LRIECO 50: INCORPORATING SPATIAL AND SEASONAL VARIABILITY IN COMMUNITY SENSITIVITY INTO CHEMICAL RISK ASSESSMENT



Jonathan F. Jupke, Tomás Duque, Javier Jarillo, Sebastian Scheu, Thomas Sinclair, Sanne van den Berg, Martin Entling, Frederik De Laender, Lorraine Maltby, Paul J. Van den Brink, Ralf B. Schäfer



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1. Executive Summary

Ecosystems are exposed to a blend of synthetic chemicals. Regulatory practices aim to protect ecosystems from unacceptable effects but current approaches largely ignore spatio-temporal variation in recipient ecosystems. In this project, we investigated whether spatio-temporal turnover in the assemblages of earthworms, spiders, macroinvertebrates, and diatoms across large scales are associated with differences in chemical sensitivity that would merit changes to current risk assessment practices.

To this end, we first established terrestrial and aquatic ecosystem types. These types divided the continuous gradients of environmental attributes that shape assemblages into distinct and non-overlapping groups such as *Boreal Wetland* or *Small Lowland River on siliceous bedrock*. For terrestrial ecosystems, we developed a new typology system purpose-built for this project, which consisted of eleven types. For aquatic ecosystems, we relied on the recently published Broad River Type system, which delineates 20 distinct types.

We compiled a large occurrence database for the four focal taxonomic groups. With this database, we inferred typical assemblages for the ecosystem types. These typical assemblages focused on taxa that occurred frequently or typically within the respective ecosystem type. As we derived typical assemblages only for those types with sufficient data coverage and merged typical assemblages with strongly overlapping species lists, we determined 25 (twelve terrestrial and 13 aquatic) of the 62 theoretically possible typical assemblages.

In the next step, we aimed to assess the sensitivity of the assemblages to four toxicants from different chemical classes (metal, insecticide, herbicide, pharmaceutical). Given data gaps for many species, we explored the potential for cross-species extrapolation. We performed a review that overviews currently existing cross-species extrapolation methodologies, and discussed i) how species sensitivity could be described, ii) which predictors might be useful for explaining differences in species sensitivity, and iii) which statistical considerations are important. We argue that risk assessment can benefit most from modelling approaches when sensitivity is described based on ecologically relevant and robust effects. Regarding which predictors are useful for explaining differences in species sensitivity, we reviewed interspecies-correlation, relatedness-based, traits-based, and genomic-based extrapolation methods. For each method, we describe the amount of mechanistic information the predictors contain, the amount of input data the models require, and the extent to which the different methods protect ecological entities. We developed a conceptual framework, incorporating the strengths and weaknesses of each method. Regardless of the method used, statistically significant models can be found, although the usefulness, applicability, and understanding of these models varies considerably.

Based on the findings from the review, we developed predictive models on species sensitivity based on their traits, relatedness, and interspecies sensitivity correlations for aquatic species. We used information on similarity concerning traits and taxonomy between the taxa to construct the models. The evaluated Modes of Action (MOA) encompassed chemicals belonging to insecticides, herbicides, metals and pharmaceuticals, thereby covering organic and inorganic chemicals, agrochemicals, and down-the-drain chemicals. Most models could be constructed for aquatic invertebrates, while only one model could be constructed for diatoms.

The available terrestrial toxicity data was insufficient for constructing models for the MOA of interest. Therefore, we conducted toxicity tests. We ran acute toxicity tests with imidacloprid as well as a copper-based fungicide for earthworms, and with lambda-cyhalothrin for spiders. The standard earthworm used for pesticide risk assessment in Europe, *Eisenia fetida*, was less sensitive than most tested field species. Non-epigeic earthworms were more sensitive than epigeic earthworms, which may affect the ecological function of bioturbation. The spiders from boreal and polar climates were more sensitive than those from cool and warm temperate climates. Furthermore, free-hunting spiders were more sensitive than web-builders, indicating that the ecological function of natural biological control may be affected by chemicals.

Based on the available empirical data and the data estimated in cross-species extrapolation, we used a hierarchical species sensitivity model to calculate sensitivity profiles for 3862 European river macroinvertebrate assemblages to four chemicals (copper, atrazine, lambda-cyhalothrin, imidacloprid). The sensitivity of assemblages to a chemical (measured as HC_5) varied by up to two orders of magnitude, but for 95% of assemblages, the inter-assemblage variation was approximately one order of magnitude. Assemblage sensitivity varied between river types, but no river type was consistently either the most or the least sensitive across the four study chemicals.

We also constructed ecological models that take into account interspecific interactions to assess assemblage sensitivity. The variable of interest is species richness - we defined the concentration at which the first species was lost as an assemblage's sensitivity. A general analysis of the model equations revealed generic descriptors that explain assemblage sensitivity. These descriptors relate to the way in which the food web is structured (e.g. predator species are more sensitive than prey species), and which trophic level is most sensitive to the chemical. We then parameterized the model to represent the typical assemblages using trait data bases, showing that the generic rules explain sensitivity of these typical assemblages. The ecological models confirm that sensitivity differences among assemblages is limited, and that these differences can be predicted from ecological theory. For WP 4: "Validation with community and single-species sensitivity tests", we performed two mesocosm experiments in collaboration with other projects. Merga and Van den Brink (2021)

investigated the effects of four applications of imidacloprid with a weekly interval. The results showed that the macroinvertebrate and zooplankton community structure changed significantly due to imidacloprid contamination in mesocosms repeatedly dosed with ≥ 0.1 and $\geq 0.01 \mu g/L$, respectively. Van de Perre *et al.* (2021) performed a similar study in sub-tropical China. At the community level, a lowest NOEC_{community} of 0.03 $\mu g/L$ was calculated for the zooplankton, phytoplankton, and macroinvertebrate communities. Under (sub-)tropical conditions, the toxic effects of imidacloprid occur at lower concentrations than found for temperate ecosystems. We found no clear relationships between the hSSD derived HC₅ values and the NOEC_{community} values, with HC₅ values being much lower than NOEC_{community} values when the study was performed in the temperate zone, but comparable when the study was performed in the temperatures, which has been shown by experiments performed by Hang *et al.* (2023) and mechanistically modelled by Mangold-Döring *et al.* (2022). This shows that hSSD can probably be used to predict the sensitivity of communities to imidacloprid within climatic regions but not beyond

After thorough discussion at the project workshop, we come to the conclusion that the spatial variation in taxonomic composition across habitats at larger spatial scales is a relevant driver of differences in sensitivity to chemicals. However, the magnitude of the differences in sensitivity found in this project would likely be captured by current assessment factors. environmental factors that drive differences in chemical sensitivity, and may therefore be more relevant when considering the need for a spatially explicit zonal risk assessment of chemicals in Europe, were outside the scope of this project.

2. Project Overview

Many sectors of European societies rely on chemicals, which are regulated in different political frameworks with the aim to protect from unacceptable effects (Galic *et al.* 2010). The effects on non-target organisms are currently assessed in a one-size-fits-all approach centred on sensitive single species tests or multispecies test systems. However, ecological assemblages in the real world vary in their composition in space and time and so may their sensitivity towards a chemical. Using a single effect threshold might thus, depending on the variability in sensitivity, result in over- or underprotection. Evaluating the spatio-temporal variation in chemical sensitivity thus is pivotal for an efficient risk assessment. The GETREAL project aimed to provide this information and discuss consequences for risk assessment.

To evaluate spatio-temporal variation in sensitivity, we compiled data on the assemblage composition of spiders, earthworms, diatoms, and macroinvertebrates in different ecosystems and seasons. The data was used to identify typical assemblages of these taxa for ecosystem types that were delineated by a terrestrial or an aquatic typology system respectively. The sensitivity of these assemblages was determined via toxicity tests, stochastic hierarchical species sensitivity distribution models (hSSD), and ecological predator-prey models. These assemblage-level sensitivity predictions were evaluated with the results of mesocosm experiments performed within the context of this project and available in the open literature. Finally, the results and consequences for risk assessment were discussed in a workshop with stakeholders.

Overall, the project was structured in five work packages (see Figure 2.1).

- 1. Characterization of European land units to derive a landscape typology
- 2. Identification of typical assemblages
- 3. Determination and prediction of species and assemblage sensitivity
- 4. Validation of assemblage-level sensitivities
- 5. Incorporating sensitivity variation in risk assessment



Figure 2.1: Conceptual diagram showing the different in- and outputs of the project and work packages, the tools used to link these and the linkages between the WP's.

3. Characterising European land units to derive a landscape typology

In working package (WP) 1, we established two typology systems: One for terrestrial and one for aquatic ecosystems. Both typology systems summarise the variation in the major environmental gradients that determine assemblage composition of our focal organism groups (i.e., spiders, earthworms, diatoms, and macroinvertebrates) into discrete and non-overlapping ecosystem types. For the terrestrial typology, we derived a new system specifically tailored to our needs in this project. For the aquatic typology system, we were able to benefit from a new pan-European typology system that was published after the start of this project and met all our demands. Both typology systems are shortly introduced below.

3.1 Terrestrial Typology System

The major environmental gradients that determine assemblages vary at different scales (Ricklefs, 1987). We used climate and land use to account for variation on a large and small spatial scale, respectively. For climate, we used the temperature component of the Holdridge life zones (HLZ) of Western Europe (Holdridge, 1967). We based the biotemperature on the mean monthly temperature from the WorldClim dataset (Fick & Hijmans, 2017), adjusting monthly temperatures below 0°C and above 30°C to the respective limit. Calculating the overall mean, yields the mean annual temperature and the four HLZ polar (0-3°C), boreal (>3-6°C), cool temperate (>6-12°C), and warm temperate (>12°C). For land use, we chose forest, open land, and wetland according to the major CORINE land cover (CLC) categories, excluding water bodies and artificial surfaces (Büttner *et al.*, 2004). The intersection of the four climatic and three land use types yields twelve different types, however the type *Polar Forest* does not occur in reality (Figure 3.1.1).



Figure 3.1.1 - Derivation of habitat typology as intersection of climate and land use categories. Note that the type *Polar Forest* (*) is non-existent and was omitted from the analysis.

3.2 Aquatic Typology System

As an aquatic typology system, we used the Broad River Types proposed by Lyche-Solheim *et al.* (2020). They are an aggregation of national Water Framework Directive (WFD) typology systems. The WFD requires all participating states to create national waterbody typology systems. The national states have considerable leeway in selecting the classification criteria and in determining the class thresholds. This has resulted in noticeable differences between the national typology systems. Lyche-Solheim *et al.* (2020) harmonised and aggregated the national types in an effort to create the first pan-European river typology system.

