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REVIEW ARTICLE

Pesticides and earthworms. A review

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Abstract Earthworms provide key soil functions that favour many positive ecosystem services. These services are important for agroecosystem sustainability but can be degraded by intensive cultural practices such as use of pesticides. Many literature reports have investigated the effect of pesticides on earthworms. Here, we review those reports to assess the relevance of the indicators of earthworm response to pesticides, to assess their sensitivity to pesticides, and to highlight the remaining knowledge gaps. We focus on European earthworm species and products authorised in Europe, excluding natural compounds and metals. We consider different organisation levels: the infra-individual level (gene expression and physiology), the individual and population levels (life-history traits, population density and behaviour) and the community level: community biomass and density. Our analysis shows that earthworms are impacted by pesticides at all organisation levels. For example, pesticides disrupt enzymatic activities, increase individual mortality, decrease fecundity and growth, change individual behaviour such as feeding rate and decrease the overall community biomass and density. Insecticides and fungicides are the most toxic pesticides impacting survival and reproduction, respectively.

Keywords Lumbricidae · Organisation levels · Plant protection products · Biomarkers · Insecticides · Fungicides · Herbicides

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1 Introduction

Intensification of agricultural practices and especially the use of pesticides (Fig. 1) often result in a loss of biodiversity (Hole et al. 2005), but the effects of pesticides on different taxa and especially on soil organisms are still not very clear. The present review focuses on earthworms because they represent a large fraction of soil living biomass in many temperate ecosystems and play an important role in soil functioning. As ecosystem engineers (Jones et al. 1994), they influence organic matter dynamics, soil structure (Fig. 2a, b) and microbial community (Edwards and Bohlen 1996; Fragoso et al. 1997; Sims and Gerard 1999). They actively participate in soil aeration, water infiltration and mixture of soil horizons, and they represent an important source of food for many other organisms like birds or moles (Fig. 2c, d) (Edwards and Bohlen 1996; Lavelle et al. 2006). As early as 1984, Callahan (1984) underlined the importance of earthworms for assessing the general impact of pollution in soil. Since then, earthworms have sometimes been used as bioindicators for soil quality and the environmental impacts of cropping systems and pollutants (Cortet et al. 1999; Paoletti 1999). Many earthworm species are easy to collect and to identify; some are easily bred (Lowe and Butt 2005; Yasmin and D'Souza 2007), so they have been adopted by the international community as sentinel species for the study of the environmental impact (Ecological Risk Assessment (ERA)) of anthropogenic contaminants, such as pesticides, hydrocarbons and metal trace elements (Edwards and Bohlen 1996; Greig-Smith 1992; Kautenburger 2006; Pierce et al. 2002; Seeber et al. 2005; Spurgeon et al. 2003). For instance, mortality and/or reproduction of *Eisenia fetida* are currently used to assess the effects of pesticides under laboratory conditions before marketing authorisation (ISO 11268-1 1993; ISO 11268-2 1998; OECD 207 1984). Often, after marketing authorisation, pesticides are no longer subject to any further evaluation by the national agencies that authorised their use. Yet in cultivated fields, non-target organisms, such as earthworms are exposed to frequent and different (e.g. insecticide, fungicide and herbicide) pesticide applications. Because of the major role they play in soil functioning, the effects of pesticides on these soil organisms should be investigated further.

Most published ecotoxicological studies on earthworms have focused on metals (Lowe and Butt 2007) while the effects of pesticides have been less studied. To date, almost 400 substances or plant protection products, also called pesticides, are authorised in Europe, including natural compounds and metals. In the scientific literature, most studies on the effects of pesticides on earthworms were made in the 1980s. Some are more recent but focus on compounds that are no longer permitted in Europe. This is the case with many studies on carbofuran (Anton et al. 1993; Ruppel and Laughlin 1977), benomyl (Stringer and Wright 1976; Wright 1977; Wright and Stringer 1973), carbaryl (Neuhauser and Callahan 1990; Tu et al. 2011), dieldrin and dichlorodiphenyltrichloroethane (Davis 1971). In Lee (1985), which is one of the major text books on earthworm biology and ecology, a review of pesticide effects on earthworms was presented. Only 13 substances out of the 84 presented are still authorised in Europe. In the same way, in Edwards and Bohlen's (1996) book, only 43 substances out of 181 are still used in Europe. For 21 of these 43, the results are insufficient to exclude adverse effects. Similarly, reviews on the effects of pesticides on soil invertebrates in the laboratory or in the field (Càceres et al. 2010; Frampton et al. 2006; Jänsch et al. 2006; Robert and Dorough 1985; Yasmin and D'Souza 2010) describe the effects of many substances that are no longer used in Europe. Recently, Tu et al. (2011) showed that 'older pesticides […] had greater

Fig. 1 Pesticide application in a field

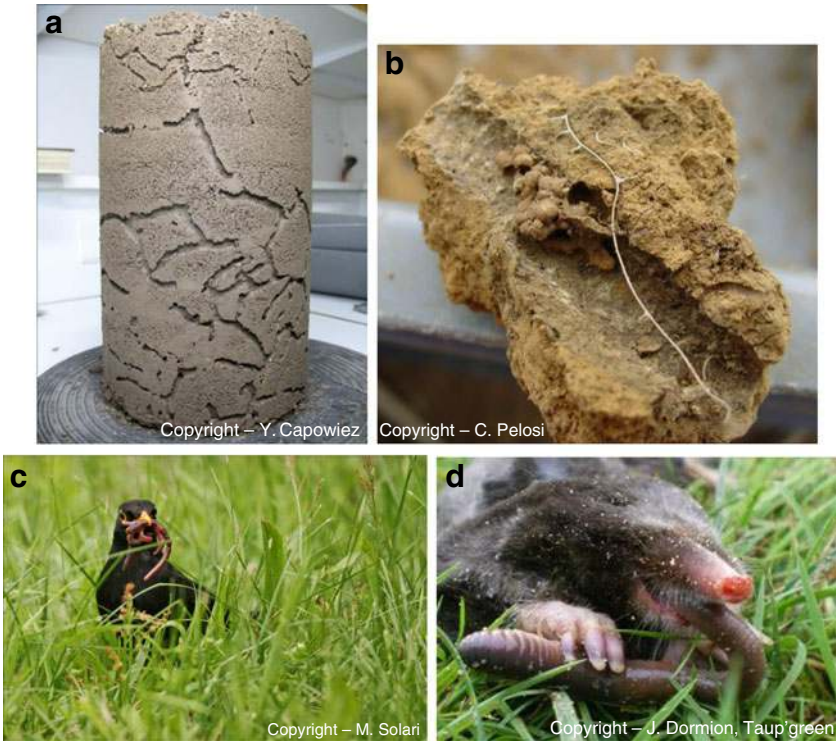

Fig. 2 Pictures showing some of the roles of earthworms in soil structure (**a**, **b**) and as a trophic resource for other organisms (**c**, **d**)

inhibitory effects on earthworms than the newer ones' and reported that 'newer pesticides are generally less toxic to non-target organisms (e.g. earthworms) because of their relatively higher selectiveness (Casida and Quistad 1998)'. However, since there is no comprehensive study summarising the effects of currently used pesticides on earthworms in European cultivated fields, it is necessary to recap the knowledge and information available on this subject.

Studies found in the literature on the effect of pesticides on earthworms were conducted either under laboratory conditions (Bauer and Römbke 1997; Cathey 1982; Rodriguez-Castellanos and Sanchez-Hernandez 2007; Brulle et al. 2010; Muthukaruppan and Paramasamy 2010) or in the field (Martin 1986; Reddy and Reddy 1992). Evaluation was achieved using different indicators that were investigated at various organisation levels. Indeed, changes in responses to the presence of a chemical compound such as a pesticide can be measured at (1) the infra-individual level, e.g. gene expression, enzyme activities, (2) the individual level, e.g. survival, fecundity and behaviour and (3) the community level, e.g. diversity and community structure. Usually, the objective of studies that are made at infra-individual and individual levels is to extrapolate the risks or effects to higher organisation levels, mainly the population level. Some responses are the direct result of a toxic effect. For instance, a contaminant may affect the expression of a gene (infra-individual level) involved in a physiological function (higher level). This has been highlighted using metal pollution for the gene expression of annetocin which is a hormone involved in reproduction of the earthworm *E. fetida* (Ricketts et al. 2004). Other responses, probably most, are indirect responses of compensation or restoration, e.g. physiological plasticity or homeostasis (Ankley et al. 2006). Indeed, animals that allocate resources to the detoxification of contaminants are likely to allocate less resource to other functions such as reproduction or growth. To provide a heuristic and comprehensive perspective of pesticide effects on earthworms, we have to consider consequences of pesticides at all these organisation levels. This might include analysing how the effects at lower levels cascade onto higher levels and even allow the early prediction of consequences at higher levels. In this review, we want to emphasise the importance of documenting pesticide effects at all organisation levels and all earthworm species that may be affected.

The aims of this review are (1) to list and assess the relevance of the different indicators used to study earthworm responses to pesticides at different organisation levels from the infra-individual to the community level (see above), (2) to assess the effects of pesticides on earthworms at these organisation levels using substances authorised in Europe and (3) to highlight the knowledge gaps. This review brings together ecotoxicologists, soil ecologists and agronomists and presents, in an accessible way, the state of knowledge on earthworms for the ecotoxicological monitoring of pesticides. It is based on the international literature but considers only earthworms species found in Europe, i.e. excluding tropical species, as well as only plant protection

products authorised in Europe, i.e. excluding natural compounds and metals.

2 Effects of pesticides at different organisation levels

2.1 Response at infra-individual level

2.1.1 Literature review

The literature review was carried out on the basis of keywords in Scopus using combinations of the following keywords: 'pesticide* earthworm* biomarker* indicator* herbicide* fungicide* insecticide* genotoxic* biochemical* cellular*' in Topics. We retrieved several hundred publications. Those which appeared relevant for the review were sorted using the titles, the abstracts and the full texts. To complete the review, starting from the selected references, authors that had produced references on the subject of interest were identified and all their publications were studied. This procedure allowed us to select a corpus of about 76 references.

2.1.2 Indicators at infra-individual level

One approach to meet the social demand for biomonitoring methods is the development of indicators at infra-individual level. Biomarkers describe effects induced by various environmental stresses at any level of biological organisation, from the cell to the ecosystem. However, the term biomarker is more commonly used in a more restrictive sense, namely infra-individual changes resulting from individual exposure to xenobiotics (Lagadic et al. 1994). This is the definition we used here. This approach considers that the most appropriate method to detect the biological effects of contaminant exposure is to investigate the effects of contaminants on biological systems. Indeed, compared with methods focusing on physical and chemical properties of soils, biomarkers are assumed to focus on the effects of the bioavailable fraction of chemicals and to integrate the putative interactive effects of complex mixtures of chemicals in the ERA. Theoretically, a biomarker can be defined from any observable and/or measurable functional response to exposure to one or several contaminants that can be characterised at the sub-individual level of biological organisation (molecular, biochemical, cellular and physiological) (Weeks 1995). Importantly, the response is assumed to indicate a departure from healthy status that cannot be detected from an intact organism (Ricketts et al. 2004; van Gestel and van Brummelen 1996; Weeks 1995). The concept of biomarker is thus based on the causal relationship between the contamination of environments by any chemical inducing a stress (e.g. pesticides, polycyclic aromatic hydrocarbons, metals) and biological changes induced by the contaminated environment. Such an approach has of course been used to investigate the ecotoxicological effects of pesticides. Paradoxically, despite the massive use of pesticides, relatively little work was identified if we restrict it to pesticides currently authorised in Europe and their effects on European earthworm species. Main biomarkers at the sub-individual level that have been investigated so far for pesticides are DNA damage, lysosomal damage and changes in enzyme activities (Table 1).

