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The relative toxicity of metaldehyde and iron phosphate-based molluscicides to earthworms

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ABSTRACT

Slugs are suppressed by cereal flour-based baits or pellets containing metaldehyde, or containing iron phosphate plus chelating agents, which are also consumed by earthworms and other invertebrates. These studies compared the effects of metaldehyde and iron phosphate alone, with those of iron phosphate plus chelating agents EDDS and EDTA, and of the chelating agents alone on earthworms.

OECD artificial soil test: Earthworms (*Eisenia fetida*) were exposed directly to the molluscicides in artificial soil. The test chemicals were: metaldehyde; iron phosphate; ethyldiaminetetraceticacid (EDTA), ethylenediaminesuccinicacid (EDDS) and mixtures of iron phosphate and these latter two chemicals. LD_{50} values were more than 10,000 mg kg^{-1} for metaldehyde and iron phosphate, 156.5 mg kg^{-1} for EDTA, 145.6 mg kg^{-1} for EDDS, 72.2 mg kg^{-1} for iron phosphate/EDTA, and 83.0 mg kg^{-1} for iron phosphate/EDDS.

Microcosm test: Four mature *Lumbricus terrestris* were exposed in each microcosm (15 cm diam. × 30 cm deep) to pellets containing: no active ingredient; metaldehyde (4%); iron phosphate/EDTA (1%) (Sluggo®); iron phosphate (3%); EDTA (3%); EDDS (3%). Metaldehyde and iron phosphate did not affect earthworm feeding, growth or mortality. Sluggo® decreased earthworm feeding and caused loss of weight and mortality. Earthworms consumed fewer pellets containing EDDS or EDTA and lost weight.

1. Introduction

Earthworms are considered to be key soil-inhabiting invertebrates due to their great importance in soil formation, fertility, and nutrient turnover (Edwards and Bohlen, 1996; Edwards, 2004). Because of this, they have been suggested as critical indicators of soil quality (Berry, et al., 1996; Blair et al., 1996), and for the same reason, earthworms were selected by the European Union (EU) and Organization for Economic Cooperation and Development (OECD) to be used as one of a set of critical assays to assess the toxicity of chemicals to the environment (Edwards, 1983, 1984; OECD, 1984). As a consequence, they have been used extensively by commercial companies and registration authorities to screen the toxicity of chemicals including pesticides. Edwards and Bohlen (1992) reviewed the effects of more than 200 biocidal chemicals on earthworms using the OECD/EU test and a range of other test methods and categorized them into four relative levels of toxicity.

Molluscs, including slugs and snails, are important pests of a wide range of crops in Europe and USA particularly in wet seasons, and are responsible for very considerable losses in crop productivity. Not many chemicals have been marketed as molluscicides and those most commonly used include: metaldehyde and some carbamates such as methiocarb and more recently, iron phosphate plus chelating agents. Because it is difficult to penetrate a mollusc's coating of slime, most molluscicides are used as poisons in baits based on wheat or barley flour, or as especially formulated flour-based pellets. However, these pellets also attract earthworms and a range of invertebrates such as beetles (*Coleoptera*), millipedes (*Diplopoda*), and woodlice (*Isopoda*), which reduce the pellets' persistence and effectiveness by removing them from the soil surface within a few days (Bieri, 2003).

Metaldehyde was introduced as a molluscicide in 1936 and was first used in slug baits in the early 1940s; it is still the commonest molluscicide used. Methiocarb (Mesurol® or Draza®) is a carbamate that has also been used for slug and snail control and which has been demonstrated to affect earthworms (Bieri et al., 1989). Henderson et al. (1989) described the molluscicidal properties of some organic complexes of iron or aluminum. These were toxic when absorbed through the crawling feet of slugs. They showed that the metals in experimental milled metal salts could kill slugs exposed in the laboratory on a glass top, but in the field the metals needed to be chelated which increased their mobility and effects. More recently, since 1990s, this has been confirmed for metal-based compounds such as iron phosphate (Bullock et al., 1992; Young,

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1996), but almost always in conjunction with chelating agents such as ethylene diamine tetracetic acid (EDTA) (U.S. Patent, 1995; Zheng et al., in press) or ethylene diamine succinic acid (EDDS) (International Patent Application, 1999), both of which make the iron more toxic to molluscs by solubilizing it. EDTA is a non-biodegradable chelant that may extract toxic heavy metals from river sediments and permit them to be taken up by plants. EDDS, which is a structural isomer of EDTA (Tandy et al., 2006) is soluble and readily biodegradable. Iron phosphate with EDTA has been marketed commercially under the trade names Sluggo®, Ferramol® and Ferro®.

