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published in
Bioavailability of Organic Chemicals in Soil and Sediment
2020

DOI (link to publisher)
10.1007/698_2020_511

document version
Publisher's PDF, also known as Version of record

citation for published version (APA)
González-Alcaraz, M. N., Malheiro, C., Cardoso, D. N., Prodana, M., Morgado, R. G., van Gestel, C. A. M., & Loureiro, S. (2020). Bioaccumulation and Toxicity of Organic Chemicals in Terrestrial Invertebrates. In J. J. Ortega-Calvo, & J. R. Parsons (Eds.), *Bioavailability of Organic Chemicals in Soil and Sediment* (pp. 149-189). (Handbook of Environmental Chemistry; Vol. 100). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/698_2020_511

Bioaccumulation and Toxicity of Organic Chemicals in Terrestrial Invertebrates

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Contents

Abstract Terrestrial invertebrates are key components in ecosystems, with crucial roles in soil structure, functioning, and ecosystem services. The present chapter covers how terrestrial invertebrates are impacted by organic chemicals, focusing on up-to-date information regarding bioavailability, exposure routes and general concepts on bioaccumulation, toxicity, and existing models. Terrestrial invertebrates are exposed to organic chemicals through different routes, which are dependent on

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Jose Julio Ortega-Calvo and John Robert Parsons (eds.), *Bioavailability of Organic Chemicals in Soil and Sediment*, Hdb Env Chem (2020) 100: 149–190,
DOI 10.1007/698_2020_511,

both the organismal traits and nature of exposure, including chemical properties and media characteristics. Bioaccumulation and toxicity data for several groups of organic chemicals are presented and discussed, attempting to cover plant protection products (herbicides, insecticides, fungicides, and molluscicides), veterinary and human pharmaceuticals, polycyclic aromatic compounds, polychlorinated biphenyls, flame retardants, and personal care products. Chemical mixtures are also discussed bearing in mind that chemicals appear simultaneously in the environment. The biomagnification of organic chemicals is considered in light of the consumption of terrestrial invertebrates as novel feed and food sources.

This chapter highlights how science has contributed with data from the last 5 years, providing evidence on bioavailability, bioaccumulation, and toxicity derived from exposure to organic chemicals, including insights into the main challenges and shortcomings to extrapolate results to real exposure scenarios.

Keywords Beneficial arthropods, Bioavailability, Biological traits, Conceptual models, Earthworms, Edible insects, Exposure routes, Pollinators

Terrestrial invertebrates are key components in ecosystems, which play crucial roles in soil structure, functions, and services [1]. Soil structure is characterized by high spatial and composition heterogeneity and is a major driver of soil biodiversity. Invertebrate functions in soils (e.g., carbon transformations, nutrient cycling, structure maintenance, biological populations' regulation) are often related to ecological and morphological traits that include size, morphology and body characteristics, feeding habits, and specific habitat location [2]. The ecology of terrestrial invertebrates, i.e., the interactions among them and their environment, is known to be threatened by many different types of pressures, which can nowadays be included within global changes. These include climate changes, chemical exposure, and biological pressures that will unbalance the ecosystem turning it into an unsustainable environmental compartment. Among the threats, organic chemical compounds are often appearing in agricultural, rural, and urban environments, mainly derived from agricultural practices, industrial activity, wastewater treatment plants (biosolids and/or effluents), or even from groundwater contamination.

1 Exposure Routes and Organismal Traits

Understanding exposure routes of terrestrial invertebrates is paramount in risk assessment, and, therefore, the European Food Safety Authority (EFSA) proposed a new testing strategy, which takes into account the relevant exposure routes for terrestrial organisms and their related effects, specifically for plant protection products [3]. Morphological and feeding traits, along with preferable habitats, discriminate terrestrial invertebrate exposure routes to chemical compounds. This exposure is also dependent on the time chemical interacts with the target organ/cell and the fate of the substance in soil (Fig. 1). The latter encompasses the bioavailable fraction of the chemical, where the fraction taken up by an organism is related to the dynamic equilibrium from the exchange of the chemical among the soil solid phase, the soil solution, and the biota. Chemicals can be taken up: (1) via direct contact with the surrounding aqueous media, through pore water, by gills or dermally; (2) by ingestion, as particle-bound chemicals, through the ingestion of soil particles or organic matter, by ingesting contaminated prey; or (3) by the respiratory tract, when volatile chemical compounds are present [4]. Within all these processes, we can distinguish between passive dermal absorption of the dissolved chemical fraction in the interstitial water, intestinal uptake of the chemical compound during gut passage (for soil and food items), and exposure through air contamination via the respiratory tract [5]. Besides being species-specific, exposure routes are also dependent on the chemical characteristics that may change the fractions within the different soil compartments (soil particle-pore water-air). For chemicals with log $K_{\mathrm{ow}} > 5$ (K_{ow}: octanol-water partitioning coefficient), uptake from soil particles may become more relevant than from pore water, especially in high organic soils, as hydrophobic chemicals tend to adsorb more efficiently to soil organic matter [6].

Soil exposure comprises the duality of exposures through pore water (dermal and/or “drinkable”) and soil particle ingestion. Dermal uptake is usually measured in terrestrial invertebrates (e.g., earthworms) by the filter paper method, where organisms are in direct contact with the study chemical. The Organisation for Economic Co-operation and Development (OECD) Guideline 207 [7] advises this initial screening test to further identify potentially toxic chemicals in soil, although it has to be noted that results obtained with this test (values in $\mu\mathrm{g\ cm}^{-2}$) cannot directly be translated to soil concentrations. The second step advised already includes a soil exposure test. This allows discrimination between main exposure routes, depending on the chemicals. In addition, as an effective exposure route, this test is also used to infer on chemical modes of action [8]. Collembolans are known to use the ventral tube to ingest water as a way to balance their fluid and electrolyte content [9]. In this sense, they have often been used to approach porewater exposure in soils, although in recent years few studies have been published on organic contaminants toxicity to collembolans specifically accounting for porewater concentration [10].

Litter exposure route is also key for some macrofauna decomposers, like isopods [3]. As major litter transformers, evaluating effects from litter contamination is crucial in rural and urban ecosystems, but there are not so many recent studies available on this exposure route [11]. Besides litter, exposure through feeding has shown recently to be important regarding, for example, plastics and fibers [12–14].

Volatile chemical compounds can potentially provide an extra route of exposure through the respiratory system [15]. When looking at the respiratory system of insects, as an example, a diffusion gradient is generated, and O_2 is dissolved in a small fraction of water in order to be exchanged by diffusion into the cells [16]. A similar pattern occurs with isopods, where air dissolves in the surrounding moisture comprising their pleopods (pseudolungs) and allowing O_2 to diffuse [17]. Considering that volatile compounds can also be trapped in this water and diffused into cells,

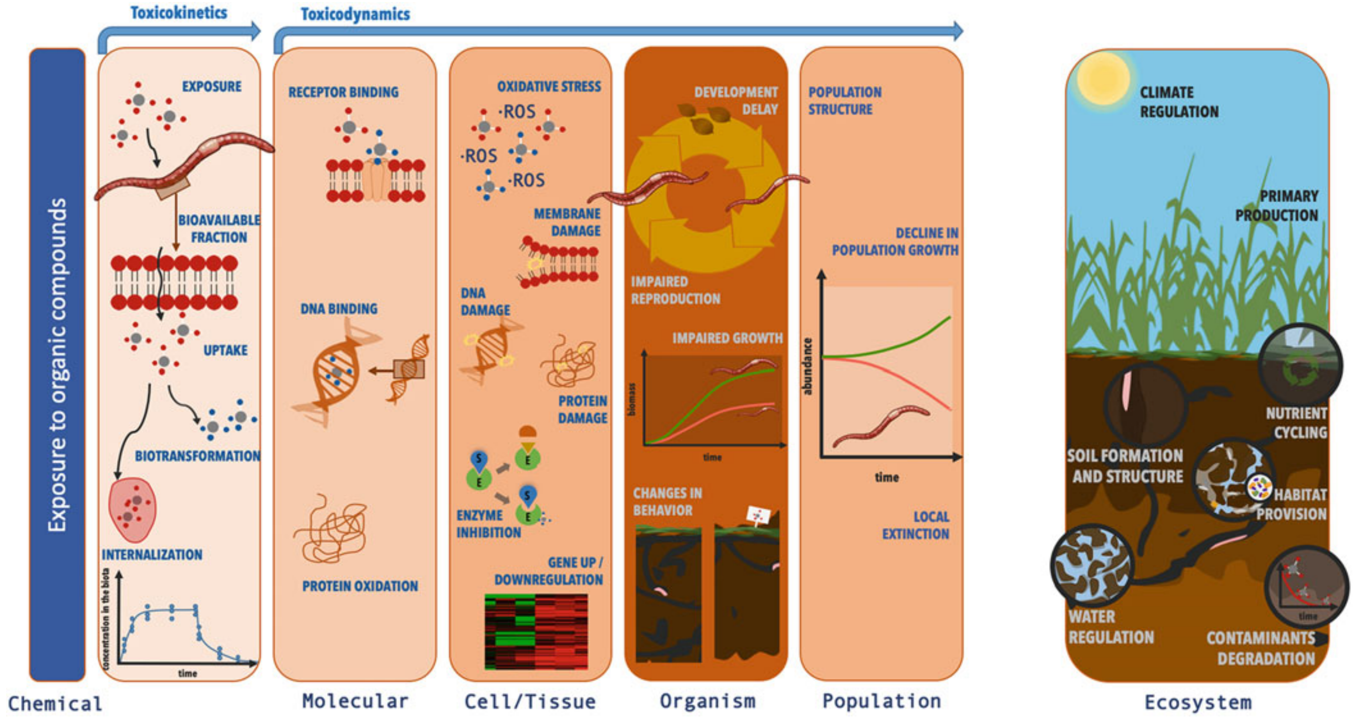

Fig. 1 Bioavailability, toxicokinetic, and toxicodynamic endpoints that have been reported as targets, at different levels of biological organization, from a potential exposure to organic chemicals, leading at a final stage to changes in ecosystem functions and services

this may be an important route to explore as it enters directly into the circulatory system of soil invertebrates. So far, no studies on this are available.