2.1.3 Effect of pesticides at infra-individual level

The bibliographic review (Table 1) shows that: (1) pesticides can cause DNA damage in earthworms; two methods can be used to demonstrate DNA damage: the micronucleus test and Comet assay, the latter being much more sensitive than the former (Casabé et al. 2007; Klobučar et al. 2011), (2) pesticides disrupt the activity level of enzymes involved in oxidative stress such as superoxide dismutase, catalase and glutathione-*S*-transferase (Booth and O'Halloran 2001; Schreck et al. 2008, 2012; Wang et al. 2012), (3) pesticides, in particular organophosphate insecticides, affect the activity of carboxylesterases (Sanchez-Hernandez and Wheelock 2009) and the activity of cholinesterase (Booth and O'Hollaran 2001; Collange et al. 2010; Denoyelle et al. 2007; Gambi et al. 2007; Hackenberger et al. 2008; Jordaan et al. 2012; Olvera-Velona et al. 2008; Rault et al. 2007; Schreck et al. 2008; Venkateswara et al. 2003),(4) earthworm lysosomal membrane stability, measured using the neutral red retention test, can be altered by pesticides (Booth et al. 2001a, b; Casabé et al. 2007; Gambi and al. 2007; Klobučar et al. 2011; Svendsen et al. 2004) and (5) sub-cellular morphology and histological alterations may be observed following exposure to pesticides (Dittbrenner et al. 2011; Venkateswara et al. 2003).

Experimental protocols of ecotoxicological studies characterising the biological response of earthworms to pesticide exposure are generally similar. Naïve individuals (see below) are exposed under control laboratory conditions, typically in microcosms (Fründ et al. 2010), to one or several levels of contaminant concentrations, using either artificially contaminated substrates or field-sampled soils. Indeed, in most studies, authors compared phenotypes of conspecific individuals differentially exposed to one or several pesticides. The use of 'naïve' organisms means that they belong to model species and/or test individuals that have never been previously exposed to contaminant and are not descended from exposed individuals. In such cases, it seems reasonable to assume that phenotypic responses observed in contaminated conditions in contrast to control conditions may not be explained by genetic differences among individuals, but rather are environmentally induced responses (i.e.

Table 1 Effects of pesticides authorised in Europe on earthworms at infra-individual level

Reference	Species	Studied biomarkers	Origin	Development stage	Authorised active substance	Family	Action	Concentrations
Booth et al. (2001b)	*Aporrectodea caliginosa*	NRRT assay	Collected in reference site and exposed	Adult	Chlorpyrifos and diazinon	Organophosphate	Insecticide	In laboratory and semi-field (earthworms in mesocosms in a field sprayed with the pesticides)
Booth and O'Halloran (2001)	*A. caliginosa*	ChE activity, glutathione-*S*-transferase activity, NRRT assay growth and cocoon production	Collected in reference site and exposed	Juvenile and adult	Chlorpyrifos and diazinon	Organophosphate	Insecticide	Diazinon—12 mg kg^{-1} Chlorpyrifos—4 mg kg^{-1}
Casabé et al. (2007)	*Eisenia andrei*	Avoidance behaviour ISO N 281 (2004), reproduction (ISO 11268–2 (1998), NRRT assay and Comet assay	Cultured	Adult	Chlorpyrifos and glyphosate	Organophosphate Phosphonoglycine	Insecticide Herbicide	Nominal concentration recommended for soya crops
Denoyelle et al. (2007)	*Allolobophora chlorotica*	ChE activity	Collected in orchards	Adult	Field study		Pesticides	In situ
Dittbrenner et al. (2011)	*Eisenia fetida*, *A. caliginosa* and *Lumbricus terrestris*	Body mass and histopathologie	*E. fetida*, *L. terrestris* (bought) and *A. caliginosa* (collected)	Adult	Imidacloprid		Insecticide	0.2, 0.66, 2 and 4 mg kg^{-1} dry weight
Dittbrenner et al. (2012)	*E. fetida*, *A. caliginosa* and *L. terrestris*	HSP 70 protein level and avoidance behaviour	*E. fetida*, *L. terrestris* (bought) and *A. caliginosa* (collected)	Adult	Imidacloprid		Insecticide	0.2, 0.66, 2 and 4 mg kg^{-1} dry weight
Jordaan et al. (2012)	*E. andrei*	Cholinesterase activity, NRRT assay, growth, reproduction and behaviour	Cultured	Juvenile	Azinphos-methyl	Organophospahte	Insecticide	Exposure range at 10–40 mg kg^{-1}
Schreck et al. (2012)	In situ focus on the most abundant species: *A. caliginosa* nocturna	Enzyme activities (glutathione-*S*-transferase, catalase and cholinesterase) communities	Collected	Adult	Field studies in vineyards	NA		In situ
Wang et al. (2012)	*E. fetida*	Enzyme activities (cellulase, superoxide dismutase and catalase activities)	Cultured	Adult	Chlropyrifos Fenvalerate	Organophosphate	Insecticide	10, 20 and 40 mg kg^{-1} dry soil
Zang et al. (2000)	*E. fetida*	Micronucleus test	Bought	Adult	Imidacloprid RH-5849		Insecticide	0.0004, 0.020, 0.100 and 0.500 μg/cm^2 for imidacloprid
		Comet assay					Insecticide	160, 320 and 480 μg/cm^2 for RH-5849

Reference	Pesticide used	Substrate	Type of adding	Number of replicates	Earthworms per replicate	Exposure period	Main results
Booth et al. (2001b)	F	Natural soil	Sprayed				NRRT reduced following exposure to chlorpyrifos or diazinon even at field rates
Booth and O'Halloran (2001)	F	OECD artificial soil	Mixed	4–8	10	1, 2 and 4 weeks	ChE activity and NRRT were more sensitive than growth in each age group for detecting exposure to the pesticides. Growth and cocoon production more sensitive when earthworms were exposed as juveniles
Casabé et al. (2007)	F	Natural soil	Sprayed			7 and 28 days	Reproduction and avoidance tests sensitive indicator of glyphosate exposure. NRRT and Comet assays revealed alterations at sub-cellular levels

Table 1 (continued)

Reference	Pesticide used	Substrate	Type of adding	Number of replicates	Earthworms per replicate	Exposure period	Main results
Denoyelle et al. (2007)	F	Natural soil	Sprayed	17 sites			Although ChE activity significantly decreased in earthworms form treated orchards, results illustrate the difficulty in obtaining reference values for the use of ChE as a biomarker in field studies
Dittbrenner et al. (2011)	M	Reference test soil	Mixed	1	8	1, 7 and 14 days	Body mass changes in *E. fetida* and *A. caliginosa*. Histological changes after 24 h of exposure at the lowest concentration
Dittbrenner et al. (2012)	M	Reference test soil	Mixed	1 8 replicates for avoidance test	8 10	1, 7 and 14 days	HSP protein quantity is not a good biomarker of imidacloprid toxicity. Significant avoidance behavior but different between species.
Jordaan et al. (2012)	F	Artificial soil	Mixed	3	10	14 days	Inhibition of cholinesterase activity. NRRT lower in exposed earthworms. No avoidance of azinphos-methyl even at concentrations as high as 50 % the LC_{50}
Schreck et al. (2012)	F	Natural soil	Sprayed				Neurotoxicity enzyme (ChE) activity in vineyards was not affected by pollutants conventionally spread on the vineyard, regardless of soil agricultural practices
Wang et al. (2012)	M	Artificial soil	Mixed			1, 3, 5 and 7 days	Alteration and changes in enzyme activities
Zang et al. (2000)	M	Filter paper test		1	6	48 h	No genotoxicity detected with the micronucleus test DNA damage detected with Comet assay

For origin—cultured (in laboratory), collected (in the field) or bought (from a supplier). For development stage—juvenile or adult. For substrate—natural and artificial refer to natural soil and artificial soil, respectively (OECD for Organisation for Economic Co-operation and Development). For type of adding—mixed (into the soil) or sprayed (at soil surface)

For pesticide use— *F* formulation, *M* pure molecule, *NA* data are not available, *ChE* cholinesterase, *HSP* heat shock proteins, *NRRT* neutral red retention time, *ISO* International Organization for Standardization, LC_{50} lethal concentration for 50 % of exposed individuals, *RH-5849* 1-*tert*-butyl-1,2-diben-zoylhydrazin

the source of phenotypic variation is mainly environmental) (Pauwels et al. 2013). Moreover, those studies are mostly based on the analysis of stress responses over a short period of time, at most equal to an individual's lifetime. Consequently, biomarkers must be considered as early markers of exposure that do not reveal long-term effects of the contaminant on the ecosystem.

It is sometimes possible to identify typical response patterns shared by different species. For example, a decrease in the neutral red retention time by lysosomes or a decrease in cholinesterase activity is frequent following exposure to organophosphate insecticide. However, it is usually difficult to identify general patterns because data for each pesticide have been recorded in one or two species only and data for each species have been recorded for only a few active substances. For example, using the Comet assay, it has been shown that some insecticides (like chlorpyrifos) used at the commercially recommended rates cause DNA damage in *Eisenia* sp. (Casabé et al. 2007) but it is not known whether this is the case for all insecticides. It is therefore not clear whether all oligochaete annelids have the same sensitivity to insecticides (probably not) and/or if all insecticides cause similar DNA damage (probably not).

In soil ecotoxicology, model species are usually chosen from species that are easy to maintain and breed in laboratory conditions and for which molecular tools are available. They do not necessarily occur naturally on polluted soils. Considering soil ecotoxicology in oligochaete annelids, model species are mostly from the genus *Eisenia*. *E. fetida* and *Eisenia andrei*, in particular, have been used in most toxicological studies (Sanchez-Hernandez 2006), although species from the *Lumbricus* genus are increasingly studied (Morgan et al. 2007). In particular, *E. fetida* is the reference earthworm in international toxicity tests (Nahmani et al. 2007a, b). In recent years, ecotoxicological investigations have benefited greatly from the emergence of molecular biology techniques, which lead to a better understanding of the mechanisms of contaminant action at molecular level (see Brulle et al. 2010). Paradoxically, although these approaches have been widely used to better understand the effects of metals, there is almost no molecular study focusing on the effect of authorised pesticides on earthworms. An interesting study was published in 2008 by Svendsen et al. but the pesticide was atrazine which is now banned.

Biomarker responses can also be measured in field-sampled organisms (Aamodt et al. 2007; Booth et al. 2000a; Denoyelle et al. 2007). Several studies deal with field-collected earthworms: this was to validate cholinesterase (ChE) activity as a biomarker of pesticide exposure. Rault et al. (2007) characterised the tissue distribution (whole body, nervous tissue and crop/gizzard), activity of ChE over two seasons in six different species of earthworm collected in an unpolluted field: *Lumbricus terrestris*, *Lumbricus castaneus*, *Aporrectodea nocturna*, *Aporrectodea caliginosa*, *Allolobophora chlorotica* and *Aporrectodea rosea*. They demonstrated that ChE has a consistent activity in any given species and varies little between species of the same genus, suggesting that ChE would be a good biomarker of organophosphate insecticide. Therefore, when earthworms belong to natural populations that have been exposed to contaminants over a long period of time, their response might be different since they may have evolved to limit the harm caused by contaminants (Pauwels et al. 2013).

