GROWTH, DEVELOPMENT AND FECUNDITY OF THE EARTHWORM *APORRECTODEA CALIGINOSA* AFTER EXPOSURE TO TWO ORGANOPHOSPHATES

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ABSTRACT

The effect on fecundity of two organophosphates, chlorpyrifos and diazinon, was assessed in the earthworm *Aporrectodea caliginosa*. Juveniles were exposed to two sub-lethal concentrations of each organophosphate, the lowest of which was a laboratory-simulated field rate, for 4 weeks and recovery was monitored for 12 weeks in organophosphate-free soil. Growth, maturation, cocoon production and hatching success in resulting adults was monitored. Growth was reduced by both pesticides during exposure but not in the recovery phase. Chlorpyrifos affected maturation rates and fecundity at the highest concentration, but diazinon had no effect at either concentration. These preliminary results suggest no effects of these pesticides at field rates, but the effects of longer term pesticide exposure on fecundity should be determined.

Keywords: earthworms, organophosphates, growth, fecundity, recovery.

INTRODUCTION

Environmental contamination by pesticides and other agrochemicals is of growing concern in New Zealand and worldwide. A variety of pesticides are used extensively in New Zealand, sometimes at higher application rates than used internationally (MacIntyre *et al.* 1989). Monitoring programmes are being developed to help predict or give early warning of ecosystem degradation. Earthworms have been selected as a suitable representative soil organism as they are key components of most soil biota and they contribute to the overall productivity of agricultural soils through their feeding, casting and burrowing activities (Culy and Berry 1995).

The use of biomarkers in environmental monitoring is now becoming a routine method for examining toxicity of chemicals (Huggett *et al.* 1992). Biomarkers are 'a biological response that can be related to an exposure to, or toxic effect of, an environmental chemical or chemicals' (Peakall 1994). Biomarkers can be used as early warning indicators of environmental contamination and potential adverse effects on populations (Peakall 1994; Day and Scott 1990) and can provide a link between the presence of a chemical and its toxic effect. However, in order to determine the long-term impacts of exposure on a population, biomarkers need to be related to higher-order effects on the organism, such as growth and fecundity.

This study is part of a multistage project to develop a suite of validated biomarkers for exposure to the organophosphates, diazinon and chlorpyrifos, that can be related to higher-order effects. Stage one of this research was evaluation of potential biomarkers in the earthworm species *Aporrectodea caliginosa* (Savigny) under laboratory conditions (Booth *et al.* 1998). Stage two was evaluation of these biomarkers in a semi-field and field experiment (Booth *et al.* 2000). Stage three is evaluation of the effects of the pesticides on maturation and fecundity and how they relate to disturbances of earthworm population densities. Fecundity in earthworms is sensitive to pesticides (Brunniger *et al.* 1994), and even though population densities of

earthworms may not be immediately affected, there can be reproductive changes, which may reduce populations in the longer term. For example, earthworms exposed to 50 mg/kg carbaryl showed no mortality, but this exposure resulted in a reduction by more than 50% in cocoon numbers which would cause a significant reduction in the population in 2-3 generations (Neuhauser 1990).

The aim of this study was to determine the effects of two organophosphates, diazinon and chlorpyrifos, on growth and maturation, and fecundity of the common pasture earthworm *Aporrectodea caliginosa*.

METHODS

Pesticides

The pesticides tested in this experiment were the organophosphates diazinon (Basudin 600EW; Ciba-Geigy NZ, Avondale, NZ) and chlorpyrifos (Lorsban 40EC; DowElanco (NZ), New Plymouth, NZ). Both pesticides are commonly used on pastures in the Canterbury region of New Zealand, mainly to control grass grub (*Costelytra zealandica*) and porina caterpillars (*Wiseana cervinata*).