2 Bioaccumulation and Toxicity

Chemical partitioning in the soil is dependent on the soil properties, and it is widely known that different soil types provide, in a general sense, different bioavailable fractions. But, the bioavailable fraction cannot be disconnected from the exposure route involved nor the organism's physiology. If this were just a question of chemistry, one would suggest that the toxicity of a chemical to plants would be similar to that for collembolans, as their exposure is mainly through pore water, which is not the case. Toxicity is surely dependent on chemical uptake (related to the bioavailable fraction), but the organism's physiology is key regarding toxicity (toxicodynamics; presence of specific receptors; metabolic capacity) (Fig. 1). In animals, the distribution of chemical compounds from their gut system to the cells is then again fractionated (bioaccessible fraction), and only a percentage reaches the target organ/cell (bioactive fraction) [18]. The mode or mechanism of action of a chemical will trigger the effects induced, according to the concentration that reaches the target.

The bioaccumulation concept is paramount to understand toxicity, by looking at the amount taken up by the organism, the loss by several processes including egestion, metabolism, transfer to offspring, and growth (e.g., molting), and how chemicals are internalized by the organism. Other factors like feeding traits, habitat use, reproduction, age, biotransformation ability, or energy demand are also crucial in determining bioaccumulation patterns [19]. Therefore, both concepts and data (bioaccumulation and toxicity) are used for the risk assessment of chemical compounds.

For organic chemical compounds, chemical persistence, expressed as their half-life, is key to understand toxicity and bioaccumulation, considering that exposure concentration may vary in time. In addition, their biotransformation through gut passage is also important regarding the observed effects, the gut microbiome being of great importance. Persistent organic pollutants (POPs) are important compounds to study as they tend to stay longer in the environment and their properties potentiate bioaccumulation and toxicity to terrestrial organisms. They are considered hydrophobic and lipophilic, having high affinity with cell membranes, tending to accumulate into lipids rather than entering the aqueous fraction in cells. Toxicity is known to be exerted through a disturbance of membrane integrity by itself and also as a path for the partitioning of pollutants into biological membranes. Also, these substances are accumulated in the lipid fraction to reduce the amount circulating in the animal's plasma, but this may potentially lead to biomagnification in trophic chains.

Bioaccumulation factors are, therefore, calculated as a ratio between the concentration of the substance in the biota, corrected for the lipid content, and the concentration in the soil [20]. This, therefore, enables comparison across species but also when dealing with different stages of animal development. For example, in the ontogeny of insects, different lipid concentrations are present throughout development [21], and, therefore, this should also be taken into account. In this way, the variation due to variable lipid content is eliminated.

In the case of nonpolar organic compounds (e.g., polycyclic aromatic compounds (PACs)), some are nonreactive or with a nonspecific mode of action (also known as baseline toxicity), which is exerted through narcosis due to the nonspecific interaction between lipophilic chemical molecules and the phospholipids in biological membranes [22, 23]. Narcosis-type effects are reversible, and toxicity thresholds can be extrapolated from organism to organism, with a correction for lipid content [22]. For reactive compounds, where specific modes of action are present (e.g., neurotoxicity, endocrine disruption, genotoxicity), mortality and sublethal effects can occur in specific taxa at low doses, with organisms eliciting lower body burdens when compared to those from nonreactive compounds.

3 Models

In the last years (or even decade), few studies are available reporting the use of models to predict bioaccumulation or toxicity of organic chemical compounds to terrestrial invertebrates. Models and tools like the equilibrium partitioning theory (EqP theory), QSARs, or DEBtox tool (Fig. 2) that require complex and expensive datasets are nowadays less frequently applied than when they were first described.

The EqP theory, described in the later 1980s and early 1990s for aquatic organisms, is a tool that enables the estimation of the internal concentration of hydrophobic chemicals in biota [24]. In soils, this relationship is determined by the porewater concentration and relates to soil properties and several constants like desorption and adsorption rate constants. The partitioning coefficient (K_p) defines the dynamic process of chemical sorption to the soil particles and desorption, which leads to the presence of contaminants in pore water. This process is mainly driven by soil properties (e.g., organic carbon content) and by the chemical K_{ow} (K_p increasing with increasing K_{ow}). Bioconcentration factors (BCF) for chemicals accumulating in organisms whose accumulation is mainly driven by porewater, therefore, depend on the chemical K_{ow} (and lipid content of the organism), with higher BCFs for chemicals with higher K_{ow}s. When relating bioaccumulation patterns in organisms to the chemical concentrations in soil, a bioaccumulation factor (BAF) is calculated. BAF is the ratio of BCF and K_p as it captures both the uptake from porewater and the sorption of the chemical from porewater to the soil solid phase. Since for nonpolar organic chemicals both the BCF and K_p are related to the K_{ow}, the BAF is independent on the K_{ow} but dependent on the organic carbon content of the soil and the lipid content of the organism. In addition, as it relates to other constants dependent on the organism's physiology and behavior, for a more accurate prediction, the EqP theory can be adapted regarding the organism tested. Using earthworms as an example, the

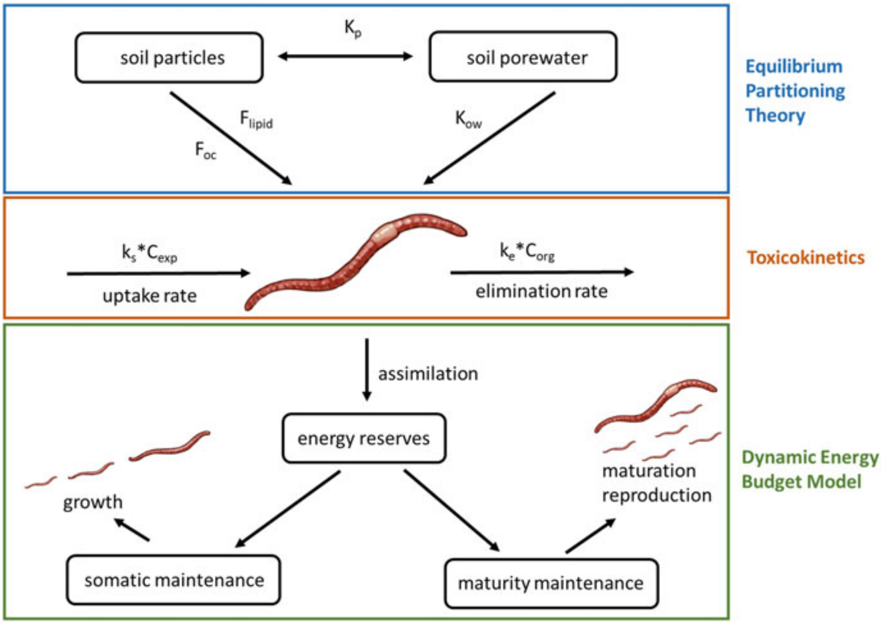

Fig. 2 Mathematical models and tools to estimate biological responses in terrestrial invertebrates, considering exposure routes (soil particles and soil porewater), uptake and elimination kinetics, and how assimilated organic chemicals change somatic and maturity maintenance. K_p partitioning coefficient, K_{ow} octanol-water partitioning coefficient, F_{lipid} lipid fraction, F_{oc} organic carbon fraction, k_s uptake rate constant, C_{exp} concentration of exposure, k_e elimination rate constant, C_{org} concentration in the organism

EqP theory can, therefore, include a dietary uptake rate constant for soil ingestion and an uptake rate constant for pore water [25], for cases where no equilibrium is reached. In addition, the metabolism, reproduction effort, and growth of organisms can also be included as rate constants.

Nowadays, to ensure all requests from the European Union regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), several approaches are being used based on intelligent test systems to decrease animal testing and use existing datasets. Software and statistical tools (e.g., in silico methods) enable the extrapolation from one chemical to similar chemical compounds, using read-across, or from quantitative structure-activity relationships (QSARs), where toxicity is related to chemical properties. In the latter case, QSARs for soil organisms can be developed as linear regression relationships between LC_x/EC_x (lethal/effective concentrations at x %) based on the dissolved fraction, bioavailable in the pore water (in mol L^{-1}), and chemical lipophilicity expressed as log K_{ow} (negative regression) [26]. For this, soil-specific coefficients like the sorption coefficient, based on the carbon-water partitioning and the organic matter fraction present, are determined in a specific soil. Solid-phase microextraction (SPME) is an effective tool to assess interstitial concentrations of organic chemicals in soils reliably. As an example, for the collembolan *Folsomia candida*, whose chemical exposure route is known to be exclusively through porewater, a QSAR was developed by Giesen and van Gestel [26] for six chloroanilines. EC_{10} and EC_{50} values for effects on reproduction were used, based on porewater concentrations measured by SPME and estimated from nominal soil concentrations and soil-water partitioning coefficients. Measured and estimated porewater concentrations were comparable only for tetra- and pentachloroaniline, with a decreasing degree of chlorination inducing a higher disparity between modeled and measured concentrations. Therefore, some extra optimizations were needed regarding the bioavailable fraction. Several QSARs for organic carbon normalized partitioning coefficient (K_{oc}) or Freundlich soil-water partition coefficient (K_{foc}) use as the independent variable the octanol-water partitioning coefficient (K_{ow}), the molecular connectivity index (MCI), or water solubility. Both K_{oc} and K_{foc} reflect the adsorption of chemicals to soil particles (affinity), where higher values indicate higher sorption. These two coefficients are derived through linear and nonlinear distribution of the coefficient, respectively [27]. In practice, K_{foc} is more appropriate for chemicals for which sorption ability depends on their concentration. More recently, Eckel [28] derived a novel calculator to estimate K_{foc} for soils. In this study, K_{foc} for 41 pesticides in 18 agricultural soils was predicted from subcooled liquid solubility, with robust estimates when compared to the existing literature. This estimation accounts for ionization of the compound and determines its solubility as a liquid at room temperature, with the final aim of achieving robust estimates for both solids and liquids and neutral and anionic compounds.

Toxicokinetic/toxicodynamic (TKTD) models simulate effects in time during an exposure scenario, accounting for the interaction between the bioavailable fraction and the uptake and elimination of the chemical in a defined organism (toxicokinetics) but also how the chemical interacts with the cellular/organ target, traducing that into effects (toxicodynamics). The General Unified Threshold model of Survival (GUTS) is a unifying TKTD framework for predicting the time course of survival, which has different assumptions, data requirements, and complexity [29]. All GUTS versions use the external concentration to estimate an individual damage dynamic and which further translates into an individual hazard state variable, resulting in simulated mortality when an internal damage threshold is exceeded [30]. The toxicodynamic component of GUTS deals with death mechanisms in different ways, assuming that (a) death rate is identical for all individuals in a population, and the threshold parameter for lethal effects is fixed – stochastic death (SD) model –, and (b) effects are distributed among individuals, and once an individual tolerance is exceeded, the organism dies immediately – individual tolerance (IT) model [30].