Thus, the measurement of infra-individual parameters has been primarily developed using model species and naïve earthworms in short-term laboratory experiments. A direct transfer of these results to natural populations that have been exposed to pesticides for generations can be envisaged, but only if caution is used.

2.2 Response at individual and population levels

2.2.1 Literature review

The literature review was carried out on the basis of keywords in ISI Web of Knowledge, using the 'All Databases' option, with the following formula: 'earthworm* and (pesticide* or herbicide* or fungicide* or molluscide* or nematicide* or insecticide*)' in Topics. We retrieved more than 1,700 publications. Those which appeared relevant for the review were sorted using the titles, the abstracts and the full texts. To complete the review, starting from the previously selected references, authors that had produced papers on the subject of interest were identified and their publications were studied. This allowed us to select a corpus of about 150 relevant references.

2.2.2 Indicators and effects at individual and population levels

Life history traits In the studies made before the 1980s, generally only mortality was assessed, using LC_{50}, i.e. lethal concentration for 50 % of exposed individuals. However, as pointed out by Neuhauser et al. (1985), 'reproduction may be inhibited or halted at chemical concentrations far below a given LC_{50}'. In aquatic ecotoxicology, it has been proven that the LC_{50} and the no observed effect concentration (NOEC) for reproduction and growth are generally similar, while in terrestrial ecotoxicology, the NOEC is often much lower than the LC_{50} (van Gestel et al. 1992). Vermeulen et al. (2001) explain that '[…] Mortality as a measure of a population's sensitivity to a chemical is regarded as neither a sensitive nor a relevant ecological parameter'. Even if molecules do not significantly affect earthworm survival, they may affect other life history traits and behaviour, resulting in the reduction of populations and/or of earthworm activity,

which may influence soil functioning (Lal et al. 2001; Luo et al. 1999; Slimak 1997). The explanation is that stress caused by the presence of a contaminant may divert energy from growth, reproduction and/or burrowing activity. Instead, energy is used to ensure the survival of the organism (Gibbs et al. 1996; Odum 1982). Many authors therefore stress the importance of studying effects of pesticides on reproduction or growth in addition to survival (Choo and Baker 1998; Yasmin and D'Souza 2010). Addison and Holmes (1995), Kokta (1992a) and Neuhauser and Callahan (1990) have suggested that cocoon production (Fig. 3) is a more sensitive indicator of pesticide-induced stress than growth in earthworms.

Using available databases that provide information on almost 400 pesticides (ANSES Agritox 2012; PPDB 2013), we found that less than 5 % of pesticides have a LC_{50} below or equal to 10 mg kg^{-1}, which is considered as moderately to highly toxic for the species *E. fetida* (PPDB 2013), i.e. one acaricide, two fungicides, four herbicides and nine insecticides. We found information on reproduction for only 97 pesticides. For more than 50 % of them, we found a NOEC <10 mg kg^{-1}, i.e. 12 insecticides, 23 fungicides, 12 herbicides, 3 nematicides and 2 molluscicides. According to these databases, insecticides and fungicides appear to be the most toxic chemicals affecting survival and reproduction respectively. Herbicides are well represented in toxic chemicals despite what some authors have said (Lee 1985). The pyrimidine insecticides seem nontoxic to earthworms and triazine herbicides appear to have a moderate effect on earthworm populations (Edwards and Bohlen 1996). The most harmful pesticide families to earthworms seem to be nicotinoides, strobilurins, sulfonylureas, triazols, carbamates and organophosphates.

Despite these data, information is lacking on the pesticide effects on earthworm reproduction and growth. Studies found in the literature focus mainly on the following substances: cypermethrin, glyphosate, mancozeb, chlorpyrifos, carbendazim and dimethoate, i.e. three insecticides, two fungicides and one herbicide (Table 2). For clarity, only publications that addressed at least reproduction and/or growth parameters, i.e. not only mortality, are listed in Table 2. Moreover, studies can be performed using different substrates, e.g. soil, water and filter paper, which may change the response of earthworms to pesticide. Only tests that were done in soil are shown in Table 2.

For a given duration of exposure, when pesticides were used at agronomic rates, only few authors found significant effects on earthworm survival (Correia and Moreira 2010; Roark and Dale 1979). In general, pesticides used at these rates did not show any effect at the individual level (Addison 1996; Bauer and Römbke 1997; Capowiez et al. 2005; Choo and Baker 1998; Vermeulen et al. 2001), or they only affected earthworm growth and reproduction (Choo and Baker 1998; Correia and Moreira 2010). For instance, the use of chlorpyrifos at agronomic rates may cause a delay in juvenile growth and a decrease in cocoon production of *A. caliginosa* (Alshawish et al. 2004; Booth and O'Halloran 2001; Booth et al. 2000b). Glyphosate may affect cocoon hatchability and therefore the number of juveniles as well as growth, thus modifying the time to maturation (Correia and Moreira 2010; Springett and Gray 1992; Yasmin and D'Souza 2007). However, the effects of a given compound may differ between studies and/or species. For instance, Casabé et al. (2007) did not find any effect of chlorpyrifos used at agronomic rates on the reproduction of *E. andrei*. Similarly, Burrows and Edwards (2004) showed that carbendazim used at agronomic rates had no effect on

Fig. 3 Cocoons of earthworms

Table 2 Effects of pesticides authorised in Europe on earthworm life history traits

Reference	Species	Studied parameters	Origin	Development stage	Authorised active substance	Family	Action	Number of concentrations	Pesticide used	Substrate
Addison (1996)	*Dendrobaena octaedra*	Survival, growth and reproduction	NA	Adult	Tebufenozide	Diacylhydrazine	Insecticide	3 (AD, 10 AD and 100 AD)	M	Leaf litter
Alshawish et al. (2004)	*A. caliginosa*	Body mass and cocoons			Chlorpyrifos	Organophosphate	Insecticide	LC_{50} and one twentieth of LC_{50} value		
					Cypermethrin	Pyrethroid	Insecticide			
					Mancozeb	Dithiocarbamate	Fungicide			
Bauer and Römbke (1997)	*Eisenia fetida*, *Aporrectodea longa* and *Lumbricus terrestris*	Mortality, biomass and reproduction	Cultured or bought	Adult	Amitrol/diuron	Triazole/phenylurea	Herbicide	1 (5× maximum AD)	F	Natural and artificial
Booth and O'Halloran (2001)	*A. caliginosa*	Growth, maturation of juveniles, cocoon production and cocoon viability	Cultured	Juvenile and adult	Chlorpyrifos	Organophosphate	Insecticide	2 (AD and 4 AD)	F	Natural
Booth et al. (2000b)	*A. caliginosa*	Biomass, cocoon production, hatching success	NA	Juvenile	Chlorpyrifos	Organophosphate	Insecticide	2 (AD and 4 AD)	F	Natural
Burrows and Edwards (2004)	*Allolobophora rosea*	Mortality and biomass	NA	Adult	Carbendazim	Benzimidazole	Fungicide	5 (AD, 3 AD, 9 AD, 27 AD and 81 AD)	F	Natural
Capowiez et al. (2005)	*Aporrectodea icterica* and *Aporrectodea caliginosa nocturna*	Mortality and weight loss	Collected	Sub-adult/adult	Imidacloprid	Neonicotinoid	Insecticide	6 (near AD+other 5)	M	Natural
Casabé et al. (2007)	*Eisenia andrei*	Reproduction (and avoidance behaviour)	Cultured	Adult	Chlorpyrifos	Organophosphate	Insecticide	1 (AD)	F	Artificial
					Glyphosate	Phosphonoglycine	Herbicide			
Choo and Baker (1998)	*Aporrectodea caliginosa trapezoides*	Survival, growth and reproduction	Collected	Juvenile and adult	Methiocarb	Carbamate	Molluscicide/ insecticide	2 (AD and 10 AD)	F	Natural
					Phenamiphos	Organophosphate	Nematicide			
Cluzeau et al. (1990)	*L. terrestris*	Survival, weight gain and reproduction	Collected	Juvenile and adult	Carbendazim	Benzimidazole	Fungicide	1 (120 % of AD)	F	Natural
					Mancozeb	Dithiocarbamate	Fungicide			
					Prochloraz	Imidazole	Fungicide			
					Propiconazol	Triazole	Fungicide			
Correia and Moreira (2010)	*E. fetida*	Growth, survival and reproduction	Collected	Adult	Glyphosate 2,4-D	Phosphonoglycine	Herbicide	5 (AD+other 4)	M	Natural
						Alkylchlorophenoxy	Herbicide			
Dalby et al. (1995)	*A. caliginosa*, *A. longa*, *A. rosea* and *A. caliginosa trapezoides*	Growth and survival	Collected	Adult	Dimethoate	Organophosphate	Insecticide/ acaricide	1 (AD)	NA	Natural
					glyphosate 2,4-D+mix	Phosphonoglycine	Herbicide			
						Aryloxyalkanoic acid	Herbicide			
De Silva et al. (2009)	*E. andrei*		Cultured	Adult	Carbendazim	Benzimidazole	Fungicide	7	M	Natural and artificial
					Chlorpyrifos	Organophosphate	Insecticide		F	

Table 2 (continued)

Reference	Species	Studied parameters	Origin	Development stage	Authorised active substance	Family	Action	Number of concentrations	Pesticide used	Substrate
		Survival, growth and reproduction								
Kreutzweiser et al. (2008)	*E. fetida* and *D. octaedra*	Survival, weight and cocoon production (and leaf consumption)	Collected and bought	Adult	Imidacloprid	Neonicotinoid	Insecticide	5 (>AD)	F	Litter
Kula and Larink (1997)	*E. andrei*, *A. caliginosa*, *Allolobophora chlorotica*, *E. fetida* and *A. rosea*	Mortality, growth and reproduction	Cultured and collected	Juvenile and adult	Dimethoate	Organophosphate	Insecticide	4	M	Natural and artificial
Liang and Zhou (2003)	*E. fetida*	Mortality and weight	NA	Juvenile	Acetochlore	Chloroacetamide	Herbicide	6 (>AD)	M	Natural
Luo et al. (1999)	*E. fetida*	Mortality and reproduction	Cultured	Adult	Imidacloprid	Neonicotinoid	Insecticide	8 (>AD)	M	Artificial
Ma and Bodt (1993)	*A. caliginosa*, *A. longa*, *E. fetida*, *Eisenia veneta*, *L. terrestris* and *Lumbricus rubellus*	Mortality and reproduction	Collected and bought	Adult	Chlorpyrifos	Organophosphate	Insecticide	LC_{50}, NOEC and EC_{50} (reproduction)	M	Natural and artificial
Martikainen (1996)	*Aporrectodea caliginosa tuberculata*	Survival and biomass	Collected	NA	Dimethoate	Organophosphate	Insecticide	5	M	Natural and artificial
Martin (1982)	*A. caliginosa*	Mortality and growth of juvenile	Cultured	Juvenile	Amitrole	Triazole	Herbicide	3	F	Natural
					Asulam	Carbamate	Herbicide			
					Glyphosate	Phosphonoglycine	Herbicide			
					Linuron	Urea	Herbicide			
					MCPB	Aryloxyalkanoic acid	Herbicide			
					Metribuzin	Triazinone	Herbicide			
					Oxadiazon	Oxidiazole	Herbicide			
					2,4-D	Alkylchlorophenoxy	Herbicide			
Mosleh et al. (2003a)	*L. terrestris*	Mortality and growth	Collected	Adult	Isoproturon	Urea	Herbicide	5 (>AD)	F	Natural
Mosleh et al. (2003b)	*A. caliginosa*	Growth rate	Collected	Adult	Cypermethrin	Pyrethroid	Insecticide	LC_{25} and LC_{50}	F	Artificial
					Metalaxyl Mn–Zn	Phenylamide	Fungicide			
Roark and Dale (1979)	*E. fetida*	Mortality, life duration and reproduction	Bought	Adult	Chlorothanil	Chloronitrile	Fungicide	1 (AD)	F	Artificial
					Mancozeb	Dithiocarbamate	Fungicide			
					Thiabenzadole	Benzimidazole	Fungicide			
					Thiophanatemethyl	Benzimidazole	Fungicide			
					Thiram	Dithiocarbamate	Fungicide			
Springett and Gray (1992)	*A. caliginosa*	Growth, survival and reproduction	Cultured	Adult	Carbendazim	Benzimidazole	Fungicide	4	F	Artificial
					Phenmedipham	Bis-carbamate	Herbicide			
	E. fetida		Cultured	Adult	Mancozeb	Dithiocarbamate	Fungicide	2 (AD+other 1)	F	Artificial

