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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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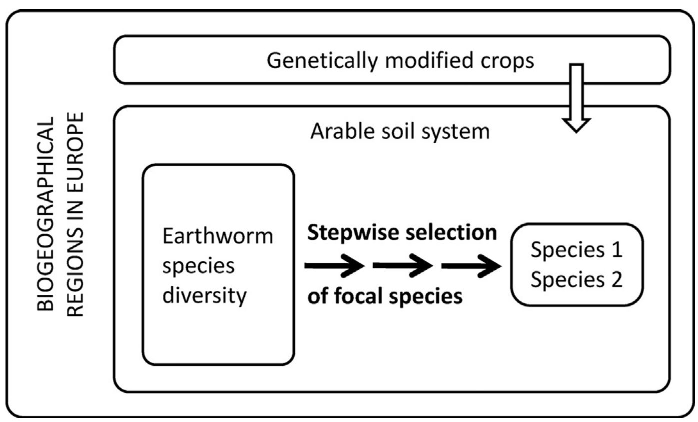
HIGHLIGHTS

- 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 species from the functional group in the specific food web. For that purpose, a literature survey was conducted to identify and select appropriate focal earthworm species which (i) are functionally of high relevance in arable soil systems of different biogeographical regions in Europe, and (ii) fulfill the requirements for an ERA test system based on life-history traits. Literature was searched with respect to the country of study, the arable cropping system, the provision of lists on earthworm species and their abundances.

2. Materials and methods

2.1. Selection matrix

A selection matrix for NTOs was prepared based on the criteria indicated in the EFSA ERA Guidance Document (EFSA, 2010). The EFSA selection process includes four steps: (i) *Identification of functional groups*; (ii) *Categorization of NTO species*; (iii) *Ranking species based on the ecological criteria*; and (iv) *Final selection of focal species.*

For ranking species (step 3) 7 criteria are listed in the EFSA ERA Guidance Document (EFSA, 2010). Based on current knowledge on earthworm diversity, occurrence and ecology in published literature, the following 3 criteria were considered in the present selection process: possible exposure to the GM plant, linkage to the production system and abundance. The dominance distribution within species communities was calculated by means of total or relative abundances of species in relation to total individual densities. The calculation of the dominance structure of the species assemblage was assessed following the classification system of Engelmann (1978). According to this system, species abundances were classified as follows: dominant (>10%), subdominant (3.2–9.9%), recedent (1.0–3.1%), subrecedent (0.32–0.99%) and sporadic (<0.32%). For the present assessment, we adopted a simplified system in which dominant and subdominant, as well as recedent and subrecedent, species were pooled. Species were ranked according to the scoring system presented in Tab. 2. Four criteria remained unconsidered since information on earthworms is still insufficient (sensitivity of species to the transgenic product, interactions with target species like larvae of *Ostrinia nubilalis* in maize residues), impact of agricultural measures on earthworms vary widely with management intensity (species vulnerability i.e. species are already threatened) or aspects are generally negligible for earthworms due to their comparatively low mobility (relevance to adjacent habitats). Additionally, the criterion 'presence' was used which indicates the frequency of literature records i.e. the number of literature sources providing information on the occurrence of an earthworm species. For each identified functional group according to step 1, the species selection process, including scoring in steps 2 and 3, was used to prioritize species in step 4.

2.2. Data collection and data assessment

Peer-reviewed papers in scientific journals which address soil biology issues, proceedings and reports of projects, and online-available species lists were screened. The literature sources were examined for data on earthworm communities in arable soils of Ireland, Sweden and Spain chosen as case studies to represent Atlantic, Boreal and Mediterranean regions of Europe, respectively. The search was originally meant to identify species associated with maize and potato, the only two crops for which an authorization for cultivation of GM crops was issued in Europe. Where no such association was available, other literature sources were utilized. Several studies, mainly older ones from before the 1990s, were published only in the respective native language (especially Spanish studies). Results of these studies were, nonetheless, considered on the basis of literature citations, like, for instance, given in Briones et al. (2009). In total, 44 literature sources (10 sources for Irish soils, 14 sources for Swedish soils, and 20 sources for Spanish soils) were collected and rated suitable to provide appropriate information, allowing the selection of focal earthworm species (see Appendix for the complete list of sources).

