on Scientific Criteria for Environmental Quality, NRCC 16740

Peterson RH (1974) Influence of fenitrothion on swimming velocities of brook trout (*Salvelinus fontinalis*). J Fish Res Board Can 31: 1757–1762

de Peyster A, Long WF (1993) Fathead minnow optomotor response as a behavioral endpoint in aquatic toxicity testing. Bull Environ Contam Toxicol 51:88–95

Post G, Leasure RA (1974) Sublethal effect of malathion to three salmonid species. Bull Environ Contam Toxicol 12:312–319

Rand GM (1977) The effect of exposure to a subacute concentration of parathion on the general locomotor behavior of the goldfish. Bull Environ Contam Toxicol 18:259–266

Rand GM, Kleerekoper H, Matis J (1975) Interaction of odour flow perception and the effects of parathion in the locomotor orientation of the goldfish *Carassius auratus* L. J Fish Biol 7:497–504

Saglio P, le Martret MA (1982) The role of pheromones in intraspecific communication in immature goldfish, *Carassius auratus* L. Olfactometric study of the behavioral activity of epidermic extracts. Biol Behav 3:221–234

Sandheinrich MB, Atchison GJ (1990) Sublethal toxicant effects on fish foraging behavor:empirical vs. mechanistic approaches. Env Toxicol Chem 9:107–119

Sherrer B (1984) Biostatistique. G Morin (ed). Quebec, Canada

Sparks RE, Waller WT, Cairns Jr J (1972) Effects of shelters on the resistance of dominant and submissive bluegills (*Lepomis macrochirus*) to a lethal concentration of zinc. J Fish Res Board Can 29:1356–1358

Steele CW (1983) Open field exploratory behaviour of fish: an underutilized tool for behavioural toxicology. Mar Pollut Bull 14: 124–125

Sur RK, Ghose KC (1978) Studies on *in vitro* and *in vivo* inhibition of fish brain cholinesterase by some insecticides. Indian J Physiol Allied Sci 32:72

Trotter DM, Kent RA, Wong P (1991) Aquatic fate and effect of carbofuran. Critic Rev Environ Contr 21:137–176

Weis JS, Weis P (1972) Behavioral changes in the goldfish, *Carassius auratus*, produced by treatment with nerve growth factor. Physiol Behav 9:367–372

———, ——— (1974) DDT causes changes in activity and schooling behavior in goldfish. Environ Res 7:68–74