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Abstract

Before their placing on the market, the safety of plant protection products (PPP) towards both human and animal health, and the environment has to be assessed using experimental and modelling approaches. Models are crucial tools for PPP risk assessment and some even help to avoid animal testing. This review investigated the use of modelling approaches in the ecotoxicology section of PPP active substance assessment reports prepared by the authorities and opened to consultation from 2011 to 2021 in the European Union. Seven categories of models (Structure-Activity, ToxicoKinetic, ToxicoKinetic-ToxicoDynamic, Species Sensitivity Distribution, population, community and mixture) were searched for into the reports of 317 active substances. At least one model category was found for 44% of the investigated active substances. The most detected models were Species Sensitivity Distribution, Structure-Activity and ToxicoKinetic for 27, 21 and 15% of the active substances, respectively. The use of modelling was of particular importance for conventional active substances such as sulfonylurea or carbamates contrary to microorganisms and plant derived substances. This review also highlighted a strong imbalance in model usage among the biological groups considered in the European Regulation (EC) No 1107/2009. For example, models were more often used for aquatic than for terrestrial organisms (e.g., birds, mammals). Finally, a gap between the set of models used in reports and those existing in the literature was observed highlighting the need for the implementation of more sophisticated models into PPP regulation.

Keywords: ecological risk assessment, pesticide package, biodiversity, pesticide approval, regulation procedures

1. Introduction

Plant protection products (PPP), frequently named pesticides, represent "*one of the most important ways of protecting plants and plant products against harmful organisms, including weeds, and of improving agricultural production"* (European Commission, 2009). In the European Union (EU), the placing of PPP on the market is subjected to the Regulation (EC) No 1107/2009 (European Commission, 2009) and relies on two main steps. First, all of the components of the PPP (active substances, synergists, safeners) have to be approved at the EU level and the co-formulant must not be on the list of unauthorized ones. Second, the commercial form of the PPP is assessed at a zonal level (within a group of Member States, namely southern, central, and northern zones) prior to its authorization in one or several Member States of the targeted zone (Fig. 1). In this paper, only the procedure for active substances will be further considered, as these are the molecules likely to impair non-target species. In the regulation, active substances are defined as the "*substances, including microorganisms, inducing a general or a specific action on undesirable organisms or plants, parts of plants or plant products*" (European Commission, 2009, article 2). To be approved, an active substance must show its efficacy towards the target species as well as its safety towards human and animal health and environment. Also, it shall not have "*any unacceptable effect on vegetal and their products, shall not induce pain on vertebrate animals and shall not induce unacceptable effects on the environment*" (e.g., on non-target species or on biodiversity and ecosystem) (European Commission, 2009, article 4). Therefore, the environmental risk assessment (ERA) of an active substance is a mandatory step, among others such as risk assessment for human health. The ERA of pesticides assesses the impact that the use of pesticides has on non-target organisms and on soil, water, and air. In this work, we focus on the assessment of PPP bioaccumulation and impact on non-target organisms which relies on a "tiered approach" (e.g. Solomon et al., 2008). In brief, ERA starts at Tier 1 and can go up to higher tiers, if needed. Increasing the tier level goes along with an increase in the experimental system complexity for both biotic and abiotic aspects to make assessment scenarios more realistic. Despite such a procedure, deleterious effects of PPP on biodiversity have often been reported in the literature (e.g., on bees (Uhl & Brühl, 2019), birds (Tassin de Montaigu & Goulson, 2020), terrestrial (Gunstone et al., 2021) and aquatic ecosystems (Beketov et al., 2013; Malaj et al., 2014)), highlighting the need to adapt the ecotoxicological assessment

of PPP within the regulation procedure to improve the protection of biodiversity (Schäfer et al., 2019; Stehle & Schulz, 2015). Previous statements already asked for (i) adopting more holistic and realistic approaches in PPP risk assessment (Möhring et al., 2020; Schäfer et al., 2019), (ii) considering the landscape scale (Streissl et al., 2018) and (iii) integrating mixture effects (Stehle & Schulz, 2015) in order to better predict the effects of PPP on the environment. According to EFSA (European Food Safety Authority), modelling approaches can help to refine risk assessment as it may improve, for example, the ecological realism and reduce the uncertainties (e.g., using higher number of species, considering trophic interactions or exposure changes according to life-cycle or landscape) (EFSA, 2009; EFSA PPR Panel, 2013, 2014).