The Dynamic Energy Budget model (DEBtox) is another TKTD tool that has been used for mechanistic models to infer on stressor effects on the life-history traits of individual organisms. This enables the extrapolation to higher and lower levels of biological organization. The advantage of this tool is the integration of a time course of effect data within one consistent framework. These data gather time point series for several endpoints like survival, reproduction, and growth (Fig. 2). For example, Jager et al. [31] infer on the modes of action of chlorpyrifos in *F. candida*, where reproduction, growth, and mortality are modeled and modes of action predicted based on the results obtained from multiple endpoints in life-cycle toxicity tests. In this specific case study, chlorpyrifos affected the process of egg production but also aging through oxidative stress. More recently, bee species responses to chemical mixtures have been compared using conceptual pharmacological models (concentration addition and independent action) and the DEBtox model [32]. The use of time series is indeed advised as changes in time may occur and can, therefore, be predicted. This is also highlighted by Hesketh et al. [33], who reported the benefits of evaluating chronic exposure instead of acute (short-term) effects for toxicity tests with the honey bee *Apis mellifera*. In this case study, DEBtox was used to infer on the potential survival up to 30 days and 90 days of summer and winter worker lifespans. Despite the advantages that this kind of modeling brings to regulation, the effort to gather data is high, and therefore not many studies have been carried out with terrestrial invertebrates.

4 Organic Chemicals and Interactions with Biota

This section includes a summary of the scientific literature of the last 5 years on the bioavailability and effects of organic chemicals on terrestrial invertebrates following the biological organization represented in Fig. 1. The information is focused on:

(a) Plant protection products (herbicides, insecticides, fungicides, and molluscicides), pharmaceuticals (veterinary and human), PACs, polychlorinated biphenyls (PCBs), flame retardants, and personal care products;
(b) Key terrestrial invertebrates for ecosystem functioning including annelids, arthropods, and mollusks;
(c) Bioaccumulation data;
(d) Effects at sub-organism level (genotoxicity and biochemical, morphological, and histological alterations);
(e) Effects at individual and population levels (life-history traits and behavior).

Soil annelids, especially earthworms, are by far the most studied group, with information covering many different organic chemicals and, in some cases, some of their main metabolites. Nevertheless, it is also referred at bioaccumulation and toxicity information on other invertebrate groups.

4.1 Plant Protection Products

4.1.1 Herbicides

Bioaccumulation of Herbicides

Bioaccumulation studies of herbicides in terrestrial invertebrates are scarce due to difficulties in their chemical determination; most of the studies are focused on earthworms. For example, greater bioaccumulation of atrazine has been found in *Metaphire guillelmi* (BAF 0.42) than in *Eisenia fetida* (BAF 0.08) [34]. The authors attributed this to the fact that *E. fetida* uptake is mainly through dermal absorption, whereas that of *M. guillelmi* is largely affected by gut processing in which physical grinding and surfactant-like materials could facilitate atrazine desorption from the soil. Tejada et al. [35] reported greater bioaccumulation of oxyfluorfen in *Allolobophora molleri* (BAF 4.0–4.5) than in *E. fetida* (BAF 3.0) and *Lumbricus terrestris* (BAF 1.0–1.5). Goto and Sudo [36] found higher bioaccumulation risk of trifluralin and pendimethalin in *Eisenia* spp. (BAF 9.1 and BAF 5.8, respectively) than in *Pheretima* spp. (BAF 0.93 and BAF 0.27, respectively) (BAFs calculated from kinetic parameters). Jing et al. [37] reported enantioselective bioaccumulation of fenoxaprop-ethyl in *E. fetida*, with a preferential accumulation of the R-enantiomer (BAF 1.4) over the S-enantiomer (BAF 0.17). For the majority of the previously referred studies, the lack of BAF standardization for earthworm lipid content and soil organic carbon makes it difficult to compare different species and herbicides.

Effect of Herbicides at Sub-Organism Level

Herbicides can cause DNA damage in terrestrial invertebrates. This has been shown, for example, for the pure active substances fomesafen and mesotrione in *E. fetida* [38] and glyphosate-based herbicides in the land snail *Cantareus aspersus* [39]. Herbicides can also alter gene expression. For example, the pure active substance 2,4-dichlorophenoxyacetic acid (2,4-D) may upregulate superoxide dismutase, glutathione S-transferases, and catalase genes expression in *Eisenia andrei* [40], while siduron-based herbicides may induce downregulation of metallothionein and the expression of heat shock protein genes in *E. fetida* [41]. In the honey bee *A. mellifera*, paraquat may downregulate glutathione S-transferase, superoxide dismutase, and peroxiredoxin gene expression levels, but not those of catalase, cytochrome P450s, and vitellogenin genes [42].

Herbicides favor the production of reactive oxygen species (ROS) [38], which can overcome the antioxidant defenses of terrestrial invertebrates, causing lipid peroxidation [40, 43, 44]. Invertebrates can counteract this through the activation of certain antioxidant enzymes (e.g., catalase, superoxide dismutase, peroxidase, glutathione peroxidase, glutathione reductase) [38, 40]. However, some studies also found decreasing activity of antioxidant enzymes or no effects after herbicide exposure [43, 45]. Herbicides can also induce changes in the activity of enzymes involved in xenobiotic detoxification (e.g., glutathione S-transferases and carboxylesterases) [43, 46] and in hydrolysis of acetylcholine neurotransmitter (acetylcholinesterase) [43, 44].

Little information exists on the possible morphological and histological alterations induced by herbicides in terrestrial invertebrates. This is the case of glyphosate-based herbicides in the earthworm *Eudrilus eugeniae* (e.g., setal anomalies, epidermal lesions, clitellar swelling) [44], as well as in the cellular ultrastructure of the hypopharyngeal glands of *A. mellifera* [47]. On the contrary, Druart et al. [48] found no effects of glyphosate-based herbicides on the male genital apparatus of the land snail *C. aspersus*.

Effect of Herbicides at Individual and Population Levels

Life-History Traits Herbicides can induce earthworm mortality, either through dermal contact in filter paper tests of short duration or through medium-/long-term exposure to soil conditions. In the case of filter paper tests, greater toxicity to *Eisenia* spp. has been found, for example, for diquat and tembotrione ($LC_{50} < 10$ μg a.i.[1] cm^{-2}), compared to glyphosate and siduron (LC_{50} ~10–100 μg a.i. cm^{-2}) or imazamox ($LC_{50} > 100$ μg a.i. cm^{-2}) [41, 49]. Herbicide metabolites can sometimes be more toxic than parent compounds (e.g., fenoxaprop-ethyl and quizalofop-ethyl metabolites for *E. fetida*) [37, 50]. In the case of earthworms exposed to herbicide-spiked soils, several species are commonly used, although most of the information refers to *E. fetida* (e.g., $LC_{50} < 10$ mg a.i. kg^{-1} d.w.[2] for terbuthylazine, ~100–500 mg a.i. kg^{-1} d.w. for acetochlor, and >1,000 mg a.i. kg^{-1} d.w. for butachlor) [51–53]. Plenty of information exists on earthworm survival in glyphosate-spiked soils. As pure active substance, glyphosate only causes adverse effects on earthworm survival (e.g., no observed effect concentration, NOEC >50,000 mg kg^{-1} d.w. for *E. fetida* in field soil; NOEC 478 mg kg^{-1} d.w. for *E. fetida* in OECD artificial soil) at levels well above the predicted environmental concentration (PEC 5.7–6.6 mg kg^{-1} d.w.) [54–56]. This trend has also been shown for its main metabolite in soil (aminomethylphosphonic acid, AMPA), with field-relevant concentrations having no effects on earthworm survival (e.g., NOEC 1,000 mg kg^{-1} d.w. vs. PEC 2.0–6.2 mg kg^{-1} d.w.) [55–57]. However, glyphosate-based herbicides may induce earthworm mortality at field-recommended application rates [58, 59]. Negative effects on survival of the enchytraeid *Enchytraeus crypticus* have been found upon exposure to atrazine from cocoon stage (LC_{10} 125 and 378 mg a.i. kg^{-1} d.w. for pure active substance and commercial formulation, respectively), while no effects have been reported upon exposure of

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[1] a.i. – active ingredient.

[2] d.w. – dry weight.

adults (NOEC $>$200 and $>$400 mg a.i. kg^{-1} d.w. for pure active substance and commercial formulation, respectively) [60]. Compared to annelids, the effect of herbicides on the survival of other terrestrial invertebrates is less studied. Several studies found negative effects of herbicides, generally at field-realistic concentrations, on the survival of ants [61], bees [62], beetles [63], collembolans [64], isopods [65], ladybugs [66], predatory mites [67], snails [58], and spiders [68]. However, there are also studies reporting no effects of realistic field concentrations of herbicides on the survival of terrestrial arthropods and mollusks [55, 69–72].

Similar to survival, most of the studies evaluating herbicides' effect on terrestrial invertebrate reproduction are focused on annelids. Glyphosate pure active substance causes no effects on earthworm reproduction at field-realistic concentrations (e.g., NOEC $\geq$470 mg a.i. kg^{-1} d.w. for *E. fetida* in artificial and field soils) [54, 55]. Its main metabolite AMPA shows a variable effect by being able to stimulate or not affect earthworm reproduction at concentrations similar to PEC values [55–57]. On the contrary, glyphosate-based herbicides have been found to negatively affect earthworm reproduction at field application rates [45, 59]. In the case of other herbicides, it is described, for example, that nicosulfuron can stimulate earthworm reproduction, oxyfluorfen can reduce it, depending on the study species, and tembotrione has no effects when applied at recommended field rates [35, 43]. The effect of herbicides on enchytraeid reproduction is also highly variable. Adverse effects are described for atrazine, especially when comparing the pure active substance (EC_{50} 161 and 236 mg a.i. kg^{-1} d.w. when exposed from adult and cocoon stages, respectively) with commercial formulations ($EC_{50} >$ 400 mg a.i. kg^{-1} d.w.) [60]. Negative effects on enchytraeid reproduction have been also reported for commercial formulations of phenmedipham (especially in acidic soils and/or with low organic matter content) [73]. On the contrary, no effects have been found for commercial formulations of metsulfuron-methyl [74]. For other terrestrial invertebrate groups, some studies have reported negative effects of realistic field concentrations of herbicides on the reproduction of beetles [63], collembolans [64], isopods [65], and snails [48]. However, most of the studies evaluating herbicide effects on arthropod and mollusk reproduction found no toxic effects [55].

