

Methoxychlor Alters the Predator–Prey Relationship Between Dragonfly Naiads and Salamander Larvae

R. L. Ingermann,^{1,3} D. C. Bencic,^{1,*} P. Verrell^{2,3}

¹ Department of Biological Sciences, University of Idaho, Moscow, ID 83844-3051, USA

² School of Biological Sciences, Washington State University, Pullman, WA 99164-4236, USA

³ Center for Reproductive Biology, Washington State University—University of Idaho

Received: 19 December 2001/Accepted: 17 April 2002

The pesticide methoxychlor (MXC; 1,1'-[2,2,2-trichloroethylidene]bis[4-methoxy]-benzene) has been widely used as a replacement for DDT due to its lower toxicity and shorter half-life (Bulger and Kupfer 1985; Cummings 1997; Danzo 1997). In Canadian rivers, MXC has been applied to wetlands to control fly and mosquito larvae, particularly in the spring and summer, with target concentrations of about 0.9 μM (0.31 mg/L; ACSCEQ 1983; ATSDR 1994). Inadvertent exposure of non-target organisms with such applications cannot be avoided and pesticides may have a variety of sublethal, but nonetheless highly deleterious, effects on amphibians. These effects include induction of anatomical deformities (Clark et al. 1998; Cooke 1970; Pawar et al. 1983), and alterations in feeding and developmental rates (Berrill et al. 1993; Marchal-Segault and Ramade 1981; Pawar et al. 1983). In addition, pesticides may influence predator-avoidance behavior. Maintenance of eggs with MXC induces an attenuated startle response in the larvae of the salamander *Ambystoma macrodactylum* at concentrations as low as 0.3 μM (Ingermann et al. 1997, 1999). This result suggests that MXC may compromise *A. macrodactylum* survival in terms of predator avoidance. Indeed, Verrell (2000) has shown that exposure of *A. macrodactylum* eggs to MXC concentrations as low as 0.03 μM leads to increased predation when larvae are combined with dragonfly naiads, a natural predator. That a pesticide can reduce the survival of an exposed, amphibian larva in the presence of an unexposed predator has been previously documented (Cooke 1970, 1971). However, we lack predator-prey studies in which both predator and prey are exposed to the pesticide. Exposure of predatory and prey terrestrial arthropods to insecticides has resulted in complex responses (e.g., Lowe and Benevicius 1981; Hamers and Krogh 1997). For example, exposure of mites (predator) and springtails (prey) to the pesticide dimethoate results in a reduction in the size of the predator population due to a reduction in availability of prey (due either to mortality of adults or reduced fecundity or fertility) rather than by a direct effect of the pesticide on the predator (Hamers and Krogh 1997). To our knowledge, similar two-species toxicity studies have not been conducted with amphibians. Berrill et al. (1993, 1997) reported that exposure of anuran tadpoles and predatory *Ambystoma maculatum* larvae to sublethal concentrations of herbicides and insecticides results in a reduced swimming response to prodding in these amphibians, with *A. maculatum* larvae being particularly sensitive. Although exposed predators and prey were not combined in these studies,

*Present address: Department of Cell and Developmental Biology, University of North Carolina, Chapel Hill, NC 27599-7090, USA

Correspondence to: R. L. Ingermann

the results suggest that, for these xenobiotics and these amphibians, the prey (anuran tadpole) might fare better than the predator (salamander larva) in response to sublethal exposures. Conceivably in such pairings, predator and prey may be equally sensitive, or the prey may be more sensitive to the deleterious effects of the pesticide than the predator, leading to enhanced predation. Therefore, it is possible that sublethal concentrations of a pesticide may have significant effects on amphibian populations by increasing their susceptibility to predation. To explore this possibility, we tested the effects of MXC on the startle response of *A. macrodactylum* larvae, on dragonfly naiad feeding and on the interaction between MXC-exposed larvae and naiads. As commercial MXC may contain contaminants with biological activities (Bulger et al. 1978), purified MXC was used in the experiments reported here.