There have been reports that pellets containing these latter molluscicides can affect earthworms, particularly *Lumbricus terrestris* L, when these pellets are applied to the soil surface. For instance, Langan and Shaw (2006) reported that earthworms took iron phosphate pellets containing EDTA that were offered to them, much more slowly than metaldehyde pellets or control pellets with no chemicals. In the same experiments metaldehyde pellets were acceptable to earthworms, and had no effects whatsoever on earthworm activity, numbers or growth, at the application rates, Langan and Shaw used, which were higher than recommended field application rates.

The present investigation aimed to compare the overall effects of metaldehyde and iron phosphate alone with those of iron phosphate combined with the chelating agents EDDS and EDTA, and the chelating agents alone, in terms of toxicity to earthworms, feeding effects, and potential influences on earthworm populations, particularly those of *L. terrestris*, in microcosm experiments, and *Eisenia fetida* in artificial soil in the OECD test.

2. Methods and materials

2.1. Organization for Economic Cooperation and Development (OECD) artificial soil earthworm toxicity test

Edwards (1983, 1984) and Goats and Edwards (1988) developed the protocol for a standard earthworm toxicity laboratory test for the European Economic Community (E.E.C.–now E.U.) and the Organization for Economic Cooperation and Development (OECD) which was adopted by both these organizations. For such laboratory tests to be reproducible and to relate to field exposure to the chemicals, it is important that the earthworms are exposed to the test chemicals in a medium that closely simulates normal low absorptive capacity field soil conditions. To minimize the variability that occurs between natural soils, a standardized artificial soil was used, with an adsorptive capacity similar to that of a typical loam soil (25 meq). The artificial soil was prepared from a mixture of the following components:

- 10% finely ground sphagnum peat (Ph 5.5–6.0).
- 20% kaolinite clay (containing >30% kaolinite).
- 70% industrial quartz sand (dominant fine sand with more than 50% of particle size of 0.05–2.0 mm).
- 1% calcium carbonate ($CaCO_3$ – pulverized to bring the pH of mixture to 6.0 + 0.5).

These components were mixed thoroughly and the artificial soil brought to 35% dry weight moisture content. The dry components were blended in the correct proportions and mixed thoroughly, in a large-scale laboratory mixer. Moisture content was determined by drying a small sample at 105°C for 1 h and re-weighing. Deionized water was added to give an overall moisture content of approximately 35% of the dry weight, and the medium was then thoroughly mixed. The complete mixture was moist but not so wet that water appeared when the artificial soil was compressed. Glass jars were used as test containers. 750 g of artificial soil which had been treated with one of the test chemicals (or a control with no chemicals) were placed in each jar. The lumbricid species *E. fetida* is designated for the standard test protocol, since toxicity results from this species are representative of other lumbricid species, and *E. fetida* can be bred readily in laboratory cultures. Ten *E. fetida* each with live weights of approximately 1 mg were added to each jar. There were four replicates of each chemical and dose (28 treatments).

Treatments were: over the range of concentrations 0 (control), 0.1, 1.0, 10.0, 100.0, 1000.0, and 10,000.0 mg of chemical kg^{-1} d.w. soil.

The treatments tested were:

1) Control – no chemicals
2) Metaldehyde
3) Iron phosphate ($Fe(PO_4)_3$)
4) EDDS
5) EDTA
6) 100% $Fe(PO_4)_3$ + 3% EDDS*
7) 100% $Fe(PO_4)_3$ + 3% EDTA*

*These are the amounts of chelating agents needed to obtain complete chelatisation of the iron phosphate.