Herbicides can affect terrestrial invertebrate growth. Several studies reported lower growth of earthworms in the presence of herbicides (e.g., 2,4-D, glyphosate, terbuthylazine) but at concentrations generally exceeding field-realistic levels [40, 55, 75]. Some herbicide metabolites can also affect earthworm growth (e.g., AMPA at concentrations $<$2.5 mg a.i. kg^{-1} d.w.) [57]. Few studies have assessed the effects of herbicides on the growth of other terrestrial invertebrates. Gomes et al. [60] found effects of atrazine on *E. crypticus* growth (variable response depending on whether it is applied as a pure active substance or commercial formulation). Druart et al. [48] and Ogeleka et al. [58] found effects of glyphosate-based herbicides on the land snails *C. aspersus* (growth stimulation) and *Archachatina marginata* (growth inhibition), respectively. Herbicides can also affect invertebrate development, in this case, most of the studies being focused on arthropods. Exposure to recommended field application rates of commercial formulations of several herbicides (e.g., 2,4-D, atrazine, glyphosate) has been reported to affect the development of the beetle *Zygogramma bicolorata* [63]. Freydier and Lundgren [66] found negative effects of commercial formulations of 2,4-D and dicamba on the development of the ladybug *Coleomegilla maculata*, while no effects of glyphosate-based herbicides have been reported on the ladybug *Harmonia axyridis* [71]. Molting can also be affected by herbicide exposure in bees [69], collembolans [64], isopods [65], and spiders [76].

Behavior Some terrestrial invertebrates can avoid herbicides. This is the case, for instance, of *E. andrei* against metsulfuron-methyl [74], *E. crypticus* against atrazine [60], the collembolan *F. candida* against glyphosate [77], and the spider *Neoscona theisi* against glyphosate [46]. On the contrary, other studies revealed non-avoidance response of terrestrial invertebrates when exposed to herbicides [45, 74, 77]. The avoidance behavior of terrestrial invertebrates against herbicides may depend on specific soil properties. Chelinho et al. [73] assessed the avoidance response of *E. crypticus* against a phenmedipham-based herbicide in soils with different pH, organic matter, and texture and found higher toxicity in sandy soils with low pH.

The effect of herbicides on terrestrial invertebrate mobility is highly variable. Decreasing adult mobility is reported for *C. maculata* after exposure to 2,4-D and dicamba pure active substances [66]. Sanogo et al. [78] found immobility effects of commercial formulations of atrazine and diuron on beetles of the genus *Crenitis*. Higher activity has been reported for the spider *Pardosa milvina* when exposed to recommended field application rates of glyphosate [79]. In the case of *A. mellifera*, higher mobility has been found upon exposure to recommended field application rates of commercial formulations of bentazone but not for metamitron [80].

The effect of herbicides on terrestrial invertebrate fodder or prey consumption is also highly variable most of the studies being focused on some arthropod groups. Field-realistic concentrations of glyphosate did not affect the consumption of sugar-spiked solutions by *A. mellifera* [81], while decreasing fodder consumption has been reported upon exposure to bentazone and metamitron [80]. Recommended field application rates of 2,4-D and dicamba did not affect the prey consumption of *C. maculata* [66]. On the contrary, recommended field application rates of glyphosate increased the consumption of prey by *P. milvina* [79].

4.1.2 Insecticides

Bioaccumulation of Insecticides

Few authors have studied bioaccumulation of insecticides in nontarget terrestrial invertebrates in recent years. Qu et al. [82] reported similar bioaccumulation of two fipronil enantiomers (R and S) in *E. fetida*, although their degradation inside the body was enantioselective with a preference toward S-fipronil. Also, bifenthrin and lambda-cyhalothrin presented different bioavailability and enantioselective bioaccumulation in *E. fetida*, where the less toxic enantiomer was preferably bioaccumulated [83]. This is in line with the study of Ye et al. [84] on fenvalerate and esfenvalerate, with the latter characterized by higher toxicity and lower BAF (BAF 1.4–1.6 for fenvalerate and 0.8–0.9 for esfenvalerate). Liu et al. [85] reported concentration- and exposure time-dependent bioaccumulation of two dinotefuran metabolites in *E. fetida*. Besides chlorpyrifos hydrophobicity, Svobodová et al. [86] emphasized the role of soil organic matter and clay content in the bioavailability and bioaccumulation of this insecticide in *E. andrei*.

Effect of Insecticides at Sub-Organism Level

Insecticides can induce genetic alterations in terrestrial invertebrates. Several studies reported DNA damage in earthworms upon exposure to sublethal concentrations of cypermethrin (*Pheretima peguana*) [87], the neonicotinoids imidacloprid and dinotefuran (*E. fetida*) [88, 89], and the keto-enol insecticide spirotetramat (*E. fetida*) [90]. This is not in accordance with Wang et al. [91] who found no DNA damage in *E. fetida* exposed to the neonicotinoid guadipyr at concentrations up to 100 mg a.i. kg^{-1} d.w. Cardoso et al. [92] found DNA damage in *F. candida* at field-recommended concentrations of dimethoate (0.4 mg a.i. kg^{-1} d.w.). Proteins related to glycolysis can be affected by low doses (e.g., <NOEC for reproduction of 43.8 mg a.i. kg^{-1} d.w.) of tebufenozide in the collembolan *Yuukianura szeptyckii* [93]. Neonicotinoids can induce gene downregulation in the brain of honey bee workers, namely, those encoding the enzymes related to glycolysis and lipids. The authors argued that such effects could further impair honey bee physiology, behavior, and survival [94].

Insecticides can induce ROS production in terrestrial invertebrates, leading to alterations in the activity of antioxidant enzymes (e.g., *E. fetida* exposed to imidacloprid) (e.g., [88, 95]). Velki et al. [96] reported species-specific biomarker responses to organophosphate and pyrethroid insecticides in soil microcosms for several earthworm species (*E. andrei*, *L. terrestris*, *Lumbricus rubellus*, and *Octolasion lacteum*). The authors indicated higher responses to the organophosphates dimethoate and pirimiphos-methyl, especially for the activity of acetylcholinesterase, carboxylesterase, catalase, and glutathione S-transferases. Although lack of correlation between biomarker responses in *E. fetida* and the presence of organochlorine insecticides in agricultural soils was observed [97], neurotoxicity of dimethoate in the isopod *Porcellionides pruinosus* was documented, alongside with oxidative stress and lipid peroxidation [98]. Oxidative damage was also described for the land snail *Cantareus apertus* upon exposure to carbaryl-contaminated food [99]. Balieira et al. [100] reported activity of the antioxidant enzymes glutathione peroxidase and catalase in *A. mellifera* exposed to imidacloprid. However, Zhu et al. [101] found no effects of imidacloprid on the activity of esterase, acetylcholinesterase, glutathione S-transferases, and invertase in honey bees surviving a spray tower experiment.

Insecticides can affect the structure and integrity of terrestrial invertebrate cells. For instance, cypermethrin negatively affected cell viability in coelomocytes of

P. peguana, alongside the micronucleus frequency and pinocytic adherence activity [87]. Leomanni et al. [99] documented alterations of hemocyte lysosomal membrane stability in *C. apertus* as a consequence of carbaryl-induced oxidative stress.

Insecticide exposure can also lead to morphological and histological alterations in terrestrial invertebrates. Saxena et al. [102] reported that carbamide (carbaryl and carbofuran) and synthetic pyrethroid (cypermethrin and fenvalerate) insecticides led to cuticular membrane damage and disintegration of circular and longitudinal muscles in the earthworms *E. andrei* and *Metaphire posthuma*. Neonicotinoids can induce disruption of the epidermis and midgut tissue in *E. fetida* [95]. The organophosphate insecticide monocrotophos can induce morphological and histological changes in *E. eugeniae* (e.g., clitellum swelling, epithelial cells damage) [44].

Effects of Insecticides at Individual and Population Levels

Life-History Traits Terrestrial invertebrate survival has been largely assessed in scientific studies on insecticides (e.g., [75, 103–106]). The survival of the isopod *Porcellio scaber* can be affected by imidacloprid and thiacloprid (LC_{50} 7.6 and 32 mg a.i. kg^{-1} d.w., respectively), but not by chlorantraniliprole [104]. Salvio et al. [107] reported no mortality of the slug *Milax gagates* upon exposure to chlorpyrifos and cypermethrin. These insecticides, however, had a lethal effect on the isopod *Armadillidium vulgare* [107]. Insecticide metabolites can be more toxic than the corresponding parent compounds (e.g., pyriproxyfen metabolites in *E. fetida*) [108]. Zhu et al. [101] evaluated the survival of *A. mellifera* workers exposed to imidacloprid in a spray tower experiment. While the concentrations >80 mg a.i. L^{-1} caused mortality, the bees continued dying even after 48 h of posttreatment time. This is one example that illustrates the situation in which short-term toxicity tests may not show possible long-term consequences of chemicals exposure. The same study underlines the importance to address different exposure duration for insecticides with different physicochemical characteristics.

Diverse effects of insecticides on terrestrial invertebrate reproduction are documented. According to Leitão et al. [106], the organophosphate ethoprophos induced lower reproductive output in laboratory bioassays with *F. candida* (EC_{50} 0.03 mg a.i. kg^{-1} d.w.), compared with *E. andrei* and *E. crypticus* (EC_{50} 8.3 and 68.5 mg a.i. kg^{-1} d.w., respectively). These authors reported negative effects of ethoprophos on *F. candida* and *E. andrei* reproduction upon exposure to soils collected from a greenhouse experiment [109]. *F. candida* reproduction was also affected by chlorantraniliprole, with lower toxicity in soils with higher organic matter content. Toxicity was not observed in the case of *E. crypticus* and the mite *Oppia nitens* reproduction [104]. de Lima e Silva et al. [105] found higher sensitivity of *F. candida* and *E. andrei* reproduction to neonicotinoids than for *O. nitens*. Both imidacloprid and fipronil affected the reproduction of *F. candida* at doses comparable to PEC values (0.230 and 0.096 mg a.i. kg^{-1} d.w., respectively) [103]. A consistent reproduction response of *F. candida* to imidacloprid was found over three generations, while for thiacloprid recovery was obtained from the second generation [110]. Such responses were explained by the persistence of imidacloprid versus fast degradation of thiacloprid. Multigenerational and transgenerational exposures to the pyrethroid etofenprox induced significant changes in egg size of *F. candida*, which can imply severe consequences at the population level [111]. Bori et al. [112] evaluated commercial formulations of imidacloprid covering from the manufacturer recommended doses to the worst-case scenario representing an excessive application (0.13–2 mg a.i. kg^{-1} d.w.). The authors found effects on *E. fetida* reproduction (EC_{50} 1.4 mg a.i. kg^{-1} d.w.; NOEC 1 mg a.i. kg^{-1} d.w.), but not on *F. candida*, and argued that adjuvants and solvents present in the formulation might have contributed to toxicity. The need for more scientific studies on ecotoxicity and risk assessment of adjuvants was also highlighted in the review by Mesnage and Antoniou [113].