MATERIALS AND METHODS

Eggs of *A. macrodactylum* Baird were collected as previously described (Ingermann et al. 1997, 1999). Dragonfly naiads, known to be the predators of many amphibian taxa (Caldwell et al., 1980), were collected in minnow traps from a pond on the Washington State University campus in Pullman, WA. (This pond also is a salamander breeding site.) EcoAnalysts (Moscow, ID) identified 110 naiads used in this study to genus of which 109 were *Aeshna* sp. and one was an *Anax* sp. Naiads were fed *Tubifex* worms *ad libitum* until the initiation of our experiments. All animals were maintained and experiments were conducted at 10°C. With the exception of the startle response experiments, all were conducted in subdued light on a seasonal timer in containers with a 23 cm² base containing 180 ml fluid. Startle response data were collected in light primarily as described previously (Ingermann et al. 1999), except that each *N* reported in the present study represents a single response by an individual larva. Prior to use, all MXC used in this study was base-washed and recrystallized from hexane (Bulger et al. 1978); the MXC vehicle was 0.4 ml dimethyl sulfoxide (DMSO)/L. MXC concentration values are based on calculations of stock solutions and, therefore, are nominal concentrations. All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Our previous studies of the effects of MXC on the startle response of larval *A. macrodactylum* were based on chronic MXC-exposures of eggs that lasted until hatch (Ingermann et al. 1997, 1999). In the current study, *A. macrodactylum* were not exposed prior to hatch; rather, they were exposed for 3 d as free-swimming larvae. At hatch, 1 to 4 larvae were transferred to a container with only aged tap water for 7 d. Water in the containers was then replaced with water, DMSO, or 0.01 to 1 µM MXC and 3 d thereafter, these solutions were replaced with 6 changes of aged water and maintained for an additional 3 d. Thirteen days after hatch, 1 to 4 larvae were tested for startle response. A total of 19-21 larvae per treatment were analyzed.

To determine the effect of MXC on naiad feeding, naiads were transferred to individual containers provided with a boiled, wooden stick for purchase and maintained without food for 7 d. Naiads were then exposed for 3 d to aged tap water, DMSO, or 0.008 to 5.0 µM MXC. Thereafter, the wooden stick was replaced and

the solution in the container was replaced with 6 changes of water. Three days after, the water was replaced twice and 5 *Tubifex* worms were added. Number of worms present after 1, 2, 4, 8 and 24 h were counted. Also, it was noted whether the naiad was clinging to the wooden stick, a normal behavior pattern. A total of 6–10 naiads per treatment were analyzed.

We conducted the following experiment to determine the effect of MXC on predator-prey interaction. Upon hatch, salamander larvae were transferred in groups of 5 to individual containers with aged tap water. At the same time, a naiad was transferred to a similar, but separate container with a wooden stick for purchase and maintained without food. Both larvae and naiads were maintained for 7 d with water. The water was subsequently replaced with water, DMSO, or 0.01 to 1 μM MXC. Containers were maintained with these solutions for 3 d, after which time solutions were replaced by 6 changes of aged water (and the wooden stick in the naiad container replaced). Three days thereafter, 5 larvae were combined with a comparably treated naiad, and numbers of larvae surviving after 1, 2, 4, 8 and 24 h were counted. Data represent 5–7 replicates per treatment.

Data on *A. macrodactylum* startle response and the consumption of *Tubifex* worms by naiads were analyzed using the Kruskal-Wallis test followed by nonparametric multiple comparisons tests as described by Zar (1984) with $\alpha = 0.05$. The naiad-larva predator-prey relationship was assessed by linear regression analysis. A value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Ambystoma macrodactylum larvae exposed to MXC *in ovo* demonstrate an attenuated startle response relative to DMSO controls (Ingermann et al. 1997). Consistent with this observation, exposure of free-swimming *A. macrodactylum* larvae for 3 d to 0.1 μM and greater MXC also resulted in a significantly attenuated startle response relative to DMSO controls (Fig. 1). Transient exposure to these concentrations resulted in a greater than 90% decrease in distance traveled in response to a startle stimulus relative to that of DMSO controls. This suggests that salamander larvae may be particularly susceptible to predation at concentrations $\geq 0.1 \mu\text{M}$, assuming that the predator is not more sensitive to MXC.

The influence of MXC on naiad feeding was evaluated. Transient, 3 d-exposure to elevated concentrations of MXC resulted in a reduced ability of naiads to feed on *Tubifex* worms (Fig. 2A). Exposure to MXC concentrations above about 0.5 μM was associated with a statistically significant reduction in naiad feeding. Naiads typically cling to submerged structures, and exposure to elevated concentrations of MXC resulted in a decreased attachment of naiads to wooden sticks provided for purchase (Fig. 2B). Exposure to MXC concentrations in excess of 0.5–1.0 μM resulted in a greater incidence of naiads found on the container bottom, often on their sides and immobile.