Their final typology system used the descriptors: altitude, bedrock geology, catchment size, region, and flow regime to discriminate between 20 different broad river types. These twenty types are Very large rivers (River Type (RT) 1); Lowland, siliceous, medium to large rivers (RT 2); Lowland, siliceous, very small to small rivers (RT 3); Lowland, calcareous and mixed, medium to large rivers (RT 4); Lowland, calcareous and mixed, very small to small rivers (RT 5); Lowland, organic and siliceous (RT6); Lowland, organic and calcareous (RT7); Mid-altitude, siliceous including organic, medium to large rivers (RT 8); Mid-altitude, siliceous, very small to small rivers (RT 9); Mid-altitude, calcareous or mixed organic, medium to large rivers (RT 10); Mid-altitude, calcareous or mixed, very small to small rivers (RT 11); Mid-altitude, organic and siliceous (RT12); Mid-altitude, organic and calcareous (RT13); Highland, siliceous and organic (RT14); Highland, calcareous and mixed (RT15); Glacial rivers (RT16); Mediterranean, lowland, perennial, medium to large (RT17); Mediterranean, mid-altitude, perennial, medium to large (RT18); Mediterranean, perennial very small to small (RT19); Mediterranean temporary and intermittent streams (RT20). The thresholds between these categories are provided in Table 3.2.1.

Table 3.2.1 - Class thresholds for broad river type descriptors.

Type descriptor	Category	Value	
Altitude	lowland	< 200 m.a.s.l	
	mid-altitude	200 - 800 m.a.s.l	
	highland	> 800 m.a.s.l	
Geology	siliceous	< 1 mEq/L	
	calcareous	> 1 mEq/L	
	organic/humic	any	
	mixed	any	
Catchment size	very small to small	< 100 km ²	
	medium to large	100 - 10.000 km ²	
	very large	> 10.000 km ²	
Region	Mediterranean		
	Rest of Europe		
Flow regime	perennial		
	temporary/ intermittent		

4. Identifying typical assemblages

For each type of the typology systems derived in WP 1, we aimed to define typical assemblages (TA) of spiders, earthworms, diatoms, and macroinvertebrates in WP 2. The TA of an ecosystem type consists of taxa that occur frequently in the ecosystems of the respective type. To identify these taxa, we compiled a database of occurrence records throughout Europe. We collected data from national monitoring agencies, the scientific community, and available prior databases.

Several criteria were applied to select spider samples. First, in the case of abundance data species lists were only used if at least two families, five species, and ten individuals were reported for a given site. Second, for presence-absence data the number of individuals was irrelevant and only the former two criteria applied. Afterwards, all selected spider data were transformed to presence-absence to avoid mixture of data types and to account for methodological differences (e.g., number of traps, sampling duration). Earthworm samples were collected with a range of different methods (e.g., hand collecting, various extraction methods) and on different scales (e.g., abundance per area in plot, abundance per volume of soil), and therefore all assemblages were converted to incidence data beforehand. Afterwards, the criteria for presence-absence data were applied. The provided habitat information was categorised according to the European Nature Information System (EUNIS; Davies et al., 2004). This allowed us to exclude transitional habitats (e.g., forest edges and hedgerows). which did not fit the land use types we defined. To avoid bias from using multiple samples from intensively sampled regions, we selected the most representative of all assemblages within a given EUNIS category on the first level and within 50 km, and omitted all other samples of the same habitat from that circumference. This was done by selecting the assemblage closest to the group centroid using non-parametric Permutational Analysis of Multivariate Dispersion (Anderson et al., 2006). Further analysis was conducted on species level for both earthworms and spiders.

For diatom and macroinvertebrates, we removed sites that were more than 500 m away from the closest river reach in the digital representation of the Broad River Types (Globevnik, 2019, Accessed 11.12.2019), as well as sites deemed disturbed (see Jupke *et al.* 2022 for details).

For the derivation of TAs, the preprocessing steps above resulted in 284 spider, 246 earthworm (see Figure 4.1), 4538 macroinvertebrate, and 1169 diatom communities available for analysis (see Figure 4.2).



Figure 4.1: Spatial distribution of spider (A) and earthworm (B) samples



Figure 4.2: Spatial distribution of diatom (A) and macroinvertebrate (B) samples

Macroinvertebrate data are often available at genus- or family-level. To include taxa with coarse taxonomic resolution (e.g., Oligochaeta) while making full use of the information

provided by taxa that are reported at species level, we used variable taxonomic levels for the macroinvertebrate data. The optimal level for each taxon was established with a hierarchical approach. For each taxon, we calculated the percentage of observations represented at each higher level, where higher level means higher resolution (e..g, species is a higher level than genus). For example, 4.12% of observations from the order Lepidoptera are at the species level, 74.77% at the genus level, 7.75% at the family level, and 13.35% at the order level. Given a threshold *X*, we hold a taxon to be optimally represented at a certain taxonomic level if less than *X*% are represented by higher levels. For example, Lepidoptera would be represented on order level if X > 4.12% + 74.77% + 7.75% = 86.64%. We used 85% as a threshold, which would mean in this case that Lepidoptera would be represented at family level. All species and genus level observations would be aggregated to family level. Observations that were made at a lower than the optimal level (e.g., family when genus is the optimal level) were included at their respective level.

For all four taxonomic groups, we focused on those ecosystem types that were best represented by our samples. To this end, we omitted six ecosystem types for spiders and earthworms (all Polar regions, Boreal forest and wetland, and warm temperate wetland), the river types 1, 3, 4, 5, 7, 11, 12, 13, 17, 19, 20 for diatoms, and the river types 6, 7, 12, 13, 17, 19, and 20 for macroinvertebrates.

As stated earlier, we defined an ecosystem type's TA as the taxa that frequently occur in the focal ecosystem type. We included all species that occurred in more than a quarter of the samples we obtained from any given ecosystem type in that ecosystem type's TA. For the macroinvertebrate which also contained observations with low taxonomic resolution, we adjusted the thresholds to half of the samples for genera and three quarters of the samples for families or lower taxonomic resolutions. For the aquatic TAs, we additionally included specific species, i.e., those species that are close to endemic to a single river type. Specific species have at least 90% of their occurrences in a single river type while occurring in more than 5% of samples of the respective river type. Only species were considered for this.

To validate the ecological relevance of the abiotically defined ecosystem types, we computed the overlap in TAs between different ecosystem types. If two TAs shared more than 75% of their taxa we regarded them as redundant, combined the two types, and determined a new TA for the combined type.

For spiders, earthworms and diatoms we derived six TAs. For macroinvertebrates we derived seven TAs. All TAs are listed in Appendix 1.

For all taxa, we lacked data from the same or spatially close sampling locations across seasons. This lack of data prohibited the determination of seasonal TAs. As a proof of concept,

we developed seasonal TAs for selected river types. Non Metric Dimensional Scaling (Kruskal 1964) indicated a large overlap between seasonal TAs and Generalised Dissimilarity Models (Ferrier et al. 2007) showed low taxonomic turnover between seasons. Hence seasonal variation seems to be lower than spatial variation.

5. Determination and prediction of species and assemblage sensitivity

5.1 Assessing and determining species sensitivities

In WP 3, the aim was to compare the sensitivity of assemblages derived in WP 2 to four different toxicants (metal, insecticide, herbicide, down the drain chemical). A necessary prerequisite for this comparison was to assign toxicity data for these compounds to the organisms from the assemblages (5.1) via toxicity tests and cross-species extrapolation. For terrestrial organisms, data scarcity prohibited the use of cross-species extrapolation and we therefore conducted toxicity tests for a range of earthworms and spiders with pesticides that were known to be acutely toxic (5.1.1 and 5.1.2). For aquatic organisms, we explored the use of cross-species extrapolation to fill data gaps (5.1.3). The modelling of assemblage sensitivity is described in 5.2.

5.1.1 Earthworm chemical sensitivity

For earthworms, we assessed the mortality effects of the insecticide imidacloprid and a copper-based fungicide (CuSO₄·5H₂O). Adult earthworms were collected by hand in winter and fall of 2020 around Landau in der Pfalz, Germany. Sampling sites were selected to cover the major ecosystems of the region, i.e., grasslands, forests, and wetlands, covering both acidic and neutral soils (Table A2.1). Additionally, Eisenia fetida (the standard earthworm used in pesticide risk assessment), an atypical soil species, was obtained from a domestic compost pile, as well as from a laboratory culture (ECT Oekotoxikologie GmbH, Flörsheim/Main, Germany). Earthworms were transported to the laboratory and kept with natural soil for one week, prior to ecotoxicological assessment, in a climate chamber at 16°C, 65% relative humidity and 16/8 light/dark cycle. The mortality tests were based on the OECD guideline 207 (1984), with the following adaptations. Instead of artificial soil, we used the standard soil LUFA 2.2 as the test substrate, which is widely used as a standard soil for the ecotoxicological assessment of soil invertebrates. Additionally, the test temperature was decreased from 20°C to 16 °C, which better reflects the temperature of the soils where the test organisms originated from. The acute effects of imidacloprid or copper were tested on earthworms from the same species and ecosystem. Survival was assessed by testing the organism's reaction to a gentle mechanical stimulus on days seven and 14 after the pesticide application. The median lethal concentrations (LC₅₀s) were calculated after seven (if 100% mortality was reached at the highest concentration) and 14 days of exposure for all tested species by fitting binomial dose-response models to the data (Ritz *et al.* 2019). Furthermore, species sensitivity distributions (SSDs) were fitted for both pesticides using the 14-day LC₅₀ values for all examined species and literature data from comparable studies, as seven-day LC₅₀s were not available for all tests. Hazardous concentrations 5% (HC₅s) were derived from these distributions, and their respective 95% confidence intervals. In addition, potential associations between earthworm chemical sensitivity in terms of LC₅₀, ecosystem characteristics and fresh weight were measured with Pearson correlation coefficients. *E. fetida*, an epigeic compost earthworm rarely found in nature, was excluded from these calculations.

In total, we ran 14 tests for each pesticide, with six species of earthworms from the genera *Allolobophora, Aporrectodea, Eisenia*, and *Lumbricus*. Earthworm 14-day LC₅₀s (Table A2.1) for imidacloprid ranged between 0.72 and 3.53 mg a.i./kg dry-weight (d.w.), and LC₅₀s for copper ranged from 200 to 433 mg a.i./kg d.w. Additional LC₅₀ values were included from the literature to fit the SSDs (Table A2.2). The HC₅ (95% CI) derived from the SSDs for imidacloprid (Fig. 4.1.1.1 a) and copper (Fig. 4.1.1.1 b) were 0.70 (0.32-1.47) and 202 (178-234) mg a.i./kg d.w., respectively.

