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Environmental risk assessment of PPP application in European soils and potential ecosystem service losses considering impacts on non-target organisms

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ABSTRACT

The use of Plant Protection Products (PPPs) is leading to high exposure scenarios with potential risk to soil organisms, including non-target species. Assessment of the effects of PPPs on non-target organisms is one of the most important components of environmental risk assessment (ERA) since they play crucial functions in ecosystems, being main driving forces in different soil processes. As part of the framework, EFSA is proposing the use of the ecosystem services approach for setting specific protection goals. In fact, the services provided by soil organisms can be impacted by the misuse of PPPs in agroecosystems. The aim of this work was to assess PPPs potential risk upon ecosystem services along European soils, considering impacts on earthworms and collembola. Four well-known (2 insecticides-esfenvalerate and cyclaniliprole- and 2 fungicides - picoxystrobin and fenamidone-) worst case application (highest recommended application) were studied; exploring approaches for linked observed effects with impacts on ecosystem services, accounting for their mode of action (MoA), predicted exposure, time-course effects in *Eisenia fetida* and *Folsomia* sp. and landscape variability. The selected fungicides exerted more effects than insecticides on *E. fetida,* whereas few effects were reported for both pesticides regarding *Folsomia* sp. The most impacted ecosystem services after PPP application to crops appeared to be habitat provision, soil formation and retention, nutrient cycling, biodiversity, erosion regulation, soil remediation/waste treatment and pest and disease regulation. The main factors to be taken into account for a correct PPP use management in crops are discussed.

1. Introduction

The use of Plant Protection Products (PPPs) has contributed to the high production of food during the last decades, making it possible to feed the increasing world population. While the worldwide rice, wheat and maize harvests doubled production yields, fertilizer and pesticide use increased 20 and 7 times, respectively (Oerke, 2006; Silva et al., 2019). Three million pesticide tons are applied annually all around the world (Pimentel, 2009), being 374 m tons sold only in the EU (EUROSTAT, 2018; Silva et al., 2019). In conventional intensive agriculture, crops can be sprayed with different insecticide and fungicides multiple times in a single year (typically up to 10 and 25 applications, respectively), in extreme cases with over 40 pesticide active substances (a.s.) applied per year (Van Droogе et al., 2001; Garthwaite et al., 2015; Mayer et al., 2020). High usage and application rates of PPPs lead to high exposure and therefore, potential for accumulative effects in organisms inhabiting soils (Morris et al., 2016). In addition to croplands where PPPs are directly spiked, effects could be exerted in adjacent agricultural areas, such as field margins, hedges, non-cropped patches, groundwater, ditches, streams and lakes, or also in areas far away due to long range transport of pesticides (EFSA, 2010). Hence, the increased use of PPPs, in extension and intensity, has simultaneously enhanced social awareness about their risks, and regulatory actions for reducing the overall use of chemical pesticides and their risks, such as those

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mentioned in the Farm to Fork Strategy under the EU Green Deal (European Union, 2020).

PPPs are subject to regulatory control in almost all jurisdictions worldwide; and the concerns include the possible impacts on biodiversity (Sud, 2020). In the EU, the principles established through the directive adopted in 1991 were revised in 2009 and updated as Regulation EC 1107/2009 (Streloke, 2011), which aims include to ensure high protection level regarding the use of PPPs. As expected, the level of conservativeness provided by this regulation and their implementing acts and guidance documents are considered excessive by some authors (Kluxen et al., 2021), while insufficient by others (Mayer et al., 2020). This regulation specifies a dual mechanisms for the scientific assessment of PPPs. The active substances are assessed at EU level through a process involving a peer-review by the European Food Safety Authority (EFSA). After the authorization of the active substance, the formulated PPPs are assessed at zonal level by groups of EU Member States, supporting the national decisions for authorising their use. EFSA provides guidance for conducting the assessments and have also published proposals for further improve the scientific assessment process (EFSA, 2018 EFSA).

The assessment of active substances and PPPs includes requirements to cover the risk for consumers, occupational exposure, and humans exposed via the environment; as well as non-target organisms, the later integrated as Environmental Risk Assessment (ERA). ERA is mandatory for all relevant non-target organisms and populations exposed to pesticides and their residues (Kluxen et al., 2021), and implemented as a set of guidance documents covering the different relevant groups. A quarter of world's biodiversity are soil living organisms and among them, invertebrate communities are especially sensitive to ecosystem changes (Velasquez and Lavelle, 2019). Hence, the absence of a proper evaluation of PPPs risk to soil invertebrates could lead to an imbalance in the ecosystems, with the consequent biodiversity loss, and the affection of the offered services (TEEB, 2008). Ecosystems Services are the benefits that society obtains from ecosystems as support (nutrient cycling, primary production), provisioning (food provision), regulatory (purification of water, climate regulation) or cultural services (recreational, educational) (Millennium Assessment Ecosystems, 2005). In this framework, soil plays a key role providing several functions and services (EC, 2006). Therefore, based on the Panel on Plant Protection Products and their Residues (PPR proposal (EFSA, 2010) and the Scientific Committee (EFSA Scientific Committee, 2016a, 2016b, c), EFSA is proposing the use of the ecosystem services approach for setting specific protection goals. However, assessing the risk derived from an Ecosystem Service loss is the biggest challenge in ERA (Ostrom, 2009). An option for addressing this challenge is to extract the relevant through the identification and analysis of available information. For pesticides, the mechanistic information includes the classification based on intended use, target group and pesticide mode of action (MoA) (Rani et al., 2021), combined with mechanistic knowledge related to the toxic effects observed in non-target organisms; some toxicological mode of actions are similar or linked to the pesticidal mode of action, while others are independents, thus both information sets should be combined. For the identification of possible environmental impacts, this information can be integrated with the ecological and landscape characteristics of the relevant assessment scenarios, including their variability.

Deciphering the pesticidal and toxicological MoA of pesticides may facilitate the identification of possible hazard pathways at different levels of biological complexity (molecular, cellular, tissue, reproductive, feeding and/or motility impairments among others). The information can then be integrated with biological and ecological knowledge in order to identify the expected sensitivities of different organisms and possible impacts on ecological functions, and lastly on ecosystem services.