Assessment of earthworm fecundity

Templeton silt loam (17% clay, 52% silt, 31% sand, 3.9% organic matter) was used for these experiments. Juvenile earthworms 3 months old and weighing between 200 and 300 mg were exposed for 28 days to the highest sub-lethal concentration (high concentration) of chlorpyrifos (28 mg/kg) or diazinon (60 mg/kg), as determined in previous experiments (Booth *et al.* 1998), and a laboratory-simulated field rate (low concentration) for each pesticide and compared with controls. The laboratory-simulated field rates were determined as an estimate of the amount of pesticide that earthworms are exposed to in the soil. Calculations assumed that the pesticide would not penetrate the soil below 13 mm (Kuhr and Tashiro 1978), therefore when chlorpyrifos is applied at the field rate of 2 litres of 40% chlorpyrifos/ha the maximum concentration in the soil is 6.15 g active ingredient (ai)/m³ which equates to 4 mg/kg. When diazinon is applied at the maximum field rate of 4 litres of 60% diazinon/ha the maximum concentration in the soil is 18.46 g ai/m³ which equates to 12 mg/kg. Pesticide was prepared as an aqueous solution, added to soil up to a moisture content of 25% and mixed thoroughly to ensure a homogeneous mixture. Dry grass-meal was added at the rate of 15 g/kg dry soil to provide food for the earthworms. To each of four replicate 500 ml jars for each treatment, 500 g of prepared soil and 10 earthworms were added. Jars were maintained at 25% moisture content and 20°C. After 4 weeks exposure, earthworms were removed from the soil, weighed and the number of mature earthworms (evidenced by a full clitellum) recorded. Recovery of the earthworms was then monitored by placing the earthworms into fresh soil without pesticide and rearing until maturity. During this recovery period, they were monitored every 4 weeks (at 4, 8 and 12 weeks after removal from contaminated soil) for weight change, numbers of mature earthworms and cocoons. Soil was wet-sieved to remove any cocoons, which were then placed into Petri dishes containing wet filter paper and maintained at 20°C for 8 weeks to monitor hatching success.

Statistics

Earthworm weight was analysed over time using repeated measures ANOVA in SYSTAT 7. The maturation data (weeks 2 and 4 only) were analysed using ANOVA in SYSTAT 7. The cocoon production and hatching success data were analysed by repeated measures ANOVA. A Bonferroni test was used to identify treatments that were significantly different ($P < 0.05$).

RESULTS

Earthworm weight was significantly reduced by exposure to both pesticides and this effect was dependant on day ($P < 0.005$). After 4 weeks exposure, earthworm weight differed from the control in all treatments except for the low dose of diazinon ($P < 0.01$) (Table 1). However, earthworm weight did not differ from the control during the recovery phase for any pesticide treatments.

Maturation of juvenile earthworms was influenced by exposure to the pesticides ($P < 0.005$). Earthworms exposed to the high concentration of chlorpyrifos matured more slowly than did control earthworms ($P < 0.005$), but no other treatments differed from the control ($P > 0.05$ for other comparisons). After 4 weeks exposure, 75–80% of earthworms exposed to the low concentration of each pesticide and the controls were mature, while only 53% and 65% of the earthworms exposed to the high concentration of chlorpyrifos and diazinon respectively were mature. All earthworms were mature 8 weeks after removal from contaminated soil.

TABLE 1: Weight of juvenile *Aporrectodea caliginosa* exposed to chlorpyrifos and diazinon for 4 weeks and recovery after removal to clean soil. Values are the mean $\pm$ SE.

Treatment	Mean weight per earthworm (mg)				
	Exposure period		Recovery period		
	Week 0	Week 4	Week 4	Week 8	Week 12
Control	278 $\pm$ 21	465 $\pm$ 22	588 $\pm$ 10	761 $\pm$ 22	762 $\pm$ 25
4 mg ai/kg chlorpyrifos	301 $\pm$ 17	417 $\pm$ 7*	573 $\pm$ 6	790 $\pm$ 16	700 $\pm$ 35
28 mg ai/kg chlorpyrifos	249 $\pm$ 3	300 $\pm$ 8*	513 $\pm$ 25	709 $\pm$ 58	737 $\pm$ 23
12 mg ai/kg diazinon	251 $\pm$ 13	438 $\pm$ 11	661 $\pm$ 30	772 $\pm$ 26	734 $\pm$ 30
60 mg ai/kg diazinon	290 $\pm$ 6	340 $\pm$ 9*	550 $\pm$ 20	728 $\pm$ 19	743 $\pm$ 41

* $P < 0.05$ for comparisons with the control.

Cocoon production was significantly affected by pesticide exposure ($P < 0.05$) and time ($P < 0.005$) (Table 2), but there was no evidence for interaction between these factors ($P > 0.05$). Earthworms exposed to the high concentration of chlorpyrifos produced fewer cocoons per adult than controls ($P < 0.05$) at 4 and 8 weeks during the recovery period, but no other treatments differed from the control ($P > 0.05$ for remaining comparisons). There was also some evidence of a dose-related suppression of cocoon production where fewer cocoons were produced at the high concentration of chlorpyrifos compared with the low concentration ($P < 0.05$). Cocoon production also recovered over time, with similar cocoon numbers in both the control and in chlorpyrifos-treated earthworms by week 12.