The relevance of modelling approaches to support the regulatory ERA of PPP, but also the associated technical and conceptual limitations that prevent their use routinely, were already highlighted (Forbes et al., 2009; Preuss et al., 2009). Modelling approaches present the advantage to assess PPP effects and risks for the environment overtime. The ultimate objective of a model is to support regulators for decision-making by (i) predicting bioaccumulation and effects of PPP on individuals, populations or communities; (ii) reducing uncertainty on risk assessment; (iii) estimating missing values such as organism sensitivity or compound physico-chemical parameters (Larras et al., 2022). Current guidance documents mainly recommend the use of such modelling approaches for risk assessment, but also to characterize the bioaccumulation potential of active substances based on their hydrophobicity or the properties of their degradation products (e.g. EFSA, 2009). Moreover, in the last decades, several publications supported the implementation of more sophisticated models in PPP regulation by providing guidance on good modelling practices (EFSA PPR Panel, 2014), pointing out among many other things the interest of toxicokinetic-toxicodynamic (TKTD) mechanistic models (EFSA PPR Panel, 2018a) or population models for bees (e.g., ApisRAM (EFSA Scientific Committee et al., 2021) or BEEHAVE (EFSA PPR Panel, 2015b)), reptiles and amphibians (EFSA PPR Panel, 2018b) as well as small mammals like common vole (Schmitt et al., 2016) with possible integration of variables at the landscape scale (e.g., ALMaSS model, Animal, Landscape and Man Simulation System, Topping et al., 2003). As a general rule, the ERA of PPP is continuously improving as demonstrated by the EFSA on-going reflection publication (EFSA, 2018).

In this context, this review aims at identifying the modelling approaches used in PPP regulation active substance assessment reports at the EU level, and the gaps between recommendations (e.g. guidances) and practices (namely, the assessment reports) in terms of model use. The modelling approaches found in these reports for the corresponding active substances were first identified, either they were implemented by the applicants or they came from scientific literature and either they were accepted or not by the RMS. Second we investigated the number of times each model category was involved in assessment reports within the past 10 years. Third, we explored which biological group of organisms were considered (e.g. which organisms are covered and if they are covered similarly). Our review is based on the investigation of the ecotoxicology sections (Volume 3 B-9 documents) of all the active substances opened to public consultation between 2011 and 2021.

2. Material and methods

2.1 Collection of assessment reports

For each active substance, a folder was collected from the public consultation archive webpage of EFSA (https://www.efsa.europa.eu/en/calls/consultations). Each folder encompassed several pdf documents related to the identity of the active substance; its physical and chemical properties; further information on the substance (intended purpose, dose); analytical methods; toxicological and metabolism studies; residues in or on treated products, food and feed; fate and behavior in the environment, and ecotoxicological studies. In this work, only the document relative to ecotoxicology (entitled Volume 3 B-9, named "assessment report" in this article) was kept for downstream analysis (Fig. 1, step 1: orange part). At least one Volume 3 B-9 document was available per active substance, but it also happened that more than one report was retrieved because: (i) additional ones exist for the representative PPP formulation, (ii) *addenda* were later provided, or (iii) an active substance was submitted a first time for approval (DAR=Draft Assessment Report) and a second time for renewal before approval expiration (dRAR=draft Renewal Assessment Report). In order to assess the time course of the use of modelling approaches in reports during the past 10 years, only those opened to public consultation from 2011 to July 2021 were selected. These documents allowed the analysis of the non-approved active substances

as well as the lastly submitted ones that are still under evaluation. Following this procedure, assessment reports were collected for a total of 317 active substances (=624 pdf documents). The investigated active substances as well as their characteristics (e.g. approval date, type of report, chemical group) are presented in Table S1.

