



Review

Searching for a more sensitive earthworm species to be used in pesticide homologation tests – A meta-analysis

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HIGHLIGHTS

- ▶ *Eisenia fetida* is less sensitive to pesticides than species found in cultivated fields.
- ▶ Sensitivity of *Lumbricus rubellus* is variable from one study to another.
- ▶ *Aporrectodea caliginosa* and *Lumbricus terrestris* are the most sensitive species to pesticides.
- ▶ *A. caliginosa* is proposed as model for ecotoxicological and homologation tests.

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ABSTRACT

Pesticide risk assessments include experiments designed to measure the effect of pesticides on earthworms using the *Eisenia fetida fetida* or *Eisenia fetida andrei* species. There is no clear consensus in the literature on the sensitivity of different earthworm species to pesticides. We performed a meta-analysis on the sensitivity of several earthworm species to pesticides to determine the most sensitive species, and to discuss their suitability for European homologation tests. A dataset including median lethal dose (LC50) values reported in 44 experimental treatments was constructed and then analyzed in order to compare the sensitivity levels of *E. fetida* with that of other earthworm species. Results showed that LC50 values reported for *Lumbricus terrestris* and *Aporrectodea caliginosa* were on average significantly lower than for *E. fetida*. Considering the relatively high LC50 values reported for *E. fetida* and the absence of this species from zones where pesticides are usually applied, the relevance of using *E. fetida* for pesticide homologation tests is questionable and we advise risk assessors to use *A. caliginosa* as model species. A new protocol based on this species could be proposed for European homologation tests but its implementation will require the definition of a new standard and take time. In the meantime, the results obtained with *E. fetida* should be interpreted with caution taking into account the low sensitivity of this species. Our study illustrates the value of the meta-analysis approach for comparing the sensitivity of different earthworm species to pesticides. It would be useful to extend the dataset presented in this paper in order to analyze the sensitivity of other aquatic or terrestrial organism groups used for pesticide homologation or ecotoxicology tests.

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1. Introduction

Intensive agricultural production has led to a deterioration in soil quality (Vitousek et al., 1997). Chemical inputs, in particular pesticides, have been applied in large quantities in western European agricultural areas, and have affected soil biodiversity (Mäder et al., 2002; Bengtsson et al., 2005). In order to limit the environmental impact of pesticides, national and European food safety agencies carry out risk assessments for pesticides in order to provide scientific support to stakeholders prior to pesticide marketing authorization. Such risk assessments include experiments designed to measure the effect of pesticides on different aquatic and terrestrial organisms, such as earthworms.

Earthworms represent a large proportion of soil organism biomass and provide important agro-ecological functions necessary for the functioning of agroecosystems. Earthworms are “one of the key organisms in environmental toxicology” (Spurgeon et al., 2003). Two earthworm species *Eisenia fetida* and *Eisenia fetida andrei* have been in European pesticide marketing authorization experiments since the early 1980s. In these experiments, adult individuals are exposed to active substances or commercial formulations of pesticides at different concentrations for fourteen days on filter paper or in an artificial OECD soil (ISO 11268-1, 1993; ISO 11268-2, 1998; OECD 207, 1984). OECD soil is a homogeneous mixture of 10% sphagnum peat, 20% kaolin clay, and 70% sand. The pH is adjusted to 6.0 ± 0.5 by the addition of calcium carbonate (OECD 207, 1984). Measurements of earthworm mortality are used to estimate LC50 values, i.e. the lethal concentrations for 50% of exposed individuals. Effects on reproduction are also sometimes studied in these experiments, but results are rarely made publically available (PPDB, 2011; ANSES, Agritox, 2012).