Metaldehyde, and iron phosphate were applied, mixed with 10 g finely ground quartz sand, and EDDS and EDTA were both applied in deionized water solution. The control and EDDS and EDTA treatments also received 10 g finely ground quartz sand. There were four replicates of each treatment. All test containers were kept at 20 °C, laid out in a complete randomly distributed pattern (CRD). Data were analyzed statistically by analysis of variance using S.A.S. software.

The LD_{50} values were estimated using Trimmed Spearman–Karber Version 1.5 (S.E.P.A. Cincinnati, Ohio) designed for analysis of mortality data from acute chronic toxicity tests.

2.2. Microcosm assays

The lumbricid species *L. terrestris* was used in these assays since this is the most common species that consumes organic material from the soil surface. The earthworms (*L. terrestris*) were exposed to molluscicide-based pellets in specially designed microcosms, consisting of 2 mm wall cylindrical PVC pipes (15 cm diameter × 30 cm deep) with a surface area of 0.0177 m^2, each filled to within 1–2 cm of the top with medium-loam sieved field soil. The soil was kept watered to field capacity, and four large adult, clitellate *L. terrestris* (weight 3 ± 0.5 g) were introduced on to the soil surface of each PVC microcosm containing the test soil. Individual earthworm weights were recorded before their introduction into the microcosms. All microcosms were covered with fine mesh gauze (1.0 mm aperture) at top and bottom to keep the earthworms from leaving the microcosms. The earthworms were acclimatized in a cool room (4 °C) for one week and allowed to establish burrows before pellets were added and maintained at 4 °C for the 14 day duration of the test in a dark room.

The molluscicide pellet application rates were based on recommended field application rates converted to the surface area of the microcosm which is 0.0177 m^2. The recommended field application rate of Sluggo® (therefore of EDTA and EDDS) is 350 pellets per m^2. We approximated this rate by applying 340 pellets per m^2 which is 6 pellets per microcosm to the nearest unit as a single application rate, and 30 pellets per microcosms as a five times application rate. The recommended application rate of metaldehyde is 38–39 pellets per m^2, so to bring this to the number of pellet units to apply to microcosms, we had to use one pellet per microcosm which is the equivalent of 57 pellets per m^2, i.e. an overdose of about 40%. For the five times application rate we used 5 pellets per microcosm.

The following pellet treatments based on these calculations were applied to the surface of the soils in the microcosm:

1) Control – no chemical treatment (1 pellet per microcosm)
2) Control – no chemical treatment (5 pellets per microcosm)
3) Metaldehyde (4% commercial formulation), 1× recommended application rate (1 pellet per microcosm)
4) Metaldehyde (4% commercial formulation), 5× recommended application rate (5 pellets per microcosms)
5) Chelated iron phosphate (1%) (Sluggo®) recommended application rate (6 pellets per microcosm)
6) Chelated iron phosphate (1%), Sluggo® 5× recommended application rate (30 pellets per microcosm)
7) Iron phosphate only (3%), recommended application rate (6 pellets per microcosm)
8) Iron phosphate only (3%), 5× recommended application rate (30 pellets per microcosm)
9) Chelating agent only (3%), EDTA pellets recommended application rate (6 pellets per microcosm)
10) Chelating agent only (3%), EDTA pellets recommended application rate (6 pellets per microcosm)
11) Chelating agent only (3%), EDDS pellets, 5× recommended application rate (30 pellets per microcosm)
12) Chelating agent only (3%), EDDS pellets, 5× recommended application rate (30 pellets per microcosm)

All treatments were replicated six times. Pellet treatments were placed on the soil surface in each microcosm, in numbers designated by the treatment, after an acclimatization period in the microcosms for the earthworms at 4 °C for 7 days. There were a total of 72 experimental units (12 treatments (including control) × 6 replications). The experimental microcosms were placed in a constant temperature chamber kept at 4 °C in a completely randomized design (CRD). This was found to be the best temperature for the tests in preliminary experiments after 21 days. The numbers of pellets removed by *L. terrestris* from each microcosm were recorded daily by counting those remaining on the surface for 14 days. After 14 days, all of the remaining pellets (if any) were removed from soil surfaces. At the end of the experiment after 21 days, the earthworms were collected from each microcosm and their condition, numbers, and wet weights recorded. The data were subjected to an analysis of variance and Fisher's protected LSD ($P \leq 0.05$) was used to determine significant differences between treatment means. Appropriate *a priori* paired treatment means were subjected to Contrast analyses using SAS statistical software. Where possible, LC_{50} values were estimated for treatment.

