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Clonal variation in acetylcholinesterase biomarkers and life history traits following OP exposure in *Daphnia magna*

Liane B. Printes*, Mark D.E. Fellowes, Amanda Callaghan

Environmental Biology, School of Biological Sciences, University of Reading, Whiteknights, P.O. Box 68, Reading, Berkshire RG6 6BX, UK

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Abstract

Two clones of *Daphnia magna* (Standard and Ruth) were exposed for 7 days to sub-lethal concentrations of acephate (5.0 and 10.0 mg/L). Survivorship, individual growth, reproduction and the population growth rate (λ) were evaluated over three weeks. Acetylcholinesterase (AChE) activity was measured on days 2, 7 and 21. Acephate exposure inhibited AChE activity but had no direct effect on life history (LH) traits. There was also no effect of clone on AChE activity, LH and λ. However, a significant interaction between clone and acephate concentration was found on both fecundity and λ. AChE inhibition at 48 h was associated with a decrease in λ in the Standard clone and an increase in λ in clone Ruth. Therefore, our findings show that genotypic variation will influence the link between AChE activity and toxic effects at higher levels of biological organisation in *D. magna*.

Keywords: *Daphnia magna*; Cholinesterase; Life history traits; Population growth rate; Organophosphates

1. Introduction

Biomarkers measure the response of an organism to contaminants in the environment, indicating that an environmental toxicant has entered the organism and elicited a toxicological effect (McCarthy and Shugart, 1990). One fundamental reason to consider using biomarkers in ecological risk assessment is their potential as a tool to provide an early warning of toxic effects at higher levels of biological organisation (Van Gestel and Van Brummelen, 1996). Increasingly, ecologists are being challenged to demonstrate that these biomarkers can provide a robust, sensitive and fairly predictable indication that populations will suffer long-term adverse effects.

Acetylcholinesterase (AChE) is the recognised target site of organophosphate (OP) and carbamate insecticides. These pesticides interfere with neurotransmission in cholinergic synapses and neuromuscular junctions by inhibiting the enzyme AChE, which cleaves the transmitter acetylcholine. The pesticides bind to the AChE active site as an ester substrate (Eto, 1974). However, hydrolysis of the pesticide is very slow, effectively resulting in a net inhibition. This results in a larger residence time of the neurotransmitter at the cholinergic receptor, leading to hyper-excitation and death. Inhibition of AChE activity is an established biomarker for OP contamination (McCarthy and Shugart, 1990; Peakall, 1992; Lagadic et al., 1994). In mammals and birds, inhibition of AChE activity higher than 50% is normally associated with a life-threatening situation (Walker, 2001), whereas inhibition of AChE activity higher than 40% has been related to behavioural and physiological effects (Grue et al., 1991; Walker, 1995). Aquatic invertebrates appear to be more sensitive to OPs than vertebrates (Giesy et al., 1999), although in fish and aquatic invertebrates, a clear-cut association between AChE activity and acute toxicity has yet to be established (Day and Scott, 1990; Bocquené and Galgani, 1991; Fulton and Key, 2001). Arguments for using AChE as a biomarker include the rapid breakdown of OPs and carbamates in the environment where their concentrations may fall below detectable levels in hours to days. The inhibition of AChE in many species can persist

*Corresponding author. Escola de Engenharia de São Carlos, University of São Paulo, Av. do Trabalhador São-Carlense, 400, Cx. Postal 292, São Carlos, São Paulo CEP 13.560-900, Brazil,
Fax: +51 16 3373 8251.
E-mail address: printes_liane@yahoo.com.br (L.B. Printes).

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for much longer than this, which offers and advantage over using analytical techniques (Fulton and Key, 2001).

The relationship between AChE activity and acute toxicity in *Daphnia magna* and *Chironomus riparius* depends on a number of factors, including the chemicals applied (Sturm and Hansen, 1999; Printes and Callaghan, 2004). Although the primary site of action of OPs is AChE, they also bind to other forms of cholinesterase as well as to enzymes that are responsible for the metabolism and detoxification of pesticides (Grue et al., 1991). Exposure has also been linked to the stimulation of oxygen consumption and the inhibition of other enzymes (Day and Scott, 1990; Glynn, 1999). Clearly OP toxicity is likely to be more complex than originally thought, depending on the interaction of the pesticide with the physiological and biochemical systems of the organism. In addition to these variables, the response of the organism itself will not be uniform, since genetic variation for traits associated with the toxic response and in detoxification will almost certainly exist. There has been some evidence for this in *Daphnia*. Significant interclonal variation in fecundity and population growth rate was reported for *D. magna* exposed to 3,4 dichloroaniline and sodium bromide (Soares et al., 1992). Another sub-lethal response, feeding inhibition, also varied significantly among clones of *D. magna* exposed to methyl-parathion (Baird and Barata, 1998). Hence, both secondary target sites and genetic variation may introduce variables that reduce the strength of a predictable association between biomarkers and population responses.