Data on species diversity, individual density and specific functional relevance were analyzed and consolidated. Those species were identified, which (i) play an important functional part in respective soil systems; (ii) are ecologically well adapted to the biogeographical regions; (iii) are expected to occur under cultivation of maize (Sweden and Spain) or potato (Ireland).

3. Results of data evaluation and discussion

The literature sources indicated the occurrence of earthworm species in Atlantic, Boreal and Mediterranean regions of Europe either by presenting whole species lists (7 sources), records of selected species (8 sources) or earthworm community compositions (29 sources). Studies on field-derived data partially indicated species numbers or abundances and/or biomasses of whole communities or species as relevant parameters for selecting appropriate focal species (Table. 1).

3.1. Step 1: Identification of functional groups being exposed to the GM plants

Earthworms are important decomposers of organic material in plant–soil systems (Lavelle and Spain, 2005; Wall et al., 2012). They contribute to important soil processes like bioturbation, formation of organo-mineral complexes during gut passage, regulation of nutrient cycling processes; and earthworms are highly involved in increasing soil fertility and improving plant growth (Edwards et al., 1995; Parmelee et al., 1998; Bertrand et al., 2015) and soil health (Wolfarth et al., 2011; Bertrand et al., 2015). Due to their burrowing activity, consumption of leaf litter and promotion of microbial activity they play an important role in soil formation (Tomlin et al., 1995; Schrader et al., 1995; Wall et al., 2012). Moreover, earthworms represent an important part of the diet of many vertebrates and other invertebrates (Edwards and Bohlen, 1996). With respect to functional differentiation within earthworms as decomposers two functional groups were identified as being exposed to GM maize and/or GM potato: (i) primary decomposers feeding directly on more or less intact plant residues; (ii) secondary decomposers consuming pre-decayed organic material (Fig. 1). Both functional groups, which are well known from literature (e.g. Lavelle and Spain, 2005), are identified to be considered for ERA.

3.2. Step 2: Categorization of NTO species from identified functional groups

According to their environmental and ecological preferences earthworms can be distinguished into aquatic, semiaquatic and terrestrial species. Generally, terrestrial earthworm species are assigned to three

Table 1
Number of literature sources [n] on earthworm parameters relevant to the selection of focal earthworm species in three biogeographical regions of Europe represented by Ireland (IR), Sweden (S) and Spain (ES).

	Number of literature sources [n]		
	Atlantic [IR]	Boreal [S]	Mediterranean [ES]
Total [n]	10	14	20
Species lists	3	2	2
Records of communities	7	8	14
Records of species	0	4	4
Crop (potato or maize)	1	0	2
Species number [n]	10	9	9
Total earthworm abundance [ind. m^{-2}]	6	5	1
Total earthworm biomass [g m^{-2}]	5	5	0
Species abundance [ind. m^{-2}]	5	4	1
Species biomass [g m^{-2}]	0	3	0
Relative abundance of species [%]	6	5	1

ecological groups, which differ strongly concerning their vertical distribution, burrowing activity and food preferences: (i) epigeic species are characterized by feeding on plant residues on the soil surface and the creation of non-permanent horizontal burrows; (ii) deep-burrowing anecic species feed on decaying plant residues from the soil surface and create permanent vertical burrows, allowing the incorporation of organic matter deeper in the soil profile; (iii) endogeic species form network-like semi-permanent burrows and feed on topsoil and associated pre-decayed organic material. The classification system allows for assigning species to intermediate groups like epi-endogeic or epi-anecic species. Epigeics and anecics are classified as primary decomposers and endogeics as secondary decomposers (see above: Step 1).

Information from total national or regional lists of earthworm species, which have been recorded in any habitat of the selected countries (Ireland, Sweden, Spain), were combined with species records specifically derived from agricultural fields to compile lists of earthworm species as complete as possible including their assignment to the ecological groups (Suppl. Tabs. 1–3).