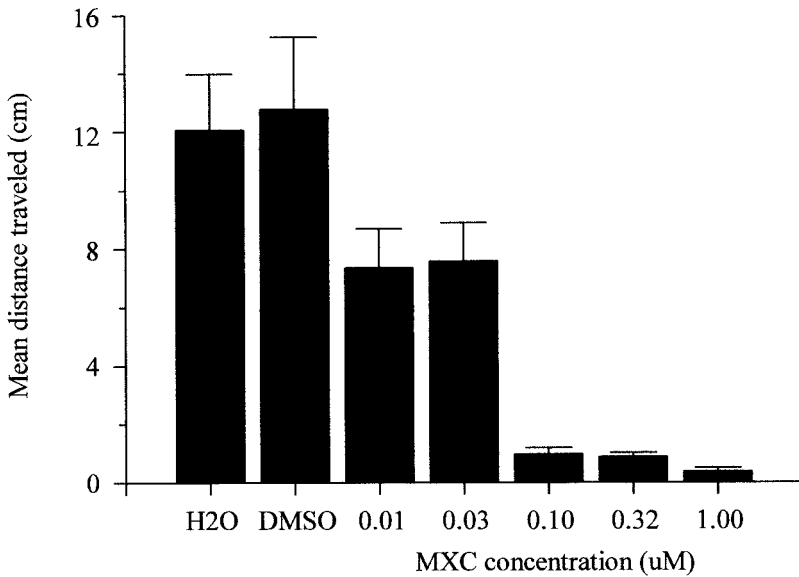


Figure 1. Mean distance traveled \pm SEM by 19-21 larvae per treatment in response to a startle stimulus as a function of the MXC concentration. Distances traveled by the H₂O, DMSO and 0.01 and 0.03 μ M MXC-exposed groups did not differ, but were significantly different from distances traveled by the ≥ 0.1 μ M MXC-exposed groups.

Deleterious effects of MXC on the feeding and perching of dragonfly naiads were not apparent with transient exposures to concentrations below about 0.4 μ M MXC, while the startle response of *A. macrodactylum* larvae was severely compromised by transient exposures to at least 0.1 μ M MXC. This suggests that naiads should be particularly effective predators of *A. macrodactylum* larvae when both are exposed to MXC concentrations between about 0.1 and 0.4 μ M. Indeed, with a transient exposure of both dragonfly naiads and *A. macrodactylum* larvae to 0.32 μ M MXC, the amphibian larvae were at increased risk of predation (Fig. 3). Furthermore, over the range of 0.01 to 0.32 μ M MXC, there was a linear relationship between the logarithm of MXC concentration and increased risk of larval predation by naiads (Fig 4). This relationship did not hold above 0.32 μ M MXC perhaps due to an MXC-induced inhibition of naiad feeding (as suggested by Fig. 2).

The results of these studies indicate that relatively low concentrations of pesticides such as MXC can influence predator-prey relationships and can have significant deleterious effects on amphibian larvae beyond any direct lethal effects. Whether larvae die from direct chemical exposure or from an inability to negotiate challenges present within their local environment, such as an impaired ability to avoid predation,