3. Results

3.1. OECD test

The effects of the molluscicides and chelating agents on earthworms in the OECD test are summarized in Fig. 1. Clearly, metaldehyde and iron phosphate had no significant effects on the earthworms at any of the concentrations tested. However, EDTA and EDDS had significant effects ($P \leq 0.05$) on earthworm numbers at concentrations between 100 and 1000 mg kg^{-1} (ppm). The combinations of iron phosphate with both EDTA and EDDS had even greater effects on the earthworm numbers. This conclusion is emphasized by the LD_{50} calculations for metaldehyde, iron phosphate, EDTA and EDDS and their mixtures, which are summarized in Table 1. This shows that metaldehyde and iron phosphate had very high LD_{50} values of more than 10,000 mg kg^{-1}, EDTA had an LD_{50} value of 156 mg kg^{-1}, EDDS of 145.57 mg kg^{-1}, iron phosphate + EDTA of 78.16 mg kg^{-1} and iron phosphate + EDDS of 82.98 mg kg^{-1}.

3.2. Microcosm test

This experiment focused on assessing the rates of disappearance of pellets, at the equivalent of the recommended application rates, for the control, metaldehyde, iron phosphate, Sluggo®, EDTA and EDDS, from the surface of the microcosms (Fig. 2a). It compared the effects of these rates of application with the equivalent of five times the recommended rates of application of pellets containing the same chemicals (Fig. 2b).

At both the recommended and five times application rates the control pellets and metaldehyde pellets both disappeared most

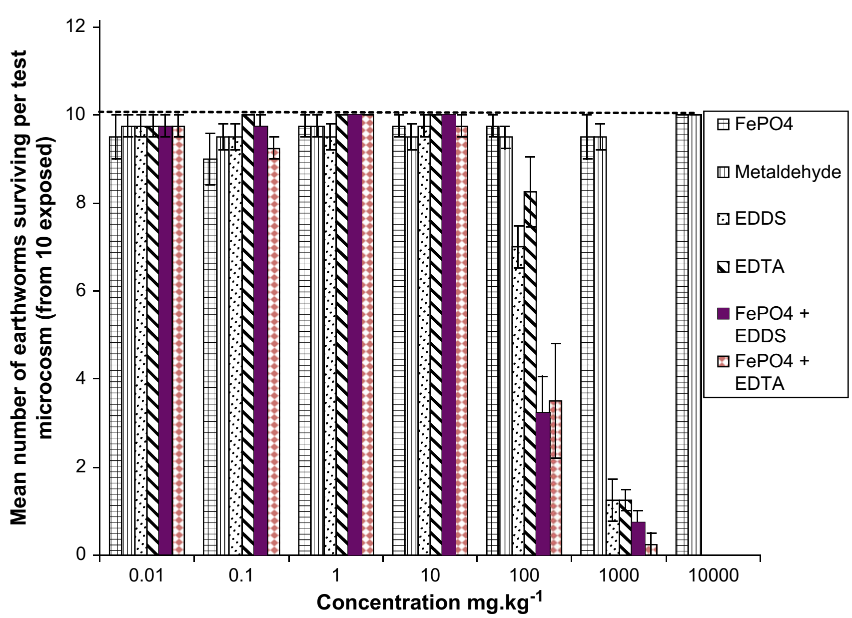

Fig. 1. Effects of molluscicides on earthworm (*Eisenia fetida*) activity in artificial soil (OECD test). Number surviving (±SE).

Table 1
Toxicity of molluscicides to earthworms (OECD artificial soil test) (calculated LD_{50} values and confidence limits).