Table 2 (continued)

Reference	Species	Studied parameters	Origin	Development stage	Authorised active substance	Family	Action	Number of concentrations	Pesticide used	Substrate
Vermeulen et al. (2001)		Growth, survival and reproduction								
Viswanathan (1997)	*E. andrei* and *L. terrestris*	Biomass, cocoon production, excretion and respiration	Cultured and bought	Juvenile	Terbuthylazine	Triazine/ microbiocide	Herbicide/ algicide	3	F	Artificial
Xiao et al. (2006)	*E. fetida*	Growth and reproduction	Cultured	Adult	Acetochlore	Chloroacetamide	Herbicide	4 (AD+other 3)	M	Artificial
Yasmin and D'Souza (2007)	*E. fetida*	Growth and reproduction	Cultured	Adult	Carbendazim Dimethoate Glyphosate+mix	Benzimidazole Organophosphate Phosphonoglycine	Fungicide Insecticide Herbicide	2 (AD and 4× maximum AD)	F	Natural
Zhou et al. (2006)	*E. fetida*	Mortality and weight	NA	Adult	Acetochlore	Chloroacetamide	Herbicide	6 (>AD)	M	Natural
Zhou et al. (2007)	*E. andrei*	Mortality, growth and fecundity (and avoidance response)	Bought	Adult for acute tests and juvenile and adult for growth tests	Chlorpyrifos	Organophosphorus	Insecticide	4	M	Artificial
Zhou et al. (2008)	*E. andrei*	Mortality, growth and fecundity (and avoidance response)	Bought	Juvenile and adult	Cypermethrin	Pyrethroid	Insecticide	5	M	Artificial
Zhou et al. (2011)	*E. andrei*	Mortality, growth and fecundity (and avoidance response)	Bought	Adult	Chlorpyrifos Cypermethrin+ mix	Organophosphorus Pyrethroid	Insecticide Insecticide	5	M	Artificial

Reference	Method of addition	Organic matter	Temperature	Moisture	Light/ photoperiod	Number of replicates	Earthworms per replicate	Duration	Results
Addison (1996) Alshawish et al. (2004)	Mixed	Leaf litter	Constant	NA	NA	20	2	12 weeks	No effect even at highest concentration Higher body mass increment for control. Toxicity chlorpyrifos (100 % hatching failure)>mancozeb (>73 % hatching failure)>cypermethrin (80 % survival and normal hatching)
Bauer and Römbke (1997)	Sprayed and mixed	Cow manure and dried leaves	10 or 20 °C	51 or 82 % of WHC	NA	4	10 *E. fetida*, 3 *L. terrestris* or 5 *A. longa*	14 and 28 days	No evidence of any effects
Booth and O'Halloran (2001)	Mixed	Dry grass	20 °C	20 to 25 %	Dark	4	10	2, 4, 6, 8, 10 and 12 weeks	At AD, no marked effect on adult growth but delay of juvenile growth; cocoon production divided by 1.8 (in 8 weeks) to 2.4

Table 2 (continued)

Reference	Method of addition	Organic matter	Temperature	Moisture	Light/ photoperiod	Number of replicates	Earthworms per replicate	Duration	Results
									(in 12 weeks). Recovery of cocoon production and viability after 4 or 8 weeks
Booth et al. (2000b)	Mixed	Dry grass	20 °C	25 %	Dark	4	10	4, 8 and 12 weeks (for recovery)	Growth reduced during exposure (not in the recovery phase). Maturation rates and fecundity affected at the highest concentration. At AD, no marked effect on juvenile maturation or reproduction but 'effects of longer term pesticide exposure on fecundity should be determined'
Burrows and Edwards (2004)	Sprayed	Wheat straw	27 °C	Artificial rainwater	12/12-h photoperiod	5	3	7, 14, 28,and 56 days	No effect at AD. Effect on biomass and mortality at high application rate
Capowiez et al. (2005)	Mixed	NA	12 °C	NA	Dark	2 (mortality) or 3 (growth)	10	7 and 14 days	No significant effect at 0.1 mg/kg but decreases in weight at 0.5 and 1 mg/kg (near PEC)
Casabé et al. (2007)	Sprayed	Cereal mixture	NA	50–60 % WHC	NA	3	6	28 and 56 days	100 % survival and no difference in cocoon production between control and treated soil. Deleterious effect on cocoon viability (number of hatched cocoons significantly reduced with glyphosate (−59 % hatchability)=≥86 % juveniles at 56 days. No significant effect of chlorpyrifos on reproduction
Choo and Baker (1998)	Sprayed and mixed	Fresh sheep dung	17 °C	25 %	Dark	10	2, 3 or 4	1, 2, 3, 4, 5 weeks and 38 days	No significant effect of methiocarb at AD but reduction of weight and cocoon production at 10 AD. Phenamiphos; 100 % mortality in 24 h on filter paper but few mortality in soil. 2.5 times less cocoons produced at AD
Cluzeau et al. (1990)	Sprayed or mixed	Hay	15 °C	80 %	NA	5	4	16 to 20 weeks and observations every 4 weeks	No more mortality than in control except for prochloraze and mancozeb (25 and 40 % mortality in 8 weeks, respectively). No effect on weight gain. Secondary sexual characteristics: slower evolution with prochloraze and propiconazole; slight regression with mancozeb and carbendazime. −15 and −13 % of cocoon production and −13 and −20 % cocoon viability with mancozeb and carbendazime, respectively=> toxicity mancozeb>carbendazim> propiconazol and prochloraz (no effects)
Correia and Moreira (2010)	Mixed	Manure bovine	20 °C	60 % WHC	Light	4	10	14, 21, 28, 42 and 56 days	Glyphosate, no mortality but −50 % weight for all concentrations in 56 days. 2,4-D, 100 % mortality with highest concentrations. At 14 days, 30–40 % mortality levels in all other concentrations. No cocoons or juveniles found in soil treated with herbicides=> severe effects on the development and reproduction
Dalby et al. (1995)	Sprayed	10 days without	15 °C	25–30 %	NA	6	1, 2 or 4	10 days or 3 weeks	No effect on survival nor growth, with or without plant cover

Table 2 (continued)

Reference	Method of addition	Organic matter	Temperature	Moisture	Light/ photoperiod	Number of replicates	Earthworms per replicate	Duration	Results
		plants and 3 weeks with plants							
De Silva et al. (2009)	Sprayed and mixed	Ground horse manure or cow manure	20 or 26 °C	50 % of WHC	Dark	4	10	28 (growth and survival) or 56 days (reproduction)	No effect on survival at 28 days. Reduction of juvenile number near AD for both substances. For chlorpyrifos, survival is more sensitive at the higher temperature (probably due to increased activity). Reproduction and growth varied inconsistently with temperature and soil types. Toxicity carbendazim>chlorpyrifos
Kreutzweiser et al. (2008)	Sprayed and mixed	Litter and leaf material	20 °C	50–70 % relative humidity	12/12-h light/dark	4	2	7, 14, 28 and 35 days	*D. octaedra*, more sensitive than *E. fetida*. Significant weight losses among survivors of *D. octaedra* at 3 mg/kg. No effects on cocoon production among survivors at 3 mg/kg. *E. fetida*, significant weight losses at 14 mg/kg=>Imidacloprid toxic for *D. octaedra* when concentration>3 mg/kg
Kula and Larink (1997)	Sprayed and mixed	Ground cattle manure	NA	50 % WHC	NA	1 (mortality) or 4 (reproduction and growth)	10 *E. fetida* or 4 *A. caliginosa*	1, 4, 8, 14 and 28 days	Juveniles and cocoon production, most sensitive parameters (>weight). Sensitivity of species, *A. caliginosa* and *A. rosea*>*A. chlorotica*>*E. fetida*. *E. fetida*, +3–4 % mortality in 8 days; no marked weight difference; −50 to 90 % cocoon production and more infertile cocoons; 10 times less juveniles. Same results for 'surface contamination' and 'total contamination'
Liang and Zhou (2003)	NA	NA	20 °C	NA	400–800 lux	3	16	6 and 14 days	Weight loss and mortality, mainly at 14 days
Luo et al. (1999)	Mixed	NA	20 °C	NA	NA	6	1	24/48 h; 3, 7 and 14 days	Sperm deformity and dose–effect relationship at concentration of >0.5 mg/kg=>can induce reduction of reproduction capacities
Ma and Bodt (1993)	Mixed	Sphagnum peat or leaves of alder	15 °C	55 % dry mass basis	NA	4	5 *L. terrestris* or 10 for others	14 days	Sensitivity *Eisenia*<*Aporrectodea*<*Lumbricus*. Reproduction more sensitive than survival. Substrate influence species sensitivity
Martikainen (1996)	Sprayed	Horse manure	13 °C	55 % WHC	Light	3	5	14 days	Biomass reduction even at sub-lethal concentration=> moderately toxic but may cause some adverse effects on the earthworms already at NOEC concentrations. Differences between substrate types
Martin (1982)	Mixed	Grass meal	20 °C	25 %	Dark	5	1	7 days	Weight loss with 100 p.p.m. oxadiazon, 2,4-D, MCPB, metribuzin and linuron. Slower growth with 100 p.p.m. asulam. No effect of glyphosate
Mosleh et al. (2003a)	Mixed	NA	14±1 °C	70–90 % relative humidity	12/12-h photoperiod	4	10	7, 15, 30, 45 and 60 days	No effect on mortality even at the highest concentration tested. Reduction of growth at all concentrations (between −3 and −27.9 %)

Table 2 (continued)