Insecticides may impair the growth and development of terrestrial invertebrates. Body weight reduction of *E. fetida* was reported upon exposure to commercial formulations of both organophosphates (field-recommended dose of 47 mg a.i. kg^{-1} d.w. and above it) and imidacloprid (0.13–2 mg a.i. kg^{-1} d.w.) [75, 112]. Body weight of *P. scaber* was not affected by chlorantraniliprole [104], but it was significantly lower in the presence of thiacloprid [105]. The molting frequency of *Y. szeptyckii* was affected by tebufenozide [93]. Yu et al. [114] reported no effects of imidacloprid on the development time, pupation, and adult emergence of the ladybug *Coccinella septempunctata*, while egg production and hatching were compromised.

Behavior Avoidance is a commonly reported endpoint in laboratory insecticide exposure studies. Bori et al. [112] found avoidance behavior of *E. fetida* against soils spiked with an imidacloprid-based formulation, while this was not the case of *F. candida*. Avoidance of the predatory mite *Hypoaspis aculeifer* was a sensitive endpoint in exposure to deltamethrin, dimethoate, and chlorpyrifos [115]. However, avoidance/preference behavior can provide false-positive results. For instance, the ryanoid insecticide chlorantraniliprole impacted *F. candida* locomotion, preventing them from avoiding the spiked soils [104].

Flight behavior of honey bees has been used as an endpoint upon insecticide exposure. Williams et al. [116] found no effects of field concentrations of the neonicotinoids thiamethoxam and clothianidin (4 and 1 μg a.i. kg^{-1} d.s., respectively) measured in pollen on the mating flight behavior of honey bee queens. These authors, however, emphasized that their observations were not aligned with other studies regarding honey bee workers (references cited by Williams et al. [116]).

The impact of insecticides on terrestrial invertebrate prey or fodder consumption may vary. Maple leaf consumption by *P. scaber* was reduced in imidacloprid-spiked soils (EC_{50} 6.7 mg a.i. kg^{-1} d.s.) [105]. Byrne et al. [117] found no effect of imidacloprid on the consumption of honey bees foraging on citrus flowers. Overmyer et al. [118] documented negative effects on *A. mellifera* feeding of thiamethoxam at concentrations >100 mg a.i. L^{-1} at the individual level and >50 mg a.i. L^{-1} at the colony level, both in the range of concentrations reported in other field studies. Wang et al. [119] reported increased food consumption and stimulated digging and foraging in invasive ants exposed to low doses of imidacloprid (0.01 μg a.i. L^{-1}) but suppression above 0.25 μg a.i. L^{-1}. These authors raised the concern that such complex behavioral changes in invasive ants might occur with other beneficial ant species upon exposure to neonicotinoids. Low levels of imidacloprid affected ladybugs by reducing their consumption of aphids, ultimately reducing adult body weight and inducing slower development, underlining the relevance of looking into effects on predatory species [120]. This agrees with Bredeson et al. [121] who reported altered quality of *Rhopalosiphum padi* aphids for the predatory species *C. maculata* upon exposure to thiamethoxam.

4.1.3 Fungicides

Bioaccumulation of Fungicides

Most of the current studies do not consider the bioaccumulation of organic fungicides in terrestrial invertebrates, probably because of their complex analysis. The available studies are only focused on earthworms, showing, for example, bioaccumulation of tebuconazole, furalaxyl, pentachloronitrobenzene, and tolclofos-methyl in *E. fetida* [86, 122–124]. Moreover, fungicide bioaccumulation can be related to specific soil properties, such as organic matter and clay content, which can increase sorption and decrease fungicide bioavailability in soils [86].

Effect of Fungicides at Sub-Organism Level

Few studies evaluated genotoxicity of fungicides on terrestrial invertebrates. Certain fungicides, like carbendazim, induced DNA damage in coelomocytes of *E. fetida* at concentrations above 0.4 mg a.i. kg^{-1} d.w. after 7 days of exposure [125]. Chlorothalonil can impact *F. candida* by altering several pathways, including detoxification and excretion, immune response, cellular respiration, protein metabolism, and oxidative stress defense [126]. In the same species, Qiao et al. [127] revealed a general downregulation of the expression levels of multiple genes when exposed to 87 mg a.i. kg^{-1} d.w. of pentachlorophenol. Fungicides can also induce transcriptional alterations in genes encoding enzymes related to oxidative phosphorylation and metabolism in bees [128].

Fungicides can increase ROS production and induce oxidative damage. For example, both pentachloronitrobenzene (0.1 mg a.i. kg^{-1} d.w.) and tolclofos-methyl (0.01 mg a.i. kg^{-1} d.w.) induced ROS production and increased lipid peroxidation in *E. fetida* despite the higher activity of the enzyme superoxide dismutase [122]. Wang et al. [129] also found alterations in the antioxidant defense system of *E. fetida* exposed to dimethomorph but only above the recommended application rates ($>$100 mg a.i. kg^{-1} d.w.). Beyond oxidative damage, other biochemical responses can be sensitive to fungicides as shown by Rico et al. [130] in *E. fetida* exposed to carbendazim, tebuconazole, and prochloraz (alterations on cholinesterase, dehydrogenase, and alkaline phosphatase enzyme activities). Morgado et al. [131] reported higher metabolic costs (energy reserves and consumption) of mancozeb exposure to early life stages of *P. pruinosus*.

In the last years, very few studies described the possible effects of fungicides on morphological and histological alterations in terrestrial invertebrates. One example is the finding that carbendazim (4 mg a.i. kg^{-1} d.w.) and prochloraz (286 mg a.i. kg^{-1} d.w.) can induce morphological changes in the body wall and gastrointestinal tract of *E. fetida* [132].

Effects of Fungicides at Individual and Population Levels

Fungicides can affect the survival, growth, and reproduction of terrestrial invertebrates. The majority of the studies used earthworms as model species, exposing them to azoxystrobin, carbendazim, chlorothalonil, dimethomorph, furalaxyl, mancozeb, pentachloronitrobenzene, prochloraz, tebuconazole, and tolclofos-methyl [106, 122, 123, 129, 130, 132, 133]. Few other soil invertebrates have been used to study fungicide effects: the enchytraeids *Enchytraeus albidus* and *E. crypticus* [106, 134, 135], *F. candida* [107, 136, 137], *P. pruinosus* [131], and *H. aculeifer* [134]. For most of these studies, a negative impact on at least one life-history parameter is described when invertebrates are exposed to fungicide-spiked soils. Fungicides can also induce effects on bees such as the timing of pupation and metamorphosis into adult bees [138], decrease in larval survival and malformations during development [139], or even negatively impact colony health [140].

Schnug et al. [141] used a soil-multi-species test system with four different collembolan species and one earthworm species exposed to picoxystrobin for 8 weeks. The authors found a lower sensitivity of *F. fimetaria* compared to the other collembolan species and that earthworm performance was correlated to both collembolan abundance and bait-lamina consumption.

4.1.4 Molluscicides

There is a lack of information, in the past 5 years, on the bioaccumulation and effects at the sub-organism level of molluscicides in terrestrial invertebrates, so only effects at individual and population levels are presented.

Effect of Molluscicides at Individual and Population Levels

Life-History Traits Several studies described the adverse effects of molluscicides on the survival of target organisms, such as slugs and snails. McDonnell et al. [142] evaluated the potential molluscicidal action to the land snail *Cornu aspersum* of several essential oils (bitter orange, cedarwood, cinnamon, clove bud, eucalyptus, garlic, lemongrass, peppermint, pine, rosemary, and spearmint) and the terpene d-limonene. The clove bud oil was the most effective (LC_{50} 0.03%), followed by pine (LC_{50} 0.08%) and spearmint (LC_{50} 0.10%) oils, while d-limonene showed the lowest toxicity. The high efficacy of the clove bud oil can be related to its high content of eugenol which has known insecticidal and herbicidal effects [142].

Among the nontarget organisms, earthworms are one of the most affected groups by molluscicidal baits [143]. However, recent studies have suggested that recommended agricultural doses of metaldehyde-based molluscicides have no deleterious effect on the survival and growth of *E. fetida* and *L. terrestris* [75, 144]. For other terrestrial invertebrates, Cardoso et al. [145] evaluated the effects of metaldehyde and methiocarb bait products to *F. candida* by exposing organisms to single and pulse (recommended application mode by manufactures) doses. The authors showed higher toxicity of metaldehyde to collembolan survival (LC_{50} 102.4 and 69.6 mg a.i. kg^{-1} d.w. for single and pulse exposure to metaldehyde, respectively; no effects of methiocarb), while methiocarb affected reproduction more (EC_{50} 58.4 and 19.8 mg a.i. kg^{-1} d.w. for single and pulse exposure to metaldehyde, respectively; EC_{50} 39.1 and 12.5 mg a.i. kg^{-1} d.w. for single and pulse exposure to methiocarb, respectively).

Behavior Molluscicides exposure may alter the feeding behavior of target organisms. This is, for example, the case of the slug *Arion vulgaris* exposed to metaldehyde, especially in less irrigated systems, as slug recovery is affected in drier environments and also because watering reduction diminishes molluscicide losses by leaching [144]. Cardoso et al. [145] found no effects of metaldehyde baits on the avoidance behavior of *F. candida.* They also found a preference response for methiocarb baits, which may indicate no adverse effects of this molluscicide or even the presence of some attractants in their composition.