2.2 Identification of modelling approaches in the reports

As it was not feasible to manually review the 624 pdf documents to identify all the used models, a set of models potentially used in reports was previously defined. They were identified based on (i) the results of a recent review on the effect modelling approaches employed in PPP ERA (Larras et al., 2022); (ii) EFSA documents related to PPP regulation (e.g., guidance documents, scientific opinions, technical reports); and (iii) several randomly-selected reports. Seven model categories of interest were identified: Structure-Activity, ToxicoKinetic (TK), ToxicoKinetic-ToxicoDynamic (TKTD), population, Species Sensitivity Distribution (SSD), community and mixture models. To illustrate their relevance under Regulation (EC) No 1107/2009, Table 1 presents the aims of the seven investigated model categories as well as some examples of input and output metrics.

As (i) a model category can encompass different models (e.g. Structure-Activity category is related to QSAR, QSPR, read-across); (ii) some assessment reports did not directly name the model but used related terms instead (e.g., Hazardous Concentration for Species Sensitivity Distribution model category); (iii) a same model could be named differently from one report to another (e.g.,“IBM” and “ABM” for population model); vi) a same model could be differently written between reports (e.g., number of compartments in a model written fully or with numbers), each of the seven model category was associated to a set of keywords to increase the chance of not missing a model. Dose-response models (static models) were not included as they represent already a keystone part of Tier 1 PPP risk assessment for most biological groups.

A total of 66 keywords (Table S2) were searched into the 624 pdf documents using the tm R-package version 0.7-8 (Feinerer & Hornik, 2008, 2020) to render the pdf files compatible to R functions.

2.3 Identification of biological groups

For each model category detection, the associated organism was manually retrieved and gathered into four aquatic (amphibians, aquatic invertebrates, aquatic primary producers, fish) and four terrestrial (birds, mammals, terrestrial invertebrates, terrestrial primary producers) biological groups.

2.4 Data analysis

As one active substance can be associated to more than one document (e.g., DAR and/or dRAR, an *addendum* or a report for its representative PPP formulation), the results were compiled at the active substance level. For example, if a given keyword was found in one or several of these documents, only one occurrence was counted (presence/absence (binary) data) for this active substance. Results at the substance and model category levels are presented in Table S1. If the year of opened consultation was of interest to collect reports, only the year of the assessment report made by the main rapporteur Member State (the one present in the label name of the reports) was considered in the downstream analysis.

All plots were performed using the R software (R Core Team, 2021) and the ggplot2 R package (Wickham, 2016).

3. Results and discussion

3.1 Characteristics of the reviewed active substances

Among the 317 inventoried active substances, 76 corresponded to three special categories: 49 were potential candidates to substitution (e.g. exhibiting carcinogen, mutagen, reprotoxic (CMR) effects), 26 were low risk substances (e.g. not CMR; no endocrine disruptor effect; microorganisms not showing resistance to anti-microbial products), and one was a basic substance (not a substance of concern; does not cause endocrine disruption, neurotoxic or immunotoxic effects; not used for plant protection purposes but nevertheless useful for plant protection) according to the active substance dataset of EFSA (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/, consulted on July 2021, the 7th). The remaining 241 active substances were considered as classical active substances. In total, 297 active substances were related to one submission while 20 active substances were related to different cases (e.g. first submission + renewal; DAR or dRAR + *addendum*).