Reference	Method of addition	Organic matter	Temperature	Moisture	Light/ photoperiod	Number of replicates	Earthworms per replicate	Duration	Results
Mosleh et al. (2003b)	Mixed	NA	23 °C	70–90 %	NA	4 (mortality) or 8 (growth)	10	7, 14, 21 and 28 days	Toxicity cypermethrin>metalaxyl. Reduction in growth rate with LC_{25}
Roark and Dale (1979)	Mixed	Corn and oats	24 °C	8.5 %	NA	4	10	84 days	High mortality after immersion in 0.1 % thiophanate methyl and 2 % thiram solutions. Decrease in longevity in treated soils
Springett and Gray (1992)	Mixed	Cow dung	20 °C	35 % (*w*/*w*)	NA	4	10	3 weeks	At low concentration, no effect of phenmedipham growth, percent fertile cocoons and number of juveniles/fertile cocoon but −35 % cocoons $worm^{-1}$ $week^{-1}$ and −43 % juveniles $worm^{-1}$ $week^{-1}$. At low concentration, no effect of carbendazim growth and reproduction
Vermeulen et al. (2001)	Mixed	Urine-free cattle manure	NA	75 %	NA	8 (acute) or 5 (sub-lethal)	10	14 days for acute and 10 weeks for sub-lethal	No significant detrimental effect on either growth reproduction whatever the dose
Viswanathan (1997)	Mixed	Alfalfa	NA			NA	NA	433 days	No reduction of juvenile number for *L. terrestris* at high doses. For *E. andrei*, reduction of cocoon production and biomass, increase of cocoon sterility with increasing duration and pesticide concentration
Xiao et al. (2006)	Mixed	Urine-free cow	20 °C	50 %	12/12-h photoperiod and 400–800 lux	5	10	7, 15, 30, 45 and 60 (growth) or 28 days (reproduction)	At AD, no significant effect on growth except after and 30 days. For concentration at >20 mg kg^{-1}, decrease of growth rates and numbers of juveniles per cocoon
Yasmin and D'Souza (2007)	Sprayed (at surface or in food)	Cow dung	NA			3	10	28 days (survival)+ 8 weeks (cocoon production)	At AD, loss of weight (−50 %) with the 3 pesticides and mix; reduction of earthworm number after 8 weeks due to reduction of produced cocoons (−14 % pour carbendazim and dimethoate, −8 % for glyphosate and −22 % for mix. Marked negative impact on growth and reproduction, even at AD. Toxicity carbendazim and dimethoate>glyphosate
Zhou et al. (2006)	Sprayed	NA	20 °C	NA	400–800 lux	3	16	6, 10, 12 and 18 days	Mortality and weight loss increase with exposure time and concentrations. Weight, more sensitive index compared with the mortality
Zhou et al. (2007)	Mixed	Cattle dung	20 °C	50 %	NA	4	10 or 20	14 and 21 days for mortality or 4 and 8 weeks for growth and reproduction	5 mg/kg, after 8 weeks, between −25 and −30 % less cocoons and −22 % juvenile viability
Zhou et al. (2008)	Mixed	Cattle dung	20 °C	50 %	NA	4	10 or 20	14 and 21 days for acute. 4 and 8 weeks for growth and reproduction	At 10 mg/kg, cypermethrin had obvious adverse impact on the juvenile growth, while at 20 mg/kg, cypermethrin caused significant toxic effects in reproduction. Juveniles more sensitive than adults

Table 2 (continued)

Reference	Method of addition	Organic matter	Temperature	Moisture	Light/ photoperiod	Number of replicates	Earthworms per replicate	Duration	Results
Zhou et al. (2011)	Mixed	Cattle dung	20 °C	50 %	NA	4	10 or 20	4 and 8 weeks	Toxicity mixture>pesticides individually, especially on chronic responses. At 5 mg/kg, mixture, significant reductions on growth and reproduction but no effect individually

For origin—cultured (in laboratory), collected (in the field) or bought (in a supplier). For substrate, natural and artificial refer to natural soil and artificial soil. For type of adding, mixed (into the soil) and sprayed (at soil surface).

2,4-D 2,4-dichlorophenoxyacetic acid, *MCPB* 4-(2-methyl-4-chlorophenoxy) butyric acid, *AD* agronomical dose; LC_{50} and LC_{25} for lethal concentration for 50 and 25 % of exposed individuals, respectively *NOEC* no observed effect concentration, EC_{50} half maximal effective concentration (i.e., concentration inducing a response halfway between the baseline and maximum after a specified exposure time), *F* formulation, *M* pure molecule, *NA* data are not available, *WHC* water holding capacity, *w/w* weight/weight, *PEC* Predicted Environmental Concentration

Lumbricus rubellus individuals while Yasmin and D'Souza (2007) recorded a decrease in the growth and reproduction of *E. fetida*. These two authors used similar concentrations of carbendazim but different commercial formulations.

According to Table 2, as soon as agronomic rates are exceeded there may be effects on mortality and almost always marked effects on reproduction and growth. If the purpose of a study is to detect an effect on earthworms, it seems that mortality is in fact the least appropriate indicator to study, followed by growth and then by reproduction (Booth and O'Halloran 2001; Kula and Larink 1997; Ma and Bodt 1993; van Gestel et al. 1992; Zhou et al. 2006).

Behaviour Markers based on behavioural patterns are generally considered to be among the most sensitive ones (Doving 1991). The advantages of behavioural markers are (1) the wide range of functions concerned, e.g. locomotion, reproduction, feeding and biological interactions, that may be linked to the individual's fitness, (2) their low specificity, i.e. they react to a wide range of pollutants and (3) their ecological relevance, i.e. effects can be related to consequences at higher biological levels. The behavioural repertoire of earthworms is rather limited compared with that of mammals, birds or insects, yet it is broad and relevant enough to address some important soil functions that are affected by their activity. Indeed, since earthworms are considered as soil ecosystem engineers, modifications of their behaviour might have important consequences for soil functioning. Four main functions were identified in the literature regarding effects of pesticide on earthworms: avoidance behaviour, burrowing behaviour, bioturbation and burial of organic matter (Table 3). The avoidance behaviour is thought to be caused by a modification of the 'habitat function' of the soil (i.e. its chemical quality). This is the basis of the normalised avoidance test (ISO 17512–1 2008). This simple test was designed to reveal significant repellence of a polluted compartment compared with a control compartment. This implies that earthworms are able to detect toxic compounds and decide to escape from them. This is the most used behavioural test for earthworms since it is very simple and cost-effective. It has been successfully used for different pesticides, mainly insecticides (Table 3) but in some cases a significant attraction of earthworms for polluted soils was observed (Mangala et al. 2009). Moreover, the avoidance test is less sensitive than other markers when used with neurotoxic pesticides (Perreira et al. 2010). One of the arguments against this test is that it is a repellence test rather than a toxicity test (Capowiez et al. 2003).

An obvious consequence of earthworm activity in the soil is, except for epigeics, the creation of burrows, which influences soil transfer properties. Burrowing is thus an

Table 3 Behavioural tests, ecological functions targeted and their use to detect effects of pesticides authorised in Europe on earthworms

Test	Laboratory or field use	Ecological function targeted	Main criterion	Pesticides (with positive responses)	Earthworm species	Reference
Avoidance	Lab.	'Habitat function; (escape)	% earthworms in the control	Methomyl	Ea	Perreira et al. (2010)
				Mancozeb	*E. fetida*	Reinecke et al. (2002)
				Carbendazim		Casabé et al. (2007)
				Glyphosate		Loureiro et al. (2005)
				Lambda-cyhalothrin		Garcia et al. (2008)
				Cypermethrin		de Sousa and de Andréa (2011)
Avoidance (vertical or horizontal)	Lab.		Length of burrows in the control	Carbendazim	*Allolobophora chlorotica*	Ellis et al. (2010)
Surface Movement	Field		Abundance		All	Christensen and Mather (2004)
Time for burial	Lab.	Burrowing	Time			
2D terraria	Lab.		Burrow length	Imidacloprid	*Aporrectodea caliginosa nocturna*	Capowiez et al. (2003)
					Aporrectodea icterica	Capowiez and Bérard (2006)
					Lumbricus terrestris	Dittbrenner et al. (2011)
3D soil core	Lab (field)		Burrow volume	Imidacloprid	*A. caliginosa nocturna*	Capowiez et al. (2006)
					A. icterica	Dittbrenner et al. (2011)
					L. terrestris	
Cast Production	Lab. and Field	Bioturbation	Cast production (g)	Imidacloprid	*L. terrestris*	Dittbrenner et al. (2010)
				Chlorpyrifos	*Aporrectodea caliginosa*	Capowiez et al. (2010)
				Methomyl		Lal et al. (2001)
Funnel Test	Lab.	Organic matter burial or decomposition	Number of straws removed		*L. terrestris*	Bieri ((1992))
Feeding activity	Lab. (field)		Leaves or manure eaten (g)	Imidacloprid	*E. fetida*	Kreutzweiser et al. (2008)
				Thiacloprid	*L. terrestris*	Tu et al. (2011)
				Thiophanate-methyl	(All)	Gomez-Eyles et al. (2009)
Litter bags	Field		Litter eaten (g)		All	Förster et al. (1996)
Bait Lamina	Field		Number of holes	Glyphosate	All	Reinecke et al. (2008)
				Chlorpyrifos		Casabé et al. (2007)
						van Gestel et al. (2003)

When no pesticide is indicated, this means that no result was found with pesticides authorised in Europe at a normal application rate

interesting measurement for ecotoxicological tests. The simplest observation that was used is the time earthworms take to burrow which is always linked to the classical experimental protocol. This is however an all or nothing kind of response. Direct observations of earthworm burrowing behaviour are difficult but studying the outcomes of this activity is possible using for instance the 2D terrarium (Evans 1947). This has been rarely used with pesticides. Capowiez et al. (2003) demonstrated that normal application rates of imidacloprid cause significant effects on the characteristics of the burrow systems, i.e. length, depth and branching rate, made by *A. icterica* and *A. nocturna*. However the links with soil function remained theoretical since measurements of transfer, i.e. water, gas or solutes, are not possible in 2D. To overcome this limitation, Capowiez et al. (2006) did the same experiment in soil cores in which the burrow systems were analysed using X-ray tomography (Pierret et al. 2002) after 1 month of incubation (Fig. 4). Significant decreases in burrow length and depth were shown to be correlated with lower gas diffusion in soil, at least for *A. icterica*. Obviously, observations in 3D are too tedious and need technical skills and thus cannot be generalised.

Another physical consequence of earthworm activities in soil is bioturbation, i.e. the disrupting and mixing of soil by animals living in, feeding from or simply passing through it (Meysman et al. 2006). Earthworms feed on soil and burrow in the soil by ingesting soil particles. After gut transfer, the soil is egested as casts, which play an important ecological role in the soil (Lee and Foster 1991). Cast production can be used as a proxy for earthworm activity thanks to its simplicity (Capowiez et al. 2010). Cast production is estimated by sieving soil in which earthworms were incubated. So far, only three insecticides (Table 3) were shown to induce significant decreases in cast production for anecic and endogeic earthworms. Moreover, it was validated by some field observations in the case of imidacloprid toxicity (Lal et al. 2001). However, under field conditions, it is difficult to attribute decreases in cast production to a modification of individual behaviour or to effects at the population level, i.e. lethality.

The last soil function associated with earthworm behaviour that provides meaningful measurements in ecotoxicology is related to burial of litter, mainly due to anecic earthworms. Unlike in aquatic ecology, these tests are still astonishingly rarely developed in soils. One of the oldest tests is known as the funnel test (Bieri 1992). It was developed for *L. terrestris*, which has a well-known surface feeding behaviour. After earthworms were incubated in funnels filled with moist soil, pesticide and straws are deposited on the soil surface and the number and location of straws at the soil surface are checked daily.

Overall, measurements based on earthworm behaviour are still poorly used, with the notable exception of the avoidance test, which is the most controversial one and the least related to a soil function. There is a need for new tools that can (1) be used routinely under laboratory conditions and (2) provide an indication of important soil functions e.g. soil water transfer or organic matter decomposition, possibly under field conditions.