4.2 Pharmaceuticals: Veterinary and Human

4.2.1 Bioaccumulation of Pharmaceuticals

Bioaccumulation studies of pharmaceuticals in terrestrial invertebrates are scarce in the recent literature, and only a few reports using earthworm species are available. Carter et al. [146] evaluated the fate and uptake of different human pharmaceuticals including the antiepileptic carbamazepine (39 μg kg^{-1} d.w.), the anti-inflammatory diclofenac (49 μg kg^{-1} d.w.), the antidepressant fluoxetine (80 μg kg^{-1} d.w.), and the lipase inhibitor orlistat (65 μg kg^{-1} d.w.) using *E. fetida*. These pharmaceuticals accumulated in the earthworms, with BAF values ranging from 2.3 for carbamazepine to more than 22 for orlistat. Soil properties (mainly pH) are also essential factors that would change the uptake and accumulation of pharmaceuticals by earthworms [147].

4.2.2 Effects of Pharmaceuticals at Sub-Organism Level

Pharmaceuticals can affect terrestrial invertebrates by inducing genotoxicity. Gao et al. [148] described alterations in the expression levels of two target genes in different segments of *E. fetida* exposed to the veterinary pharmaceutical albendazole for 14 days. Regarding human pharmaceuticals, Chen et al. [149] reported effects of diclofenac on neural metabolic processes in *F. candida* at 200 mg kg^{-1} d.w., as well on the upregulation of immunity-related genes.

Pharmaceuticals can also induce biochemical alterations in terrestrial invertebrates. For human pharmaceuticals, Oliveira et al. [150] described increasing lipid peroxidation levels and inhibition of the enzyme acetylcholinesterase in *F. candida* exposed for 96 h to the antiepileptic carbamazepine (4 mg kg^{-1} d.w.) and the antidepressant fluoxetine (0.4 mg kg^{-1} d.w.), respectively. Using the same compounds, but on a multigeneration approach, Oliveira et al. [151] also found increasing oxidative stress and impaired neurotransmission in *F. candida*, especially following carbamazepine exposure at field-realistic concentrations. For veterinary pharmaceuticals, Guimarães et al. [136] observed that the antioxidant mechanisms of *F. candida* were dynamically activated along with generations when exposed to 1 mg kg^{-1} d.w. of ivermectin.

4.2.3 Effects of Pharmaceuticals at Individual and Population Levels

Life-History Traits Human pharmaceuticals can affect terrestrial invertebrates with adverse effects on survival, growth, and reproduction. For example, this was the case of *F. candida* exposed to fluoxetine, carbamazepine, and diclofenac [149–151]. Pino et al. [152] evaluated the lethal toxicity of a battery of 18 human pharmaceuticals such as nonsteroidal anti-inflammatory drugs, blood lipid-lowering agents, β-blockers, and antibiotics to *E. fetida*. From all the tested compounds, ibuprofen (LC_{50} 64.8 mg kg^{-1} d.w.) showed the highest acute toxicity to earthworms, followed by diclofenac (LC_{50} 90.5 mg kg^{-1} d.w.) and simvastatin (LC_{50} 92.7 mg kg^{-1} d.w.).

Veterinary pharmaceuticals are also the focus of different studies using terrestrial invertebrates. A battery of pharmaceuticals (ivermectin, fipronil, fluazuron, and closantel) has been evaluated using *F. candida* in tropical Brazilian soils [153]. The results confirmed higher chronic toxicity of fipronil (EC_{50} 0.19 mg kg^{-1} d.w.) and ivermectin (EC_{50} 0.43 mg kg^{-1} d.w.), followed by fluazuron (EC_{50} 3.07 mg kg^{-1} d.w.). Closantel did not show severe effects on *F. candida*. Alves et al. [154] reported adverse effects of fluazuron on the reproduction of *E. andrei* and *F. candida* (EC_{50} 20.8 mg kg^{-1} d.w. and 4.48 mg kg^{-1} d.w., respectively). The same species have been used to assess the effects of nicarbazin and monensin used in the poultry industry [155]. Monensin showed the highest toxicity, especially in terms of collembolans reproduction (EC_{50} 101 mg kg^{-1} d.w.) [155].

Behavior The few available studies in this field indicate that some terrestrial invertebrates can avoid pharmaceutical-spiked soils. This is, for example, the case of *F. candida* against carbamazepine, using a light avoidance innovative test at very low concentrations (AC_{50} 0.04 mg kg^{-1} d.w.) [151]. Alves et al. [154] found avoidance response of *F. candida* and *E. andrei* against fluazuron (AC_{50} 1.73 and 4.78 mg kg^{-1} d.w., respectively), highlighting the higher sensitivity of this behavioral response compared to reproduction (EC_{50} 20.8 mg kg^{-1} d.w.).

4.3 Polycyclic Aromatic Compounds

4.3.1 Bioaccumulation of Polycyclic Aromatic Compounds

Bioaccumulation of PACs has long been regarded as an environmental concern. Early toxicokinetic studies confirmed the bioaccumulative potential and identified main uptake routes (e.g., [156, 157]). Soil properties and aging time were found crucial for PAC bioaccumulation in earthworms, leading to marked differences in BAF and toxicokinetic parameters [158]. A peak-shaped accumulation curve was reported for phenanthrene and pyrene, resulting from the degradation and desorption, with consequent reduction of PAC bioavailability [158]. BAFs were significantly higher for soils with high total organic carbon, ranging between 2.1–37.2 for phenanthrene and 2.0–26.1 for pyrene. The distribution of accumulated PACs within soil organisms is another topic explored in recent years. A hierarchical method for extending whole-organism toxicokinetic studies was described, by addressing sub-organism, tissue, and subcellular fractionation of phenanthrene in *E. fetida* [159]. Phenanthrene partition varied dynamically with exposure concentration and through time, probably distributed by the earthworm circulatory system [159]. Heterogeneous distribution at organ level may reflect not only the main routes of exposure but also the ability of earthworms to transport PACs toward less susceptible body locations or where detoxification takes place [160]. These processes are species-specific and valuable for explaining general or endpoint-specific differences in sensitivity to PACs [160]. Ecophysiology traits might mediate PAC exposure, leading to different BAFs, as shown by Zhang et al. [160] for *E. fetida* (BAF 8.64), *Pheretima guillelmi* (BAF 107), and *M. guillelmi* (BAF 350). No differences were, however, found between *E. fetida* and another endogeic earthworm species (*Aporrectodea caliginosa*), which highlights the complex and sometimes conflicting results of PAC bioaccumulation within the soil compartment. Bioaccumulation of field-relevant PAC mixtures has also been assessed, including field-contaminated soils, soil amendments, or relevant mixtures/formulations containing multiple PACs (i.e., lubricants, oils). Rorat et al. [161] assessed PAC bioaccumulation in *E. andrei* exposed to sewage sludge in vermicomposting experiments for 5 weeks. Body concentrations in earthworms depended on the vermicomposting mixture used, leading to distinct accumulation patterns of individual PACs, even though total PAC mixtures did not show evidence of bioaccumulation (BAF 0.07–0.74)

[161]. Recent studies reported increased bioavailability of PACs from biochar-amended soils. For instance, Malev et al. [162] reported PAC bioaccumulation in *E. andrei* after exposure to a biochar-soil matrix. Prodana et al. [163] found increased levels of naphthalene-type metabolites in earthworm tissue upon exposure to soil amended with woodchip biochar particles.

4.3.2 Effect of Polycyclic Aromatic Compounds at Sub-Organism Level

Some PACs can be genotoxic to terrestrial invertebrates. Benzo[a]pyrene induced DNA damage to coelomocytes of *E. fetida* at 1 mg kg^{-1} d.w. [164]. A similar result was reported for *E. andrei* in Sforzini et al. [165]. The genotoxicity caused by some PACs (including benzo[a]pyrene) has been attributed to a biotransformation product by microsomal monooxygenases cytochromes P450 [166]. PACs were also linked to genotoxic effects arising from exposures to environmentally relevant complex mixtures (e.g., oil-contaminated soil [167]). Benzo[a]pyrene decreased lysosomal membrane stability in coelomocytes and chloragogenous tissue of *E. andrei* and increased neutral lipid accumulation and lysosomal/cytoplasmic volume ratios [165]. Alterations in ROS-scavenging enzymes and oxidative stress levels have also been reported. Duan et al. [164] found changes in ROS-scavenging enzymes (superoxide dismutase and catalase) in *E. fetida* after 14 days of exposure to benzo[a]pyrene, but not lipid peroxidation at concentrations below 500 mg kg^{-1} d.w. For the same species and chemical, Ye et al. [168] denoted an increase in superoxide dismutase and peroxidase activities and failure to reach a new homeostasis status after 56 days at 10 mg kg^{-1} d.w. Glutathione S-transferases alterations were reported for phenanthrene and fluorene in *E. fetida* [169]. Recent OMICS have highlighted important differences in toxicity pathways elicited by PACs to soil organisms, as shown by Roelofs et al. [170] for *F. candida* and *E. crypticus* after exposure to phenanthrene. Whereas no strong induction of biotransformation pathways was observed in *E. crypticus*, upregulation of genes encoding all phases of biotransformation/detoxification (I/II/III) was found in *F. candida*. Similarly, Holmstrup et al. [171] found upregulation of genes related to biotransformation/detoxification and general stress handling proteins (i.e., Hsp70) in *F. candida* exposed to phenanthrene.

4.3.3 Effect of Polycyclic Aromatic Compounds at Individual and Population Levels

Most of the recent work on the toxicity of PACs to terrestrial invertebrates has been conducted with earthworms (e.g., [172], collembolans [173], and, to a lesser extent, mites [115] and isopods [174]). Overall, collembolans are the most sensitive group, particularly when considering survival (e.g., LC_{50} values generally one order of magnitude lower than those for enchytraeids) (see [175, 176] and references therein). Earthworms have generally proved lower sensitivity than collembolans but higher than enchytraeids. However, Gainer et al. [177] showed greater sensitivity of earthworms to lubricating mixtures including PACs and aliphatic compounds probably related to a higher uptake due to their bigger size. Deviations from nonpolar narcosis might occur for sublethal endpoints, indicating that more specific responses might be present [178]. This makes it difficult to predict species-specific sublethal responses and compels a case-by-case analysis of their ecotoxicological importance. For instance, earthworm growth inhibition was a sensitive endpoint for some PACs [137], and so was biomass variation in terrestrial isopods [174]. Phenanthrene-contaminated soils triggered avoidance responses of *E. fetida* [173] and *H. aculeifer* [115], but not of *E. crypticus* and *F. candida* [173]. Again, slightly different results can be obtained for mixtures containing PACs, such as lubricating oils, which caused strong avoidance responses of *E. fetida*, *F. candida*, *O. nitens*, and *H. aculeifer* with only *E. crypticus* showing no response [179].