The species list for Irish soils comprises 30 species belonging to 3 families (Acanthodrilidae, Lumbricidae and Sparganophilidae). Twelve species belong to the endogeic, 3 to the anecic, and 14 to the epigeic ecological group. One species was classified as aquatic. In total 6 species (4 endogeic and 2 anecic) were recorded under cultivation of potato (Suppl. Tab. 1). The species list for Swedish soils includes 22 species, all belonging to the family Lumbricidae, whereof 8 species are endogeic, 2 anecic, 11 epigeic and 1 epi-endogeic (Suppl. Tab. 2). The list of earthworm species for Spanish soils comprises 75 species belonging to 7 families (Acanthodrilidae, Criodrilidae, Haplotaxidae, Hormogastridae, Lumbricidae, Megascolecidae and Ocnerodrilidae) (Suppl. Tab. 3). Of these species, 27 are classified as endogeic, 6 as anecic and 19 as epigeic. Moreover, 3 semiaquatic and 2 aquatic species are included. For 18 species no records on respective ecological groups could be found. In total, 8 out of the 75 species (4 endogeic and 4 epigeic) are described occurring under cultivation of maize (Suppl. Tab. 3).

As a first step, the earthworm species were categorized regarding their relevance in arable soils which means a potential exposure to GM crops. Aquatic and semiaquatic species as well as species of unknown ecological classification were categorized as being irrelevant (Suppl. Tabs. 1–3). It is expected that exposure and potential impacts of GM crops differ between the terrestrial ecological groups according to their behavioral and functional differences. There is evidence that individual densities of epigeic earthworm species, compared with anecic and endogeic species, are usually very low in arable soils (Kladivko, 2001; Whalen and Sampedro, 2010). These species may only become abundant in arable systems with low management input and intensity (Whalen and Sampedro, 2010). However, we focused on maize and potato which both (like many other annual crops) need considerable management intensity in most cases. Therefore, epigeic species were categorized as being less relevant (Suppl. Tabs. 1–3). The species within the remaining two groups of endogeic and anecic earthworm species were categorized as potential species of high relevance with respect to an exposure to GM maize and/or GM potato (Fig. 1; Suppl. Tabs. 1–3).

3.3. Step 3: Ranking species based on the ecological criteria

For further prioritization among the remaining anecic and endogeic species, literature data were surveyed for information on dominance distribution of these species in arable soils and their occurrence under cultivation of maize and potato. However, only three literature sources indicated the earthworm community structure under cultivation of potato in Ireland (1 source) and maize in Spain (2 sources). With regard to Swedish soils, no literature data on the species composition in maize fields were available (Table 1) since the crop has only recently been introduced in the country. For this reason, data on the dominance distribution of species under cultivation of other crops (wheat, barley, oats and rye) were considered as well. These crops are quite comparable to maize in terms of input and intensity of management measures and demands for site conditions. In total, 12 literature sources on field-derived data (Ireland: 6; Sweden: 5; Spain: 1) presented the dominance of species or indicated their abundances [ind. m^{-2}] or relative abundances [%] of species in combination with total individual densities [ind. m^{-2}].

Species, which are assumed to be generally representative for earthworm communities in arable soils, were identified by (i) total species records, (ii) occurrence under cultivation of maize (Spain), other annual crops (Sweden, see above) or potato (Ireland), and (iii) information on their dominance distribution as presented in Suppl. Tabs. 4–6. These data were used within a scoring system for species ranking which was applied on the following criteria: (i) exposure to GM crop; (ii) linkage to the production system; (iii) abundance based on the dominance distribution; and (iv) presence in literature sources (for details see Tables 2–4). Species ranked relevant allow for consideration of specific impacts of GM crop residues on species of different ecological groups and functions.