This paper reports the results of a study that measured the effects of long-term exposure and post-exposure of two *D. magna* clones to sub-lethal doses of the OP acephate. The biomarker AChE was linked to effects at the population level evaluated by measuring life history (LH) and population growth rate (λ). The population growth rate integrates potentially complex interactions among LH traits into a single parameter and provides an ecologically relevant measure of toxic response (Sibly, 1996; Forbes and Calow, 1999).

2. Material and methods

2.1. Test species

Two genotypes of *D. magna* were selected for this study. The standard laboratory IRCHA Clone type 5 (Standard) was obtained from the Water Research Centre (WRC, Medmenham, UK). The second clone, Ruth, was obtained from The University of Sheffield (Sheffield, UK). Genetic differences between the two clones (i.e. DNA profile) were confirmed by the use of the random amplified polymorphic DNA (RAPD) method (results not shown). The two clones of *D. magna* were individually cultured in 50 ml beakers kept under semi-static conditions at $20\pm2\,^{\circ}C$ with a photoperiod of 16:8 h light:dark, at a light intensity of 800 lx. They were maintained in American Society for Testing and Materials (ASTM) hard water (ASTM, 1980) with organic additive (Baird et al., 1989). The cultures were fed daily with distilled water suspensions of the unicellular green algae *Chlorella vulgaris*. Appropriate volumes of the concentrated suspension were added to each *Daphnia* culture to produce a concentration of 1 mg of organic carbon per litre of culture medium. A suspension of dried baking yeast was given as food supplement, the volume added to the cultures were calculated to provide between 0.04 and 0.05 mg/L (dry wt) of yeast.

2.2. Insecticide preparation

The pesticide tested was the OP acephate (98.4%, PS-738, Greyhound Company, UK). Acephate is a derivate of the phosphoramidothiolate methamidorphos (Eto, 1974) and has low toxicity to *Daphnia* (Tomlin, 1994).

Two nominal concentrations of acephate were selected based on previous work (Printes and Callaghan, 2004) and preliminary evaluations. They were initially chosen to give levels of AChE inhibition of ~30 and 50% following 48 h of exposure. The concentrations of acephate for both clones were 5 and 10 mg/L. One main stock solution of 100 g/L was prepared in absolute ethanol. The subsequent working stock solutions of 10 and 20 g/L of acephate were obtained by diluting the main stock in absolute ethanol. All test concentrations were obtained by adding 0.5 µl of each working stock solution per ml of ASTM water, without the organic additive. Control animals were kept in ASTM water only. Preliminary evaluations have shown no detrimental effects on *D. magna* mobility or AChE activity by the addition of pure ethanol (0.5 µl/ml) in ASTM water.

2.3. Experimental design

One experiment was performed to compare AChE activity, LH and λ in both clones of *D. magna* exposed to acephate. For each *D. magna* clone, 15 juveniles ($\leqslant$24 h) from the 3rd brood were placed in a 1000 ml beaker with 750 ml of the test solution or ASTM water without the organic additive. Three sets of beakers were simultaneously placed for analyses up to 48 h, 7 and 21 days. A total of three replicates per treatment were analysed at each time interval. In order to obtain the required number of organisms for the 48 h AChE assays, two sub-replicates per replicate were used; six beakers per treatment were established and the survivors from the two sub-replicates were pooled together for AChE analyses. The survivorship recorded for each treatment in the first time interval is therefore the mean value of the two sub-replicates. The chemicals were added to culture water in the beginning of the experiment only. All beakers were randomly placed in two columns of shelves, and kept in the dark during the first 48 h to follow the OECD recommendation for *Daphnia* acute immobilisation test (OECD, 1984).

Following 48 h of exposure, all surviving animals were transferred to Eppendorf tubes and snap frozen in liquid nitrogen for AChE activity measurements. The remaining two sets (7 and 21 days) were uncovered and subjected to the same environmental conditions described for the *Daphnia* cultures. All organisms were fed daily and left in the same test solutions or culture water for 7 days. At the end of the 7th day, all surviving animals from the second set of beakers were snap-frozen for AChE analyses. The remaining animals from the third set of beakers were transferred to clean ASTM water with organic additive. The culture medium was then replaced every 3 days. For this third group, survivorship and LH traits (individual growth, age at maturity and number of juveniles produced) were recorded until the 21st day of the experiment. In the end of the 21 days, the surviving adults were snap-frozen per replicates for further AChE analyses. All samples were stored in a $-70\,^{\circ}C$ freezer.

2.4. Analysis of LH traits and population growth rate

Body length was determined, from day 0, by taking a transverse measurement from the top of the head to the base of the tail spine under a stereomicroscope. Measurements were taken in *small eyepiece units* (s.e.u.) and converted to mm. The magnification used was 30 times up to the second week and 15 times from the second to the third week. Three individuals per replicate were measured every other day. Age at maturity was defined as the age at releasing of the first clutch of eggs into the brood chamber. Newborn juveniles were removed and counted daily. Population