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Selection of focal earthworm species as non-target soil organisms for environmental risk assessment of genetically modified plants

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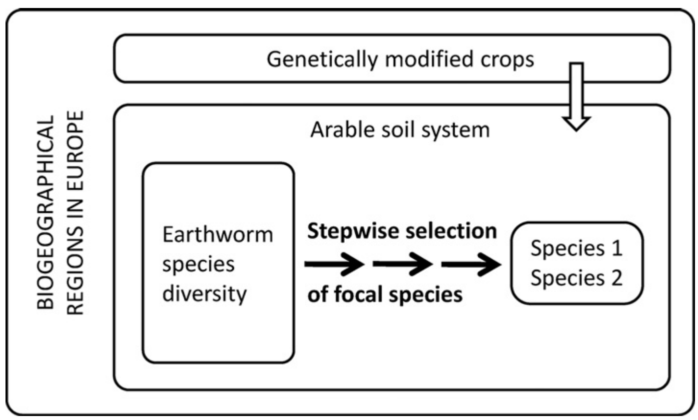
HIGHLIGHTS

- GM-crops and their potential environmental risks are still controversial in the EU.
- Earthworms are important non-target organisms in arable soils.
- Focal species are selected based on literature data following a four-step procedure.
- Selection highly representative for EU biogeographical regions under maize or potato.
- Selected focal species are recommended for testing based on life-history traits.

GRAPHICAL ABSTRACT



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ABSTRACT

By means of a literature survey, earthworm species of significant relevance for soil functions in different biogeographical regions of Europe (Atlantic, Boreal, Mediterranean) were identified. These focal earthworm species, defined here according to the EFSA Guidance Document on the environmental risk assessment (ERA) of genetically modified plants, are typical for arable soils under crop rotations with maize and/or potatoes within the three regions represented by Ireland, Sweden and Spain, respectively. Focal earthworm species were selected following a matrix of four steps: Identification of functional groups, categorization of non-target species, ranking species on ecological criteria, and final selection of focal species. They are recommended as appropriate non-target organisms to assess environmental risks of genetically modified (GM) crops; in this case maize and potatoes. In total, 44 literature sources on earthworms in arable cropping systems including maize or potato from Ireland, Sweden and Spain were collected, which present information on species diversity, individual density and specific relevance for soil functions. By means of condensed literature data, those species were identified which (i) play an important functional role in respective soil systems, (ii) are well adapted to the biogeographical regions, (iii) are expected to occur in high abundances under cultivation of maize or potato and (iv) fulfill the requirements for an ERA test system based on life-history traits. First, primary and secondary decomposers were identified as functional groups being exposed to the GM crops. In a second step, anecic and

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endogeic species were categorized as potential species. In step three, eight anecic and endogeic earthworm species belonging to the family Lumbricidae were ranked as relevant species: *Aporrectodea caliginosa*, *Aporrectodea rosea*, *Aporrectodea longa*, *Allolobophora chlorotica*, *Lumbricus terrestris*, *Lumbricus friendi*, *Octodrilus complanatus* and *Octolasion cyaneum*. Five out of these eight species are relevant for each biogeographical region with an overlap in the species. Finally, the earthworm species *Ap. caliginosa* (endogeic, secondary decomposer) and *L. terrestris* (anecic, primary decomposer) were selected as focal species. In the Mediterranean region *L. terrestris* may be substituted by the more relevant anecic species *L. friendi*. The selected focal species are recommended to be included in a standardized laboratory ERA test system based on life-history traits.

1. Introduction

Within the European Union, the environmental risks associated with genetically modified (GM) plants still remain a controversial issue and this is considered the cause that currently limits the surface cultivated in Europe with GM crops. In 2014, in Spain more than 130,000 ha were cultivated, while in other European countries currently growing GM crops (Portugal, Romania, Czech Republic and Slovakia) the total surface was less than 20,000 ha (James, 2014). Irrespective of being genetically modified or not, the total area harvested for maize was 18.75 million ha and for potato 5.61 million ha in Europe in 2014 (http://faostat3.fao.org/compare/E; accessed 20 Dec. 2015). Since 1995, environmental risk assessment (ERA) for GM crops in Europe has been carried out by the European Food Safety Authority (EFSA), which issues scientific opinions on the request for commercial use of GM crops for food and feed, import and processing and cultivation in Europe. When a GM event is approved by the European Commission, based on new scientific evidence related to the safety of a GM product, EU Member States can invoke safeguard clause measures or emergency measures in order to provisionally restrict or prohibit the commercial use of previously authorized GM organisms on their territory (Devos et al., 2014). So far, safeguard clause and/or emergency measures have been invoked by Austria, France, Greece, Germany, Hungary, Italy, Luxemburg, Portugal and Bulgaria for several GM maize, oilseed rape and potato events for a total of 37 requests. Due to the controversies generated by such requests, a change in the legislation has now given the possibility for the Member States to restrict or prohibit the cultivation of GM crops in their territory based on scientific as well as on socio-economic ground (Directive EU 2015/412).