Among soil indicator organisms, *Eisenia fetida* earthworms and *Folsomia sp*. collembolans are widely used as non-target species to address the potential environmental risks of pesticides to agricultural in-soil fauna (EFSA, 2017). Both are complementary, as earthworms represent the non-arthropod soil macrofauna group, while collembolans represent the mesofauna arthropod group. In this sense, EFSA panel has already developed concrete proposals covering different guidelines (EFSA, 2014), including standardized toxicity tests such as those promoted by OECD, for instance the Acute Toxicity (OECD-207) and Reproduction (OECD-222) tests with *E. fetida/andrei*; and Collembolan Reproduction test with *Folsomia* sp. (OECD-232) (Fountain and Hopkin, 2005). Moreover, these species are considered by EFSA Panel as key driver organisms in soil functioning as well as in ecosystem services development. In fact, these species are considered key organisms for the maintenance of soil functions and for the maintenance of supporting and provisioning services (Schroder, 2008; Pelosi et al., 2014; FAO and ITPS, 2017; Ockleford et al., 2017; Schon et al., 2017). Therefore, the impacts upon these communities should be addressed in the assessment of potential Ecosystem Service losses derived from PPP application in soils.

Most of the soil functions and the ecosystem services derived from soil fauna depend on specific conditions such as climate, pH, organic matter (OM) content or the diversity of plants and organisms inhabiting soils (Pereira et al., 2018); and these may vary along European soils. Thus, it has to be highlighted at the view of previous works, that environmental and ecological variabilities among Euroregions should be considered as they play a key role in assessing exposure and effects of PPPs. In the present work, the toxicity data of pesticides on non-target organisms was related with the predicted soil concentrations (PECs) obtained in previous works (Urionabarrenetxea et al., 2022). Moreover, the risk upon ecosystem services along European soils was assessed, considering impacts upon non-target organisms (*E. fetida* earthworms and *F. candida* collembolan) after 4 PPP (2 insecticide- esfenvalerate and cyclaniliprole-, and 2 fungicide- fenamidone and picoxystrobin-) worst case application into crops. For that, PPPs MoA, exposure, time-course effects and landscape variability were taken into account.

2. Materials and methods

2.1. Review of PPP mode of action and time course effects upon non-target organisms

Data regarding the pesticidal and toxicological MoA of esfenvalerate, cyclaniliprole, fenamidone and picoxystrobin was retrieved from available scientific literature and technical reports published in academic and environmental agency websites and databases (EFSA, and, 2013, 2015a, 2015b, 2015c). In addition, exposure routes (of mentioned compounds) for target organisms were studied in order to extrapolate effects to non-target species. Information about geographical distribution of non-target organism according to characteristics of soil substratum, and modes of feeding and behavior were obtained from EFSA reports and scientific papers (Verhoef et al., 1983; Detsis, 2000; Fountain and Hopkin, 2005; Ogungbemi and van Gestel, 2018; Holmstrup and Martin, 2019).

Ecotoxicological endpoints for *E. fetida* and *Folsomia sp*. at different PPP exposure times (7, 14, 28 and 56 days) were retrieved from ecotoxicological studies performed according to official guidelines from FAO and European Commission and assessed by EFSA (EFSA, and, 2013, 2015a, 2015b, 2015c). For each of the studies: test conditions (soil, pH, and temperature), compound purity, identified endpoints (NOEC, LOEC, LC_{50}) and observed effects were considered taking into account the most restrictive values. Chronic tests and acute toxicity tests were taken into account in the bibliographical survey (Fig. 1).

2.2. Linking PPP exposure and potential effects to non-target organisms and ecosystem services along European soils

PECs obtained in previous works (Urionabarrenetxea et al., 2022) were estimated thought PERSAM software (Persistence in Soil Analytical Model version V2.0.1) for 1 × 1 km surface squares (by overlapping up to 62 datasets: including meteorological datasets, crop datasets, soil

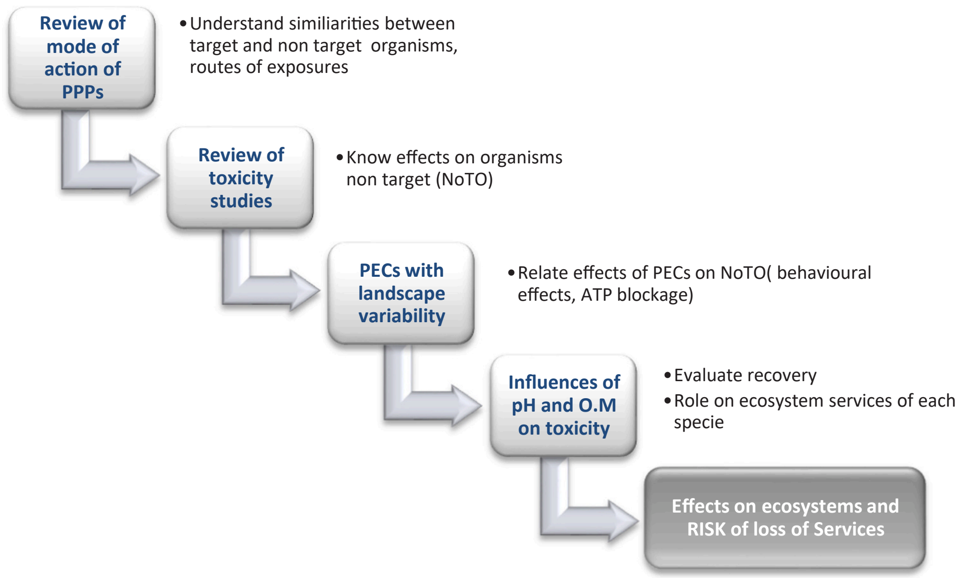

Fig. 1. Process diagram. Activities to relate mode of action of PPP and the link with in-soil fauna ecosystem services.

datasets, etc.) according to EFSA Guidance (Urionabarrenetxea et al., 2022). In the mentioned work, pesticides input was set trying to find worst-case scenario according good agricultural practices (GAP) authorised for each pesticide (fenamidone and cyclaniliprole application on tomatoes and potatoes; tomatoes and spring cereals for picoxystrobin and esfenvalerate). Additionally, the influence of each compound characteristics (e.g. pka, MoA) and European landscape variability (pH, organic matter, temperature) on toxicity was considered (Fig. 1).