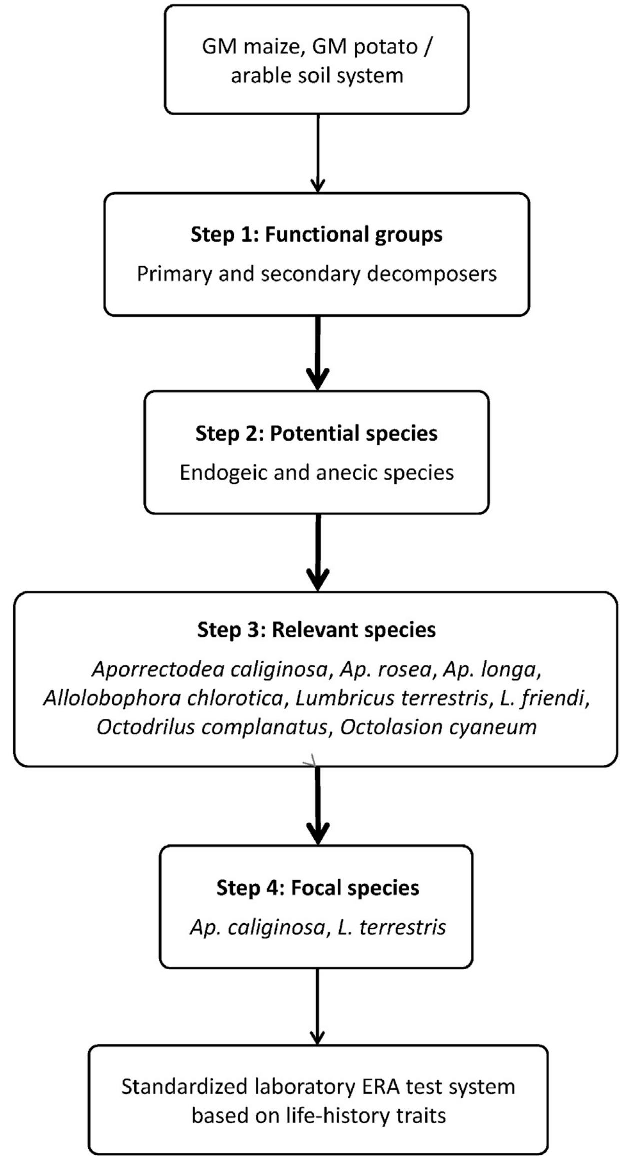

Fig. 1. Matrix of results presenting the step-by-step process of focal species selection for earthworms as non-target organisms (NTOs), which are recommended for inclusion in a laboratory ERA system to test for GM crops in Europe (adapted from Fig. 5 in the EFSA ERA Guidance Document for NTOs (EFSA, 2010)). GM = genetically modified; ERA = environmental risk assessment.

Table 2
Ranking anecic and endogeic earthworm species from Ireland. The lower the score, the higher the relevance of species for focal species selection. Species ranked as most relevant are marked in grey.

Family resp. species	Ecological group	Exposure	Linkage	Abundance	Presence	Ranking
Acanthodrilidae						
Microscolex phosphoreus (Dugès, 1837)	endogeic	2	2	3	3	2.5
Lumbricidae						
Allolobophora chlorotica (Savigny, 1826)	endogeic	1	1	1	1	1.0
Allolobophora cupulifera (Tétry, 1937)	endogeic	2	2	3	3	2.5
Allolobophoridella eiseni (Levinsen, 1884)	endogeic	2	2	3	3	2.5
Aporrectodea caliginosa (Savigny, 1826)	endogeic	1	1	1	1	1.0
Aporrectodea icterica (Savigny, 1826)	endogeic	2	2	3	3	2.5
Aporrectodea limicola (Michaelsen, 1890)	endogeic	2	2	1	2	1.8
Aporrectodea longa (Ude, 1885)	anecic	1	1	1	1	1.0
Aporrectodea rosea (Savigny, 1826)	endogeic	1	1	1	1	1.0
Lumbricus friendi (Cognetti, 1904)	anecic	2	2	1	2	1.8
Lumbricus terrestris (Linnaeus, 1758)	anecic	1	1	2	1	1.3
Murchieona minuscula (Rosa, 1906)	endogeic	1	1	1	2	1.3
Octolasion cyaneum (Savigny, 1826)	endogeic	2	2	2	2	2.0
Octolasion tyrtaeum (Savigny, 1826)	endogeic	2	2	1	2	1.8
Prosellodrilus amplisetosus (Bouché, 1972)	endogeic	2	2	1	3	2.0

Ranking criteria and their scoring.
Exposure to GM crop:
1: recorded under cultivation of model crop (potato or maize).
2: recorded under cultivation of other crops.
Linkage to the production system:
1: recorded in arable cropping system.
2: recorded in other ecosystem or no information on sampling location.
Abundance:
1: dominant in at least 1 literature source.
2: recedent in at least 1 literature source.
3: sporadic or no information on dominance.
Presence:
1: recorded in >6 literature sources.
2: recorded in 4–6 literature sources.
3: recorded in 1–3 literature sources.