Molluscicide	LD_{50} (Oral) (mg kg^{-1})	Confidence limits
Metaldehyde	>10,000	–
Iron phosphate	>10,000	–
Ethylene diamine tetracetic acid (EDTA)	156.46	−136.01 +179.98
Ethylene diamine disuccinic acid (EDDS)	145.57	−113.67 +186.41
Iron phosphate + EDTA	78.16	−63.91 +95.58
Iron phosphate + EDDS	82.98	−69.16 +99.55

rapidly. Iron phosphate pellets disappeared at a slightly slower rate than metaldehyde, whereas those containing Sluggo®, EDTA or EDDS disappeared much more slowly. At the end of the experiment, the microcosms were emptied, earthworms were sorted from the soil, counted and weighed (Fig. 3). There was virtually no earthworm mortality over the 14 days of the experiment, but there were considerable differences in earthworm weights, although none of them differed significantly ($P \leq 0.05$), from the control earthworm mean weights. The earthworms that were exposed to Sluggo® (recommended application rate) gained significantly less weight ($P \leq 0.05$) than those exposed to iron phosphate only, as did those exposed to five times the recommended application rate of Sluggo®.

4. Discussion

The data presented here from both the microcosm test and the OECD earthworm toxicity test support the conclusions reached by Langan and Shaw (2006) that commercial molluscicide pellets, based on iron phosphate and chelating agents such as EDTA