In order to assess the potential effects on ecosystem services, the Millennium Ecosystem Assessment (MEA, 2005) document was used, which listed ecosystem services in-crop and off-crop areas, and PPP potentially affected taxa for each service. From this list, services potentially affected (directly or indirectly-e.g. via trophic interactions-) were selected; choosing only the ones exhibiting a maximum importance (+++/+++) for the different spatial areas (in crop, off crop). Later, and having in mind the MoA of the PPP and time-course effects (2.1 section), the potential impact of the selected pesticides upon ecosystem functions were determined. As PPP potentially affected taxa *E. fetida* and *Folsomia sp.* were selected, representative for the impact upon earthworms and collembolans.

In a final step, a summarizing matrix was elaborated combining the pesticide, the non-target organism, a summary of the ecotoxicological endpoints (apart 2.1), the expected toxic effects for worst case PPP application in European soils and ecosystem functions and services affected.

3. Results and discussion

3.1. Pesticidal and toxicological mode of action of selected substances

The MoAs of the different pesticide active substances selected for this work are reported in Table 1. The MoA can be defined as the functional change after the exposure of a living organism to a substance (Grant et al., 2010; Aliferis and Jabaji, 2011; Sparks and Nauen, 2015; Sabzevari and Hofman, 2022); and represent the early or intermediate effects that triggers the toxicity of the active substance upon target or also non-target organisms. Although the pesticidal MOA focuses on the effects on the pest, it may also infer possible toxicity mechanisms in non-target organisms; therefore, both the pesticidal and the toxicological MOAs were combined. Physiological differences and phylogenic considerations provide information on the possible relevance of each MoA to the non-target organisms addressed in this study. Esfenvalerate

Table 1
Summary of the Mode of Action of each PPP selected for the present work.

Type of PPP	Mode of Action	Refs
Esfenvalerate Insecticide (pyrethroid)	• Disruption Na channels by preventing the closure of the voltage-gated sodium channels in the axonal membranes. Hence, nerves cannot repolarize, leaving the axonal membrane permanently depolarized, thereby paralyzing the organism • Induces change in temperature and stomach disruption	Soderlund et al. (2002); Bal-Price et al. (2017); Casida and Durkin, (2013); Abreu-Villaca and Levin (2017)
Cyclaniliprole Insecticide (diamide)	• Enters in the organism through ingestion and by absorption through cuticle • Acts on the ryanodine receptors[a] located in the endoplasmic reticulum, inducing muscle paralysis by releasing the intracellular calcium necessary for muscle contraction	EFSA (2015a);Troczka et al. (2017);Opper et al. (2010)
Picoxystrobin Fungicide (strobilurin)	• Inhibits mitochondrial respiration (block electron transfer between cytochrome b and cytochrome c1)[b] • disrupts the energy cycle within the fungus by halting production of ATP. • Antisporulant	Paramasivam and Chandrasekaran (2013); Tentu and Tentu (2016).
Fenamidone Fungicide (imidazolinone)	• Inhibits mitochondrial respiration by blocking electron transport at ubihydroquinone: cytochrome-c-oxidoreductase (Complex III).	

[a] Ryanodine receptors: responsible of Ca mobilization in the cell. Similar for some insects, mammals & arthropods.
[b] Part of the cytochrome bc1 complex, located in the inner mitochondrial membrane of fungi and other eukaryotes that are present in a lot of organism from different species.

belongs to the II group of pyrethroids chiral compounds, characterized by disruption of sodium channels in mammals, inhibiting their normal functioning (Qi and Casida, 2013; Bal-Price et al., 2017; Abreu-Villaca and Levin, 2017). This impairs animal behavior, usually due to hyperactivity, convulsions, lethargy, paralysis and finally leading to death (Awoyemi et al., 2019; Wang et al., 2020). Invertebrate sodium channels have similar biophysical properties to those of mammals, acquired before the evolutionary separation of the invertebrates from the vertebrates (Catterall, 2000); then, similar responses should be expected. Alike, the insecticide cyclaniliprole (diamine class) acts on the ryanodine receptors (Tsukamoto et al., 2021) located in the endoplasmic reticulum of insects, inducing muscle paralysis by releasing the intracellular calcium necessary for muscle contraction (Qi et al., 2013; EFSA, 2015; Troczka et al., 2017). Indeed, ryanodine receptors are similar in insects, mammals and arthropods, leading to incapacity to increase pesticide efficiency without affecting non-target organisms (Casida and Durkin, 2013). Moreover, it is well known that calcium mobilization is a universally conserved (throughout the species) activation mechanism, essential in the signaling of immune cells (Opper et al., 2010). Thus, it is highly probable that cyclaniliprole behaves in a similar manner in invertebrates, causing a general paralysis of the organism.

Regarding fungicides, picoxystrobin is a preventive and curative fungicide belonging to strobilurin group of chemicals (Bartlett et al., 2002; Jia et al., 2018) and fenamidone (imidazolinone class) is a systemic foliar fungicide. Both inhibit cytochrome bc1 complex in the inner mitochondrial membrane (also in fungi and other eukaryotes) responsible for cellular respiration of water molds and other fungal pathogens (Paramasivam and Chandrasekaran, 2013; Tentu and Tentu, 2016). Once the inhibition occurs, the electron transference between cytochrome b and cytochrome c1 is blocked (Jia et al., 2018) producing the disruption of the energy cycle and ATP production (Bartlett et al., 2002). This alteration might affect individuals reproductive activity that itself implies enormous energy costs. So, it may happen that ATP generation blockage could impact on the reproductive fitness of the individual, decreasing the possibility to allocate this energy on individual growth or development.