From 2011 to 2021, the number of reviewed active substance per year increased up to 2017, then decreased drastically up to 2021 (Fig. 2). It has to be underlined that the query was performed over half of 2021, meaning that other active substances may have been opened to public consultation later this year. During the 10 investigated years, 44 % of the reviewed active substances were associated to at least one of the seven model categories. The use of modelling varied between years from one active substance in 2021 to 33 active substances in 2017. There is a clear time course evolution of submission types. Among the collected reports, only first submissions occurred up to 2012, but it still represented the majority of the submissions in 2014. Since then, approval renewal constituted the majority of reports reviewed annually and the only type collected in 2021. One explanation could be the reassessment program of the European Commission that requested to prioritize the renewal of already approved substances (Official Journal of the European Union, 2016). According to the Article 5 of the Regulation (EC) No 1107/2009, a first approval shall not exceed 10 years but it can also run 7 years, 15 years or an unlimited time for potential candidates to substitution (Article 24), low risk (Article 22) or basic substances (Article 23) respectively. Regarding the time-course use of modelling approaches in assessment reports, presence-absence data do not unravel a trend (Fig. 2) despite EFSA have published scientific opinions to promote the use of effect modelling for PPP ERA during the last decades (e.g. good modelling practice (EFSA PPR Panel, 2014), TKTD for aquatic organisms (EFSA PPR Panel, 2018a)). However, we may expect that it takes time to see the repercussion of such publications on regulatory reports. First, the constitution and review of a report can take several years. Second, sophisticated models may require a transfer of knowledge between the academics and the regulators. Thus, we can assume that upcoming reports will contain the sophisticated models covered in such scientific opinions.

3.2 Characterization of the models used in reports

After a manual check of all the detections, our query provided at least one consistent match for 37 keywords and all of the seven model categories (Table S2). The model categories are contrasted in terms of objectives, methodology and outputs (Table 1), and their use in the context of the Regulation (EC)

No 1107/2009 (European Commission, 2009) responds to different scenarios. For example, modelling approaches may help (i) to refine the risk assessment (e.g., TKTD models account for realistic time-variable exposure, population modelling accounts for ecological factors relevant at the population level, SSD assumed to reduce uncertainty by considering many species sensitivity); (ii) to characterize metabolites properties when needed (Structure-Activity); (iii) to deal with active substances with specific physico-chemical properties (e.g., food-web modelling for hydrophobic active substances). Among the model categories that were not found in reports, we noticed for example the absence of physiologically based pharmacokinetic (PBPK) models. Also, population models were only represented by agent-based models despite the consideration of keywords related to structured and unstructured models. In addition, among the community models, only those related to trophic transfers were detected despite the search of the "community model" term in a preliminary step. Thus, this category is restricted to food web/chain in this paper. However, one can consider that community models can support more than this goal, especially for the assessment of ecological recovery.

The number of model categories detected in the reports of the 317 active substances ranged from zero to five with a clear decrease in the number of active substances from the lowest to the highest number of model category (Fig. 3A). For the majority of the active substances (n=176), none of the seven targeted model categories were detected, probably meaning that the risk was already acceptable at Tier 1, or that the risk assessment was refined without the use of modelling approaches, or that no metabolite needed to be investigated with modelling approaches. Such results are mostly dependent on the current methods used in Tier 1 level and one could assume that considering also transgenerational responses may provide different results. A total of 81 active substances were associated to one model category, 44 to two model categories, 12 to three categories, three to four categories, and one to five categories.

Among the model categories, SSD was the most detected one (n=87 active substances) (Fig. 3B), which was not surprising as they are keystone models in ecotoxicology since decades (Aldenberg & Jaworska, 2000). The main limiting factor for the use of SSD models on some biological groups is the availability of sensitivity data (cf. requirement for aquatic organism in the guidance document (EFSA PPR Panel,

2013)). The existence of computational turnkey tools could ease their accessibility and promote an harmonized way to use them (e.g., the use of the ETX 2.0 program (Van Vlaardingen et al., 2004) for dicamba, lenacil or metribuzin, among other active substances).