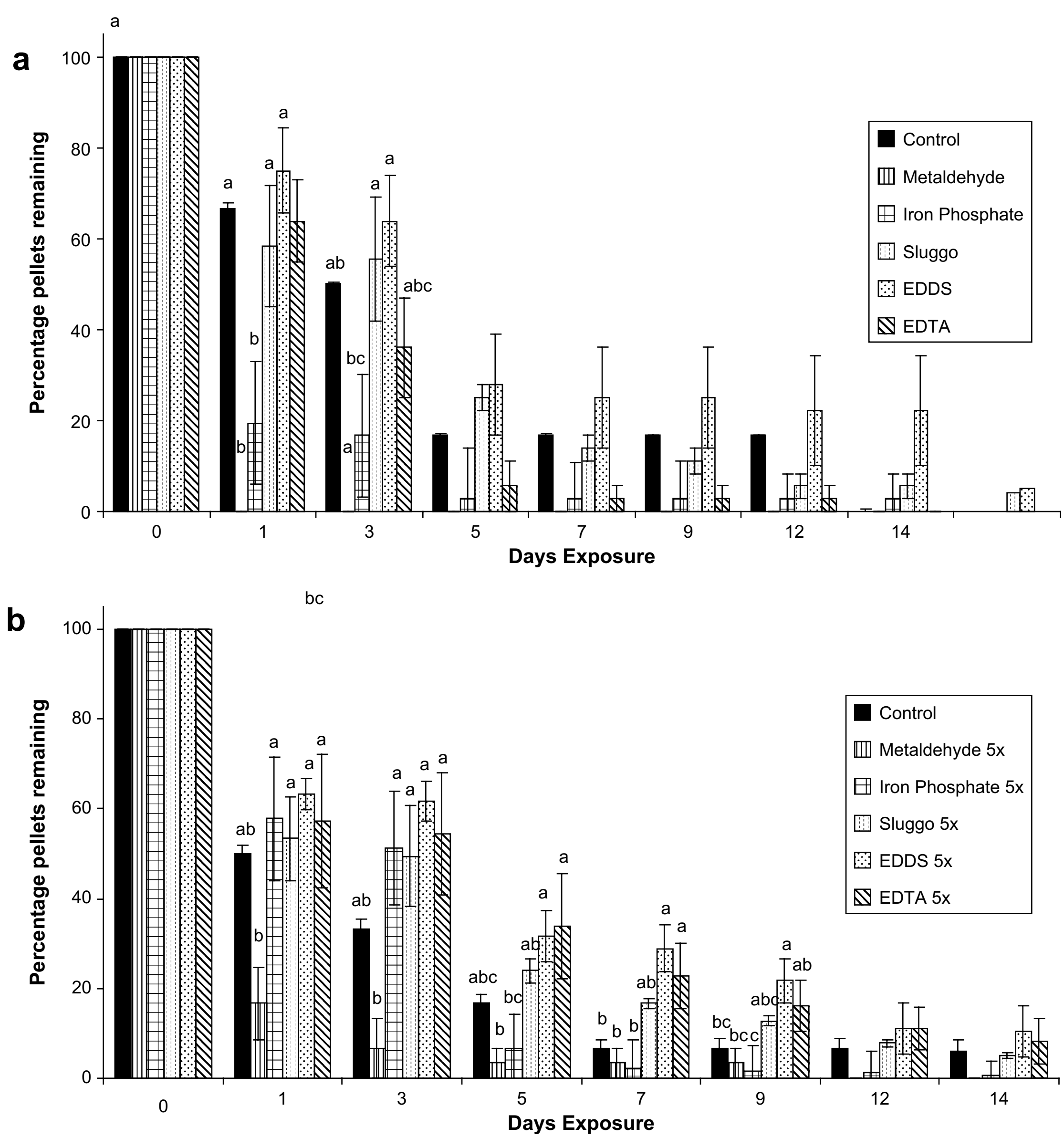

Fig. 2. (a) Percentage of pellets remaining per microcosm, recommended application rate 1×. For each date, significant differences ($P \leq 0.05$) between treatments are indicated by suffixes a, b, c, and; those with different letters are significantly different. Those with no letters are not significantly different. (b) Percentage of pellets remaining per microcosm, recommended application rate 5×. For each date, significant differences ($P \leq 0.05$) between treatments are indicated by suffixes a, b, c, and; those with different letters are significantly different. Those with no letters are not significantly different.

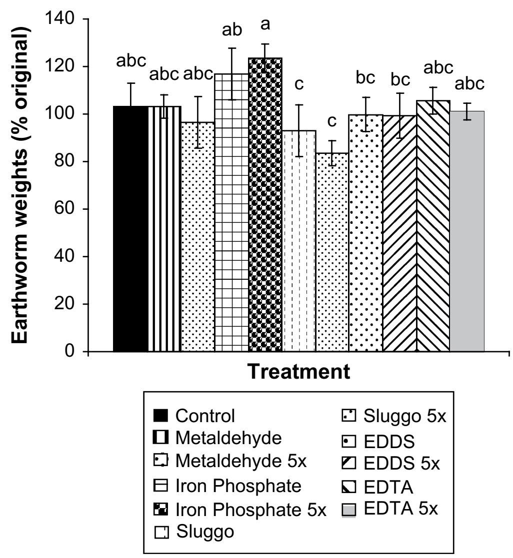

Fig. 3. Percentage *L. terrestris* weights in response to molluscicide pellet applications to microcosms. Columns with some letter a, b, or c are not significantly different ($P \leq 0.05$).

(e.g. Sluggo®), affected earthworm activity significantly, in terms of numbers of pellets consumed, although the experimental techniques that they used differed from those used in our experiments. Langan and Shaw (2006) used a Daniel funnel technique which exposed single individual *L. terrestris* to molluscicide pellets (Bieri, 1992).

Although they exposed the earthworms to larger numbers of pellets than the recommended field application rate, clearly neither metaldehyde nor control (placebo) pellets had any effects on earthworm activities, whereas Sluggo® had detectable negative impacts on the survival, activity and growth of *L. terrestris*. The manufacturers do not state the composition of the iron phosphate-based molluscicides Ferro® and Ferramol® in their patent applications. However, it appears that Sluggo® is based on iron phosphate, plus a chelating agent, ethylene diamine tetracetic acid (EDTA) (edetic acid) (United States Patent Application, 1995) or in later formulations ethylene diamine succinic acid (EDDS) (International Patent Application, 1999); these solubilize the iron and make it more toxic to molluscs. Analyses of Sluggo® by the Catalyse Analytical Laboratory, Marseille, France (in lit.) gave an iron content of 0.319% which corresponds to 1% iron phosphate and a value of 1.4% for EDTA. Zheng et al. (in press) reported that EDTA complexed with iron as Fe(III) EDTA and when snails (*Helix aspersa*) were fed Fe(III) EDTA pellets (Sluggo®/Ferramol®) they died rapidly whereas snails feed on iron phosphate alone were unaffected and could be maintained on this diet indefinitely.