4.4 Polychlorinated Biphenyls

4.4.1 Bioaccumulation of Polychlorinated Biphenyls

Understanding the bioaccumulation patterns has long been a priority for PCBs due to their high stability and hydrophobicity. However, bioaccumulation studies with PCBs in terrestrial invertebrates were almost exclusively conducted with earthworms. In recent years, the main focus is on understanding PCB bioaccumulation patterns under a wide range of exposure conditions. Differences in the toxicokinetics of PCBs were found for natural soils with markedly distinct properties, including different uptake and elimination rate constants and time to reach internal steady-state concentrations [158]. Moreover, earthworm density and, mostly, feeding activity can also mediate bioaccumulation of PCB 153, with non-fed earthworms showing twofold higher BAFs than fed individuals [180]. Assessing stereoselective bioaccumulation of chiral PCBs in earthworms has been a recent line of research. For example, significant stereoselectivity for PCBs 91, 95, and 149 during uptake and elimination phases has been shown in *E. fetida*, leading to variable enantiomer fractions over time [181, 182]. This indicates that toxicokinetics is partly driven by biological processes. An additional line of bioaccumulation-related research has focused on assessing the efficiency of soil amendments in the remediation of PCB-contaminated soils. Although promising as a remediation tool for PCBs, variable biota bioaccumulation patterns highlight the complexity related to product properties, application doses, protocols, and time, among others (e.g., [183, 184]).

4.4.2 Effect of Polychlorinated Biphenyls at Sub-Organism Level

Ecotoxicity studies evaluating sub-organism level effects of single PCBs in terrestrial invertebrates are scarce and most date back to the 1990s. Most of these studies focused on earthworm coelomocyte immunoassays as surrogates for mammalian toxicology and reported, among others, decreased immunocompetence and macrophage-related functions (e.g., [185]). There is a paucity of new approaches on PCB toxicity to terrestrial invertebrates. Recent studies denoted the induction of DNA damage to coelomocytes of *E. fetida* exposed to soil spiked with a standard PCB mixture at 0.25 mg kg^{-1} d.w. [186]. Dose-dependent increases of the ROS-scavenging enzymes (catalase, superoxide dismutase, and peroxidase) were also found in PCB-spiked soils, without signs of lipid peroxidation [186]. Similarly, Shen et al. [187] also found increased activity of antioxidant enzymes in earthworms exposed to field soils contaminated with PCBs.

4.4.3 Effect of Polychlorinated Biphenyls at Individual and Population Levels

As for sub-organism approaches, few ecotoxicity studies at the individual level have been conducted in recent years. Duan et al. [186] found growth inhibition in *E. fetida* exposed to a standard PCB mixture, with significant effects registered at lower concentrations than for effects on oxidative stress enzymes [186].

4.5 Flame Retardants

4.5.1 Bioaccumulation of Flame Retardants

There is a lack of recent information on the bioaccumulation of flame retardants in terrestrial invertebrates. A higher bioaccumulation potential was found for perfluoroalkyl substances, compared to halogenated flame retardants, in *E. andrei* exposed to an agricultural soil amended with anaerobically digested municipal waste and composted sludge [188]. Huang et al. [189] reported bioaccumulation of decabromodiphenyl ether (DecaBDE) in *P. guillelmi*. Using ^{14}C labeled-DecaBDE, these authors found that DecaBDE extractable fraction may lead to underestimating the total bioaccumulated DecaBDE. Low bioaccumulation potential of tri-n-butyl phosphate (TBP) in the earthworm *Perionyx excavatus* was reported by Wang et al. [190]. These authors also detected TBP biotransformation products, revealing specific detoxification mechanisms in *P. excavatus* for this xenobiotic.

4.5.2 Effects of Flame Retardants at Sub-Organism Level

Liang et al. [191] reported that 2,2′,4,4-tetrabromodiphenyl ether (BDE-47) and decabromodiphenyl ether (BDE-209) altered energy- and amino acid-related metabolism and the nerve activity in *E. fetida*. Shi et al. [192] reported the upregulation of superoxide dismutase and heat shock protein Hsp70 gene expression upon exposure of *E. fetida* to hexabromocyclododecane and tetrabromobisphenol A (TBBPA), with the latter inducing higher effects. Dechlorane plus, a polychlorinated flame retardant, induced oxidative stress and genotoxicity in *E. fetida* [193].

4.5.3 Effects of Flame Retardants at Individual and Population Levels

TBBPA induced higher mortality for *M. guillelmi* than for *E. fetida* [194]. The authors argue that this difference could be related to the distinct exposure routes of both earthworm species, as *M. guillelmi* is more exposed to TBBPA through soil particle ingestion while *E. fetida* mainly through dermal uptake. Shi et al. [192] reported increased *E. fetida* body mass upon exposure to TBBPA. As reviewed by Rothenbacher et al. [195], the most sensitive endpoint for TBBPA was *E. andrei* reproduction (EC_{50} 0.12 mg kg^{-1} d.w.) and has been used to derive the predicted no effect concentration of 0.012 mg kg^{-1} d.w. Since the early 2000s, there are no updates regarding the endpoints of interest for the risk assessment of TBBPA.

4.6 Personal Care Products

4.6.1 Bioaccumulation of Personal Care Products

Similar to the majority of previously referred compounds, only a few studies cover the bioaccumulation of personal care products in terrestrial invertebrates. Most of the recent studies focused on the antimicrobial agent triclosan and its main soil metabolite (methyl-triclosan), with special attention to earthworm bioaccumulation. For instance, Chevillot et al. [196] assessed the bioaccumulation of these compounds in *E. andrei* exposed to both a triclosan-spiked soil (BAF 2.6 and 0.5 for triclosan in juveniles and adults, respectively; no detection of methyl-triclosan) and a soil amended with biosolids from a wastewater treatment plant containing triclosan (BAF 2.0–2.5 for triclosan and methyl-triclosan). Macherius et al. [197] also evaluated the bioaccumulation of triclosan and methyl-triclosan in different earthworm species of a soil amended with biosolids. The parent compound showed higher BAFs compared to the metabolite (4.2–13.9 for triclosan and 1.2–5.1 for methyl-triclosan). Both studies concluded that the presence of methyl-triclosan in earthworm tissues is also related to triclosan methylation inside the organism. Havranek et al. [198] evaluated the bioaccumulation of triclosan, galaxolide, and tonalide in the earthworm *Dendrobaena veneta* exposed to a soil amended with contaminated sludge. The authors found the higher transfer of triclosan from the sludge to the earthworms (transfer factor 0.8) than those of galaxolide (transfer factor 0.1) and tonalide (transfer factor 0.02). These results could be explained from the possible excretion and/or metabolization of galaxolide and tonalide in earthworms compared to triclosan. Rivier et al. [199] described greater bioaccumulation of triclosan, compared to galaxolide and tonalide, in *A. caliginosa* exposed to a soil amended with contaminated sludge.

4.6.2 Effect of Personal Care Products at the Sub-Organism Level

Personal care products can induce genotoxicity in terrestrial invertebrates. Some authors indicate that triclosan can induce DNA damage to earthworm coelomocytes (e.g., *E. fetida*; EC_{50} 8.9 mg kg^{-1} d.w.) [200], while others describe no effects [196]. Triclosan can also alter the transcriptional expression levels of some genes as described by Lin et al. [200] for the heat shock protein Hsp70 gene in *E. fetida* (upregulation after triclosan exposure; EC_{50} 1.8 mg kg^{-1} d.w.). Novo et al. [8] evaluated the effect of an organic UV filter (4-hydroxibenzophenone, 4-OHBP) on the transcriptional expression levels of endocrine, stress, and energy-related genes in *E. fetida*. Exposure to 4-OHBP induced an increase of the ecdysone receptor gene (endocrine-related gene), while it decreased the genes CuZn superoxide dismutase (oxidative stress-related gene) and glyceraldehyde-3-phosphate dehydrogenase (energy metabolism-related gene).

Personal care products can also induce alterations at the biochemical level. Ma et al. [201] indicated that triclosan could stimulate the antioxidant defense machinery of *E. fetida* (e.g., enzymes superoxide dismutase, catalase, and peroxidase). Despite the induced antioxidant activity, it may not be enough to protect organisms from oxidative damage as indicated by the increased lipid peroxidation. Wang et al. [202] evaluated the effects of triclosan on the activity of the enzymes superoxide dismutase, catalase, and peroxidase of the land snail *Achatina fulica*. Increasing enzyme activity levels were found upon exposure to low concentrations. However, catalase and peroxidase activity inhibition occurred at high concentrations leading to increased lipid peroxidation.

4.6.3 Effect of Personal Care Products at Individual and Population Levels

Personal care products can negatively affect terrestrial invertebrate survival. This is, for example, the case of *F. fimetaria* and *A. fulica* exposed to triclosan [202, 203]. However, there are also studies indicating no effects of triclosan on earthworm survival [196, 198]. Besides the variable effects reported on survival, triclosan generally alters reproduction. Lin et al. [200] described reduced reproduction in *E. fetida* exposed to triclosan. Chevillot et al. [196], however, found positive effects of triclosan on *E. andrei* reproduction. Personal care products can induce both increased (e.g., *D. veneta* exposed to triclosan, galaxolide, and tonalide; *E. andrei* exposed to triclosan) and decreased (e.g., *E. fetida* and *A. fulica* exposed to triclosan) invertebrates' growth [196, 198].

4.7 Mixtures

Agricultural practices are a good example of complex exposures that vary in their composition in time and concentration, where pesticides are applied in pulses, in a sequence, or simultaneously. This leads to a complexity of effects due to TKTD processes that vary depending on the mode of action of the substances, the organisms' physiology, and sensitivity to the substances. In addition, there are several processes and interactions that may occur leading to differences in responses: (1) chemical and physicochemical interactions, affecting exposure and bioavailability; (2) physiological interactions at uptake sites, interfering with the quantity taken up by organisms; (3) physiological and biochemical interactions during internal processing leading to a certain amount of substance available at the molecular target site; and (4) interactions at the target site(s), leading to different processes on intoxication.

Several models have been used to predict mixture toxicity, some based on old pharmacological models: the concentration addition and independent action models, which differ regarding the concept of the similarity or dissimilarity of chemical modes of action, respectively. These two models assume that there is no chemical interaction inside the organism and that chemicals may act as dilutions of each other (concentration addition) or are response additive, measuring the joint probability of effect from all chemicals in the mixture (independent action) [204].