Our results confirm that the standard test organism E. fetida is less sensitive to pesticides than other earthworm species (Fig. 5.1.1.1; Pelosi et al. 2013). Protecting the ecosystem services provided by these soil invertebrates requires the inclusion of more ecologically relevant and sensitive earthworms in the risk assessment (Forbes et al. 2021). Moreover, the lower limit of the HC₅ is useful as a proxy for potential mortality risk for earthworms under field conditions when compared with measured and recommended field concentrations. In European vineyards, concentrations of copper in topsoil and subsoil were reported up to 600 mg/kg (Komárek et al. 2010), and even 1600 mg/kg around the study area (Steinmetz et al. 2017), which is characterised by long-term intensive viniculture. This shows that the soils in areas which are heavily contaminated with copper, such as vineyards, may exert acute toxicity to earthworms. Imidacloprid concentrations in agricultural soils were reported up to 0.65 mg/kg after one month of application in crops (Donnarumma et al. 2011). The most sensitive species, i.e., A. chlorotica and A. rosea, may have a mortality risk in such conditions. In addition, effects on sublethal endpoints, such as reproduction, typically occur at much lower concentrations than the LC₅₀. Nevertheless, information on sublethal effects for other substances and earthworm species is scarce compared to acute data, only around 16% of earthworm toxicity studies addressed sublethal endpoints (EPA, 2022). Although laboratory culturing of fieldcollected earthworm species may be challenging, the mineral dweller *A. caliginosa* is a promising candidate to evaluate the chronic effects of pesticides (Bart *et al.* 2018). Furthermore, the update of the ISO 11268-2 (under development) will incorporate environmentally relevant species, e.g., *A. caliginosa* and *D. rubidus*, for testing pollutant effects on earthworm reproduction.



Figure 5.1.1.1. Species sensitivity distributions for imidacloprid (**a**) and copper (**b**) calculated from multiple earthworm species sensitivity (red line). Black points (data from the present study) and open points (literature) represent the 14-day LC_{50} values of earthworm species. Species names are aligned by sensitivity in ascending order from bottom to top on the y-axes. Dashed lines enclose the parametric bootstrap (95% CI; 1000 iterations). Blue transparent lines display all parametric bootstrap samples. The open triangle marks the HC_5 value and the black square its lower limit.

5.1.2 Spider chemical sensitivity

On spiders, we aimed to assess the acute effects of imidacloprid, which was also chosen as the model compound for aquatic organisms. However, as imidacloprid displayed almost no acute toxic effects in range finding experiments, we switched to the insecticide lambdacyhalothrin, as additional data were available for this compound.

We collected adult female spiders with cocoons or visually pregnant in the field. Collection was done between spring 2020 and autumn 2021 by hand, with pitfall traps, or using an inverted leaf-blower. Sampling sites were selected to cover the four main western European Holdridge life zones (HLZ), polar, boreal, cool temperate, and warm temperate (Holdridge, 1967). Since spider communities are differentiated mainly by local moisture and shading (Entling *et al.* 2007), three main habitat types were selected in each HLZ: open space, wetland, and forest. Hence sampling was specifically designed to follow the terrestrial typology system introduced in 3.1. Collection was done in Germany, France, Italy, Switzerland, and Denmark (Table A2.3). None of the collected spiders are threatened species or under special protection in their respective country.

Female spiders were transported to the laboratory, transferred individually into containers with moistened plaster and fed *ad libitum* twice per week with a mixture of fruit flies (*Drosophila hydei* and *D. melanogaster*) and tropical springtails (Collembola spp.). Once spiderlings hatched, they were kept for approximately one week together with their mother in the container and more springtails were offered as food. Then, the mother spider was preserved in ethanol and identified to species level. Week-old spiderlings were individually transferred into glass jars with moistened plaster. Juveniles were fed *ad libitum* with springtails twice per week, and if the spiderlings were large enough to cope with this prey, fruit flies were incorporated into the diet. Spiderlings were raised for pesticide testing until they reached an age of approximately one month.

Ecotoxicological assessment design was based on the pesticide residue exposure assay described by Aukema *et al.* 1990 and Tahir *et al.* 2011, with modifications to allow dose-response calculations. First, two days before the ecotoxicological assessment, spiderlings of uniform age were individually transferred into glass jars with moistened plaster, and no food was provided. Juveniles were then stored in a climate chamber at 20±1°C, 100% relative humidity, and 16/8 light/dark cycle. Generally, 70 spiderlings were used for one test; however, for some species, juvenile hatching and survival rates were lower, and the tests were done

with fewer individuals. In most cases, we used seven concentrations of lambda-cyhalothrin in a geometric series, including one control, for each species and ecosystem of origin, i.e., where the mother spider was collected, with ten replicates per concentration. One replicate consisted of one juvenile placed onto a filter paper treated with 1mL aliquot of the desired test concentration in a glass Petri dish (90 mm Ø). Ultra-pure water was used for the control. Petri dishes with the spiderlings were placed back in the climate chamber, and survival was visually assessed after 24 hours of the application. Spiderlings were classified as alive, dead, or paralyzed (Baatrup & Bayley, 1993).

For all tested species, binomial dose-response models were fitted to the data, and LC_{50} s after 24 hours of exposure were calculated following Ritz *et al.* 2019. Moreover, we fitted separate SSDs for warm temperate, cool temperate, and polar + boreal climate (Table A2.4). Polar and boreal climates were combined, because fewer species were available from these zones and locations were only marginally below or above the biotemperature limit between the two zones. We derived the HC₅s from the SSDs together with their 95% CI. In addition, potential associations between spider chemical sensitivity, traits and habitat characteristics were measured with Pearson correlation coefficients. The correlation matrix compared spider LC₅₀ values with habitat, climate, hunting mode, weight and body length.

In total, we performed 34 toxicity test, including 28 spider species from the families Lycosidae, Linyphiidae, Gnaphosidae, Theridiidae, Pisauridae, Clubionidae, Thomisidae, and Zoropsidae. Spider LC_{50} s ranged between 6.69 and 21.42 ng a.i./cm² for boreal and polar climate, between 12.81 to 191.22 ng a.i./cm² for cool temperate species, and between 12.43 and 91.44 ng a.i./cm² for warm temperate spiders (Table A2.3). Spider HC₅s (95% CI) derived from the SSDs (Fig. 4.1.2.1) for boreal and polar, cool and warm temperate climates were 5.99 (4.39-9.17), 9.66 (5.23-21.61), and 8.08 (4.49-17.69) ng a.i./cm².

A proxy of potential mortality risk for spider species under field conditions can be inferred when comparing acute endpoints, such as LC_{50} and HC_5 , with measured and recommended field concentrations. The LC_{50} s of 89% of species tested and the HC_5 s derived for each climate zone (Fig. 5.1.2.1) were below the recommended application rate of lambda-cyhalothrin (75 ng a.i./cm²). Our results suggest a high mortality risk to spiders under field conditions, which can partially explain the effects of lambda-cyhalothrin observed in field studies (Niehoff *et al.* 1994; Rodrigues *et al.* 2013), where abundance and diversity of spiders were reduced after lambda-cyhalothrin application. In addition, different LC_{50} s values were derived from spiders with similar traits, for example *Piratula* spp. were eight times more resistant than *Pardosa* spp. (Table A2.3). This may be explained by the mode of action of lambda-cyhalothrin, which is

neurotoxic due to its effects on sodium, chloride, and calcium channels (He *et al.* 2008). To understand why some species are more sensitive than others, toxicogenomic studies including multiple spider species are required.

Despite the lethal effects of insecticides on spiders, and considering that sublethal effects, e.g., on behaviour, mobility, and predatory performance (e.g. Tahir *et al.* 2015; Laino *et al.* 2021), occur at lower concentrations than those tested in this study, spiders are not yet included as standard test organisms in the European risk assessment of pesticides (EFSA PPR, 2015). Instead, the EFSA uses other organisms as standard non-target arthropods for pesticide testing, such as the wasp *Aphidius rhopalasiphi* and the predatory mite *Typhlodromus pyri*, which prey on smaller organisms than spiders. Spiders need to be considered in the risk assessment framework to safeguard them and their ecological functions.



Fig. 5.1.2.1. Species sensitivity distributions for boreal and polar (**a**), cool temperate (**b**) and warm temperate (**c**) calculated from multiple spider sensitivity (red line). The 24-hour LC_{50} values of spider species are represented by habitats, open space (black points), forest (open circles), and wetland (black diamonds). Species names are aligned by sensitivity in ascending order from bottom to top on y-axes. Dashed lines enclose parametric bootstrap (95% C.I; 1000 iterations). Blue transparent lines display all parametric bootstrap samples. The black triangle marks the HC₅ value and the black square its lower limit.

5.1.3 Sensitivity modelling

In addition to the acute toxicity tests, we aimed to develop predictive models of species sensitivity toward chemicals based on their traits, relatedness, and interspecies sensitivity correlations. To this end, we first reviewed currently available approaches for sensitivity modelling, and evaluated their merits and pitfalls (Van den Berg et al., 2021). Based on this review, we decided to favour models that include traits and relatedness. However, we expected that the model's performance would differ between taxonomic groups due to differences in the availability of trait, relatedness (taxonomic or phylogenetic tree), and sensitivity data. Therefore, when data on traits were insufficient (as was expected for spiders and earthworms), we constructed relatedness-based models.

When constructing sensitivity models for different combinations of chemicals and taxonomic groups, two important factors should be considered. First, the response for which most ecotoxicological data is available may vary between taxonomic groups. Consider, for example, that effects on invertebrates are most frequently determined as mortality at the level of the individual, whilst for diatoms most frequently the growth rate or a functional response (e.g., Chlorophyll a density) is measured at population level. Second, the chemical under study determines the response that most likely provides an accurate description of sensitivity. For example, although a 72-h LC₅₀ might accurately reflect the effect of chlorpyrifos on aquatic invertebrates, a 72-h mortality test would likely give inaccurate results for fluoxetine. This is due to differences in the Mode Of Action (MOA) between the chemicals, which can be sublethal rather than lethal and manifest itself only after a longer exposure duration. To obtain the most accurate description of sensitivity, we constructed models for all possible responses present in the ECOTOX database (e.g., morality, behaviour, reproduction, growth, immobilisation). Sensitivity models were constructed according to Van den Berg et al. (2020). and details can also be found in Van den Berg et al. (2019) and Rubach et al. (2010). Figure 5.1.3.1 provides an overview of the modelling process. Several public databases were utilised to obtain data on MOA, toxicity, chemical properties, traits, and taxonomy.



Figure 5.1.3.1: Schematic overview of the modelling process.