3.2. Time course effects of PPP upon non-target organisms

At the individual level, survival, mortality (LC_{50}), fecundity, reproduction or growth are the main endpoints used to assess the toxicity of PPPs in soil macroinvertebrates (Van Gestel et al., 1989, 2012). Behavior can be also taken into consideration in earthworms, with four main functions potentially affected by PPPs: avoidance behavior, burrowing behavior, bioturbation and burial of OM (Pelosi et al., 2014). However, in studies regarding pesticides effect on soil invertebrates summarized by Jänsch et al. (2006), 89% of studies used abundance and/or biomass as endpoint, followed by mortality (10%); while few studied behavior or development (< 1%). Among the most used tests, chronic test aiming sublethal effects must be highlighted, which are very sensitive and realistic for the prediction of environmental effects because exposure concentrations are usually quite low (Rombke et al., 2007). Both type of tests (acute and chronic) were available for *E. fetida* and *Folsomia sp*, although less information was found for collembollans. Exposure concentration, time course effects and toxicity tests applied to assess the toxicity of the selected PPP have been summarized in Tables 2 and 3, for *E. fetida* and *Folsomia* sp., respectively. Soil toxicity tests reproduce the exposure conditions expected under realistic conditions and do not provide continuous exposure levels: The concentration reaches a maximum shortly after the application and then decreases with the dissipation of the active substance. The effects are assumed to be related to the parent compound although the role of metabolites is not investigated during the same experiment. The dissipation under field conditions may vary significantly for some active substances, and therefore the test conditions and the soil type used in the studies are relevant but may not represent real exposure. In addition, the endpoints, and the timepoints for measuring these endpoints are selected withing the study design; as it is not feasible to observe the test organisms on hourly or daily basis. All these elements were considered in the assessment. Laboratory toxicity tests have been designed for measuring specific endpoints at defined timelines. The main aim is to identify the NOEC/LOEC or ECx to be used in the hazard characterization. The assessment of possible impacts on ecosystem servicesrequires a more comprehensive approach, considering the effect, concentration and time relationships. The possibility for extracting this kind of information from existing soil toxicity standard studies was explored. Despite the limitations, relevant information on concentration and time related responses could be extracted and is summarized in Tables 2 and 3.

3.2.1. Insecticides: esfenvalerate and cyclaniliprole

The studies in EFSA reports regarding esfenvalerate toxicity on *E. fetida* reported exposure concentrations ranging 0.07 and 50 mg a.s. /Kg soil. All toxicity tests (except Stabler 2009, in EFSA, 2013) were carried at 10% of sphagnum peat and 19–20 °C conditions, considering a product purity of around 5%. The lowest NOEC reported was 1.132 mg a.s. /Kg of soil. Effects (mortality and severe weight loss) were detected at medium exposure levels (3.125–50 mg/Kg, 7–14 d) while higher concentrations (>50 mg/Kg) exerted mortality at shorter exposure periods (7 d; Table 2A). Nevertheless, other sub-lethal effects such as difficulties to reach maturity have been detected in earthworms at low esfenvalerate exposure levels; even lower concentrations than those evaluated in reviewed toxicity assays (Schnug et al., 2014). This controversy deserves further research to establish accurate toxicity endpoints at different biological complexity levels.

Regarding collembolans, exposure concentrations in toxicity tests varied from 0.349 to 5.62 mg a.s./Kg, with a NOEC stablished at 0.349 mg a.s./Kg. Effects on reproduction were detected at high exposure levels (0.698–5.62 mg/Kg for 56 d, Table 3A).

The effects produced by cyclaniliprole in *E. fetida* (Lührs, 2011; 2012; Lührs, Meinerling 2012, EFSA, 2015a) were assessed at exposure concentrations ranging 2.89–957.1 mg a.s./Kg soil. NOEC was estimated at 957.1 mg a.s./Kg (Table 2B) so no evidence for lethal and sublethal effects was observed in all the toxicity studies reviewed for *E. fetida*. This might be due to the low solubility of the compound making it barely available for organisms. Although, oxidative stress glimpses have been observed in earthworms after diamide exposure with the same MoA (Liu et al., 2018a, 2018b), more information is needed to understand the toxicity mechanisms of the compound in these organisms. Meanwhile, the range of cyclaniliprole exposure concentrations for *Folsomia* sp. was between 0.625 and 10 mg a.s./Kg with the NOEC stablished at: 2.5 mg a.s./Kg soil. For reproduction and mortality, two ECs were determined as toxicity endpoints: 2.5 mg a.s. /Kg for reproduction and 5 mg a.s. /Kg for mortality. Exposures up to 5 mg/Kg did not cause mortality although a significant reduction in the number of juveniles was recorded after 56 d (Table 3B). At concentrations higher than 5 mg/Kg, enhanced mortality and a significant reduction in the number of juveniles were observed in *Folsomia* sp. (Ganßmann, 2012; EFSA, 2015a).

3.2.2. Fungicides: Picoxystrobin and Fenamidone

A concentration range between 0.16 and 10 mg/Kg was studied for picoxystrobin, estimating the NOEC for *E. fetida* at 0.63 mg/Kg. The reproductive capacity appeared to be affected after 56 d at 1.25–2.5 mg/Kg, while mortality was recorded at higher concentrations ($LOEC_{reproduction}$: 3.2 mg/Kg; LC50 6.1 mg/Kg) Table 2C). The toxicity of picoxystrobin for *F. candida* was tested in a range of exposure concentrations between 2.5 mg and 40 mg a.s/Kg (Lührs, 2012 retrieved from EFSA, 2015b). NOEC was established at 20 mg a.s/Kg, while significant differences on reproductive capacity were only observed after 56 d of exposure to the highest dose (40 mg/Kg, Table 3C).