Structure-Activity models were identified for 66 active substances, mainly to derive EC50 for various organisms (cf. section 3.5), estimate estrogen receptor binding affinity, octanol/water partition coefficient, or acid dissociation constant of metabolites. As for SSD, Structure-Activity models benefit from different tools (e.g., ECOSAR for metrafenone, mefentrifluconazole or DS TOPKAT for rescalure), which ease their handling in a harmonized framework. In addition, the higher use of Structure-Activity models in reports can be explained by the fact that they were already recommended for aquatic ecotoxicology, and birds and mammals in guidance documents since the early 2000s (EFSA, 2009; EFSA PPR Panel, 2013; European Commission, 2001). Read-across models were also detected to extrapolate organism sensitivity among substances of similar mode of action or among metabolites.

TK models were also often detected (n=46 active substances). Usually, they were fitted on experimental data to estimate various parameters such as uptake rate, depuration rate, bioaccumulation metrics or depuration half-time (see for example the assessment report of epoxiconazole or 1,4-dimethylnaphtalene, among other active substances). Depending on the number of involved compartments (e.g., one-compartment if the organism is considered as a whole, n compartments if organs or tissues are considered), different TK models were found: Single First Order, Double First Order in Parallel or First-Order Multi-Compartment kinetics. Among TK models, the absence of PBTK models frequently used in human risk assessment could be explained by the fact that they are still in development for species used in ERA (Grech et al., 2019).

The four remaining model categories *i.e.* TKTD models (n=8), mixture models (n=6), community models (n=5) and population models (n=4) were rarely detected in reports. The three first model categories were found acceptable in most of cases (Table S3). In our collection, issues regarding model reliability were pointed out for one case of population and community model. While being more recently developed (Jager et al., 2011; Kooijman, 2009), TKTD models such as GUTS and DEBtox models are of high interest for ERA (Brock et al., 2021; EFSA PPR Panel, 2018a). They are ready to use in practice in a regulatory context for few organisms like aquatic macroinvertebrates, fish and macrophytes, and

their potential is mentioned for other organisms like non-target arthropods (EFSA PPR Panel, 2015a). In the report collection, the detection of mixture models corresponded to PPP representative formulation reports to predict for example the joined toxicity of several active substances (e.g., formulation SIGNUM (BAS 516 07 F) containing pyraclostrobin and boscalid). Consequently, it could be expected that mixture models will be more often found in PPP registration reports at the Member State level. The 1st of October 2019, the European court of justice requested that procedures leading to the placing of a PPP on the market must consider the cumulative effects of the active substances of the PPP as well as their cumulative effects with other compounds of the product (Court Of Justice of the European Union, C-616/17 – Case Blaise and Others ECLI:EU:C:2019:800). Therefore, mixture models could be especially of interest for the commercial PPP rather than for the active substances. Finally, population and community models were the less detected ones. While population models were developed in research area on various organisms such as for example small mammals (Topping et al., 2003), fish (David et al., 2019) or bees (Crall et al., 2019), they were only detected in a few reports (more details in Table S3). It could be due to the latest development of models ready to be used under PPP regulation (e.g. Schmitt et al., 2016) and to the delay among their development, their validation and their appearance in reports. Recent scientific opinions highlighted the potential of population modelling at the landscape scale for assessing pesticide effects on non-target arthropods (EFSA PPR Panel, 2015a) as well as for reptiles and amphibians (EFSA PPR Panel, 2018b). Finally, dynamic community and ecosystem models have not been detected in assessment reports.

3.3 Temporal evolution of model use in reports

Finally, the time course of the use of each model category was investigated. In the last 10 years, SSD, Structure-Activity and TK models remained the most used models, despite some fluctuations probably due to the between-year variability in the number of reviewed active substances (Fig. 3C). The other models are still rarely involved. We may also expect an increase in the next years in the usage of the latest models such as TKTD and population models as suggested by the increasing interest of the EFSA PPR. This interest is demonstrated in EFSA scientific opinions dealing with modelling (EFSA PPR Panel, 2014, 2018a) as well as in guidance documents (e.g. EFSA PPR Panel, 2013). Indeed, these

models are increasingly referenced in regulatory documents or publications from meetings like Modelink (e.g., Hommen et al., 2016). Based on our review, it seems that guidance documents and transfer of knowledge is a crucial step for new modelling approaches to be implemented. The recommendations on good practices in modelling (EFSA PPR Panel, 2014) as well as the recent publications going in this direction for PPP ERA (Arlos et al., 2020; Raimondo et al., 2021; Roeben et al., 2020; Tarazona et al., 2021), constitute one step further in the acceptance of model categories like population models or TKTD in assessment reports.