With regard to Irish soils, those species which occur under cultivation of potato (6 species) and are recorded in at least 80% of total literature sources (5 species: *Allolobophora chlorotica*: 100%; *Aporrectodea caliginosa*: 100%; *Aporrectodea longa*: 80%; *Aporrectodea rosea*: 100%; *Lumbricus terrestris*: 90%) were ranked relevant species for the Atlantic region (Table 2). Four out of five species were described as dominant in at least one literature source (Suppl. Tab. 4). In the following, *Al.* stands for *Allolobophora* and *Ap.* stands for *Aporrectodea* to clearly distinguish between both genera.

For Swedish soils no information on species distribution under cultivation of maize was available. Therefore, those endogeic and anecic earthworm species, which were described as dominant in literature sources in other arable crop systems (wheat, barley, oats and rye) (Suppl. Tab. 5), and which were recorded in at least 50% of total literature sources for Swedish soils (*Ap. rosea*: 100%; *Ap. caliginosa*: 79%; *Ap. longa* and *L. terrestris*: 71%; *Al. chlorotica*: 50%), were ranked relevant species for the Boreal region (Table 3).

With regard to anecic species in Spanish soils, the circum-Mediterranean species *Octodrilus complanatus* (recorded in three literature sources (15%), and described as dominant in one source) and *Lumbricus friendi* (recorded in 13 literature sources: 65%) (Díaz Cosín et al., 1992; Rodriguez et al., 1997) were ranked relevant species (Table 4). The species *L. terrestris* was excluded since it was described only to occur in very low densities in Spanish soils (Briones et al., 2009). Concerning the endogeic species, *Ap. caliginosa* (50%) and *Ap. rosea* (45%) were ranked relevant species (Table 4), because both species were recorded under the cultivation of maize and were described as dominant (Suppl. Tab. 6). Furthermore, *Octolasion cyaneum*, which was recorded under maize and listed in 8 sources (40%), was also ranked relevant (Table 4). In the following, *Octod.* stands for *Octodrilus* and *Octol.* stands for *Octolasion* to clearly distinguish between both genera.

Based on consolidated literature data, 8 earthworm species (3 endogeic and 2 anecic species for each biogeographic region), typically occurring in Irish, Swedish or Spanish arable soils, were finally ranked relevant (Tables 2–4; Fig. 1). They belong to the Oligochaeta family Lumbricidae (*Ap. caliginosa*, *Ap. rosea*, *Ap. longa*, *Al. chlorotica*, *L. terrestris*, *L. friendi*, *Octod. complanatus*, *Octol. cyaneum*). These species might be vulnerable when already threatened (e.g. mechanically or chemically) in an agro-ecosystem. Whether earthworms are seriously threatened or not depends on the intensity of agricultural management measures like, for instance, the choice of the tillage system (van Capelle et al., 2012) as well as type, quantity and frequency of pesticide application (Pelosi et al., 2014). Therefore, the last criterion (vulnerability) was declined for ranking species.

3.4. Step 4: Final selection of focal species

For the final selection of focal species the following additional practical criteria were considered: (i) availability of species (field collection or purchase from a commercial supplier) (Fründ et al., 2010); (ii) knowledge on cultivation and breeding (Lowe and Butt, 2005); and (iii) suitability for testing under laboratory conditions (Fründ et al.,

Table 3
Ranking anecic and endogeic earthworm species from Sweden. The lower the score, the higher the relevance of species for focal species selection. Species ranked as most relevant are marked in grey.

Family resp. species	Ecological group	Exposure	Linkage	Abundance	Presence	Ranking
Lumbricidae						
Allolobophora chlorotica (Savigny, 1826)	endogeic	2	1	1	1	1.3
Allolobophora cupulifera (Tétry, 1937)	endogeic	2	2	3	3	2.5
Allolobophoridella eiseni (Levinsen, 1884)	endogeic	2	2	3	3	2.5
Aporrectodea caliginosa (Savigny, 1826)	endogeic	2	1	1	1	1.3
Aporrectodea limicola (Michaelsen, 1890)	endogeic	2	2	3	3	2.5
Aporrectodea longa (Ude, 1885)	anecic	2	1	1	1	1.3
Aporrectodea rosea (Savigny, 1826)	endogeic	2	1	1	1	1.3
Lumbricus terrestris (Linnaeus, 1758)	anecic	2	1	1	1	1.3
Octolasion cyaneum (Savigny, 1826)	endogeic	2	1	2	1	1.5
Octolasion tyrtaeum (Savigny, 1826)	endogeic	2	1	2	2	1.8

Ranking criteria and their scoring.
Exposure to GM crop:
1: recorded under cultivation of model crop (potato or maize).
2: recorded under cultivation of other crops.
Linkage to the production system:
1: recorded in arable cropping system.
2: recorded in other ecosystem or no information on sampling location.
Abundance:
1: dominant in at least 1 literature source.
2: recedent in at least 1 literature source.
3: sporadic or no information on dominance.
Presence:
1: recorded in >6 literature sources.
2: recorded in 4–6 literature sources.
3: recorded in 1–3 literature sources.