In the work of Morgado et al. [205], a multiple biomarker approach was used to infer on possible time-dependent mechanisms of chlorpyrifos and mancozeb mixtures in the terrestrial isopod *P. pruinosus*. At recommended doses for agriculture practices, isopods revealed impaired detoxification and oxidative stress-related enzymes, although with some ability to recover and with juveniles showing higher stress upon exposure than adults. This difference regarding age or state was seen especially for energy-related parameters, showing associated metabolic costs.

The ladybug *C. maculata*, a beneficial insect in cropland, is prone to be exposed to pesticide mixtures. In the study of Freydier and Lundgren [66], second instars of ladybugs were exposed to nonlethal effects of 2,4-D and dicamba applied as pure active ingredients and in commercial formulations. The commercial formulations were more toxic than the active ingredients, showing adjuvants increase the efficacy of these compounds in nontarget species. Effects were observed at the survival level of organisms, growth, and the proportion of males produced. Although the authors conclude that dicamba did not increase the lethality of 2,4-D to ladybug larvae, no clear conclusion was derived regarding the interaction pattern occurring when these two formulations were mixed. This highlights the need for complex experimental designs, in order to cover a high range of exposure doses, which enables the prediction of toxicity using the already mentioned conceptual models and deriving interaction patterns like synergism or antagonism.

In the study of de Santo et al. [206], three soil invertebrates, *E. andrei*, *E. crypticus*, and *P. minuta*, were exposed in a laboratory trial to the herbicide metsulfuron-methyl and also to its mixture with mineral oil (as adjuvant).

The herbicide at the recommended dose did not represent any harm to the test species, but when used along with the mineral oil, effects on reproduction were observed for the three species. The combination of the herbicide and the mineral oil did not affect the feeding activity of soil fauna, in a field trial.

Besides mixtures of two, three, or four organic compounds, studies with more complex mixtures are scarcer. One example is the long-term study of Chevillot et al. [207] where *E. fetida* was exposed to complex mixtures of 7 neonicotinoids, 54 pesticides (including the previous 7 neonicotinoids), and 69 organic compounds (54 pesticides and 15 pharmaceuticals), using artificial soil at relevant field measured concentrations. Bioaccumulation of neonicotinoids under a joint exposure to low concentrations of multiple organic compounds was related to other individual (e.g., decrease in reproduction) and molecular (e.g., DNA damage) adverse effects.

Considering the predictions from the IPCC-Intergovernmental Panel on Climate Change, deviation of mixture toxicity from the expected patterns has also been highlighted due to changes in exposure conditions (e.g., soil moisture, temperature). In the study of Morgado et al. [131], the isopod *P. pruinosus* was exposed to chlorpyrifos and mancozeb at different soil moisture contents (mimicking drought and flood scenarios). Moisture did not affect the mixture toxicity, where additivity was the more parsimonious pattern observed. However, soil moisture content did influence the effects of individual pesticides and, as a consequence, of the pesticide mixture itself, with the major contribution for toxicity arising from the interaction of each pesticide with in the soil mixture.

In the study of Bednarska et al. [208], the earthworm *E. fetida* was exposed to chlorpyrifos, copper, and different temperatures (10 and 20°C). Chlorpyrifos significantly affected acetylcholinesterase activity, while Cu induced low levels of effect with no potentiation in joint exposures. The assimilation rate constant for chlorpyrifos was higher at 20°C for the single chlorpyrifos exposure, but also under co-exposure with Cu, the elimination rate constant behaved similarly, being only significant for chlorpyrifos single exposure.

5 Bioaccumulation in Edible Terrestrial Invertebrates: Link to Human Exposure

One of the major concerns for the next 30 years is how to feed the 9 billion people that the world is expected to have in 2050 [209]. Oceans are overfished, the land is overexploited, and climate change and water scarcity may lead to the search for innovative food production solutions [210]. The farming of edible insects has been presented as one of the best sustainable solutions, challenging the reuse of sub-products and other wasted feedstocks, reintroducing these components into the food value chain [209, 210]. Insects have a high content of nutrients and proteins, and their use as food has valuable environmental advantages over conventional meat, producing nutritional food sources with low environmental impact.

The data available on the transfer of chemical contaminants from different substrates to the insects is minimal, and there is a need to comply with the applicable food safety regulations, especially for residues of pesticides, veterinary pharmaceuticals, and PACs in insects, that could be taken up and accumulated by terrestrial invertebrates [211–213]. The majority of the studies evaluating the potential accumulation are on the black soldier fly (*Hermetia illucens*), one of the most used insects for food and feed for animals and humans.

The accumulation of veterinary pharmaceuticals may occur, as reported by Charlton et al. [211], who detected nicarbazin in *Musca domestica* growing on poultry manure. However, other studies report the opposite, with no accumulation of different antibiotics and one antiepileptic in *H. illucens* larvae grown in a composting system to produce organic fertilizer [213]. In order to combat infections and diseases in the rearing systems, antimicrobial agents should be used for prevention. Consequently, there is a need to find the right equilibrium between avoiding the toxic effects of the drugs for rearing insects and the need to control possible insect infections [214]. Insects used for food and feed are also prone to pesticide accumulation. Results indicate that pesticides with the higher log K_{ow} tend to bioaccumulate in edible insects, while those with a lower log K_{ow} tend to be readily excreted by the insects [212]. Fungicides were efficiently metabolized and degraded by *Tenebrio molitor* after exposure to substrate contaminated with metalaxyl, epoxiconazole, benalaxyl, and myclobutanil [215, 216]. Different PACs (benzo[a]pyrene, benzo [a]anthracene, benzo[b]fluoranthene, and chrysene) were also found in the fly larvae [211], but no maximum limits for PACs in animal feed are set.

Nowadays, this line of investigation is crucial, and more studies are needed for a better comprehension of how insects that serve for food and feed accumulate toxic compounds that could be biomagnified at higher levels in the trophic chain and, eventually, negatively impact humans. Because of that, joint efforts are needed to update the legislation for these types of food sources, as already is in place for other "traditional" food sources.

6 Final Remarks

Soil risk assessment of organic chemicals remains a challenge for the years to come. From the scientific literature addressing the bioaccumulation and toxicity of these compounds to terrestrial invertebrates, in the last 5 year period, the main gaps and research needs identified are related to:

- Biodiversity beyond standardized species. The majority of the studies available focused on groups of organisms used in the standardized laboratory tests (i.e., earthworms, collembolans, predatory mites, and honey bees), but little information exists on other terrestrial invertebrates with crucial roles in soil structure and functioning such as ants, beetles, ladybugs, snails, and spiders.

- Ecological relevance of dermal contact bioassays. A large number of studies evaluated the toxicity of organic chemicals through filter paper contact tests and/or topical applications. As soil exposure conditions are not considered, the outcome of these studies cannot be used by regulators for soil risk assessment or for specific chemical risk assessment (e.g., plant protection products).
- Ecotoxicological endpoints required. For soil risk assessment, LC_x and EC_x values are critical endpoints to derive insight into the hazard and risk of organic chemicals. Still, nowadays, risk assessors prefer to be informed on NOEC and LOEC as valuable endpoints, which are scientifically unprecise and biased and that could be replaced by EC_{10} or EC_{20}s. The majority of the studies do not report either of these ecotoxicological endpoints, which are of particular importance for new emergent organic chemical compounds.
- Inconsistency in units' reporting. The consensus is missing among researchers in reporting the units of ecotoxicological endpoints, which hampers their use in soil risk assessment. Moreover, reporting details on compound application methods, soil properties including bulk density and thickness of the soil layer to which a compound is applied, would allow for the conversion of units.
- Broader concentration ranges for low levels of biological organization. The growing number of studies covering effects at the sub-organism level represents a step further in understanding the modes of action of organic chemicals. However, the complexity of this type of study often hinders the inclusion of several test concentrations, not allowing regulators to consider them for soil risk assessment since no ecotoxicological endpoints can be derived.
- Scarcity of bioaccumulation studies for terrestrial invertebrates. The existing models and tools on bioaccumulation and toxicity of organic chemicals to terrestrial invertebrates are requested under the REACH regulation. The general lack of scientific literature on the toxicokinetics of organic chemicals in terrestrial invertebrates is primarily associated with the relatively high costs of chemical analysis and the absence of well-established and/or standardized analytical chemical methods and protocols for specific organic compounds and residues in the soil matrix and animal tissues. BAFs reported in the current literature are very often not standardized for organism lipid content and soil organic carbon content, being one of the limitations when comparing the results of different studies and for different test species. Beyond soil risk assessment, the knowledge of the bioaccumulation of organic chemicals, alongside the necessary optimization and development of quantification methods, could directly contribute to food safety regulations regarding the use of edible terrestrial invertebrates.
- Information on mixture toxicity. Most of the currently available studies on the mixture toxicity of organic chemicals consider the approach based on concentration addition and/or independent action recommended by ECHA. Albeit the advances, most of the studies focus on earthworms and binary and/or ternary mixtures, emphasizing the need to address the effects on other terrestrial invertebrates and for more complex mixtures. The latter should also cover commercial formulation components (e.g., adjuvants). Further complexity arises from climate change predictions, whose effects might potentially interact with the toxicity of

mixtures of organic chemicals, but such research is up to now scarce. Mixture toxicity studies in terrestrial invertebrates have generally been focused on individual-level endpoints. Additional research at both lower and higher levels of biological organization would improve one's ability to predict potential deviations from additivity by, respectively, improving the mechanistic knowledge on mixture toxicity and assessing the ecological significance of such deviations at the community or ecosystem level.

- Higher-tier studies. Although their long-known importance, not enough effort has been put on developing integrated approaches that account for species interactions and soil ecosystem functioning (e.g., microcosm and mesocosm studies) in the context of organic chemical exposure. Likewise, the soil compartment is still behind aquatic counterparts in terms of the development and application of modeling approaches to extrapolate the results of laboratory toxicity experiments to the field for organic chemicals. Such higher-tier studies are critical for improving the ecological realism of soil toxicity assessments and extrapolating the effects from laboratory to field conditions.

Acknowledgments The authors acknowledge funding to the ECOCENE (POCI-01-0145-FEDER-032471) and METOXCLIM (POCI-01-0145-FEDER-029557) projects funded by FEDER, through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES, and to FCT/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020) through national funds. Rui Morgado was granted by FCT with a postdoctoral grant (SFRH/BPD/123384/2016). Catarina Malheiro was granted by the Doctoral Programme in Biology and Ecology of Global Change of the University of Aveiro with a Ph.D. grant from FCT (PD/BD/135577/2018).

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