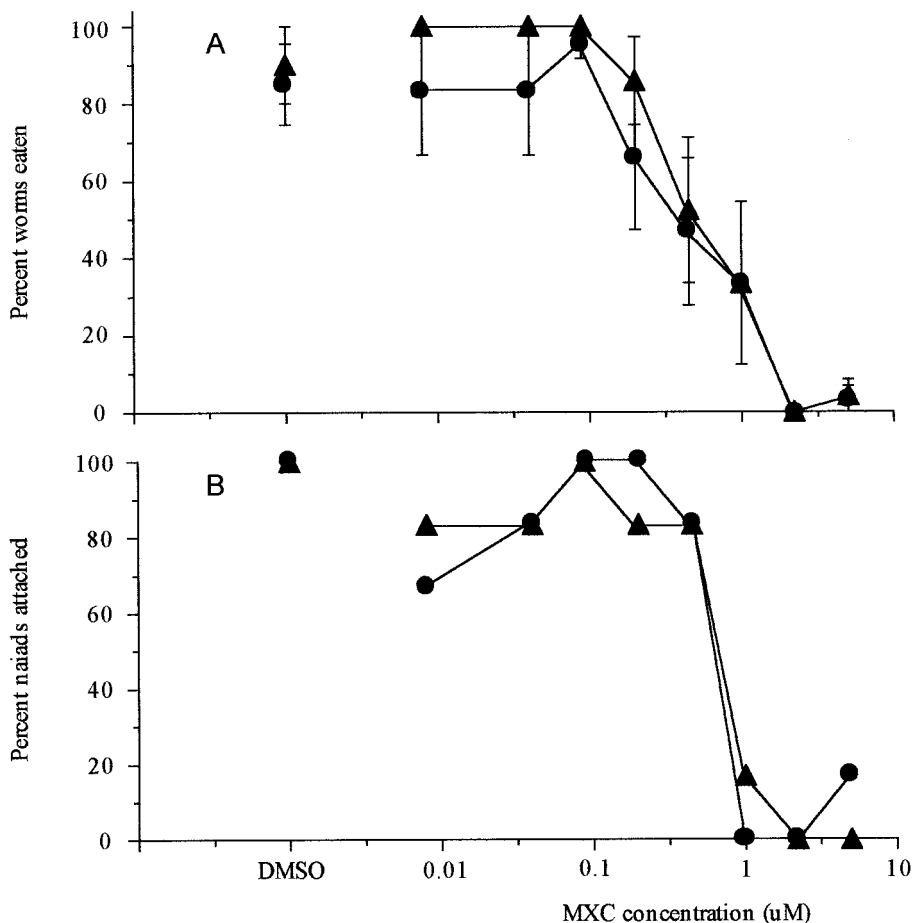


Figure 2. **A)** Percent of *Tubifex* worms eaten by naiads after 2 h (●) and 4 h (▲) as a function of the logarithm of the MXC concentration. Initially, 5 unexposed worms were combined with a single, MXC-exposed or control dragonfly naiad. Naiads exposed for 3 d to at least 0.2 μM MXC consumed statistically fewer worms than did DMSO controls after 2 h; naiads exposed to at least 0.45 μM MXC ate statistically fewer worms than did DMSO controls after 4 h. **B)** Percent of dragonfly naiads attached to sticks after 2 h (●) and 4 h (▲). For A and B: $N = 10$ for DMSO group; $N = 6$ for all other groups; values for water and DMSO were not different in A, 100% of naiads in water were attached (data not shown).

is inconsequential relative to the stability and/or persistence of an amphibian population. Our data underscore the importance of testing for indirect, sublethal effects of xenobiotic exposure on both individuals and populations. Analyses of the effects of xenobiotics on predator-prey interactions are prime targets for further investigation.

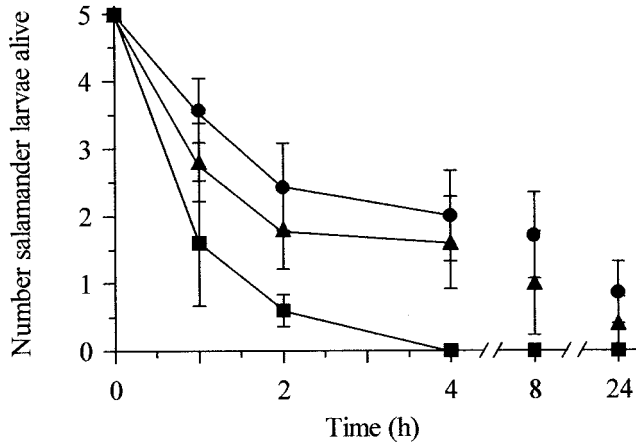


Figure 3. Number of salamander larvae alive as a function of time after 5 larvae were combined with a single dragonfly naiad. Both larvae and naiads had previously been exposed to DMSO (●), 0.03 μM (▲), or 0.32 μM MXC (■). Values of the 0.32 μM group are significantly different from the DMSO control group at 1, 2, 4 and 8 h; DMSO and 0.03 groups differ at 8h; no other comparisons are significantly different. $N = 7$ for DMSO; $N = 5$ for both MXC-exposed groups.

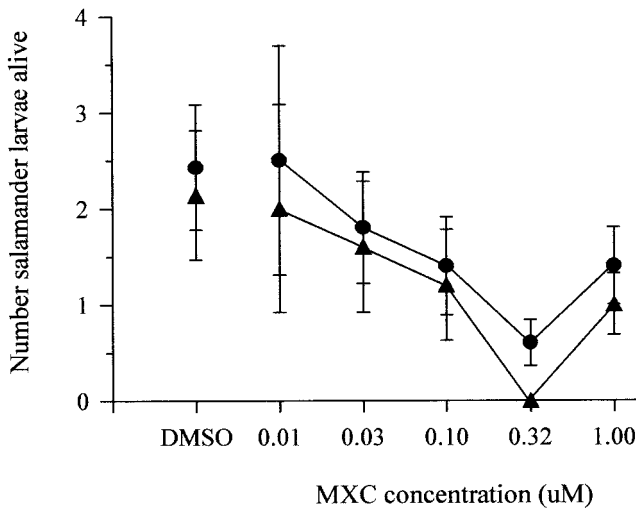


Figure 4. Number of *A. macrodactylum* larvae alive (of 5 initial) versus the logarithm of the MXC concentration after being combined with a single dragonfly naiad for 2 h (●) or 4 h (▲). There was a significant correlation between MXC concentration and *A. macrodactylum* mortality at 2 and 4 h between 0.01 and 0.32 μM MXC. Within this range, the equations of the line generated to the 2 h and 4 h data are: number alive_{2h} = $-7.26 - 1.22 \log [\text{MXC}]$ and number alive_{4h} = $-8.23 - 1.30 \log [\text{MXC}]$, $N = 19$ for each, with $r^2 = 0.22$ and 0.24 , and with 95% confidence intervals in the slopes being $[-2.40, -0.03]$ and $[-2.50, -0.11]$, respectively.

Acknowledgments. We thank Julie Fronzuto and Joshua Woolsey for assistance in collecting dragonfly naiads and salamander eggs. This study was supported by the University of Idaho Research Council.

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