3.4 Active substances and modelling in reports

As previously indicated, the use of modelling in reports is mainly justified by the need to refine the risk assessment, to assess metabolite properties and by the relationship between the physico-chemical properties of active substances and their effects. Thus, the fact that an active substance does not pass Tier 1 may be linked to its status (candidate to substitution vs. low risk), chemical group or biochemical target.

First, active substances that are candidates to substitution appeared logically more often associated with effect modelling approaches (34 out of 49 active substances) than the low risk active ones (1 out of 26). Second, based on the Pesticide Properties Database (http://sitem.herts.ac.uk/aeru/ppdb/index.htm), a chemical group was attributed to 305 active substances (out of 317), leading to 109 chemical groups (Table S1). The 15 groups gathering the highest number of active substances are presented in Figure 4A. We noticed a lower use of modelling in reports for several chemical groups of active substances such as the "Micro-organism derived" (7 active substances related to modelling out of a total of 64), the "Plant derived" (2 out of 12) and the "Inorganic compounds" (1 out of 9). Although micro-organisms and plant derived substances are not a chemical group in the strict sense of the term, they are still interesting to be considered as they are also covered by the Regulation (EC) No 1107/2009 and submitted to the same ERA procedure as conventional active substance chemical groups. The same trend was observed for the "unclassified" group (4 out of 15) gathering all of the active substances for which no group was assigned. Conversely, at least one model category was used in most active substances related to the sulfonylurea active substance chemical group (12 out of 17), the carbamates (11 out of

15), the triazoles (6 out of 9), the organophosphates (5 out of 7) and the pyrethroids (7 out of 8) (Fig. 4A).

Third, a similar analysis was performed using the universal R4P classification (R4P, 2019) which provides a class mainly informing on the interaction between the active substance and the biological target. One class was attributed to 277 active substances, and 19 classes were identified (Fig. 4B). We noticed a high diversity of classes, with contrasting results in terms of modelling use. For example, the microbial pesticide group, composed by the highest number of active substances (n=41), as well as the integrity of cellular membrane class (mostly derived from plants and microorganisms) were not or weakly related to modelling approaches according to the set of used keywords. The stimulation of the plant defense class also displayed such pattern (1 out of 7 active substances). In addition, cellular division or cytoskeleton and carbohydrate metabolism classes concerned only a low number of active substances with modelling approaches. A similar pattern was observed for most of the active substances with the unknown target class as well as for those not appearing in the classification (7 out of 40 active substances). More than half of these substances were related to microorganisms, plant hormones, animals or unknown chemical groups. Only a few active substances (e.g. 1,4-dimethylnaphthalene) without clear target used modelling approaches. Inversely, classes such as nervous system or muscle (25 out of 37 active substances), photosynthesis (11 out of 17 active substances), amino acid and proteins biosynthesis (20 out of 28 active substances) or lipid metabolism (8 out 10 active substances) were associated to a high number of active substances for which at least one modelling category was used.