Data on MOA were obtained from Barron *et al.* (2015) and Busch *et al.* (2016). The US Environmental Protection Agency (EPA) ECOTOX database (USEPA 2019) was selected as the source of toxicity data, and further complemented with our own toxicity data. Data on solubility were obtained by batch-running all CAS numbers available in the MOA database in the EPI (Estimation Programs Interface) Suite program WSKOWWIN (USEPA 44 2018). Data on molecular weight were obtained by extracting SMILES (Simplified Molecular-Input Line-Entry System) of all MOA CAS numbers from the SMILECAS database (also available through EPI Suite), and calculating the molecular weight based on these SMILES using the rcdk package in R (version 3.4.5, Guha 2007). Different trait databases were exploited for the different taxonomic groups, and only traits for which we hypothesised a relationship with chemical sensitivity went into the model construction process (Table *5.1.3.1*).

Finally, to facilitate the cross-linking of information among the different databases, and to construct the relatedness-based predictors, the Taxonomy database of the NCBI (Benson *et al.* 2009, Sayers *et al.* 2009) was used to extract the scientific names, along with the taxonomic rank and unique id, of all the species present in the ECOTOX and all the traits databases. For this we used the taxize package in R (version 0.9.94, 55 Chamberlain and Szöcs 2013).

Table 5.1.3.1: The selected sensitivity-related traits for each taxonomic group that entered the model construction process, and their references.

Taxonomic Group	Sensitivity-related traits	Reference
Aquatic invertebrates	Size related, life cycle duration, nr. of cycles per year, pH preference, salinity preference, dispersal mode, respiration mode, feeding mode, temperature tolerance, water content, exoskeleton thickness, lipid content, shape of organism	Tachet <i>et al.</i> 2000 Usseglio- Polatera <i>et al.</i> 2000 Rubach <i>et al.</i> 2012
Diatoms	Length, width, thickness, biovolume, size class, habitat, life form, growth form, colony shape, guild	Rimet <i>et al.</i> 2019
Spiders	Size, niche width, hunting strategy, guild, web shape, microhabitat, prey specialisation, lifestyle (diurnal versus nocturnal)	Entling <i>et al.</i> 2007 Entling <i>et al.</i> 2010 Cardoso <i>et al.</i> 2011
Earthworms	Body length, ecotype (lifestyle), fresh weight	Bouché 1972 Hedde <i>et al.</i> 2012 Köhler 2014 Krück 2018

Relatedness-based predictors were constructed by i) constructing a taxonomic tree, ii) converting it to Phylogenetic Eigenvector Maps (PEMs), and iii) extracting species scores that subsequently served as predictors of relatedness in model construction (Griffith and Peres-Neto 2006, Guénard *et al.* 2013). These relatedness-based predictors (indicated by a **V** in Table 5.1.3.2) were combined with the traits and sensitivity data. When sufficient toxicity data was available for the selected chemical, we used LC_{50} or EC_{50} values as a direct metric of sensitivity. However, when available toxicity data was insufficient, we calculated the Mode Specific Sensitivity (MSS) value of each species, which is the average relative sensitivity of a species to a group of chemicals with the same MOA (described in Rubach *et al.* 2010). Finally, single and multiple linear regressions were performed between sensitivity (LC_{50} , EC_{50} , or the MSS values) and species traits and relatedness predictors. This was done both at the species and at the genus level, to determine the appropriate taxonomic level of the different taxonomic groups to best explain the variation in sensitivity to the different chemicals.

Table 5.1.3.2: Overview of the sensitivity models. The V variables indicate the taxonomic predictors. Best performing, significant models were found at genus level, unless otherwise indicated. MSS indicates Mode Specific Sensitivity, and N.A. indicates not applicable.

Chemical	MOA	Aquatic Invertebrates	Diatoms	Spiders	Earth worms
Insecticide - Imidacloprid	Neurotoxicity ^a , Neuroactive ^b	$LC_{50} \sim length$ (r ² = 0.68, arthropods only)*	Insufficient data	Not constr	ructed
Herbicide - Atrazine	Narcosis ^a , Photosynthesis Inhibition ^b	$\begin{array}{l} MSS_{LC50} \sim V14 - \\ V2 + respiration \\ mode + V4 (r^2 = \\ 0.47) \end{array}$	EC ₅₀ ~ V1 + V9 + V13 + V17 (r ² = 0.61)		
Metal - Copper	lono, Osmoregulatory-, Circulatory Impairment	$MSS_{LC50} \sim Velocity$ preference + Life cycles per year (r ² = 0.48)	Insufficient data		
Down the drain - Fluoxetine	Neuroactive ^b	Insufficient data	Insufficient data	N.A.	N.A.

^aMOA classification according to (Barron *et al.* 2015) ^bMOA classification according to (Busch *et al.* 2016) *Model construction performed at species level

We constructed models for a limited set of MOAs (Table 5.1.3.2). The evaluated MOAs encompassed chemicals belonging to insecticides, herbicides, metals, and chemicals acting as narcotics, thereby covering organic and inorganic chemicals, agrochemicals, and down-the-drain chemicals. Not all models could be constructed, due to limited data availability.

5.2 Predicting assemblage sensitivity

5.2.1 Predicting assembly sensitivity with hSSDs

We used an hierarchical species sensitivity distribution (hSSD) model based on taxonomic relatedness to predict toxicity data for untested species and generate assemblage-specific sensitivity profiles (Craig, 2013). This novel approach allows us to calculate the sensitivity of specific assemblages to toxic chemicals and to explore how the chemical sensitivity of freshwater macroinvertebrate assemblages may vary across river types and complements the trait-based approach described in section 5.1.3. We used existing toxicity data and the hSSD model to predict toxicity values for all taxa in 3862 European river macroinvertebrate

assemblages and then constructed assemblage-specific species sensitivity distributions to derived the chemical concentration that was hazardous to 5% of the species in the assemblage (i.e., assemblage HC₅). This procedure was repeated for each of the four study chemicals (i.e. copper, imidacloprid, lambda-cyhalothrin, and atrazine) (Figure 5.2.1.1).



Figure 5.2.1.1: Approach used to calculate assemblage-specific HC₅ values.

Acute toxicity data were extracted from the US EPA ECOTOXicology Knowledgebase (Olker et al, 2022 available at http://www.epa.gov/ecotox/). EC_{50} (immobility) or LC_{50} (mortality) values for aqueous exposure durations of 1-7d were extracted for macroinvertebrates.

The hSSD model is a Bayesian model that uses a Markov chain Monte Carlo (MCMC) sampler to predict a suite of sensitivity predictions for a taxon, each the result of a single run using all of the taxa in the total species pool. The MCMC had a burn-in of 2500 runs per chemical and the predicted sensitivity values were calculated from 10,000 runs post-burn. The average predicted EC_{50} value for each taxon was calculated from a geometric mean of 10,000 runs. The predicted toxicity values were allocated to the 3862 study assemblages based on their taxonomic composition and a log-normal SSD was fitted. The concentration that was hazardous to 5% of species in the assemblage was calculated for each chemical and assemblage (Alderberg and Jaworski, 2001).

Assemblage-specific HC₅ values varied by up to two orders of magnitude, but for 95% of assemblages (i.e. excluding the 2.5% most sensitive (lowest HC₅) and 2.5% least sensitive (highest HC₅) assemblages), the variation was between 5.5 and 13.7 fold, depending on the chemical (Figure 5.2.1.2).



Figure 5.2.1.2: Variation in assemblage-specific HC₅ values for 3862 European river macroinvertebrate assemblages to four study chemicals. Vertical lines denote the 2.5th and 97.5th percentiles.

When we compare the observed spatial variation in sensitivity to the current standard SSD approach, we find that the current approach would be protective for all assemblage exposed to copper or lambda-cyhalothrin and to 95% and 97% of assemblages exposed to atrazine and imidacloprid, respectively (Figure 5.1.2.3).



Figure 5.1.2.3: Variation in assemblage-specific HC₅ values for 3862 EU river macroinvertebrate assemblages to four study chemicals. Red vertical lines denote the HC₅ as derived by current standard SSD. The associated numbers provide the percentage of assemblages that have a lower hSSD HC₅ than the SSD HC₅.

Assemblages were grouped by river type (section 4) and the mean assemblage HC_5 compared (Figure 5.2.1.4). There was significant variation in mean HC_5 values across river types but the relative sensitivity of assemblages in specific types was chemical-dependent. For example, whereas assemblages in very large rivers were least sensitive to atrazine, imidacloprid, and lambda-cyhalothrin, they were most sensitive to copper.



Figure 5.2.1.4: Variation in mean assemblage-specific HC₅ values across river types. Within chemicals, bars that share the same letter are not statistically different.

5.2.3 Predicting assembly sensitivity with an ecological model

We built community models of interacting macroinvertebrate taxa, and used trait data to parameterize these models. Doing so allowed us to predict the sensitivity of the typical assemblages and to examine if and why these sensitivities differed. The models are based on sets of differential equations, where population growth is the difference between gains (consumption) and losses (mortality and predation). We focus on bi-trophic communities of predators and prey, using the classic Lotka-Volterra formalism. Effects of chemicals are implemented by writing model parameters as a function of the chemical concentration.

Once the basic model equations were defined, we first carried out mathematical analyses of these equations to test if there exist general "rules" that predict assemblage sensitivity, only based on some aggregate assemblage properties and so regardless of specific parameter setting. Specifically, we analytically derived the maximum chemical concentration that allowed the taxa to persist. From these maximum chemical concentrations, we estimate the resistances of the prey (or victim, Ω_V) and the predators (Ω_P) as

$$\Omega_V = \tau_V \left(\frac{b}{m} - 1\right) , \qquad (5.2.3.1)$$

$$\Omega_P = \tau_P \frac{\left(\frac{b}{m} - 1\right) \frac{\lambda \eta}{1 + (n_V - 1) \alpha} - \frac{d}{m}}{\frac{\tau_P}{\tau_V} \frac{\lambda \eta}{1 + (n_V - 1) \alpha} + \frac{d}{m}} < \Omega_V , \quad (5.2.3.2)$$

where τ_V and τ_P denote the tolerances of the prey and the predators, in the absence of species interactions within the community; *b* and *m* denote the birth and mortality rates of the prey species, while *d* represents the mortality rate of the predators in the absence of the prey; λ denotes the attack rate of the predators, and η the conversion efficiency between attacked prey and the birth of new predators; α represents the strength of competition between the prey species; and n_V holds for the number of prey species.

These analytical computations confirmed that general rules indeed exist to predict assemblage sensitivity. For example, we found that predators are generally less resistant than prey taxa, or that predator resistance decreases with the number of prey taxa (Eq. 5.2.3.2).

