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As the Worm Turns: *Eisenia fetida* Avoids Soil Contaminated by a Glyphosate-Based Herbicide

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As appreciated by Darwin (1881), earthworms (Annelida: Oligochaeta) play crucial roles in the formation and maintenance of fertile soils. Thus, they may be considered 'keystone' species as targets of the anthropogenic contamination of terrestrial ecosystems (Greig-Smith et al. 1992). Also, earthworms increasingly are being considered as sentinels, or biomarkers, of soil health and integrity (Keddy et al. 1995). Contamination with pesticides is known to negatively impact earthworm activity in natural soils, as measured by decreased removal of surface litter and reduced numbers of worm castes (e.g., Keogh and Whitehead (1975). But, are such consequences of exposure to contaminants due directly to the death of worms or to their migration into adjacent unaffected areas (Slimack 1997; Yeardley et al. 1996)?

The skin of oligochaete worms is supplied richly with chemoreceptors (Jamieson 1981), and many species exhibit a capacity for considerable locomotor activity. Thus, the ability of earthworms to both detect contaminated soil and then move away from it seems likely. In a laboratory study, Reinecke et al. (2002) observed that the worm *Eisenia fetida* (a model species in studies of annelid toxicology) migrates away from soil contaminated by the fungicide Mancozeb.

Here we report the results of three laboratory experiments designed to test for acute effects on *E. fetida* of exposure to a glyphosate-containing herbicide. This herbicide, Ortho Groundclear Total Vegetation Killer (hereafter referred to as Groundclear), contains 5% glyphosate by mass (as isopropylamine salt), and is touted by its manufacturer as effective for the long-term suppression of unwanted plant growth. Our data suggest that exposure to this formulation at the nominal concentration recommended for application (one part Groundclear to four parts water) results in low to negligible acute toxicity in *E. fetida*. However, Groundclear appears to alter locomotor activity in a way that may compromise survival.

MATERIALS AND METHODS

Eisenia fetida is a small clitellate earthworm that is maintained as a large breeding colony in our laboratory. This colony resides in a closed plastic bin of 50 L in

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volume at a temperature of 16 C. The substrate consists of commercial potting soil that is purported to be pesticide-free. Domestic vegetable material regularly is buried in the substrate to provide worms with ample access to food.

The experiments reported here were preceded by a pilot study in which worms were exposed to five concentrations of Groundclear, plus a water control, for 124 hr. These concentrations ranged from nominal to 1/10,000th of nominal in 10-fold decrements, and five replicates of each of these six treatments were included (N = 30 worms per treatment).

Mortality was the only end-point examined in this pilot study. Six worms died, all within 48 hr and only in those replicates exposed to the nominal concentration of Groundclear. Based on these preliminary results, we chose to conduct three experiments to determine the effects of acute (up to 48 hr) exposure of *E. fetida* to Groundclear at ecologically-realistic concentrations (nominal and 1/10th of nominal).

In the first experiment, 20 worms were placed into a plastic box that contained 2 L of commercial potting soil. Twenty one such boxes were created, and each was randomly assigned to one of three treatments: application of 82 ml of aged tap water (control), application of 82 ml of a nominal solution of Groundclear in aged tap water, or application of 82 ml of 1/10th the nominal solution of Grounclear. These solutions resulted in a final soil moisture of approximately 40% by mass at a temperature of 16 C (with pH ranging between 6.5 and 6.8).

These boxes were examined visually 2 hr after application of the solutions. They were then left for 22 hr, at which time they were emptied and the soil carefully examined for dead worms. Soil and worms were then returned to their boxes, and left for an additional 24 hr before being checked for a final time.

In the second experiment, a plastic box was taken, and separated into two halves by a plastic divider. On one side was placed 1 L of potting soil moistened with aged tap water, and on the other was placed 1 L of soil moistened with a nominal solution of Groundclear. The plastic divider was then removed and a piece of white thread was placed on the surface of the soil to mark the division between control and treated sides. Twenty worms were then placed at the mid-point where the two soil types were adjacent. Boxes were left undisturbed for 24 hr (N = 7 replicates), at which time the numbers of worms present in each soil type were counted.

In the third experiment, worms were placed in potting soil moistened either with aged tap water (control) or a nominal solution of Groundclear. After 10 hr of exposure, worms were removed and separated into groups of 10. Each group was placed at one end of a plastic box 30 cm in length and floored with a paper towel that had been moistened with aged tap water. A 40 watt white bulb then was positioned at the end of the box in which the worms had been placed, and we counted the number moving > 20 cm away from the light/heat source within 30 min. Water (control)- and Groundclear-exposed worms were tested in a pairwise fashion, $N = 7$ replicates for each of the two treatments.