All these results suggest that active substances related to microorganisms and natural derived compounds rarely fit to one of the three conditions mentioned at the beginning of this section and requiring modelling (i.e. to refine the risk assessment, to account for the physico-chemical properties or to estimate metabolite properties). These chemical substances mostly belong to the biopesticides which do not exist in Regulation (EC) No 1107/2009 but which corresponds to a set of crop protection methods that have been defined by the article L 253-6 of the French Rural and Maritime code. In general, biopesticide active substances appeared less associated to the use of such modelling approaches than the conventional active substances, meaning that the risk linked to biopesticides may be more easily acceptable in Tier 1. However, as several biopesticides can be nevertheless problematic (Robin &

Marchand, 2019), we may also assume that if the risk is not acceptable, it may be refined with other methods than modelling, which will not be detected in our study. It is also worth noticing that living microorganism active substances are also subjected to other criteria (e.g., infectiveness or pathogenicity) that may require other types of modelling which were not investigated in this work. Conventional pesticides have been more often studied with modelling, potentially meaning that they pose a higher risk to non-target species or that they produce metabolites that have to be characterized. Among them, some categories and modes of action of active substances have been investigated since decades because of the ecological risk they pose to non-target species in the environment. Consequently, more data are available, what eases the use of models, for example for SSD approaches.

3.5 Non-target organisms addressed by modelling

The non-target organisms (gathered into biological groups) which were addressed in modelling approaches were investigated to determine if some models dealt specifically with some groups. The Figure 5 illustrates the percentage of active substances (hereafter, called edge value), based on the total number of active substances using at least one model category, involving each model category to each biological group. In the reviewed reports, all of the identified biological groups dealing with modelling were involved at least once in a SSD. Most of the SSD models were used for non-target terrestrial plants (NTTPs, edge value=11%), aquatic primary producers (macrophytes and microalgae), aquatic invertebrates (including sediment organisms), and fish. That echoes the high number of herbicides and insecticides needing modelling as demonstrated in Figure 4. SSDs is a well-accepted method in ERA, and its use is recommended in the PPP regulation since the early 2000s for NTTPs (European Commission, 2002) and aquatic organisms (European Commission, 2001). In compliance with the need to reduce animal testing, it seems logical that the SSD approach (that requests lots of organism sensitivity data) appears less used for biological groups such as birds, mammals, reptiles and amphibians. Structure-Activity models were widely associated with aquatic organisms such as invertebrates (edge value=12%, e.g. chironomids, crustaceans, especially *Daphnia* or *Americamysis bahia*), primary producers (edge value=11%, especially microalgae) and fish, in order to estimate their

sensitivity (e.g. EC50) to active substances. In the reports, Structure-Activity also allowed to estimate the bioaccumulation potential of active substances in fish. In this perspective, TK models are classically used for fish (edge value=13%) and terrestrial invertebrates (e.g. earthworms, spiders and coleopterans) to calculate bioaccumulation metrics of active substances in organisms and also, under steady-state, uptake and elimination rates (Ratier et al., 2022). Body Burden Models were exclusively used for birds and small mammals. First and second order models are classically used, associated with models ranging from one to five compartments depending on the targeted level of complexity. Kinetic parameters are especially interesting when the TK models support the estimation of active substance bioaccumulation in bird and mammal preys.

The category of TKTD models (gathering DEBtox and GUTS) was associated to only three aquatic biological groups: microalgae (primary producers), invertebrates and fish. The use of population models remained marginal, involving only one terrestrial (*Folsomia candida*) or aquatic invertebrate (*Chaoborus crystallinus*) as well as small mammals (vole). Finally, community models (not shown in Fig. 5 as they gather more than one of our biological groups) were applied to trophic food chains for aquatic (5 active substances) or terrestrial (3 active substances) set of species.

One of the main results depicted in Fig. 5 is the strong imbalance in modelling coverage between aquatic and terrestrial biological groups in assessment reports. In the reviewed reports, aquatic groups appeared especially well supported in terms of model diversity and usage frequency. Kattwinkel et al. (2015) also identified such imbalance regarding the number of studies dealing with aquatic and terrestrial population recovery in PPP risk assessment. The gap between aquatic and terrestrial groups could be due to data availability, because test characteristics and ethical issues are not the same between algae/aquatic macroinvertebrates and birds or mammals. According to the logic of the tiered-approach, one may also suggest that it is because aquatic organisms may need more often risk refinement than terrestrial organisms. In addition, the targeted models were maybe so far more suitable to aquatic organisms. However, as the reasons remain unclear, it could be of high interest to investigate such question to better characterize the limits of PPP ERA in regulation procedures.