While the analyses are powerful in their generality, they also were based on a number of simplifying assumptions that will not hold in natural settings. These are

 all prey species having the same dynamical parameters (birth and mortality rates, competition strengths) and sensitivities to the chemical (in the absence of species interactions);

- all predator species having the same dynamical parameters (mortality and attack rates, biomass-conversion efficiencies) and sensitivities to the chemical (in the absence of species interactions);
- all prey and predator species located in a unique patch (no spatial model).

We parameterized the developed models to represent as precisely as possible the typical assemblages. To do so, we have categorised the taxa within the assemblages as either prey or predators (using the globi database, Poelen *et al.* 2014), and parameterized the taxa interactions among prey using traits (obtained from the Tachet database, Tachet *et al.* 2000). We assume that the presence of the chemical increases the mortality rate of the taxa, with a slope given by the (isolated) taxon tolerances provided by the hSSDs. Then, we simulated community dynamics at different chemical concentrations and determined the maximum concentration at which a taxon persists. This maximum concentration, which can differ among taxa, is the resistance of the taxon (Fig. 5.3.2.1). Moreover, the sensitivity of the full assemblage is estimated as the smallest chemical concentration leading to the first extinction of a taxon within the community (Fig. 5.3.2.2). We have done this analysis for the seven previously-derived different TAs, and for three different chemicals: imidacloprid, atrazine, and copper.



Figure 5.3.2.1: Tolerances to Imidacloprid of taxa from the Typical Assemblage for small mid-altitude rivers. In blue bars, chemical concentrations that let the taxa within the assemblage to persist in the simulated community dynamics (Ω). In red dots, tolerances as predicted by the hSSD (τ).



Figure 5.2.3.2. In blue dots, fraction of lost taxa from the Typical Assemblage (TA) for small mid-altitude rivers when we increase the concentration of Imidacloprid in the community dynamics simulations. The red line shows the fraction of taxa within the TA that should be affected by the presence of the chemical, as derived from the hSSDs.

We found that the simulations for the real typical assemblages confirm the general rules obtained through our analyses. In particular, we find that:

- Predator taxa are less resistant than prey taxa. Hence, chemical exposure is expected to affect community structure (increase the prey/predator ratio)
- Community structure affects the assemblage resistance (e.g., too many prey taxa reduce the assemblage resistance) (Fig 5.2.3.3)
- The species sensitivity distribution within the community affects the assemblage resistance (generally, if prey are more sensitive than predators, the TA resistance decreases) (Fig. 5.2.3.4)

These results show that the difference in assemblage resitance, albeit limited, can be explained by a limited number of aggregate properties. For instance, the TA of large siliceous lowland rivers is most sensitive to Copper because, in this TA, predators are much less sensitive than the prey taxa. Conversely, the TA of calcareous lowland rivers is generally robust to chemical effects because of the limited number of prey species within the assemblage.

We finally compared the assemblage sensitivities from the community models to the sensitivities obtained by the hSSDs in 5.2.1. We found that the difference between these two methods is greatest for the TA from large mid-altitude rivers, specially for atrazine and copper, possibly caused by the large number of prey taxa when compared to the other assemblages (Fig. 5.2.3.3).



Fig. 5.2.3.3. For the six typical assemblages containing at least one predator, resistance of the typical assemblage (measured as the smallest chemical concentration that can remove one taxon) depends on the number of prey taxa within the typical assemblage. Confirming the general analytical result obtained with the simpler models, assemblage resistance ('TA resistance) decreases with the number of prey taxa.



Fig. 5.2.3.4. For the six typical assemblages containing at least one predator, resistance of the typical assemblage (measured as the smallest chemical concentration that can remove one taxon) depends on the relative sensitivity of the predator vs. the prey taxa. Confirming the general analytical result obtained with the simpler models, assemblage resistance ('TA resistance) decreases with the ratio (tolerance of the predator)/(tolerance of the prey), τ_P/τ_V .

6. Validating assemblage-level sensitivities

In WP 4, we aimed to validate the results obtained from the modelling (5.2) with results from community and single-species sensitivity tests. To this aim, we first conducted multispecies microcosm experiments (6.1 and 6.2) and compiled literature data from micro- and mesocosm experiments (6.3), which were subsequently compared to the results from modelling in WP 3 (6.4).

6.1 Multispecies microcosm experiments

We performed two mesocosm experiments in collaboration with other projects. Merga and Van den Brink (2021) investigated the effect of imidacloprid on structural (invertebrates and primary producers) and functional (organic matter decomposition and physicochemical parameters) characteristics of tropical freshwaters using acute single species and mesocosm studies performed in Ethiopia. Their acute toxicity test showed that Cloeon dipterum (96-h $EC_{50} = 1.5 \mu g/L$) and Caenis horaria (96-h $EC_{50} = 1.9 \mu g/L$) are relatively sensitive arthropods to imidacloprid. The mesocosm experiment evaluated the effects of four applications of imidacloprid with a weekly interval and the results showed that the macroinvertebrate and zooplankton community structure changed significantly due to imidacloprid contamination in mesocosms repeatedly dosed with ≥ 0.1 and $\geq 0.01 \mu g/L$, respectively. The largest responses were found for C. dipterum, C. horaria, Brachionus sp. and Filinia sp. Chlorophyll-a concentrations of periphyton and phytoplankton significantly increased in the $\geq 0.1 \mu g/L$ treatments levels, which were indirect effects as a result of the release of grazing pressure. A significant, but quantitatively small, decrease of organic matter decomposition rate was observed in mesocosms treated with repeated doses of 1 µg/L (TWA112d of 2.09 µg/L). No recovery was observed for the macroinvertebrates community during the study period of 21 weeks, but zooplankton recovered after nine weeks. Merga and Van den Brink (2021) observed spatio-temporal related toxicity differences between tropical and temperate aquatic taxa, with tropical taxa generally being more sensitive.

Van de Perre *et al.* (2021) performed a similar study in sub-tropical China. A mesocosm experiment was conducted under sub-tropical conditions to assess the effects of imidacloprid on the structure (macroinvertebrates, zooplankton and phytoplankton) and functional endpoints of an aquatic ecosystem and to compare the results with similar temperate and (sub-)tropical mesocosm studies. Imidacloprid (0, 0.03, 0.3 and 3 μ g/L) was applied to 13 mesocosms weekly over a period of four weeks. At the community level, a lowest NOEC_{community} of 0.03 μ g/L was calculated for the zooplankton, phytoplankton, and

macroinvertebrate communities. The highest sensitivity to imidacloprid (NOEC < $0.03 \mu g/L$) were observed for *Gerris* sp., *Diaptomus* sp., and *Brachionus quadridentatus*. Imidacloprid induced population declines of the larger zooplankton species (*Diaptomus* sp. and Ostracoda) resulted in increased rotifer abundances and shifted the phytoplankton community to a graze resistant gelatinous cyanobacteria dominated ecosystem. These cyanobacteria blooms occurred at all different concentrations and could pose an important public health and environmental concern. Although there are some differences in species and community sensitivity between the present and the other (sub-)topical mesocosm studies, it can be observed that all show a similar general community response to imidacloprid. Under (sub-)tropical conditions, the toxic effects of imidacloprid occur at lower concentrations than found for temperate ecosystems.

6.2 Complementary toxicity tests

Additional toxicity tests were performed by Merga and Van den Brink (2021) to assess the difference in sensitivity between mayflies from temperate and tropical regions. We also reviewed the literature and summarised all information in Table 6.2.1.

	Acute toxicity		Chronic toxicity		Mesocosm NOEC		
Species					values		Country
	96h LC ₅₀	96h EC ₅₀	Toxicity		Exposure		
	(µg/L)	(µg/L)	endpoint	(µg/L)	NOE	C	
					duration	(µg/L)	
A. Macroinvertebrate	es						
Cloeon dipterum	26.3a	1.0a	28-d EC10	0.033a	-	-	Netherlands
(WG)							
Cloeon dipterum	34b	25b	-	-	-	-	Netherlands
(WG)							
Cloeon dipterum	-	-	-	-	28-d	< 0.09f	Spain
Cloeon dipterum	2.7e	1.5e	-	-	14-d	< 0.01e	Ethiopia
Cloeon sp. (WG)	1152c	23.1c	-	-	-	-	Canada
Cloeon sp.	0.024d	0.0055d	-	-	9-d	< 0.03d	Bangladesh
Caenis horaria (SG)	6.68a	1.8a	28-EC10	0.024a			Netherlands
Caenis horaria (WG)	28b	6.0b	-	-	-	-	Netherlands
Caenis sp. (WG)	< 21.8c	< 21.8c	-	-	-	-	Canada
Caenis sp.	-	-	-	-	-	7.5g	Germany
Caenis horaria	3.4e	1.9e	-	-	14-d	< 0.01e	Ethiopia
Chironomidae sp.	-	-	-	-	49-d	17.3g	Germany
Chironomus dilutus	-	-	14-d LC20	0.47k	-	-	Canada

Table 6.2.1. Acute and chronic toxicity, and mesocosm study no observed effect concentration (NOEC) values of imidacloprid for macroinvertebrate and zooplankton taxa often reported in the literature.