RESULTS AND DISCUSSION

The data obtained in our first experiment provide little evidence that acute exposure to Groundclear is directly lethal to *Eisenia fetida*. Of the three treatments tested, mortality was observed only with exposure to a nominal concentration of Groundclear (Table 1). However, the numbers of worms that died were low after both 24 hr (2 of 140 worms in total) and 48 hr (5 of 140) of exposure (H = 0.30, 2 df, $P > 0.05$, two-tailed Kruskall-Wallis one-way ANOVA for data at 48 hr).

Exposure to a nominal concentration of Groundclear influenced the locomotor activity of worms. In the water (control) and 1/10th Groundclear treatments, all worms remained buried in the soil and thus not visible for 48 hr. However, large numbers of worms emerged onto the surface within 2 hr in all seven replicates exposed to the nominal concentration of Groundclear (exact counts were not made in order to avoid disturbance, although we did notice that the emerged worms tended to form one or two knot-like aggregations). Migration to the surface was complete for this treatment after 24 hr of exposure (H = 11.37, 2 df, $P < 0.05$, two-tailed Kruskall-Wallis one-way ANOVA). All surviving worms in this treatment were buried when checked at 48 hr (Table 2).

This rapid but temporary migration to the surface of contaminated soil suggests that exposure to a nominal concentration of Groundclear may elicit an avoidance response in *E. fetida*. We tested this in a second experiment by monitoring the movement of 20 worms into either 'clean' soil or adjacent contaminated soil presented simultaneously. Numbers of worms moving into 'clean' soil ranged from 18 to 20 (median = 19.0), which is significantly different from a null expectation of 10 (W = 21, $P = 0.05$, two-tailed Wilcoxon signed-ranks test).

Further evidence for an apparent stimulation of avoidance responses by exposure to a nominal concentration of Groundclear was obtained in our third experiment, in which we measured avoidance of a bright light by worms that had been exposed to either water (control)- or Groundclear-treated soil for 10 hr. The median number of worms moving > 20 cm within 30 min was 2.5 (range 1-4) for the water (control) and 8.0 (range 7-9) for the Groundclear treatments, respectively, a significant difference (z = 3.07, $P = 0.0021$, two-tailed Mann-Whitney U test).

Although we were unable to measure body burdens, we suggest that acute exposure to a concentration of Groundclear recommended for application as a broad-spectrum herbicide may compromise the survival of earthworms even though its direct toxicity appears low. (Of course, we cannot address here

Table 1. Effects of one control and two experimental treatments on the survival of *E. fetida* over a period of 48 hr.

	Treatment:		
Time	Water control	Nominal Groundclear	1/10th nominal Groundclear
24 hr	All alive in all replicates	All alive in 6 replicates; two dead in 7th	All alive in all replicates
48 hr	All alive in all replicates	All alive in 4 replicates; two, two and one dead in other 3	All alive in all replicates

potential chronic effects of exposure or species differences in responses, e.g., Ma and Bodt 1993). Such acute exposure appears to stimulate avoidance activity in *E. fetida*. To the extent that uncontaminated soil is available in the vicinity, worms may be able to migrate to a 'safe' area (at least temporarily). Of greater likely negative consequence in nature would be movement of worms onto the surface of contaminated soil. So exposed, worms might experience increased susceptibility to such indirect sources of mortality as predation, UV radiation and/or desiccation.

Whether the responses reported here might impact earthworms and, perhaps, other components of the soil biotic community in nature is unclear. However, we can imagine that possible negative effects would be most probable with repeated applications of Groundclear and, perhaps, other glyphosate-based formulations (see Springett and Gray 1992). Repeated applications may become increasingly likely in agricultural landscapes given the increasing availability of glyphosate-resistant, or Roundup-ready, crops. As noted by Darwin (1881), earthworm activity is crucial for the formation and maintenance of fertile soils, and so we should be very cautious of engaging in activities that may compromise these roles. Work is planned involving surface applications to soil mesocosms that will further explore effects of contamination at the community level.

Finally, our results provide support for the growing appreciation that behavior patterns may be especially sensitive end-points in ecotoxicological studies (e.g., Ingermann et al. 2002). Many animals, including earthworms, may respond both strongly and rapidly even to concentrations of contaminants that are not lethal directly (Smith and Logan 1997).

Table 2. Effects of one control and two experimental treatments on surface-oriented migration of *E. fetida* over a period of 48 hr.

Time	Water control	Treatment: Nominal Groundclear	1/10th nominal Groundclear
2 hr	All buried in all replicates	Many on surface in all replicates	All buried in all replicates
24 hr	All buried in all replicates	All on surface in 6 replicates; 18 on surface in 7th	All buried in all replicates
48 hr	All buried in all replicates	All survivors buried in all replicates	All buried in all replicates

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