Studies regarding fenamidone effects in *E. fetida* ranged

Table 2
Exposure concentration, time course effects and toxicity tests applied to assess the toxicity of Esfenvalerate (A), Cyaniliprole (B), Picoxystrobin (C) and Fenamidone (D) on *Eisenia fetida*; with indication of the biological endpoints reported (NOEC, LOEC, EC, LCX) Legend: a.s., active substance.

(A) Esfenvalerate

Test conditions: 10% sphagnum peat, T: 19-20º·C (all references retrieved from EFSA, 2013).

Exposure	Day 7	Day 14	Day 28	Day 56	Reference protocols	Endpoint summary
0.07 -1.123 mg a.s./Kg soil	No effect	No effect	No effect	No effect	Teixeira (2003) Chronic. 56d OECD 222	NOEC: 1.123 mg a.s./Kg soil LOECmortality >5 mg a.s./Kg LC50 (7 days):13.6 mg a.s./Kg LC50 (14 days):10.625 mg a.s./Kg
3.125 - 50 mg a.s. /Kg of soil	Mortality rise up to 100% since 7 d	Weight loss above 125 mg/Kg			Whuthrich (1991) ATT, 14 d OECD 207	
5 - 50 mg a.s. /Kg of soil	Mortality.	Mortality Weight loss			Petto (1994). ATT. 14 d. OECD 207	

(B) Cyclaniliprole

Test conditions: sphagnum peat 5 -10%, temperature was 18-22º· C (all references retrieved from: EFSA, 2015a)

Exposure	Day 7	Day 14	Day 28	Day 56	Reference protocols	Endpoint summary
2.89 - 46.3 mg a.s. /Kg soil	No effects	No effects			Lührs (2012). ATT 14 d, OECD 207, ISO 11268-1	NOEC: 957.1 mg a.s./Kg soil
From 90.92 to 957.1 mg a.s./Kg soil	No effects	No effects	No effects	No effects	Lührs, Meinerling, 2012 OECD 222 ISO 11268-2	
957.1 mg a.s./Kg soil	No effects	No effects			Lührs, 2011 ATT 14 d. OECD 207. ISO 11268-1	

(C) Picoxystrobin

Tests conditions: sphagnum peat concentrations 10%; temperature, 22°C (retrieved from: EFSA, 2015b)

Exposure	Day 7	Day 14	Day 28	Day 56	Reference protocols	Endpoint summary
0.16 - 1.25 mg a.s./Kg soil	No effects	No effects	No effects	No effects	Friedrich (2003). ISO 11268-2. Jackson & Coulson (1998). ATT 14d. OECD 207	NOEC: 0.63 mg a.s./Kg soil ECreproduction: 1.25 mg a.s./Kg soil LOECmortality: 3.2 mg a.s./Kg soil LC50: 6.1 mg a.s./Kg soil
1.25 - 2.5 mg a.s./Kg soil	No effects	No effects	No effects	Reproduction	Friedrich, 2003. ISO 11268-2 (1998)	
5 mg a.s./Kg soil	No effects	No effects	Mortality (12.5%) but not sign. to control.	Reproduction		
3.2 - 10 mg a.s./Kg soil	Mortality. Behavioral alter.	Mortality. Behavioral alter.	Mortality. Behavioral alter.		Jackson & Coulson, 1998. ATT 14d. OECD 207	

(D) Fenamidone

Tests conditions: Sphagnum peat: 5-10%; temperature: 18-22·°C (all references retrieved from: EFSA, 2015c).

Exposure	Day 7	Day 14	Day 28	Day 56	Reference protocols	NOEC
0.628 mg a.s./Kg soil	No effects	No effects	No effects	No effects	Gossmann & Luehrs, 1998 ISO 11268-2 (Draft 1995), BBA VI 2-2 (1994)	NOEC: 0.628 mg a.s./Kg soil ECreproduction: 1.247 mg a.s./Kg soil ECmortality: 4.99 mg a.s./Kg soil
1.247 mg a.s./Kg soil	No effects	No effects	No effects	Reproduction		
2.495 mg a.s./Kg soil	No effect	No effects	Lose weight trend	Reproduction		
4.99 mg a.s./Kg soil	No effects	No effects	Mortality	Reproduction		
9.98 mg a.s./Kg soil	No effects	Weight loss		Reproduction		

Table 3
Exposure concentration, time course effects and toxicity tests applied to assess the toxicity of Esfenvalerate, Cyclaniliprole, Picoxystrobin and Fenamidone on *Folsomia* sp.; with indication of the biological endpoints reported (NOEC, LOEC, EC, LCX) Legend: a.s., active substance.

(A) Esfenvalerate
Test conditions: %5 sphagnum peat, %20 Kaolin clay, % 74,7 fine quartz sand, %0,3 calcium carbonate. T=18-22·C (retrieved from: EFSA, 2013)

Exposure	Day 7	Day 14	Day 28 (adults)	Day 56 (juveniles)	Reference, test protocols	Endpoint summary
0.349 mg a.s./Kg	No effects	No effects	No effects	No effects	Luhrs (2010). OECD 232	NOEC: 0.349 mg a.s./Kg soil LOECreproduction: 0.698 mg a.s./Kg soil
0.698 mg a.s./Kg	No effects	No effects	No effects	Effects on reproduction		
1.405 mg a.s./Kg soil	No effects	No effects	No effects	Effects on reproduction		
2.811 mg a.s./Kg soil	No effects	No effects	No effects	Effects on reproduction		
5.62 mg a.s./Kg	No effects	No effects	No effects	Effects on reproduction		

(B) Cyclaniliprole
Test conditions: %5 peat, 30g wet weight/100 ml glass beaker, Feed: after day 0 and 14: 2mg granulated dry yeast per test vessel (retrieved from: EFSA, 2015a)

Exposure	Day 7	Day 14	Day 28 (adults)	Day 56 (juveniles)	Reference, test protocols	Endpoint summary
From 0.625 to 2.5 mg a.s. /Kg soil	No effects	No effects	No effects	No effects	Ganßmann, 2012 OECD-232 ISO 11267-1999	NOEC: 2.5 mg a.s./Kg soil ECreproduction: 5 mg a.s./Kg soil ECmortality: 10 mg a.s./Kg soil
5 mg a.s. /Kg soil	No effects	No effects	No effects	No mortality Reduction in number		
10 mg a.s. /Kg soil	No effects	No effects	Mortality	Mortality. Reduction in number		