Chironomus riparius	-	-	10-d LC10	1.64l	-	-	Czech
							Republic
Chironomid larvae	-	-	-	-	28-d	0.3d	Bangladesh
Chironomini	-	-	-	-	10-d	< 0.2f	Spain
Chironomidae sp.	-	-	-	-	28-d	0.01e	Ethiopia
Culicidae sp.	-	-	-	-	28-d	0.01e	Ethiopia
Notonecta tiguttata	-	-	-	-	140-d	157h	Japan
Notonecta sp.	-	-	-	-	16-d	0.03d	Bangladesh
Notonectidae sp.	-	-	-	-	28-d	0.01e	Ethiopia
Micronecta sp. (SG)	28.2a	10.8a	-	-	-	-	Netherlands
Trichocorixa sp.	450c	63.1c	-	-	-	-	Canada
(WG)							
Corixidae sp.	6.8e	3.6e	-	-	28-d	< 0.01e	Ethiopia
Gerris sp.	-	-	-	-	16-d	0.03d	Bangladesh
Gerris sp.	-	-	-	-	58-d	< 0.03n	China
Gerris latiabdominis	-	-	-	-	120-d	49i	Japan
Gerridae sp.	-	-	-	-	28-d	< 0.01e	Ethiopia
Plea minutissima	37.5a	36a	28-d LC10	2.03a	_	-	Netherlands
(SG)							
Plea minutissima	287h	189b	_	_	-	_	Netherlands
(WG)	2010	1000					Notificitatio
Plea minutissima	68e	36e	-	-	42-d	0.01e	Ethiopia
Hydaticus sp.	-	-	-	-	28-d	< 60j	Portugal
Dytiscidae sp.	-	-	-	-	28-d	< 0.01e	Ethiopia
Planorbella pilsbryi	-	-	28-d EC10	16m	-	-	Canada
Hippeutis cantori	-	-	-	-	58-d	0.03n	China
Planorbidae sp.	-	-	-	-	28-d	0.1e	Ethiopia
Physidae sp.	-	-	-	-	28-d	0.1e	Ethiopia
B. Zooplankton							
Diaptomus sp.	6.5d	0.0386d	-	-	16-d	< 0.03d	Bangladesh
Diaptomus sp.	-	-	-	-	58-6	< 0.03n	China
Keratella sp.	-	-	-	-	9-d	< 0.03d	Bangladesh
Keratella quadrata	-	-	-	-	42-d	1f	Spain
Keratella sp.	-	-	-	-	44-d	0.01e	Ethiopia
Keratella tropica	-	-	-	-	58-d	0.03n	China
Polyarthra sp.	-	-	-	-	16-d	< 0.03d	Bangladesh
Polyarthra sp.	-	-	-	-	9-d	< 0.01e	Ethiopia
Brachionus sp.	-	-	-	-	28-d	0.03d	Bangladesh
B. quadridentatus					58-d	< 0.03n	China
Brachionus sp.	-	-	-	-	23-d	< 0.01e	Ethiopia
Filinia sp.	-	-	-	_	23-d	0.3d	Bangladesh
Filinia sp.	-	-	-	_	58-d	<0.01e	Ethiopia
Trichocerca sp	-	_	_	_	23-d	0.3d	Bangladesh
Trichocerca sp	_	_	_	_	23-d	< 0.01e	Ethionia
Cyclops sp	_	_	_	_	20 u 9-d	0.3d	Banaladesh
Cyclopoida	_	_	_	_	17-d	0.00 1f	Snain
Afrocyclops sp	_	_	_	_	30-d	 ⊂ 0 01≏	Ethionia
Diantomus en	_	_	_	-	58-d	< 0.010	China
Naunliue	-	_	_	-	0-d	< 0.03H	Bangladach
Naupilus	-	-	-	-	9-u 2 d	0.030 Ef	Dariyiduesii
	-	-	-	-	-o-u	31	JUAID

-
Naupul	lii.	
nuupu		

23-d < 0.01e

< 0.01e Ethiopia

Note: Note: a = Roessink et al. (2013); b = Van den Brink et al. (2016); c = Raby et al. (2018); d = Sumon et al. (2018); e = Merga and Van den Brink (2021); f = Rico et al. (2018); g = Colombo et al. (2013); h = Kobashi et al., (2017); i = Hayasaka et al. (2012); j = Pereira et al. (2017); k = Cavallaro et al. (2017); l = Chandran et al. (2018); m = Prosser et al. (2016); n = Van de Perre et al. (2021). WG = winter generation; SG = summer generation. Reference f and j are from Mediterranean, d and n are from sub-tropical and e from tropical climate zones. The others are from temperate climate zones.

The findings of Lemessa and Van den Brink (2021) and Sumon *et al.* (2018) indicated that tropical aquatic invertebrates are more sensitive to the neonicotinoid insecticide imidacloprid than their temperate counterparts. Lemessa and Van den Brink (2021) demonstrated that the mayfly *C. dipterum* (96-h $LC_{50} = 2.7 \mu g/L$, 96-h $EC_{50} = 1.5 \mu g/L$ and 14-d NOEC < 0.01 $\mu g/L$) was found to be the most sensitive arthropod relative to the other tested tropical species. Sumon *et al.* (2018) even reported much lower acute toxicity values for *Cloeon* sp. (4d-EC₅₀ = 0.0055 $\mu g/L$) tested in sub-tropical Bangladesh. Relative to the acute toxicity data from temperate studies reported by Roessink *et al.* (2013) (96-h $LC_{50} = 26.3 \mu g/L$) for summer generation and by Van den Brink *et al.* (2016) (96-h $LC_{50} = 34 \mu g/L$) for winter generation of *C. dipterum*, the effect value measured in Lemessa and Van den Brink (2021) is 10 to 12 times lower. Relative to the results for *Cloeon* sp. sampled in spring in Canada (96-h $LC_{50} = 1152 \mu g/L$ and $EC_{50} = 23.1 \mu g/L$) by Raby *et al.* (2018) again the results of Lemessa and Merga (2021) of 96-h LC_{50} and EC_{50} are 427 times and 15 times lower, respectively (Table 6.2.1.).

6.3 Review of micro- and mesocosm tests

In Table 6.2.1., the univariate, taxon-level results of the micro- and mesocosm tests are summarised while Table 6.3.1. summarises the community-level results for the micro- and mesocosm experiments for which this was possible. This table also shows, like Table 6.2.1. that the effect concentrations are much lower in (sub-)tropical systems (Sumon *et al.*, 2018; Merga and Van den Brink, 2021; Van de Perre *et al.*, 2021) compared to Mediterranean (Rico *et al.*, 2018) and temperate (Colombo *et al.*, 2013) ones.

Table 6.3.1. Summary of the NOEC_{community} (μ g/L), lowest NOEC_{taxon} (μ g/L) days of first, and lowest observed NOEC_{community} and the recovery days based on five imidacloprid mesocosm studies.

	Sumon et al.	Merga and Van	Van de Perre et al.,	Rico et al., 2018	Colombo et
	(2018)	den Brink (2021)	2021		al., 2013
Country	Bangladesh	Ethiopia	China	Spain	Germany
Macroinvertebrates					
NOECcommunity					
Lowest	0.03	0.01	0.03	1	7.5
Consistent	0.3	0.01	> 3	1	7.5
NOEC _{taxon}					
Consistent	< 0.03	< 0.01	< 0.03	0.2	7.5
Zooplankton					
NOECcommunity					
Lowest	0.03	< 0.01	0.03	5	NA
Consistent	0.3	< 0.01	0.03	25	NA
NOECtaxon					
Consistent	< 0.03	< 0.01	< 0.03	1	NA
Phytoplankton					
NOECcommunity					
Lowest	>	NA	0.03	NA	NA
Consistent	>	NA	0.3	NA	NA
NOEC _{taxon}					
Consistent	0.03	NA	< 0.03	NA	NA

NA: Not applicable; *Days relative to the day after the last treatment

6.4 Validation of sensitivity models

Table 6.4.1. provides an overview of the calculated median HC_5 values (HC_{5_50th}) and the NOEC_{communities} observed in the mesocosm studies.

Table 6.4.1. The lowest and consistent NOEC community and NOEC_{taxon} based on the response of the macroinvertebrate community as observed in micro- and mesocosm experiments together with their HC_5 value calculated by the hSSD method based on the macroinvertebrate community composition in the micro- and mesocosm experiments.

Reference		Sumon <i>et al.</i> , 2018	Merga & Van den Brink, 2021	Van de Perre <i>et al.</i> , 2021	Rico <i>et al.</i> , 2018	Colombo <i>et al.</i> , 2013
Country		Bangladesh	Ethiopia	China	Spain	Germany
NOEC _{comm}	Lowest	0.03	0.01	0.03	1	7.5
	Consist.	0.3	0.01	> 3	1	7.5
NOEC _{taxon}	Consist.	< 0.03	< 0.01	< 0.03	0.2	1.4
HC5_50th(this study)		0.0079	0.0033	0.0255	0.0064	0.0024

The table shows that the NOECs are the lowest in the tropical countries, but the calculated HC_5 values were the lowest in a temperate study. Apparently, the sensitivity of the macroinvertebrate community is governed by other factors besides the community composition. Huang *et al.* (2023) performed experiments investigating the effect of temperature on the toxicity of imidacloprid on *Gammarus pulex* and found that temperature impacted the toxicokinetics and the chronic toxicity of both imidacloprid. For imidacloprid, the uptake rate and biotransformation rate increased with temperature, and mortality and food consumption inhibition was enhanced by temperature. This was also mechanistically modelled by Mangold-Döring *et al.* (2022) showing that both the toxicokinetics as the toxicodynamics of imidacloprid in macroinvertebrates is affected by temperature.

This can (partly) explain the observed higher sensitivity of (sub-)tropical macroinvertebrate communities compared to Mediterranean or temperate ones.

7. Incorporating sensitivity variation in risk assessment

We analysed to which extent differences in the assemblages translate into functional differences in terms of ecosystem services for the different organism groups (7.1). Moreover, we assessed whether a zonal assessment as used in exposure assessment of pesticides would yield to differences between the zones (7.2). Finally, the results were disseminated and discussed at a stakeholder workshop (7.3).

7.1.1 Macroinvertebrates

Freshwater macroinvertebrates provide multiple ecosystem services (Macadam & Stockan 2015; Vaughn 2018). Among them is the provision of potable drinking water to which they contribute through nutrient cycling (Li *et al.* 2021). The rate of nutrient cycling is affected by the trait composition of the community and the prevalence of traits like *shredding* (decomposition of coarse particulate organic matter into fine particulate organic matter (fPOM)), *burrowing* (rerelease of nutrients from sediment (Yang *et al.* 2021)) and *filtering* (building biomass from fPOM, e.g., Covich *et al.* 1999).

Nutrient cycling is also important for building macroinvertebrate biomass and hence food production for higher trophic levels. Additional functional traits that we linked to to the ecosystem service of food production are *body size* (larger organisms have more biomass) and *voltinism* (more generations per year produce more biomass). Lastly, the service of food production is directly linked to the ecosystem service of recreation though the maintenance of stable fish stock required for fishing. Higher recreation can also be provided through the presence of charismatic taxa. We used the percentage of sites with the presence of Dragonflies (Odonata) as a proxy of charismatic taxa and hence recreation.

Thus the effect traits we identified were: shredding, burrowing, filtering, body size, and voltinism. Additionally, we used the relative occurrence of dragon flies as a proxy of recreation.

Regarding the integration of ecosystem services into risk assessment we posed two questions:

- 1. Do the effect trait profiles differ between the typical macroinvertebrate assemblages?
- 2. Can these differences be related to sensitivity patterns of the hierarchical SSDs?

The presence of Odonates did not show pronounced patterns (Figure 7.1.1.1).



Figure 7.1.1.1 - Fraction of sites with Odonates present in the seven river types for which we derived typical macroinvertebrate assemblages. The dashed orange line is the mean fraction across river types.