The data from our OECD artificial soil test showed clearly that, when earthworms, such as *E. fetida*, were exposed to metaldehyde and iron phosphate, by feeding on artificial soil which contained these chemicals, they were not killed by concentrations even as high as 10,000 ppm. By contrast, when they were exposed to EDTA or EDDS they were affected by concentrations as low as 100 ppm (Fig. 1) with LD_{50} values of 156.46 mg kg^{-1} for EDTA and 145.57 mg kg^{-1} for EDDS. When they were exposed to iron phosphate chelated with EDTA and EDDS the toxicity was even greater (LD_{50}, 78.16 mg kg^{-1} for iron phosphate and EDTA and 82.98 mg kg^{-1} for iron phosphate and EDDS) (Table 1). Clearly, both the chelating agents and their complex with iron phosphate increased the toxicity of earthworms from this form of exposure considerably.

The data from earthworm exposure to pellets containing these same chemicals from the microcosm experiment confirm these conclusions. Earthworms took pellets, containing metaldehyde and iron phosphate at recommended application rates, from the soil surface in the microcosms even more readily than control pellets containing no chemicals (Fig. 2a). They also took pellets containing metaldehyde and iron phosphate at five times the application rate more readily than the control pellets (Fig. 2) but they removed significantly fewer pellets containing Sluggo®, EDTA or EDDS at either the recommended application rate or at five times this rate.

Although there was no significant mortality of *L. terrestris* in response to any of the chemicals in the pellets over the 14 days of the experiment (Fig. 3) the pellets containing Sluggo®, at the recommended rate and five times this rate, retarded weight gains by the earthworms significantly ($P \leq 0.05$) (Fig. 3) compared to those which took pellets containing iron phosphate alone.

These data together provide clear evidence that molluscicides containing iron phosphate combined with either EDTA or EDDS can have adverse effects on earthworm activity or growth and may possibly be toxic to them. This also suggests that if these key soil-inhabiting invertebrates are affected adversely by iron phosphate-based molluscicides, then other soil-inhabiting invertebrates such as beetles, millipedes and woodlice may also be affected: this needs further study. Bieri (2003) showed the metaldehyde had no effects on: carabid or staphylinid beetles, mites, or the earthworms *L. terrestris* or *E. fetida*.

These data also raise the issue as to whether iron phosphate molluscicides containing chelating agents such as EDTA are acceptable for use by organic farmers. Tamm and Speiser (2006) from the Swiss Institute of Organic Agriculture first raised this topic. They concluded that although iron phosphate alone was acceptable for organic agricultural use, when it was combined with EDTA they were not willing to give approval for organic farming since this chemical does not occur naturally. They also stated that EDTA is inappropriate for organic farming use because it binds with metal ions and increases their mobility and availability in the environment, so that they can penetrate more easily into groundwater or can be taken up by plants (Ovieda and Rodriguez, 2003).

Another important issue is the level of mammalian toxicity of iron phosphate-based molluscicides containing EDTA or other chelating agents (Tamm and Speiser, 2006), especially since if chelating agents increased the uptake of iron from soils into crops they may be fed upon by humans. There also have been reports from the USEPA of mortality or illness of domestic animals that consumed iron phosphate-based molluscicides. For instance, EPA (2008) reported 5 domestic animal deaths, 8 major domestic animal incidents and 106 moderate and minor domestic animal incidents from the use of iron phosphate slug and snail baits marketed in the USA up to May 7, 2008. It is important to be aware of the mammalian toxicity of molluscicides with iron phosphate and EDTA or EDDS. EDTA has been reported to have an oral mammalian toxicity of 30 mg kg^{-1} to rats (Tamm and Speiser, 2006) and mice (Safety Data for ETDA, 2008) compared with an oral toxicity of metaldehyde of 630 mg kg^{-1} to rats and 250–1000 mg kg^{-1} to dogs (Berg, 1986). However, the mammalian toxicity of EDDS does not appear to have been published.

Clearly, molluscicides containing iron phosphate and EDTA or EDDS chelating agents may present significant environmental hazards to earthworms, domestic animals and humans and these issues need further investigation. The registration statuses of these chemicals in USA and Europe should be reviewed in light of these new data and conclusions.

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