In addition to the marketing authorization tests, both subspecies of *Eisenia fetida* are often used in ecotoxicological studies dealing with the effects of pesticides on earthworms (Ma and Bodd, 1993; Yasmin and D'Souza, 2007). According to Yasmin and D'Souza (2007), *Eisenia fetida* “is especially appropriate for the toxicity tests because it can be easily bred on a variety of organic wastes with short generation times”. However, there are at least two reasons to question the relevance of using this species as a reference for toxicity. Firstly, *E. fetida* is not found in mineral soils (Lowe and Butt, 2007) and is thus very uncommon in the cultivated fields where pesticides are applied. Other species such as *Aporrectodea caliginosa* or *Lumbricus terrestris* are present in cultivated fields and are thus exposed to pesticides. *L. terrestris* is more commonly found in no-tilled plots since it is sensitive to plowing (Chan, 2001; Pelosi et al., 2009). Both are ecologically important in terrestrial ecosystems of many temperate regions (Bouché, 1992; Bauer and Römbke, 1997). These species are not frequently used in ecotoxicological studies and the main reason seems to be that they are less easily bred than *E. fetida* (Cortet et al., 1999).

Secondly, some authors found that *E. fetida* is comparatively less sensitive to contaminants than other earthworm species (Roberts and Dorough, 1985; Kula, 1995; Fitzgerald et al., 1996). Stenersen (1979) reported that *E. fetida* could tolerate pesticide concentrations up to one hundred times higher than the lethal concentration for *A. caliginosa*. Consequently, the use of *E. fetida* as a reference species for ecotoxicological test may lead to an underestimation of earthworm mortality due to pesticides.

The lower sensitivity of *E. fetida* was not confirmed by Callahan et al. (1994) who found that four species *E. fetida*, *Allolobophora tuberculata*, *Eudrilus eugeniae* and *Perionyx excavatus* all had similar sensitivity to 62 different chemicals. These authors concluded that *E. fetida* is a representative model for these species. Pizl (1988) highlighted a strong correlation between LC50 values of *E. fetida* and those of *Lumbricus rubellus*, which is also often used in ecotoxicological tests (Spurgeon et al., 2003; Lowe and Butt, 2007). Haque and Ebing (1983) and Heimbach (1985) showed that *E. fetida* is of comparable sensitivity to pesticides as *L. terrestris*. Since no clear consensus can be found in the literature on the sensitivity of the different earthworm species to pesticides, it is useful to synthesize and analyze the available experimental data in order to identify the most sensitive species.

Hence, the aim of this paper was to make a meta-analysis of the sensitivity of several earthworm species to pesticides and metabolites in order to determine which species is the most sensitive and to discuss which would be most suitable for use in European homologation tests.

2. Materials and methods

2.1. Literature search

A systematic literature review was conducted to find publications dealing with earthworms exposed to pesticides. The literature search was carried out on the basis of keywords in ISI Web of Knowledge, using the “All Databases” option, with the following formula: ‘(earthworm* or lumbric*, aporrectod* or eisen* or dendrobaen* or allolobophor* octalas*) and (pesticide* or herbicid* or fungicid* or mollusc* or nematocid* or insecticid*)’ in Topics.

From a corpus of more than 1800 references, a first selection was made using titles and abstracts. The full texts were examined when information was considered consistent for meta-analysis. To complete the search, starting from the previously selected references, authors that had written articles on the subject as well as books and journals of interest were identified and their articles were examined.

We only considered publications which provided data on *E. fetida* and another species in the same study in order to compare the sensitivity of the species in the same conditions i.e. the same earthworm development stage, active substance, type of substrate, pesticide addition, applied organic matter, and experiment duration. In order to be as representative as possible of natural conditions, we only included data from natural or artificial soil tests, excluding results from filter paper or immersion tests. Papers dealing with tropical earthworm species were also excluded. It was decided to focus on lethal effects because there were not enough studies on biomass or on chronic effect of cocoon production that suited the constraints we set. Finally, we selected a corpus of 15 publications, i.e. 11 papers and 4 studies in a book chapter (Appendix A).

2.2. Data extraction

Data from the fifteen selected publications were entered into a relational database including several variables: author(s), year of the study, species, development stage i.e. juvenile or mature, active

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