To summarise, studies on the effect of pesticides at the individual level generally concern earthworm life history traits and behaviour and are conducted under laboratory-controlled conditions. The existing studies cannot be used to reliably rank compounds for their toxicity because the ranking varies from one study to another. Progress could be made with tests based on earthworm behaviour.

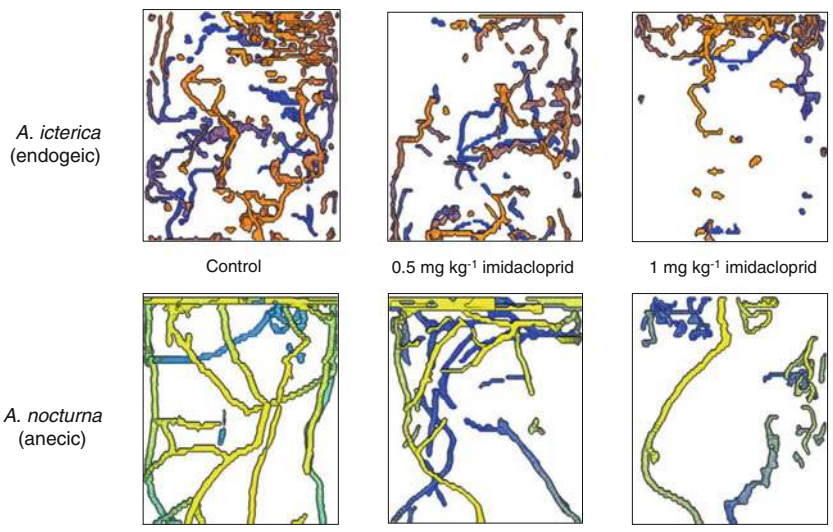

Fig. 4 Effect of different concentrations of imidacloprid on the digging behaviour of two earthworm species (adapted from Capowiez et al. 2006)

2.3 Response at community level

2.3.1 Literature review, data extraction and analysis

In order to assess the responses of earthworms to pesticides at the community level, all the combinations of the terms: earthworm*, density, biomass and community AND pesticide*, fungicide*, herbicide*, insecticide* and molluscicide* were used in the Web of Science database. To assess the effects of pesticide management on earthworms at the community level, the following combination of terms were used: earthworm*, density, biomass and community AND organic, conventional, reduced, integrated AND cropping and farming. Only studies made at field scale (Fig. 5) in European Union, and with currently authorised compounds were retained. Unpublished studies from government libraries or technical institutes were not retained.

A meta-analysis was employed to compare case studies (Hedges et al. 1999). Meta-analytical techniques allow one to determine whether individual studies share a common 'effect size' (see next paragraph), or, in other words, whether there is a single overall effect size that describes the magnitude of the experimental effects (e.g. alternative vs. conventional farming). This technique is well adapted to our objective since many confounding factors can blur the site-specific response of earthworms to pesticides. So, in addition to recording community densities and biomass in plots, site characteristics (i.e. site latitude, soil type and soil occupancy) and sampling details (i.e. sampling year, season, method and volume) were considered and included in the database. We aimed at exploring the influence of site characteristics (latitude and soil type), the sampling procedure (season and method), the type of farming practices (organic, reduced or integrated) and the type of crop. Crops were divided into five groups according to the level of available information: cereal, non-cereal, grassland, ley or unknown.

We used a response ratio defined as ln (treatment mean/control mean) where conventional and alternative pesticide use are regarded as the control and treatment, respectively (Hedges et al. 1999). This metric, termed the 'effect size', has become commonly used in meta-analysis (Mosquera et al. 2000). It is designed to measure relative differences, often appropriate in ecological studies.

Many indices are used by community ecologists to describe the three dimensions of biodiversity, i.e. structure, composition and function. However, in the context of ecotoxicology, such indices are rarely computed (Decaëns et al. 2008; Hedde et al. 2012; Pelosi et al. 2009a) and community parameters are mainly restricted to density and biomass in most studies on pesticide effects. This approach may have prevented the exploration of the whole earthworm community response.

Two questions raised in this section: (1) is it possible to distinguish a general response of earthworm communities to a restricted set of pesticides? And (2) do conventional and alternative, i.e. no or low pesticide use, cropping systems have different earthworm communities?

2.3.2 Effect of pesticides at community level

Regarding the first of these questions, it is not yet possible to identify a general response of an earthworm community to a set of pesticides. The effects of currently EU-authorised compounds per se on a community are rarely addressed. Amongst the few studies, we may cite Römbke et al. (2004) and Iglesias et al. (2003). Römbke et al. (2004) extracted intact soil columns from the field and exposed real earthworm communities to a fungicide, the carbendazim applied in the formulation Derosal®. Sixteen weeks after application of the chemical, decreases were observed in the abundance as well as the biomass of the earthworm community. However, the experimental design was not suitable to evaluate effects on diversity. The authors calculated EC_{50} values (i.e. half maximal effective concentration values) for the effect of carbendazim on earthworm abundance (2.04–48.8 kg active ingredient ha^{-1}) and on biomass (1.02–34.6 kg active ingredient ha^{-1}). On the other hand, in a field study, Iglesias et al. (2003) did not find any effect on earthworm density of formulated metaldehyde (Caraquim®) at the manufacturer's recommended rate.

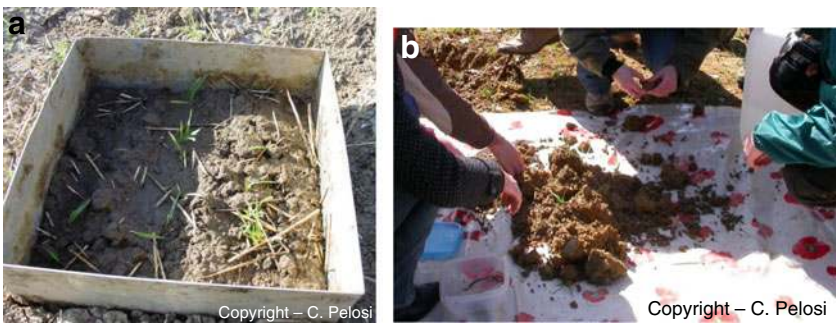

Fig. 5 Illustration of the earthworm sampling method combining **a** a chemical extraction and **b** hand-sorting

To address the second question, nine articles that met our criteria (Table 4) were studied in detail. Total earthworm density (individuals per square metre) and/or biomass (in grammes per square metre) were used as response variables. Conventional farming was compared with organic farming (six studies) or other strategies, i.e. reduced inputs, integrated or bio-dynamic farming. Studies covered an 18-year period from 1990 to 2008. In total, 68 pairs of plots reporting earthworm biomass and 82 reporting earthworm densities were collected. The mean overall effect sizes were 0.17 (±0.15) and 0.38 (±0.20) for earthworm density and biomass respectively (Fig. 6). These values differed significantly from 0 for both density (t=2.20; p<0.030) and biomass (t=3.63; p<0.001). This means that, on average, using little or no pesticides is beneficial for earthworm communities. The two-fold higher effect size recorded for biomass probably reveals that changes in species composition also occurred in favour of larger individuals, e.g. anecic earthworms. These species play an important role in soil behaviour since they influence key soil properties such as structural stability and fertility (Fig. 2a, b), via production of casts and porosity as well as via the enhancement of organic matter mineralisation. Changes in earthworm composition should thus play a major role in ecological intensification of agroecosystems.

To help explain these overall effect sizes, six factors that may influence the results (Lavelle and Spain 2001) were recorded from the literature, i.e. site latitude, soil type, sampling season, sampling method, type of alternative practice and crop. Unfortunately, soil type and sampling season were not included in the analyses because some studies did not report data season by season and most studies did not report data on soil characteristics in a similar way (e.g. soil type or soil texture). Latitude had no effect on the effect size of alternative practice on biomass and density. Similarly, the type of alternative practice did not affect these two effect sizes, whereas the sampling method, i.e. hand-sorting, chemical extraction and combined methods did. Chemical extraction and combined methods induced a significantly positive effect size, i.e. 0.51 and 0.41 for biomass and 0.20 and 0.19 for density, respectively. Conversely, effect sizes calculated from hand-sorting collections were less than 0.06 and did not differ from the null hypothesis. The extraction method is known to be important when describing earthworm populations (Pelosi et al. 2009b): in particular, irritant solutions are used for a better quantification of anecic earthworms. Again, this result suggests a beneficial effect of reducing the use of pesticides on the composition of earthworm communities, with more numerous anecic earthworms in alternative systems. Moreover, the efficiency of the earthworm extraction method was also found to be affected by the sampling date (Marinissen 1992) because variations in precipitation and temperature strongly influence earthworm activity and development (Edwards and Bohlen 1996; Lee 1985). Unfortunately, we cannot discuss this point due to the lack of consistency in the sampling date collections among studies.

The type of crop resulted in different effect sizes (Fig. 6). Of the five types of crop, only cereals induced a significantly positive effect size on biomass (effect size=0.48; t=0.27; p=0.01) and density (effect size=0.23; t=5.24; p<0.01). Unplowed plots, i.e. leys and grassland, presented lower effect sizes than plowed plots. This may be explained by a greater effect of soil tillage than the use of pesticides on earthworm communities. Indeed, soil tillage is known to be one of the main determinants of earthworm community assembly (Chan 2001). Non-cereal crops exhibited the highest effect sizes on both biomass and density, but the scatter in the data made them non-significant. As non-cereal crops included numerous crops, e.g. oilseed rape, linseed,

Table 4 List of selected paper retained for meta-analysis on pesticide effects on earthworm communities

Author	Location	Soil	Topic	Year of sampling	Season	Sampling method*	Sampling volume
Pelosi et al. (2009b)	France	Luvisol	Organic vs. conventional	2005 to 2007	Fall	F+HS	40×40×20 cm
Blakemore (2000)	UK	Clay-loam soils	Organic vs. conventional	2000/2001	Fall and winter	HS	25×25×20 cm
Scullion et al. (2002)	UK	Several soil type	Organic vs. conventional	1995 to 1997	Fall spring and early summer	HS+F	30×30×30 cm
Scullion et al. (2007)	UK	Various soil type	Organic vs. conventional	1995 and 1997	Fall spring and early summer	HS+F	30×30×30 cm
Tarrant et al. (1997)	UK		Reduced inputs vs. conventional	1993 and 1994	Spring and fall	F+HS	50×50×NA cm
Irmler (2010)	Germany	Mainly eutric cambisols and haplic luvisols	Organic vs. conventional	2001 to 2008	Spring and fall	HS	0.1 m^2×35 cm
Eltun et al. (2002)	Norway	Drained brown earths	Organic vs. integrated vs. conventional	1994 and 1995	Spring and fall	? (HS)	NA×NA×25 cm
Siegrist et al. (1998)	Switzerland	Chromic luvisol	Organic vs. biodynamic vs. conventional	1992	Fall	HS	0.064 m^2×NA cm
Hutcheon et al. (2001)	UK	Sandy clay	Integrated vs. conventional	1990 to 2000	Spring and fall	F	0.25 m^2×NA cm