(C) Picoxystrobin
Test conditions: sphagnum peat= 5%; temperature 18- 22°C (retrieved from: EFSA, 2015b)

Exposure	Day 7	Day 14	Day 28 (adults)	Day 56 (juveniles)	Reference, test protocols	Enpoint summary
From 2.5 to 20 mg a.s. /Kg soil			No effects	No effects	Lührs (2012)	NOEC: 20 mg a.s./Kg soil ECreproduction: 40 mg a.s./Kg soil
40 mg a.s. /Kg soil			No effects	Significative differences on reproductive capacity respect to control (29% reduction).	ISO 11267 (1999) OECD 232 (2009)	

(D) Fenamidone
Test conditions: sphagnum peat= 5%; temperature= 20-22 °C (retrieved from: EFSA, 2015c)

Exposure	Day 7	Day 14	Day 28 (adults)	Day 56 (juveniles)	Reference, test protocols	Endpoint summary
99,8 mg a.s. /Kg soil			No effects	No effects	Frommholz (2011). OECD 232 (2009)	NOEC: 99.8 mg a.s./Kg soil

concentrations between 0.628 and 9.98 mg a.s./Kg (Table 2D) with a lowest NOEC at 0.628 mg a.s./Kg. Weight loss, impacts on reproduction and mortality effects were recorded, following time/concentration (exposure level) dependent trend: effects were observed in low-medium concentrations ($EC_{reproduction}$: 1.247 mg a.s./Kg) at longer periods (28–56 d); while, exposure to higher concentrations ($EC_{mortality}$: 4.99 mg a.s./Kg) exerted impacts at shorter periods (14 d). For *F.candida,* 99.8 mg a.s./Kg concentration used in the unique test available did not produce behavioral nor reproductive effects; so this concentration was established as NOEC (Table 3D). The lack of available data on *Folsomia sp.* should be improved with future studies.

Among the studied compounds, effects produced by fungicides in

E. fetida were more easily detected in comparison with insecticides (Bunemann et al., 2006; Jänsch et al., 2006; Pelosi et al., 2014). In contrast, *Folsomia sp*. seems to be less affected by fungicides, while insecticides affected reproductive capacity after chronic exposures.

3.3. Potential ecosystem services loss derived from the PPP application in European soils

Agricultural landscapes provide several important ecosystem services, which could be significantly affected by the massive use of PPPs. The main effects would be mostly associated with biodiversity losses, affecting organisms playing main roles (e.g. bioturbators, shredders) and root biota (Coleman et al., 2004). The EFSA framework for setting specific protection goal based on ecosystem services includes the protection of biodiversity and specific considerations for assessing biodiversity in the agricultural context (EFSA, Scientific Committee, 2016b). Earthworms and collembolans must be highlighted due to their important role on several ecosystem functions in crop and off crop areas (EFSA, 2010). Earthworms contribute to gene pool and biodiversity, play a key role in soil formation and retention, nutrient cycling, erosion regulation, soil remediation (or waste treatment) and habitat provision (EFSA, 2010; Wang et al., 2012; Ockleford et al., 2017). Meanwhile, collembolans are important for the maintenance of pest and disease regulation (food support), the nutrient cycling, biodiversity (Filser, 2002; Ockleford et al., 2017), habitat provision, soil formation and retention (EFSA, 2010). The proposed relevant attributes as survival, growth and reproduction (EFSA Scientific Committee, 2016b), which are in fact the endpoints measured in the laboratory ecotoxicity tests.

It should be noted in that according to current (FOCUS, 1997) and EFSA (2017) soil exposure guidance, current EU assessments are based on a soil depth of 5 cm (or 20 cm in the case of soil incorporation), not on the worst-case 1 cm selected by the authors (Urionabarrenetxea et al., 2022).

For esfenvalerate concentrations in Europe between 1.123 and 3.125 mg a.s./Kg no ecotoxicological data was reported in EFSAs list of endpoints for *Eisenia* sp. (Table 2). Although no effects were detected at lower concentrations, no impacts on ecosystem services could be discussed due to lack of data in the mentioned range. In some northern spots (Finland, Sweden, Estonia, Latvia and Lithuania), concentrations from 5 mg a.s./Kg onwards could be expected (Urionabarrenetxea et al., 2022), and thus mortality and weight loss could occur (LOEC>5 mg a.s./Kg). From this concentration on, supporting, regulating, and provisioning services could be affected; principally by affecting habitat provision, soil formation and retention, nutrient cycling, erosion regulation, soil remediation/waste treatment and biodiversity (Table 4). Thus, in those spots with concentrations > 5 mg a.s./Kg soil (Urionabarrenetxea et al., 2022), a specific soil management could be required in order to recover a proper soil functioning. Meanwhile, esfenvalerate could induce reproduction impairment in collembolans for PECs in Europe ranging 1.343–5.565 mg a.s./Kg soil (Urionabarrenetxea et al., 2022). Thus, pest and disease regulation, biodiversity, habitat provision, soil formation and retention, or nutrient cycling carried out by *Folsomia* sp. could be affected; impacting soil supporting, regulating and provisioning services (Table 4).

Regarding published ecotoxicological data cyclaniliprole is not expected to exert deleterious impacts upon earthworms after a worst case PPP application in European soils; however, multiple impacts could be expected for collembolan. Due to the lack of ecotoxicological data between 2.5 and 5 mg a.s./Kg soil concentrations (Table 3), effects could not be estimated in collembolans at 3.381 mg a.s./Kg soil (Urionabarrenetxea et al., 2022). Concentrations ranging 5.66–7.957 mg a.s./Kg (spots in Germany, Poland, Estonia, Latvia and Lithuania; Urionabarrenetxea et al., 2022) would exert reproductive impairment in collembolans. Meanwhile, concentrations ranging 10.24–14.82 mg a.s./Kg soil (hot spots in Finland, Estonia and Latvia) (Urionabarrenetxea et al., 2022) could suppose significant collembolans mortality. In these

Table 4
Potential in-soil fauna ecosystem services loss derived from the PPP worst case application in European soils based on ecotoxicological endpoints (illustrated in Tables 2 and 3) and PECs.

