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Phthalate Esters Reduce Predation Efficiency of Dragonfly Larvae, *Odonata; Aeshna*

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Sublethal exposure to persistent organic chemicals cause effects different than levels resulting in acute toxicity. Examples of sublethal effects are disturbed enzymatic functions, damage to the nervous system, reduced reproduction, etc. These effects may result in altered behavior, which may reduce the fitness of the organism.

Behavioral changes are difficult to study in vertebrates and in highly specialized invertebrates (mostly evolutionary late developed species) because of large natural variation in behavioural patterns. The behaviour of insects, however, is strongly governed by genetic constraints (instincts). Variations of even complex behavioral patterns are limited and specific stimuli produce specific reactions (Wittenberger 1981).

Phthalate esters are one of the most produced chemical groups in the world and are used mainly as plasticizers (Thuren 1986). Of the phthalates DEHP (di(2-ethylhexyl)phthalate) seems to exhibit properties typical of organic micropollutants (a high octanol/water partitioning ratio, the ability to accumulate in organisms, persistent to a certain degree, and a low acute toxicity (Streufert et al. 1980)). The compound reduces reproduction in *Daphnia magna* (Sanders et al. 1973) and bioaccumulation occurs in invertebrates (arthrophods, Mayer & Sanders 1973). Since phthalate esters are lipophilic they tend to become attached to particles in the aquatic environments and consequently are found in high levels in the sediment of the lakes and rivers (Malisch et al. 1981). Benthic organisms are, therefore, more exposed to this substance than those living in the water column.

An aquatic laboratory system was constructed to study the behavior (predation efficiency) of dragonfly larvae (*Aeshna*) exposed to sediment-bound DEHP. Dragonfly larvae were chosen since the predation behavior of these animals is easily studied (shooting out the labium, Figure 1, Corbet 1963).

MATERIALS AND METHODS

Sediment and dragonfly larvae (*Aeshna* , late instars, lenght 15-40 mm) were collected during spring in a eutrophic pond in southern Sweden. The larvae were acclimatized to room temperature (22^{o}C$\pm$1^{o}).

DEHP (5 g disolved in 100 mL of ethanol) was carefully mixed into 10 L of sediment. The sediment (loss of ignition 40% dw at 105^{o}C) was left for 3 days to equlibrate with the phthalate. An estimated level of 500 ppm DEHP (fresh weight) was thus obtained in the sediment.

The contaminated sediment (5 L) was added to each of two glass-aquaria (60 L). A third aquarium (with uncontaminated sediment mixed with 50 mL ethanol) was kept as a reference. After two days, water (40 L) was carefully poured onto the sediment so as not to disturb the sediment surface. Straws of reed were placed in the sediment; the dragonfly larvae will use these as a base for predation and to reach the water surface when emerging. The aquaria were gently aerated and each of them was divided into three visual compartments by inkmarks on the glasswalls. About 20 larvae were added to each system. After an acclimatization period of three weeks the experiment was started.

Chaoborus larvae were chosen as prey organism . The movements, behavior and size of these midge larvae are similar to the natural prey of Odonates (*Diptera* larvae, Warren 1915; Chutter 1961; Pritchard 1964).

The experiments were carried out at $22^{o}\pm1^{o}C$ and the aquaria were illuminated between 0400 and 2100 h (L:D=17:7) to simulate northern European summer conditions. Both temperature and light cycle are essential for the development of Odonata larvae (Corbet 1963; Norling 1976).

Five minutes before behavioral observations, about 200 *Chaoborus* larvae were added to each aquarium. Each section (visual compartment) within the aquarium was then studied for 10 min. The number of successful/unsuccessful extensions of the labium (=strike) by dragonfly larva was recorded (Figure 1). The ratio of successful strikes to the number of total strikes was defined as a measure of predation efficiency. In order not to disturb the Odonata larvae, no light was allowed outside the aquaria. Every aquarium was observed 8-10 times for 40 days (in total 13 h and 30 min).

After 9 weeks larvae and sediment were sampled, wrapped in aluminium-foil and frozen at $-20^{o}C$.

Statistical evaluation and significance tests were carried out by analysis of variance on the data (Table 1). Differences in predation efficiency of the Odonata larvae between different aquaria were tested by one tailed Chi-square test. The difference in phthalate levels of exposed and non-exposed dragonfly larvae were tested by Mann-Whitney U-test (one-tailed, Siegel 1956).

Extraction, clean-up and analysis by capillary gas-chromatography with electron-capture detection of the samples was performed according to Thurén (1986).

RESULTS AND DISCUSSION

Odonata larvae exposed to DEHP caught significantly fewer prey per effort than did larvae not exposed to the phthalate (Table 2). In 100 trials exposed larvae caught 53 prey while non-exposed dragonfly larvae caught 66 prey. The results are based on 803 extensions of the labium in which 449 resulted in successful captures.

The concentration of DEHP in exposed and non-exposed Odonata larvae was significantly different ($p<0.002$, 14.7 (n=8,SD=13.9) and 2.9 ug g^{-1} (n=5, SD=0.6) fresh weight respectively).

The sediment in the two experimental aquaria contained 587 and 623 ug DEHP g^{-1} fresh weight (n=2 for each system). The reference sediment contained 0.4 ug DEHP g^{-1} fresh weight (n=2).