For the following analyses we used the extensive macroinvertebrate trait database published by Kunz *et al.* (2022). For each trait we computed a *mean trait value*. This is the mean value for each trait in a typical assemblage. The trait database we used is fuzzy coded. This means that each trait has multiple modalities (e.g., for the trait *functional feeding type* there are the modalities *filterer*, *shredder*, *predator*...). Values for each modality vary between 0 and 1. For a single taxon, the sum of modalities for a single trait is 1. Thus the individual values indicate a proclivity of taxa towards trait modalities. A value of zero indicates that a modality is never expressed, whereas a value of one shows that the taxon always expresses the modality. Values between 0 and 1 can indicate: 1) uncertainty regarding the trait expression of this taxon, 2) that the taxon can express multiple modalities, 3) changes in trait modalities between life stages, or ,4) for higher taxonomic levels (e.g., genus, family, order)that the different species within this group express different modalities. One last important consideration for trait data is the paucity of information for certain taxa and trait modalities. Even for the common taxa in the TAs data is only available for some of them. The percentage of taxa within each typical assemblage, with trait data, is thus an important information.

For shredders, we had a good data coverage but observed no conspicuous pattern (Figure 7.1.1.2).



Figure 7.1.1.2 - Mean shredding trait value of typical macroinvertebrate assemblages. The dashed orange line represents the mean value across typical assemblages. The numbers to the right show the fraction of taxa for which proclivity toward shredding was known.

The differences between typical assemblages were more pronounced for the trait modality filtering where data coverage was still good (Figure 7.1.1.3). Filterers were most common in the typical assemblage of very large rivers and absent from that of lowland calcareous rivers.





For burrowing the data coverage was poorer than for shredding and filtering (Figure 7.1.1.4). The differences between the typical assemblages with regards to this trait were minor. The

only typical assemblage that deviates notably from the overall mean, is that from lowland, siliceous small rivers. It has fewer burrowing taxa than other TAs.



Figure 7.1.1.4 - Mean burrowing trait value of typical macroinvertebrate assemblages. The dashed orange line represents the mean value across typical assemblages. The numbers to the right show the fraction of taxa for which proclivity toward burrowing was known.

For the last two traits, body size and voltinism, we used a slightly different approach. The modalities of these traits have an intrinsic order: small (<9 millimetres (mm)), medium (9-16 mm), large (> 16 mm) and semivoltine, univoltine, multivoltine. We coded the modalities with the numbers 1 to 3 where 1 corresponds to small and semivoltine, respectively. The mean trait value still is the mean of all taxa within the TAs but it now ranges between 1 (all taxa are small/ semivoltine) and 3 (all taxa are large/ multivoltine).

The low data coverage for body size (0.31 - 0.76) prohibits interpretation of the pronounced differences with confidence (Figure 7.1.1.5).



Figure 7.1.1.5 - Mean body size trait value of typical macroinvertebrate assemblages. The dashed orange line represents the mean value across typical assemblages. The numbers to the right show the fraction of taxa for which the body size category was known.

Voltinism showed the strongest differences between typical assemblages (Figure 7.1.1.6). The data coverage was lower than for shredding and filtering but higher than for body size. Multivoltine taxa seem to be more prevalent in larger rivers. The typical assemblage of very large rivers has the highest mean trait value and lowland, siliceous small rivers the lowest.



mean trait value Figure 7.1.1.6 - Mean voltinism trait value of typical macroinvertebrate assemblages. The dashed orange line represents the mean value across typical assemblages. The numbers to

the right show the fraction of taxa for which the voltinism category was known.

Based on these trait patterns we conclude that, taking data availability into account, the only conspicuous pattern in the trait profiles is the increase of voltinism with river size. Turning to our second question, can this pattern be connected to the sensitivities predicted by the hSSDs? Very large rivers had the highest sensitivity towards copper (Figure 5.2.1.3). This is in line with previous results that macroinvertebrates with higher voltinism tend to be more sensitive to heavy metals (Clements *et al.* 2019). Thus, heavy metals might pose a larger threat to food production, and hence to recreation, in larger rivers that tend to harbour more multivoltine taxa. This should, however, be regarded as a hypothesis and requires substantiation in future studies.

voltinism

7.1.2 Diatoms

We conducted a literature search for connections between diatom community trait composition and ecosystem service provision. While diatoms provide different services like primary production, nutrient removal, and biogenic sediment stabilisation (Decho 2000), studies that directly linked their levels to community trait composition are lacking. The same verdict was reached by Brown *et al.* (2020) in their effort to evaluate ecosystem service-based approaches for environmental risk assessment. Meanwhile, studies highlighting the ecological relevance of the diatom guild system proposed by Passy (2007) are legion (e.g., Jamoneau *et al.* 2022; Lindholm *et al.* 2018; Tison-Rosebery *et al.* 2022). Hence, we highlight the link between diatom community trait composition and levels of ecosystem service provision as an important current research and knowledge gap.

7.1.3 Earthworms

Earthworm chemical sensitivity to imidacloprid differed significantly between epigeic and nonepigeic earthworms (F(1,6) = 17.44; p < 0.01; Table 7.1.3.1). The LC₅₀s of non-epigeic earthworms were generally twice as low as those from epigeic earthworms (Fig. 7.1.3.1). Earthworm sensitivity to copper increased significantly with increasing soil pH of their ecosystem of origin (F(1,6) = 11.66; p = 0.01; Table 7.1.3.1). Thus, earthworms obtained from extremely acidic soils were approximately twice as resistant to copper than those sampled in neutral soils (Fig. 7.1.3.2).

While the sensitivities of earthworms showed no clear differences between ecosystem types, they varied with soil pH. Ontogenetic traits acquired during development may explain the observed differences with soil pH. For example, a reduced sensitivity to copper in earthworms from highly acidic soil may be an adaptation to such low pH values (<5.5), in which heavy metals such as copper are mobilised (Fernández-Calviño *et al.* 2008). In addition, pesticide uptake in earthworms is mainly through direct contact and oral ingestion. Uptake varies among different species, as well as their toxicokinetic and toxicodynamic traits, and these dynamics mainly determine organisms' sensitivity to pesticides (Ashauer & Jager, 2018). Thus, toxicogenomic experiments (cf. Short *et al.* 2021) combined with earthworm populations from different habitats could clarify the differences in earthworm sensitivity and habitat relationships observed in this study.

To protect a region-specific soil community and the ecological roles of its constituent species would require considering the soil characteristics in risk assessment. The sensitivity of ecosystem service provision might vary together with that of individual earthworm species. Non-epigeic earthworms contribute considerably to bioturbation (Lee & Foster, 1991) and are more sensitive than epigeic earthworms (Fig. 7.3.1.1), especially to insecticides (Pelosi *et al.* 2014). Thus, pesticide application potentially reduces population sizes of non-epigeic earthworms more strongly than those of epigeic earthworms, and may consequently lower bioturbation.

Table 7.1.3.1: Comparison between earthworm traits, habitat characteristics, and chemical sensitivity for imidacloprid and copper. Statistically significant differences (p<0.05) have an asterisk.

Covariate	LC ₅₀ Imidacloprid			LC ₅₀ Copper		
	df	F	<i>p</i> -value	df	F	<i>p</i> -value
Ecotype	1	17.45	0.005*	1	0.01	0.91
Weight	1	0.23	0.65	1	0.04	0.85
Habitat	2	1.54	0.29	2	0.31	0.74
pН	1	1.10	0.34	1	11.66	0.001*



Figure 7.1.3.1. Comparison between epigeic and non-epigeic earthworm imidacloprid $LC_{50}s$. Different letters show significant differences (*p*<0.05).



Figure 7.1.3.2. Comparison between copper LC_{50} s of earthworms sampled in extremely acidic, slightly acidic, and neutral soils. Different letters show significant differences (*p*<0.05).

7.1.4 Spiders

Chemical sensitivity differed between free-hunting and web-building species (F(1,26) = 3.35; p = 0.08; Table 7.1.4.1). The LC₅₀s of free-hunters were on average 34% lower than those from web-builders (Fig. 7.1.4.1). In addition, spider sensitivity was significantly higher for species from boreal to polar climate than for cool (p<0.001; Table 7.1.4.2) and warm temperate climates (p = 0.04; Table 7.1.4.2). Boreal and polar spiders were five and three times more sensitive to the insecticide than cool and warm temperate species, respectively (Fig. 7.1.4.2). All other tested relationships were not statistically significant (p>0.28; Table 7.1.4.1).

The chemical sensitivity of spiders differed depending on the species' hunting mode, where free-hunters were more susceptible than web-builders (Fig. 7.1.4.1). Our results confirm previous research (Pekár, 1999) on the effects of direct spray of insecticides on spiders. This outcome may be explained with the behaviour and foraging mode of the species tested. First, free-hunters presumably had more contact with lambda-cyhalothrin in the applied area due to their higher mobility in comparison with web-builders, which may result in higher insecticide uptake. On the other hand, webs can provide efficient shelter to spiders and therefore organisms could have had less direct contact with the insecticide. The relationship between foraging mode and spider sensitivity may affect the ecosystem service of biocontrol, as free-hunters play a larger role in pest suppression than web-builders (Michalko *et al.* 2019).

In addition, our results showed that climate was related to spider sensitivity to lambdacyhalothrin (Fig. 7.1.4.2). Specifically, spider sensitivities may be related to temperature, as it is one of the abiotic parameters which clearly differentiates each climate zone. Although we are not aware of other studies that have investigated the relationship between climate and spider sensitivity, previous research focused on interactions between temperature, humidity, and spider sensitivity under laboratory conditions (Akkerhuis *et al.* 1997; Everts *et al.* 1991). Temperature was negatively correlated with sensitivity of spiders coming from the same habitat. Though our ecotoxicological assessment was performed at constant temperature, we cannot exclude that the habitat temperature influenced the spider sensitivity. Ontogenetic traits acquired during the development of female spiders in the field may explain the differences we observed in our experiment. The inclusion of temperature as a stressor in ecotoxicological assessment of spiders coming from different climates will help to understand the relationship obtained.

Covariate	Log LC ₅₀		
	df	F	<i>p-</i> value
Habitat	2	0.57	0.57
Climate (HLZ)	2	5.27	0.01*
Hunting mode	1	3.35	0.08
Weight	1	1.20	0.28
Body length	1	0.001	0.97

Table 7.1.4.1: Comparison between spider traits, habitat characteristics, and chemical sensitivity. Statistically significant differences (p<0.05) have an asterisk.



Figure 7.1.4.1. Comparison between spider chemical sensitivity (in a logarithmic scale), and their hunting mode.

Table 7.1.4.2: Comparison of spider chemical sensitivity between climate zones. Statistically significant differences (p<0.05) have an asterisk.

Climate 1	Climate 2	<i>t</i> -value	<i>p</i> -value
Cool temperate	Warm temperate	1.36	0.37
Boreal & Polar	Warm temperate	-2.52	0.04*
Boreal & Polar	Cool temperate	-4.20	<0.001*



Figure 7.1.4.2. Comparison between spider chemical sensitivity (in a logarithmic scale), and their climate of origin. Different letters show a statistically significant difference (p<0.05).