Pesticide – organism	Effects upon NT-organisms along Europe after worst case PPP application	Ecosystem function	ecosystem services
Esfenvalerate ***Eisenia fetida***	For PECs 1.123–3.125 mg as/Kg) (inside main European concentrations) effects are uncertain due to the lack of data For 3.876–4.721 mg a.s./Kg soil concentrations in some C and N European soils (Germany, Lithuania, Latvia and Estonia) no effects are expected At > 5 mg a.s./Kg concentrations in N soil spots (Finland, Sweden, Estonia, Latvia and Lithuania), mortality and weight loss could happen	Uncertainty — Habitat provision, Soil formation & retention, Nutrient cycling, Erosion regulation Soil remediation /waste treatment Biodiversity	[a] - Supporting Regulating Provisioning
Esfenvalerate ***Folsomia* sp.**	For all concentrations estimated along Europe (1.343–5.565 mg a.s./Kg soil) effects on reproduction are expected	Pest & disease regulation, Habitat provision Soil formation & retention, Nutrient cycling Biodiversity	Supporting Regulating Provisioning
Cyclaniliprole ***Eisenia fetida***	No effects were expected for all European soils	-	-
Cyclaniliprole ***Folsomia* sp.**	Predominant PECs along Europe were ≈ 3.381 mg a.s./Kg so no effects could be expected For PECs ranging 5.669–7.957 mg a.s./Kg soil in some C and N European spots (Germany, Poland, Estonia, Latvia and Lithuania), effects upon reproduction could be expected For PECs ranging 10.24–14.821 mg a.s./Kg in some N European spots (Finland, Estonia and Latvia) effects upon mortality could be expected	- -Pest & disease regulation, Habitat provision Soil formation & retention, Nutrient cycling Biodiversity	- Supporting Regulating Provisioning
Picoxystrobin ***Eisenia fetida***	Mortalities expected in European concentration ranges (14.58–61.63 mg a.s./Kg)	Habitat provision, Soil formation & retention, Nutrient cycling, Erosion regulation Soil remediation /waste treatment Biodiversity	Supporting Regulating Provisioning
Picoxystrobin ***Folsomia* sp.**	For main concentrations in Europe (14.58 mg a.s./Kg soil) no effects are expected No data is available in EFSA databases for 24.91–33.64 mg a.s./Kg soil range; so effects for this agricultural areas	- Uncertainty	- [a]

(*continued on next page*)

Table 4 (*continued*)

Pesticide – organism	Effects upon NT-organisms along Europe after worst case PPP application	Ecosystem function	ecosystem services
	(spots in Germany, Poland, Estonia, Latvia and Lithuania) is uncertain		
	For concentrations ranging 42.97–61.63 mg a.s./Kg soil (spots in Finland, Estonia and Latvia) reproductive effects are expected.	Pest & disease regulation, Habitat provision Soil formation & retention, Nutrient cycling Biodiversity	Supporting Regulating Provisioning
Fenamidone *Eisenia fetida*	For all European concentrations (15.95–65.02 mg a.s./Kg) mortalities are expected	Habitat provision, Soil formation & retention, Nutrient cycling, Erosion regulation Soil remediation /waste treatment Biodiversity	Supporting Regulating Provisioning
Fenamidone *Folsomia* sp.	No effects were expected for all European soils	-	-

[a] The lack of information in the official sources makes impossible an accurate estimation. More empiric information is needed in order to relate effects and Ecosystem Service loss.

specific spots within northern Europe, reproductive and lethal effects upon collembolans could impact on supporting, regulating and provisioning services (by affecting pest and disease regulation, habitat provision, soil formation and retention, nutrient cycling and biodiversity) (Table 4).

Matching ecotoxicological data (Table 2) and picoxystrobin PECs, potential mortalities for earthworms are expected in European concentrations (14.58–61.63 mg a.s./Kg; Urionabarrenetxea et al., 2022), affecting soil ecosystem services; principally supporting, regulating and provisioning ones (Table 4). For collembolans no effects were expected for main (most frequent) picoxystrobin concentrations along Europe (14.58–20 mg a.s./Kg soil) (Urionabarrenetxea et al., 2022), whereas reproductive impairment could be expected in agricultural areas with 42.97–61.63 mg a.s./Kg soil (Urionabarrenetxea et al., 2022); affecting pest and disease regulation, habitat provision, soil formation and retention, nutrient cycling and biodiversity (Table 4). It must be highlighted that the lack of ecotoxicological endpoints (Table 3) for European concentrations ranging 24.91–33.64 mg a.s./Kg (mainly in certain areas of Germany, Poland, Estonia, Latvia and Lithuania (Urionabarrenetxea et al., 2022) made impossible the estimation of the potential Ecosystem Service losses.

Fenamidone worst case application showed concentrations ranging 15.95–65.02 mg a.s./Kg soil in Europe (Urionabarrenetxea et al., 2022). For this concentration range, lethal and chronic effects could be expected (Table 2) affecting earthworms role on habitat provision, soil formation and retention, nutrient cycling, erosion regulation, soil remediation /waste treatment and biodiversity (Table 4). Meanwhile, no impacts on collembolans communities are expected along European soils after worst case fenamidone application (Table 4).

In conclusion, main affected ecosystem services by earthworm and collembolan (in off-crop areas and in-crop areas) would be habitat provision, soil formation and retention, nutrient cycling, and biodiversity (Table 5). Additionally, impacts on earthworms would affect erosion regulation and soil remediation/waste treatment; while, impacts on

Table 5

The most important ecosystem services in agricultural landscapes (according to EFSA, 2010) due to PPP disposal. ecosystem services affected by impacts upon terrestrial macroinvertebrates marked in red.