HS hand-sorting, *F* formalin extraction, *NA* data are not available

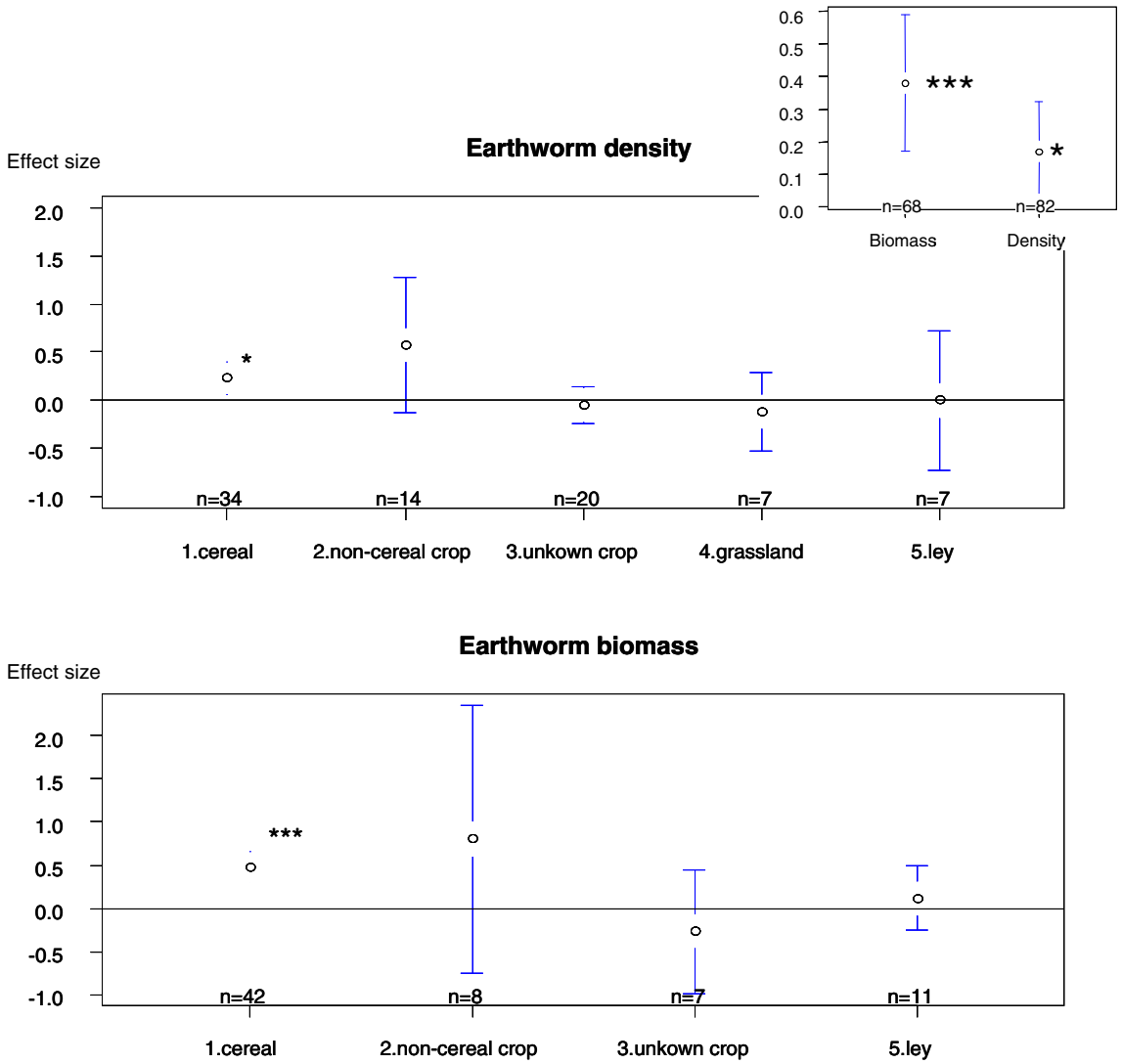

Fig. 6 Effect size of agricultural systems with low/no pesticide use compared with conventional ones on earthworm community (mean and confidence interval, p=0.95). *Upper-right sub-figure* corresponded to overall effects size on biomass and density. The two remaining sub-figures represented the effect size of culture type on density and biomass. ***p<0.001; **p<0.01; *p<0.05

beans and potatoes, this scatter may be due to the type of crop. More data are therefore needed to explain the effect sizes of alternative use of pesticides for each crop. Finally, this analysis shows that many sources of variation cannot be investigated in the present work. For instance, the previous crop, the soil tillage or the plot microclimate probably influence earthworm community dynamics and their response to alternative systems.

2.4 Synthesis

While there is much published literature on the effects of pesticides on earthworms at different organisation levels, it remains in our opinion difficult to draw general conclusions about these effects. Many questions are still unresolved and we have suggested some tentative answers to some of them: are there differences in the sensitivity to pesticides between the three earthworm ecological groups (epigeic, endogeic and anecic) or between species within these groups? Are some categories of pesticides/compounds more harmful than others to earthworms? Are some earthworm functions or traits, e.g. survival, growth, fecundity, mobility or feeding rate, more sensitive to pesticides? The difficulty in answering all these questions comes from at least two problems. Firstly, the response of earthworms to a given pesticide might be different at different organisation levels and very little effort has been made to link the responses to these different levels. Secondly, the studies were carried out in many different experimental conditions and the response of earthworms is likely to depend on environmental conditions in the field and on controlled conditions in laboratory experiments. This last point is developed in the next section.

3 Sources of variability in earthworm response to pesticides

At all organisation levels, the responses of earthworms to pesticides may vary between studies depending on the toxicity of the tested compound, but also in terms of biological material used and physico-chemical conditions and duration of exposure.

3.1 Biological models

Whatever the exposure conditions, effects on organisms depend on species, development stage, age and origin of individuals as well as the body part or tissue which is considered. Firstly, the different species of earthworms (Fig. 7) do not have the same sensitivity to pesticides. Ma and Bodt (1993) found *A. caliginosa* to be more susceptible than *E. fetida* to chlorpyrifos, and *Lumbricus* species even more sensitive. The use of *E. fetida andrei* and *A. caliginosa* respectively in the studies of Casabé et al. (2007) and Booth and O'Halloran (2001) may explain the differing effects of chlorpyrifos on reproduction. Pesticide marketing authorisation tests are performed on the species *E. fetida*, often used as a biological model in ecotoxicological studies (Ma and Bodt 1993). This species is easy to breed, with short generation times (Yasmin and D'Souza 2007) but is not common in the natural environment (Lowe and Butt 2007) and is on average less sensitive to pesticides than species present in cultivated fields (Pelosi et al. 2013). Besides, a distinction between the two *E. fetida* sub-species, i.e. *E. fetida fetida* and *E. fetida andrei*, is rarely made, although some authors have found differences between them (Lowe and Butt 2007). As shown in Tables 1, 2 and 3, the species that are commonly used in tests are *E. fetida fetida*, *E. fetida andrei* and *A. caliginosa*, followed by *L. terrestris*. Studies are often conducted on the same set of species, so that it remains difficult to predict effect of pesticides on whole communities of earthworms, encompassing epigeic, endogeic and anecic earthworms. The ecological group to which a species belongs partly determines its living and behaviour that affect the exposure of the earthworms to contaminants (Tomlin 1992; van Gestel 1992a). Culy and Berry (1995) explain that earthworms which feed at the soil surface are more affected by insecticide granules than those feeding in deeper soil layers. As the agrochemical concentration is higher in surface layers, earthworm activity may be reduced in these layers (Keogh and Whitehead 1975). For instance, individuals of *L. terrestris*, being anecic and thus living deep in the soil, are however highly exposed to pesticides because they feed on the soil surface (Edwards and Bohlen 1996; Lee 1985). Moreover, Baveco and De Roos (1996) pointed out that *L. terrestris* was more sensitive to exposure to pesticides than *L. rubellus*. *L rubellus* being an epigeic earthworm, thus likely to be more exposed to pesticides than *L. terrestris*, this answer is unexpected.

Secondly, the age and development stage of earthworms may influence their sensitivity to pesticides (Lowe and Butt 2007). Many authors have shown that juvenile earthworms are more sensitive to pesticides than adults (Booth and O'Halloran 2001; Spurgeon and Hopkin 1996; Zhou et al. 2008). Ecotoxicological risk assessment using only adult specimens may thus underestimate the effects of chemicals on populations (van Gestel and Weeks 2004). According to recommendation No. 5 of Greig-Smith (1992), when growth is of interest it is preferable to use juveniles for ecotoxicological tests. In order to assess the effects of a pesticide on earthworms, van Gestel and Weeks (2004) also recommend using juveniles because it is possible to follow their weight gain. If the aim of a study is to quantify pesticide effects on a population of earthworms, all the development stages have to be considered.

Moreover, earthworms used in ecotoxicological studies may be purchased, collected in the field, laboratory cultured or from unknown origin. Each origin presents advantages and drawbacks (Lowe and Butt 2007). For instance, laboratory cultures of earthworms permit the production of cohorts of known age and history as well as the use of juveniles but they may lead to the production of individuals adapted to laboratory conditions (artificial selection) or which are inbreeding or unhealthy. According to Lowe and Butt (2007), species selection has often been based on commercial availability or on field-collected earthworms and it is difficult to determine whether the earthworms have already been exposed to a contaminant. They thus recommend using earthworms that have been bred under known laboratory conditions.

Finally, the earthworm body part used in tests varies from one study to another and infra-individual/individual studies on the whole organism or on a specific tissue may give different results (Gao et al. 2008; LaCourse et al. 2009).

3.2 Physico-chemical conditions and duration of exposure

Different methods are used to study the effect of pesticides on earthworms: immersion tests, injection tests, forced feeding tests, feeding on treated food or laboratory soil tests with artificial or natural soils (Edwards and Bohlen 1996; OECD 207 1984; Reinecke 1992; Robert and Dorough 1985). Contact filter paper tests (Edwards 1983; OECD 207 1984) are commonly used but only short-term effects can be measured in such tests and sub-lethal effects, such as reduced reproduction, are not addressed (Choo and Baker 1998). The contact filter paper test is used for the measurement of infra-individual and some individual parameters. Moreover, Heimbach (1984) as well as Neuhauser et al. (1986) found that contact filter paper tests provide results which are different from those obtained with soil tests.