Very commonly, the main concern presented by Member States relates to a possible threat to biodiversity, namely to non-target organisms (NTOs) (Arpaia, 2010), in receiving environments for which no specific data were generated for risk assessment.

The number of species present in any agro-ecosystem makes it impossible to carry out a detailed study including all these species. It is therefore necessary to make a choice of a few species that can be considered representative for the specific receiving environment. Many possible criteria to make such selection have been suggested, and proposals were drafted to support ERA with conventional ecotoxicological models (Romeis et al., 2008), exotic species models (Orr et al., 1993) or ecological models (Andow and Hilbeck, 2004).

The GMO Panel of EFSA proposed a risk assessment approach for European environments based on the selection of focal species representative of functional groups within a tiered approach (EFSA, 2010). The main criterion adopted in the Guidance Document, is the analysis of functional biodiversity in agro-ecological habitats and the possible interference of the biodiversity's normal functioning caused by GM crops. Particular emphasis is given to the receiving environments for which the ERA is conducted. Therefore, the species selection process is aimed at the determination of “focal species” based on ecological criteria and practical considerations (e.g. species availability, suitability for laboratory testing) which lead to the final choice. In particular, it is indicated that experiments are conducted using species relevant to specific European environments and agricultural settings. Recent data suggest that the sensitivity of European species to various Cry toxins is different when compared to surrogate species selected in other environments (EFSA, 2011). Cry toxins are crystal proteins produced during the sporulation phase by *Bacillus thuringiensis* Berl. strains which have a rather specific toxic action against selected groups of insects upon ingestion. In the case of a Cry1f-expressing maize, for instance, the EFSA re-issued a scientific opinion when data on toxicity of this protein to European non-target Lepidoptera species became available (EFSA, 2011). In the previous opinion (EFSA, 2005), considering toxicity data obtained using the surrogate American species *Danaus plexippus* L., risk management options for the maize event 1507 were not included.

In arable soils, earthworms represent crucial non-target organisms (Icoz and Stotzky, 2008). They are important members of the soil biota community and are often considered as the keystone group within soil food webs (Lavelle and Spain, 2005; Wall et al., 2012). Due to their high ecological significance in plant litter decomposition, earthworms might be affected via GM-induced expression of specific proteins like the Cry1Ab protein in Bt maize. Degradation of this protein from litter material is accelerated by earthworm activity (Schrader et al., 2008; Emmerling et al., 2011). Furthermore, GM crops may differ from the near-isoline in the amount of major plant components such as cellulose, lignin, fructose or soluble carbohydrates (Escher et al., 2000; Flores et al., 2005; Poerschmann et al., 2005; Saxena and Stotzky, 2001). A near-isogenic line is the original variety transformed with techniques of genetic engineering. Even though, due to segregation, the plants used in biosafety experiments are not exactly identical to the plant that was transformed this is recognized and accepted in all regulatory systems for genetically modified plants as the most dependable comparator to assess differences and similarities with genetically modified lines (EFSA, 2010). Such differences in plant components affect nutritional parameters of plant material (Clark and Coats, 2006) and the decomposability of plant residues in soil (Flores et al., 2005; Hönemann et al., 2008; Zwahlen et al., 2007). Thus, earthworms are closely associated to GM crops and their compounds by residue degradation, and they contribute to numerous important ecosystem functions and services like for instance soil formation, water supply, nutrient cycling (Lavelle et al., 2006; Bertrand et al., 2015). According to the combination of both issues earthworms represent appropriate non-target organisms in the context of GM crop risk assessment (EFSA, 2010).

Whereas an EFSA database (http://www.efsa.europa.eu/it/supporting/pub/334e.htm) on non-target arthropod species provides bio-ecological information to support ERA of GM crops in the EU, such an information system does not exist for earthworms. Previous risk assessment studies on earthworms usually focus on the common laboratory species *Eisenia fetida* (e.g. Ahl Goy et al., 1995; Clark and Coats, 2006). As this species occurs rarely in European arable soils and therefore may only be of limited value for risk analyses, a reliable test system should base on focal earthworm species. Focal species are, according to the EFSA ERA Guidance Document for NTOs (EFSA, 2010), defined as species with a high potential exposure linked to a significant functional importance in soils of a specific biogeographical region under cultivation of a respective crop. The focal species approach addresses that standardized laboratory species frequently lack ecological relevance, an often critical point in previous risk assessment studies (Lövei and Arpaia, 2005). Thus, this approach suits to select non-target