Ecosystem Service category	In crop areas	Off crop areas
Provisioning	Food Fibre and fuel	Food Biodiversity Fresh water
Regulatory	**Pollination** Pest & disease regulation Erosion regulation	**Pollination** Pest & disease regulation Erosion regulation Water regulation Soil remediation/waste treatment
Cultural	**Education & inspiration**	**Education & Inspiration** Recreation & ecotourism Cultural heritage Aesthetic value
Supporting	**Primary production** Photosynthesis Habitat provision Soil formation & retention Nutrient cycling Water cycling	**Primary production** Photosynthesis Habitat provision Soil formation & retention Nutrient cycling Water cycling

Note: Only the services exhibiting a maximum importance (+++/+++) after PPP application according to EFSA (2010). ecosystem services affected by impacts upon soil macroinvertebrates (earthworms and collembolan) marked in red.

collembolans will affect pest and disease regulation. Overall, a higher risk for ecosystem service losses was observed in northern soils, especially in hot spots with significantly higher PECs (Finland, Sweden, Estonia, Latvia and Lithuania). Soils in northern Europe are characterized by cold climates, low pH and high OM contents leading to strong bindings between soil and pesticides, thus these are accessible to soil living organisms by oral route (Zou et al., 2018; Xu et al., 2019; Ogungbemi and van Gestel, 2018).

Moreover, the impact of PECs upon Ecosystem Service loses could be conditioned by the species habitat, role and behavior in the soil. In fact, the multiple habitats covered by different earthworm species enhance ecosystem services resilience. Epigeic earthworm species (e.g. *Lumbricus rubellus, Eiseniella tetraedra, E. fetida*) are more susceptible to environmental changes and pollutants (including pesticides) exposure than the anecic species; principally due to their close habitat to surface (Ockleford et al., 2017; Paoletti, 1999). In addition, epigeic species feed on humus, allowing incorporating PPPs attached to OM through dietary route, while, anecic species feed principally on soil column being exposed only in cases with soluble PPPs. Therefore, anecic organisms could fill the absent role (in part) of epigeic organisms affected by the use of pesticides. This scenario could occur in soils with esfenvalerate application, where high mortalities could be expected at surface due to the low solubility and the high affinity to OM, making difficult PPP leaching to deeper soil layers (Ogungbemi and van Gestel, 2018). This is a key factor for risk managers when protecting soil ecosystem services; especially when managing highly lipophilic compounds.

Folsomia sp. features high reproductive rates, and a well-developed exoskeleton that minimizes the possible effects exerted by pesticides. But their ventral tube enables water and oxygen exchange with the environment (Lock and Janssen, 2003; Fountain and Hopkin, 2005); making them particularly vulnerable to contamination via pore water (Filser et al., 2014; Ogungbemi and van Gestel, 2018). Moreover, these animals live on soil surface and could be exposed to pesticides for long periods; especially when sprayed PPPs are poorly lipophilic compounds, enabling high PPP concentrations in pore water. Only their ability to move quickly to buffer (clean) zones (Verhoef and Van Selm, 1983;

Detsis, 2000; Tsiafouli et al., 2005; Holmstrup, 2019) could enhance resilience, favoring preservation or recovery. This factor (pollution avoidance and migration to clean zones) should be considered regarding the management of large agricultural areas with pesticide disposal. Collembolans are considered one of the most abundant species in soil (Fountain and Hopkin, 2005), but their limited habitat in the shallow layers of the soil makes them less resilient than earthworms. Collembolan move/avoid to off-crop areas when pesticides are applied to in-crop areas, but may move back to the in-crop areas once PPP levels in soil decline due to the degradation. Thus, designing wild field margins in crops could be helpful to allow collembolans migrate to these zones. This crop management allows potential off-crop to in-crops area recolonization after PPP degradation (recovering lost ecosystem services). Besides, the scarce ecotoxicological information available on the effects of the selected PPPs to collembolans makes difficult to assess properly a robust managing strategy; being required further ecotoxicological studies in order to evaluate more accurate thresholds and effect ranges. Relevant information could be extracted from existing laboratory toxicity tests, but their capacity would be improved by study designs focusing on setting the concentration/time/response relationship instead of a NOEC and LOEC. In fact, regulatory agencies such as EFSA or USEPA are promoting the use of Benchmark dose approaches, which has been also applied to soil organisms including the assessment of pesticide mixtures (Yang et al., 2018).

The degradation time of PPPs should be also considered when assessing the impacts on Ecosystem Service losses. For instance, slow degradation times could pose significant effects on soil fauna for long periods. This could be crucial when managing PPPs causing chronic effects as reproductive impairment. Esfenvalerate, cyclaniliprole and picoxystrobin showed the slowest degradation times while fenamidone showed to be the fastest degrading compound. Although the later degradation period is the lowest among the selected PPPs, it should be desirable to minimize at maximum the degradation time in order to avoid effects on non-target species that could impact ecosystem services.

4. Conclusions

Ecosystem services provided by soil fauna impacted after PPP application to crops are identified: habitat provision, soil formation and retention, nutrient cycling, biodiversity, erosion regulation, soil remediation/waste treatment and pest and disease regulation. Moreover, spatial variability among European agricultural soil (pH, OM, temperature), PPPs physicochemical properties (MoA, Kom, solubility…), and non-target species behavior, habitat and role in ecosystem seemed to be the main factors to be taken into account for a correct PPP use management in crops. A change in the study design of laboratory toxicity tests, increasing the number of concentrations and intermediate time measurements, would facilitate the implementation of this approach in regulatory risk assessments.

CRediT authorship contribution statement

Erik Urionabarrenetxea: Data extraction and treatment, methodology, writing, original draft preparation. Carmen Casas: Data extraction, methodology, writing. Nerea Garcia-Velasco: Methodology, revision, writing. Miguel Santos: Methodology, writing, revision. Jose V. Tarazona: Conceptualization, writing, revision. Manu Soto: Conceptualization, methodology, writing, supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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