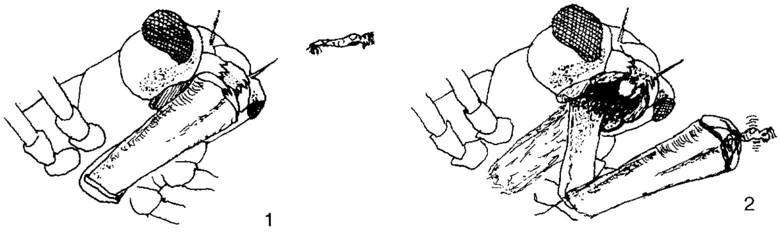

Figure 1. The studied predation behavior: 1. The Odonata larva observes and locates the prey. 2. The labium is shot out and captures the prey.

The effects of organic compounds on the behavior of invertebrates have been fragmentarily studied. The observed effects have been restricted to manifested anomalies in, for instance, net-spinning of spiders (Johanson 1967; Witt 1971) and Trichoptera larvae (*Hydropsyche*, Petersen & Petersen 1983).

The predatory behavior of dragonfly larvae is easily studied. The predator remains mainly immobile usually hanging on to a straw of reed, fixing the prey with the eyes by diminutive motions of the body and the head. When the potential prey is within reach, the labium is extended and grasps the prey. If the capture is successful, handling of the prey starts. If the attack fails, the labium is folded back to prepare for the next strike.Consequently, a definite yes or no for a successful strike is obtained by the observer. The method is therefore regarded as objective.

The predation efficiency of dragonfly larvae exposed to DEHP was lower than for non-exposed larvae. The physiological reasons for this effect are unknown. However, an effect on vision is a reasonable explanation because of the optic specialization in Aeshnidae (highly developed eyes). In late instars sight is very important for the larvae to achieve a correct judgement of the prey location before extending the labium (Corbet 1963). An effect on the nervous system and thus coordination seems also reasonable, in view of the attack behavior (Corbet 1963).

Lipophilic persistent compounds deposited in sediment have been shown to accumulate in aquatic insects (Larsson 1983;1984). The uptake may be a result of partitioning of the compounds from interstitial water and/or sediment particles to the organisms by ingestion of contaminated sediment. The level of DEHP in dragonfly larvae was low compared with the high concentration in the sediment. The highly lipophilic character of DEHP (log P for octanol/water partitioning = 9.5) makes the compound less available from the sediment (Oliver 1984). Therefore equilibrium is not attained until after chronic exposure (several months, and therefore not obtained in this experiment).

The low level of DEHP in the larvae may indicate a metabolism of DEHP in the insects. However, DEHP seems not to be easily metabolized in invertebrates (Metcalf et al. 1973; Södergren 1982). Therefore, the low level is probably a result of a slow uptake rate.

Tab 1. Data from the three model systems. The predation efficiency of dragonfly larvae was measured as the quotient of successful strikes (resulting in a caught prey) to the number of total strikes. (Effic.= predation efficiency)

Time	Non-exposed system			DEHP-exposed system 1			DEHP-exposed system 2		
(day)	Outshots			Outshots			Outshots		
	Total	Successful	Effic.	Total	Successful	Effic.	Total	Successful	Effic.
1	22	16	0.73	26	15	0.58	34	22	0.65
2	31	25	0.81	37	25	0.68	20	17	0.85
3	23	15	0.65	49	24	0.49	48	29	0.60
4	35	20	0.57	16	7	0.44	20	9	0.45
5	16	9	0.56	43	29	0.67	32	13	0.41
6	26	16	0.66	39	24	0.62	36	14	0.39
7	27	19	0.70	63	32	0.51	29	10	0.35
8	22	13	0.59	30	13	0.43	10	3	0.30
9	-	-	-	26	10	0.38	16	8	0.50
10	-	-	-	27	12	0.44	-	-	-

Acute toxicity tests with both vertebrates and invertebrates (Mayer & Sanders 1973; Peakall 1975; Streufert et al. 1980) indicate that DEHP is toxic only at high levels (>10 mg L^{-1}). However , from an ecotoxicological point of view such a statement is not satisfactory. Short-term studies are of limited value as shown by Cairns (1981), Moriarty (1983), and Collvin (1985). In their opinion, LC_{50}-tests have to be supplemented with more subtle variables, as growth rate, reproductive success, condition, behavior, etc. The results of this study show that a compound does not need to be acutely toxic to effect organisms. Even low levels of presumed non-toxic chemicals may have a large ecological impact.

Tab 2. Difference between the systems testedby chi-square analysis.The predation efficiency was significantly lower (non-exposed system/exposed system 1, chi-square=7.864, p<0.01; non-exposed system/exposed system 2, chi-square=9.966, p<0.01; exposed system 1/exposed system 2, chi-square=0.403, p>0.5) in DEHP-exposed Odonata larvae than in non-exposed larvae.

	Non-exposed system	DEHP-exposed system 1	DEHP-exposed system 2	Total
Number of successful strikes	133	191	125	449
Number of unsuccessful strikes	69	165	120	354
Total	202 (n=8)	356 (n=10)	245 (n=9)	803

Reduced predation efficiency of insects has a large ecological impact. The fitness of Odonata larvae may decrease, resulting in a decreased competitive capacity toward other insects. Thus, the prey (midge larvae) may become more abundant if not suppressed by their main predators (in fish-free ponds). Furthermore, chironomid larvae (common prey of Odonata larvae) accumulate phthalates from the water (about 300 times the concentration in the water) without being affected (Streufert et al. 1980). The chironomids will thus mediate a transport of phthalates to the dragonflies, thereby increasing their own competitive capacity.

Two factors of principal interest, probably not restricted to Odonata larvae, have been indicated above. First, predatory insects important in the aquatic food web may be negatively affected by phthalates released to the environment. Second, organic chemicals such as phthalates may induce behavioural changes in invertebrates, changes not easily observed in aquatic ecosystems, but nevertheless serious for the ecological fitness of the organisms.

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