2010). Finally, the earthworm species *Ap. caliginosa* (endogeic, secondary decomposer) and *L. terrestris* (anecic, primary decomposer) were selected as focal species to be included in a standardized laboratory ERA test system (Fig. 1). Both species are most likely to be available in sufficient numbers in the field. Knowledge on life-history traits and cultivation of *Ap. caliginosa* (Jensen and Holmstrup, 1997; Lowe and Butt, 2005) and *L. terrestris* (Butt et al., 1994; Lowe and Butt, 2005) is available. In the Mediterranean region *L. terrestris* may be substituted by the more relevant anecic species *L. friendi* due to their higher availability in arable cropping systems (see step 3) and a life-history performance comparable to *L. terrestris* (Butt and Briones, 2011).

4. General discussion and conclusions

Earthworms are well documented from many parts of the world. However, only some information on earthworm communities in Irish, Swedish or Spanish arable systems was specifically linked to the cultivation of maize or potato. Therefore, literature data on earthworm abundances and species diversity from comparable cropping systems were considered as well for the literature survey and included into the selection process. For Europe, earthworms have been considered as surprisingly under-recorded taxa (Rutgers et al., 2016 and references therein) although they are known to deliver numerous ecosystem goods and services (Lavelle et al., 2006; Bertrand et al., 2015).

Endemic species, such as *Hormogaster elisae*, *Dendrobaena madeirensis*, *Prosellodrilus pyrenaicus*, *Scherotheca campoii* or *Xana omodeoi* which occur in high abundances in arable soils in central Spain (Briones et al., 1994; Díaz Cosín et al., 1992; Novo et al., 2009), were excluded during selection to ensure the applicability of an ERA test system in different countries or sites within one biogeographical region. For this reason, only species of global (*Al. chlorotica*, *Ap. caliginosa*, *Ap. longa*, *Ap. rosea*, *L. terrestris*, *Octod. complanatus*, *Octol. cyaneum*) (Blakemore, 2006a, 2006b; de Jong, 2011; Klinkenberg, 2012) or Mediterranean (*L. friendi*) (Csuzdi and Szlavecz, 2003) distribution were ranked relevant species. However, region-specific conditions and a region-specific composition of an earthworm community might require the need to select an endemic species additionally, if *Ap. caliginosa* or *L. terrestris* are missing or less abundant.

Besides the 3 biogeographical regions Atlantic, Boreal and Mediterranean, which were considered in the present literature survey, there are other important European regions (e.g. Continental Europe, Balkans). In both these regions the earthworm species *Ap. caliginosa* (endogeic, secondary decomposer) and *L. terrestris* (anecic, primary decomposer) are very common in arable cropping systems as well (Lee, 1985; Rutgers et al., 2016). It is acceptable to argue then that both the suggested species are also potentially exposed to GM maize and GM potato in these regions. Consequently, *Ap. caliginosa* and *L. terrestris* can be recommended as focal species in ERA test systems on GM crops for whole Europe.

Tests designed to assess acute toxicity over short-term exposure may not predict effects of chronic exposure, like sublethal direct or indirect effects on non-target species over several generations (Birch et al., 2007). To meet this need for risk assessment under chronic exposure conditions, a test system should include long-term survival, growth and reproduction of focal earthworm species as main components of their fitness and relevant life-history performance traits to conclude on potential long-term effects and changes in ecological functions (Pey et al., 2014; Violle et al., 2007). GM crop risk assessment protocols should be based on biomass, cocoon production, percentage of cocoon hatching, as well as survival, biomass, growth and development of offspring as measurable endpoints, which characterize the population turnover rate. In order to measure these parameters properly under long-term experimental conditions these species should be cultivable and suitable for testing under laboratory conditions.