Finally, amphibians (the aquatic stage of two species) were included only once in a SSD model (combined with fish sensitivity data), probably because this biological group is optional in reports and

because animal testing have to be reduced. Moreover, there is no optimal risk assessment method currently available for amphibians (and reptiles), despite a recognized vulnerability to PPPs (EFSA PPR Panel, 2018b). Similarly, no model related to bees was found, although the ApisRAM model (EFSA Scientific Committee et al., 2021) is under development and should be implemented in assessment reports in a near future.

4. Conclusions

This review aimed at investigating the use of modelling approaches for ecotoxicological risk assessment of PPP before their approval for placing on the market. The most frequently detected models aimed to reduce uncertainty of species sensitivity (SSD), to estimate active substance kinetics parameters in organisms or their bioaccumulation capacity (TK), and to handle metabolites with the aim to derive organism sensitivity data, as well as physical and chemical parameters (QSAR). This reflects a global model usage for lower tiers assessment, meaning that modelling is in practice rarely used for population, community, or landscape purposes in PPP regulation yet. The explanation may be that (i) no risk refinement was needed at higher tier; (ii) data were lacking in order to correctly fill in the inputs of the models; or (iii) models were not validated enough for regulation purpose even though their relevance for this type of problem is widely proven. Thus, to date, the potential of modelling approaches for risk assessment is poorly exploited under PPP regulation maybe due to a supplementary need of recommendations in model evaluation, or due to the delay between the publication of the EFSA documents and their applicability into the assessment reports. It has to be underlined that the use of modelling may also depend on the end-user ultimate purpose, as refined risk assessment is only needed when the risk was unacceptable at lower tiers. Additionally, EFSA publications (EFSA PPR Panel, 2014, 2018a) clearly established how it became crucial to implement more sophisticated models into PPP regulation to further strengthen ERA.

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Table 1: Examples of aims, input and output data of the seven model categories in the context of their use under the Regulation (EC) No 1107/2009.

	Structure-Activity	TK	TKTD	Population	SSD	Community	Mixture
Aims in Regulation (EC) No 1107/2009	Single-species sensitivity extrapolation Metabolites characterization	To estimate bioaccumulation or internal concentration	To estimate organism response over a time-dose gradient	To estimate population viability (level effects and recovery)	To extrapolate in-situ community sensitivity	To estimate chemical trophic transfer (food chain or food web models)	To predict the joint toxicity of the combination of n components
Inputs (to inform parameters or variables)	Log P, molecular structure, SMILES	Quantity in media (water, food, …) QSAR, *in vitro* experiment, assessment on *in vivo* data		Ecological scenario (contamination scenario, abiotic environment, population life history), Toxicological data	Several ECx (depends on the guidance document)	Quantity/concentration of active substance Uptake and elimination rates	ECx (at least 2)
Outputs	ECx, LCx, … BCF, BAF, BSAF, …	Time course of internal concentration (BCF, organs...)	Time course of internal concentration and resulting effect over time	Population size and structure (asymptotic growth rate, net reproductive rate, sex-ratio…), Time to recovery, Extinction probability, Spatial occupancy	HCx	BAF, BSAF, …	ECx mix

BAF: Bioaccumulation factor; BCF: Bioconcentration factor; BSAF: Biota-*sediment* accumulation factor; EC_x: Effective Concentration leading to the inhibition of X% of an endpoint; HCx: Hazardous Concentration theoretically impairing x% of the species of *in situ* communities; LC_x: Lethal Concentration leading to the mortality of X% of a population; QSAR: Quantitative Structure-Activity Relationship; SMILES: Simplified molecular input line entry system.