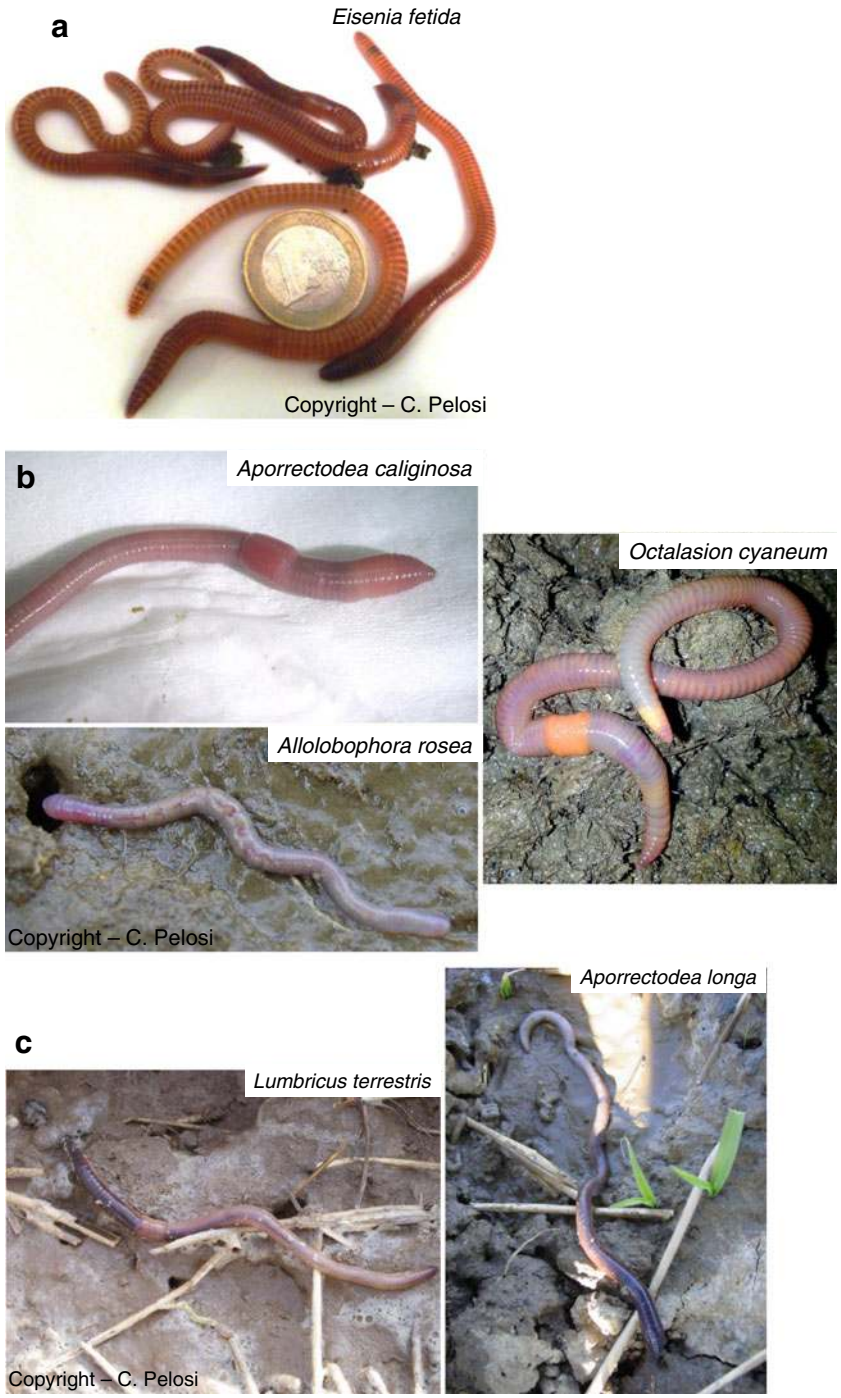

Fig. 7 Different earthworm species from the three ecological groups (Bouché, 1972): **a** epigeic, **b** endogeic and **c** anecic

Böstrom and Lofs-Holmin (1982) explained that '[…] the number of methods used until now equals the number of papers presented on the subject'. van Gestel (1992b) suggested that organic matter influences the bioavailability and thus the toxicity of pesticides to earthworms. This author proposed to use the chemical adsorption coefficient, i.e. K_{oc} (OECD 2001), to extrapolate results from one soil to another.

Studies on pesticide effects on earthworms can be, as already mentioned in this study, performed under laboratory or field conditions. Whatever the environment of the study, bioavailability of chemicals in soils is highly dependent on soil properties (van Gestel and Weeks 2004). For instance, toxicity in soil is influenced by both pH and organic matter content (van Gestel and van Dis 1988). In the same way, Högger and Ammon (1994) observed a 50 % decrease in earthworm activity when pesticide was incorporated into soil and 90 % when it was placed on the soil surface. Consequently, environmental parameters may influence the sensitivity of earthworms to pesticides. Under field

conditions, sampling method and weather conditions influence earthworm exposure as well as sampling efficiency (Högger and Ammon 1994; Roberts and Dorough 1985). Under laboratory conditions, density of earthworms, substrate type, e.g. artificial soils like Organisation for Economic Co-operation and Development or natural soils, as well as temperature, light, moisture conditions, and the methodology used to add the pesticide and duration of the exposure are not always specified, or sometimes inappropriate, often far from field conditions (Greig-Smith 1992; Lowe and Butt 2007; Reinecke 1992) and varying from one study to another (Edwards and Bohlen 1996; Lowe and Butt 2007).

Finally, the magnitude of the observed or measured earthworm response to pesticides may be influenced by the duration of exposure. At the infra-individual level, it is possible that the response occurs before or after the biomarker measurement. In this case, no response may be seen if it is simply delayed (Zhang et al. 2013). For this reason, ideal biomarkers exhibit time and dose-dependent variations (Brulle et al. 2006). Another point is that exposure durations used under laboratory conditions are not representative of field conditions, where earthworms are continually exposed. Finally, whatever the organisation level, studies have very rarely used the same exposure duration, making it difficult to compare them.

4 Knowledge gaps

4.1 Representativeness

The first identified knowledge gap is linked to the lack of representativeness of the studied combinations of earthworm species and pesticides. More studies are needed on species that are common in European cropping systems and on pesticides that are authorised nowadays. More combinations of earthworm species and pesticide should be studied to have a more comprehensive sampling and to be able to reply with more certainty to general questions such as: What are the most harmful types of pesticide for earthworms? Are endogeic earthworms more or less sensitive to pesticides than anecic?

4.2 Difficulties in scaling up from infra-individual to community levels

According to van Gestel and Weeks (2004), 'there should be a correlation (linkage) between a biochemical marker response and deleterious changes to the population or community. In this case, a sub-cellular biomarker may, for example, act as an early warning of effects at the population level'. Booth and O'Halloran (2001) highlight the usefulness of biomarkers in risk assessment, explaining that biomarker responses occurred at similar or lower concentrations than those causing an adverse effect on cocoon production and cocoon viability. However, few studies have attempted to link the effects on earthworms at different organisation levels and this remains an important issue for risk assessment. One of the only attempts was made by Ricketts et al. (2004) with metals on the species *E. fetida*. They studied the expression level of the gene coding annetocin, a neuropeptidic hormone involved in the induction of egg-laying behaviour of earthworms, under high concentrations of zinc and lead. They concluded that annetocin was a promising biomarker in earthworm ecotoxicology since it is involved in earthworm reproduction.

4.3 Difficulties in scaling up from laboratory to field

Some authors have shown that results from laboratory tests cannot be extrapolated easily to field conditions (Lowe and Butt 2007; Svendsen and Weeks 1997) but many others asserted that results from laboratory and field are comparable (Culy and Berry 1995; Heimbach 1992; Holmstrup 2000). van Gestel (1992a) estimated that field contaminant concentrations that affect earthworm populations are in agreement with effect levels determined in laboratory studies. This controversy highlights the complementarity between laboratory tests and field studies and the need for both approaches (Svendsen et al. 2005). However, field tests are rare due to difficulties with experimental design and interpretation of results. Few sub-lethal studies with exposures similar to situations in the field have been conducted (Kokta 1992b; Venter and Reinecke 1987), and many authors advocate microcosm studies using soils similar to natural soils (Addison and Holmes 1995; Brulle et al. 2011).

The reasons for the difficulties in extrapolating the results from laboratory to field are numerous. First of all, few studies compare the effects of pure compounds with commercial formulations as applied in fields. De Silva et al. (2010) showed that the latter are more harmful than pure compounds because of the toxic effects of adjuvants on earthworms. Secondly, some authors (Booth et al. 2000b; Booth and O'Halloran 2001; Yasmin and D'Souza 2007) suggested that earthworms recover their normal growth and reproduction rates between 4 and 8 weeks after their removal from pesticide-treated soil, but in the field they would not normally be removed from soil exposed to pesticides. This continuous exposure can have consequences from generation to generation if the compounds are persistent in the soil or if there is a transmission of some deleterious effects from parents to offspring, i.e. trans-generational effects. Brunninger et al. (1994) published one of the few studies dealing with effects of pesticides on several generations of earthworms. They studied the effect of carboruran and terbuthylazine on three generations of *E. andrei*. Exposure

of the first generation to carbofuran decreased cocoon production in all generations while terbuthylazine harms parents but benefits the F1 generation. It appears therefore that effects of pesticides on an earthworm species over several generations depend on different factors like the compound concerned and generation. Lastly, laboratory ecotoxicological tests generally do not take into account repeated applications of several pesticide cocktails, i.e. multi-contaminations and chronic exposure. There is a lack of studies on the effects of repeated agronomic doses on earthworms at different organisation levels. Besides, the effects of the many breakdown products of pesticides that enter the soil are mostly ignored as well as multi-contamination effects, e.g. synergistic, antagonistic or neutral interactions (Paoletti 1999). Yasmin and D'Souza (2007) explain that 'only a few studies describing the toxicity impact of chemical mixtures on earthworms have been published thus far, all of which focus on metals'. These authors studied the effect of three pesticides, i.e. carbendazim, dimethoate and glyphosate, alone and in combination, on the growth and reproduction of *E. fetida*. They showed synergistic adverse effects of the mixture compared with single pesticides. Also, Zhou et al. (2006) reported that the combination of acetochlor and methamidophos resulted in synergistic toxic effects on *E. fetida*. Conversely, according to Springett and Gray (1992), glyphosate and captan in combination have a smaller effect than glyphosate alone. All these contradictions and knowledge gaps highlight the need for further research into long-term earthworm exposure to mixtures of commercial formulations of pesticides.

5 Conclusions

From this review, we conclude that there are two main challenges: (1) the first is about knowledge, i.e. determining the real effects of pesticides on cropping systems and (2) the second concerns the methodology required to obtain this knowledge and extrapolate it to new pesticides, i.e. designing robust tests based on short-term experiments and standardised laboratory conditions that are able to predict the real field effects of new pesticides. So far, these two challenges cannot be met because we lack experiments assessing the effects of the same pesticides on the same earthworm species at different organisation levels to derive the links between the responses at these different levels. We believe that responses observed at infra-individual or individual levels have an impact on higher organisation levels (populations, communities) but there is currently no strong proof. Moreover, we also lack studies based (1) on European species and pesticides still authorised in Europe, (2) on species that are really found in cropping systems (and not only epigeic species of the genus *Eisenia*) and (3) on realistic conditions in terms of soil, pesticide dose and experimental duration. Another limitation of most studies is that the effect of mixtures of pesticides and chronic exposure to these mixtures are insufficiently studied, while earthworm populations face such conditions in cropping systems and it has been shown that response to mixtures of contaminants is very hard to predict from responses to the isolated contaminants. Moreover, long-term exposure of earthworm populations to contaminants could trigger the evolution of strategies to resist these contaminants. This suggests that this long-term response should be studied and that the provenance of earthworms used in experiments (which is often not clear) can be very influential: if earthworms come from populations that have been exposed to the tested pesticides for some generations they may respond differently to them. A final point that we want to highlight is the complementarity between i) laboratory studies which elucidate the mechanisms involved in the earthworm response to pesticides, and ii) field studies able to assess the state of earthworm communities in real conditions.

A broader challenge is to determine the impact of agricultural practices on earthworm populations in cropping systems and to design cropping systems that are more favourable to them and the functions they provide, which are proven to be important for the sustainability of soil fertility and plant production (Lavelle et al. 2006). Here, the difficulty is that earthworm density or biomass has often been compared between contrasting cropping systems, e.g. organic vs. non-organic, which involve many differences in cultural practices. All these practices are likely to interact in their effects on earthworms. For example, both pesticides and tillage affect earthworms so that that complete factorial experiments combining tillage and pesticides should be carried out. It is possible that a given pesticide only affects earthworms with frequent tillage because tillage incorporates the pesticide more quickly into the soil profile or because earthworms weakened by the pesticide are no longer able to avoid the effect of tillage through rapid movement or by making galleries. As far as we know, no such experiments have been done.

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