Table 4
Ranking anecic and endogeic earthworm species from Spain. The lower the score, the higher the relevance of species for focal species selection. Species ranked as most relevant are marked in grey.

Family resp. species	Ecological group	Exposure	Linkage	Abundance	Presence	Ranking
Acanthodrilidae						
Microscolex dubius (Fletcher, 1887)	endogeic	2	2	2	2	2.0
Microscolex phosphoreus (Dugès, 1837)	endogeic	2	2	2	2	2.0
Hormogastridae						
Hormogaster elisae (Álvarez, 1977)	endogeic	2	2	1	2	1.8
Lumbricidae						
Allolobophora chlorotica (Savigny, 1826)	endogeic	2	2	3	2	2.3
Allolobophora oculata (Hoffmeister, 1845)	endogeic	2	2	3	3	2.5
Aporrectodea caliginosa (Savigny, 1826)	endogeic	1	1	1	1	1.0
Aporrectodea georgii (Michaelsen, 1890)	endogeic	2	2	3	2	2.3
Aporrectodea icterica (Savigny, 1826)	endogeic	2	2	3	3	2.5
Aporrectodea molleri (Rosa, 1889)	endogeic	2	2	3	2	2.3
Aporrectodea opisthosellata (Graff, 1961)	endogeic	2	2	3	3	2.5
Aporrectodea rosea (Savigny, 1826)	endogeic	1	1	1	1	1.0
Aporrectodea terrestris (Savigny, 1826)	anecic	2	2	3	3	2.5
Dendrobaena madeirensis (Michaelsen, 1891)	endogeic	1	1	3	1	1.5
Lumbricus centralis (Bouché, 1972)	anecic	2	2	3	3	2.5
Lumbricus friendi (Cognetti, 1904)	anecic	2	2	3	1	2.0
Lumbricus terrestris (Linnaeus, 1758)	anecic	2	2	3	2	2.3
Murchieona minuscula (Rosa, 1906)	endogeic	2	2	3	2	2.3
Octodrilus complanatus (Dugès, 1828)	anecic	2	2	1	3	2.0
Octolasion cyaneum (Savigny, 1826)	endogeic	1	1	3	1	1.5
Octolasion tyrtaeum (Savigny, 1826)	endogeic	2	2	3	1	2.0
Postandrilus bertae (Díaz Cosín et al., 1985)	endogeic	2	2	3	3	2.5
Prosellodrilus amplisetosus (Bouché, 1972)	endogeic	2	2	3	3	2.5
Prosellodrilus fragilis (Bouché, 1972)	endogeic	2	2	3	3	2.5
Prosellodrilus idealis (Bouché, 1972)	endogeic	2	2	3	3	2.5
Prosellodrilus praticola (Bouché, 1972)	endogeic	2	2	3	3	2.5
Prosellodrilus pyrenaicus (Cognetti, 1904)	endogeic	2	2	3	3	2.5
Scherotheca campoii (Lainez & Jordana, 1983)	endogeic	2	2	3	3	2.5
Scherotheca gigas aquitania (Bouché, 1972)	anecic	2	2	3	3	2.5
Scherotheca occidentalis (Michaelsen, 1922)	endogeic	2	2	3	3	2.5
Megascolecidae						
Amynthas corticis (Kinberg, 1867)	endogeic	2	2	3	3	2.5
Amynthas morrisi (Beddard, 1892)	endogeic	2	2	3	3	2.5
Ocnerodrilidae						
Eukerria saltensis (Beddard, 1895)	endogeic	2	2	3	3	2.5
Ocnerodrilus occidentalis (Eisen, 1878)	endogeic	2	2	3	3	2.5

Ranking criteria and their scoring.
Exposure to GM crop:
1: recorded under cultivation of model crop (potato or maize).
2: recorded under cultivation of other crops.
Linkage to the production system:
1: recorded in arable cropping system.
2: recorded in other ecosystem or no information on sampling location.
Abundance:
1: dominant in at least 1 literature source.
2: recedent in at least 1 literature source.
3: sporadic or no information on dominance.
Presence:
1: recorded in >6 literature sources.
2: recorded in 4–6 literature sources.
3: recorded in 1–3 literature sources.

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Appendix A. Literature sources used for selecting focal earthworm species

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Appendix B. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.12.165.

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