Hatching success was significantly reduced by pesticide exposure and this depended on time ($P < 0.05$) (Table 2), i.e. at week 4, hatching success appeared to be lower than at other time points. However, while hatching success appeared to be reduced for 28 mg/kg chlorpyrifos, no treatments were significantly different from the control, and there was no evidence for differences between the two concentrations of each pesticide ($P > 0.05$ for both pesticides).

TABLE 2: Cocoon production and hatching success during the recovery period of adult *Aporrectodea caliginosa* derived from juveniles exposed to chlorpyrifos for 4 weeks. Values are the mean $\pm$ SE.

Time	Concentration of chlorpyrifos (mg ai/kg)					
	Control		4		28	
	No. cocoons per adult	Hatching success	No. cocoons per adult	Hatching success	No. cocoons per adult	Hatching success
Week 4	4.00 $\pm$ 0.9	61 $\pm$ 6%	4.52 $\pm$ 0.4	47 $\pm$ 6%	2.38 $\pm$ 0.6*	34 $\pm$ 12%
Week 8	8.13 $\pm$ 1.4	79 $\pm$ 4%	6.97 $\pm$ 0.8	88 $\pm$ 5%	4.97 $\pm$ 0.8*	94 $\pm$ 2%
Week 12	5.34 $\pm$ 0.9	79 $\pm$ 5%	5.7 $\pm$ 0.8	67 $\pm$ 7%	5.10 $\pm$ 1.6	60 $\pm$ 5%

* $P < 0.05$ for comparisons with controls.

DISCUSSION

Aporrectodea caliginosa is the most common earthworm species found in pasture and arable land in New Zealand and its presence in the topsoil is very important for soil productivity (Martin 1977). The pesticide-induced effects on earthworm weight were apparent only immediately after the exposure phase of the experiment (week 4), at which point earthworms were placed in fresh soil, and by week 8 growth had returned to control levels in all pesticide-treated groups. This indicates that the impact of the pesticides on earthworm growth can be reversible. *Eisenia fetida* showed a similar recovery in weight 8 weeks after termination of exposure to carbaryl (Neuhauser 1990). Despite this apparent recovery, some 'flow-on' effects were observed in the group of earthworms that had been exposed to the high concentration of chlorpyrifos. These earthworms matured significantly more slowly. Furthermore, cocoon production in the resulting adults was reduced by 40% compared with controls, and this effect lasted well into the recovery phase, with full recovery of cocoon production occurring by week 12. These results indicate that the effects on cocoon production are longer-lasting than those on growth, but they also suggest that, if the contamination is removed, earthworms will show a complete recovery over time as measured by the life-table parameters used in this study.

There was a marked increase in cocoon production in control earthworms after 8 weeks in clean soil, which may be related to the maturity of the earthworms, as it has previously been shown that newly mature individuals produce fewer cocoons than do older adults (Spurgeon and Hopkins 1996). It is possible that the significant reduction in cocoon production in the chlorpyrifos-treated earthworms may have been a direct result of the chlorpyrifos-induced lag in maturation. A decline in cocoon production was observed between weeks 8 and 12 in all groups. This is probably due to overcrowding of the resulting adult earthworms in the jars. For future experiments, earthworms will be maintained in 1 kg of soil rather than in 500 g.

No long-lasting impacts on fecundity were observed when earthworms were exposed to the laboratory-simulated field rate (low concentration) of each pesticide. This experiment focussed on the reversibility of adverse impacts if the contamination was removed. In real life, the earthworms would not be physically removed, and exposure to contaminated soil would be continuous until the pesticide degraded. Furthermore, depending on weather conditions, these pesticides are likely to persist in the soil for longer than 4 weeks (Kuhr and Tashiro 1978), and newer products such as sustained-release pellets can continue releasing low levels of pesticide into the soil over a much longer time frame. To clarify the apparent lack of effects at field simulated rates, further experiments investigating longer term exposures on earthworm fecundity are underway.

ACKNOWLEDGEMENTS

The authors acknowledge the Foundation for Research, Science and Technology CO9614 for funding this research, and thank Heather Chapman, Steve Wratten and Christine Bezar for advice and editorial comments.

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