7.2 Zonal assessment

Our comparison of the sensitivity of TA from different ecosystem types hinges on the representativeness of the chosen typology systems. To validate our results, we hence repeated the hSSD modelling for another typology system: the EFSA risk assessment zones. Authorization of new plant protection products follows a zonal approach in Europe. The three zones (Northern, Central, Southern) are defined in Appendix 1 of the regulation EC No 1107/2009 and shown in Figure 7.2.1. Formulations that use authorised active substances only need to be assessed by a single state per zone (zonal evaluation). National authorizations can be based on assessments for states of the same or a different zone (mutual recognition). The EFSA zones present a natural choice as comparison for us, because they are already deeply embedded in the current risk assessment paradigm.



Figure 7.2.1: Risk assessment zones in Europe: Northern (blue), Central (green), and Southern (red).

The modelling followed the same steps we laid out in 5.2.1. We computed the assemblage sensitivity (HC₅) for 3862 assemblages of freshwater macroinvertebrates. The sensitivities were then grouped by the risk assessment zone in which the assemblage was sampled. We found statistically significant differences in copper sensitivity (Figure 7.2.2). Assemblages from the Northern zone were less sensitive than those from the southern zone which in turn were less sensitive than assemblages from the central zone. This is potentially linked to the prevalence of very large rivers, which is high in the central zone when compared to the Southern and Northern zones. The predicted HC₅s did not show statistically significant patterns for the other three chemicals. Mean sensitivities were all within one order of magnitude. The findings concur with those for the broad river types and hence further strengthen our confidence in the results.



Figure 7.2.2: Variation in mean assemblage-specific HC₅ values across risk assessment zones. Within chemicals, bars that share the same letter are not statistically different.

7.3 Stakeholder workshop

We conducted a stakeholder workshop to discuss and disseminate findings abstained in the other WPs and build consensus on the potential implementation and added value of a more spatially defined risk assessment for future chemical regulation. The workshop brought together 35 key stakeholders from European policy and regulatory authorities, chemical industry, academia and NGOs. The workshop was held in the Azimuth hotel in Cologne from the 27th to 28th June in 2022 and had the agenda as outlined below.

Day 1

Until 14:00	Arrival	
14:00 – 14:15	Opening and Project Overview	Ralf Schäfer
14:15 - 14:45	Spatiotemporal variation in terrestrial and aquatic assemblages across Europe	Jonathan Jupke, Sebastian Scheu

14:45 - 15:40	Modelling and testing the sensitivity of assemblages towards chemicals	Javier Diaz, Frederik de Laender, Tom Sinclair, Lorraine Maltby, Tomas Duque, Paul van den Brink
15:40 - 16:10	Coffee break	
16:10 – 16:45	Spatiotemporal variation in sensitivity	Ralf Schäfer, Jonathan Jupke, Sebastian Scheu, Tomas Duque, Martin Entling, Javier Diaz, Frederik de Laender, Tom Sinclair, Lorraine Maltby, Paul van den Brink
16:45 – 17:30	EFSA position on how to consider spatiotemporal variation in risk assessment	Domenica Auteri, Alessio Ippolito
17:30 – 17:45	Preparation of break-out groups	
18:15	Walk to guided tour from hotel	
18:30 - 20:00	Guided walking tour of Colognes historic city center	
20:00	Dinner	

Day 2

9:00 - 10:30	Discussion in break-out groups	
10:30 - 11:00	Coffee break	
11:00 - 12:00	Discussion in break-out groups	
12:00 - 13:30	Business Lunch with	

presentations of break out group results and wrap up	

As can be seen from the agenda, the first day mainly consisted of the presentation of the results of ECO50 and a presentation of participants from EFSA on how to consider spatiotemporal variation in risk assessment. On the second day, the participants discussed the following questions in three break-out groups:

- How important is it to consider spatial variation when predicting chemical sensitivity?
- Under which circumstances would spatial variation be important?
- What are the challenges of quantifying spatial variation in sensitivity and which approaches should be considered in future studies?
- Should we have a zonal (i.e. spatially separated) risk assessment for current chemical risk assessment focusing on structural endpoints?
- Would the conclusion change for a chemical risk assessment using traits to estimate ecosystem services?

One break-out group consisted of experts related to aquatic risk assessment (hereafter Aquatic break-out group), one break-out group consisted of experts related to terrestrial risk assessment (hereafter Terrestrial break-out group) and one break-out group consisted of experts with mixed background and was conducted online (hereafter Mixed break-out group). Each break-out group had 2-3 members of the project team as participants.

The responses were reported to the groups by rapporteurs and are detailed in Appendix 3. All three groups agreed that for the cases considered in the project spatial variation seemed less relevant than other factors when predicting chemical sensitivity. These other factors comprise a range of environmental factors that are not captured by the broad scale habitat typologies (and the typologies would be severely inflated in their number if representing smaller scale spatial environmental variation). The Aquatic break-out group discussed that spatial variation can, in contrast to the project focussing on assemblages, also be defined on the one hand as the variation in environmental conditions across ecosystems that may influence bioavailability and on the other hand as the landscape context itself that may influence the recovery potential.

Regarding the question under which circumstances spatial variation would be important, the Mixed break-out group stressed that it would be important if the conservation status is linked to one of the habitat types from the typologies. The Aquatic group suggested that where

landscapes are associated with particular chemical pollution that spatial variation, defined as discussed before by this group, would become important.

All three groups identified several challenges of quantifying spatial variation and made a range of suggestions for future studies. All groups identified the lack of toxicity data from species from different habitats and ecosystems as a challenge. It was also suggested that multiple stressors need to be considered as these may vary between habitats and that it would be important to quantify the importance of different factors that determine the sensitivity of assemblages in real world ecosystems.

For the question of zonal assessment, the Mixed break-out group suggested that the current data would not suggest a zonal assessment, but this could be more important in other contexts. In particular, the more ecological the risk assessment becomes, the more relevant the spatial dimension would be. The spatial dimension could be captured through environmental variables. The Aquatic break-out group discussed that the spatial dimension is to some extent implicitly considered currently and that its implementation would be most straightforward in pesticide risk assessment but more complicated in other risk assessments.

Regarding the question related to ecosystem services, the Mixed and Aquatic group suggested that if moving towards more functional assessments the spatial variation would probably be lower than for structural responses. However, the Aquatic group also raised the issue that an ecosystem service-based assessment would need to be spatially explicit.

Overall, it was largely consensus among the groups that on the basis of the current results spatial variation in assemblage sensitivity seems a rather subordinate factor to consider when aiming to predict the sensitivity of assemblages and that other factors, which may also exhibit strong spatial variation, are more relevant.

8. Conclusions und Outlook

In the GETREAL project, we strove to answer whether risk assessment of chemicals should consider spatio-temporal differences in community composition. More specifically, we asked if ecological communities in recipient ecosystems differ in their sensitivity towards chemicals in patterns than can be predicted by large scale ecosystem typologies.

We found the expected spatial differences in taxonomic composition between typical assemblages of spiders, earthworms, diatoms, and macroinvertebrates. For earthworms, we found that endogeic earthworm species were more sensitive than epigeic taxa but no spatial pattern in sensitivities was found. For spiders, we found that spiders from boreal and polar climates were more sensitive than those from cool and warm temperate climates. For macroinvertebrates, when the most and least sensitive assemblages were excluded, the variation in hSSD-predicted assemblages sensitivity was between 5.5 (Atrazine) and 13.7 fold (lambda-cyhalothrin). Sensitivity differed between types but in an unpredictable and chemical-dependent fashion. Taking the observed variation between communities into account, current SSD approaches would be protective for 95 - 100% of macroinvertebrate assemblages. In the ecological model, sensitivity was positively correlated with the number of prey species in the community but predators were generally more sensitive than prey. The predicted sensitivities of hSSD and ecological models were largely consistent and confirmed by the mesocosm experiments.

At the workshop, the consensus between the project team and stakeholders from academia, industry, and regulatory bodies was that the variation in sensitivity that we found in this project does not merit explicit consideration within and changes in current risk assessment practices. This project thus comes to the conclusion that the spatial variation in taxonomic composition reflected in broad habitat typologies at large spatial scales is not a main predictor of differences in sensitivity to chemicals. However, our focus was on spatial variation, whereas multiple environmental factors that vary with space were out of scope of this project. However, these factors may influence chemical sensitivity either through affecting the bioavailability of a chemical or moderating the sensitivity of organisms and several studies discussed in section 6 suggest that they can be a relevant driver of sensitivity. For example, the toxicity of heavy metals, such as copper, is largely driven by their free ion form. If the cations are sequestered in complexes or bound to particles they are much less toxic. This sequestration and binding depends on alkalinity, pH, and salinity among others, all of which may vary strongly across ecosystems. Hence, even assemblages that are taxonomically very similar can differ in their sensitivity depending on the environmental conditions. Whether real assemblages differ in their sensitivity could be evaluated in mesocosm studies that focus on the sensitivity of

communities sampled from different ecosystem types. In light of these considerations, a spatially-explicit regional risk assessment might still be necessary if the current risk assessment moves further into realistic landscape scenarios and explicitly integrates environmental conditions.

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Appendix 1: Typical Assemblages

Terrestrial typical assemblages: Spiders

Ecosystem type	Spider species
Boreal - Open space	Agyneta rurestris, Alopecosa pulverulenta, Bathyphantes gracilis, Erigone atra, Erigone dentipalpis, Oreonetides vaginatus, Pardosa amentata, Pardosa monticola, Pardosa nigriceps, Pardosa palustris, Savignia frontata, Tenuiphantes tenuis
Cool temperate - Forest	Agroeca brunnea, Bathyphantes gracilis, Centromerus sylvaticus, Ceratinella brevis, Clubiona terrestris, Coelotes terrestris, Diplocephalus latifrons, Diplocephalus picinus, Diplostyla concolor, Haplodrassus silvestris, Histopona torpida, Inermocoelotes inermis, Macrargus rufus, Micrargus herbigradus, Microneta viaria, Neriene clathrata, Pachygnatha listeri, Palliduphantes pallidus s.l., Pardosa lugubris s.l., Robertus lividus, Saaristoa abnormis, Tenuiphantes alacris, Tenuiphantes cristatus, Tenuiphantes flavipes, Tenuiphantes tenebricola, Trochosa terricola, Walckenaeria cucullata, Walckenaeria dysderoides
Cool temperate - Open space	Agyneta rurestris, Alopecosa cuneata, Alopecosa pulverulenta, Bathyphantes gracilis, Centromerus sylvaticus, Dicymbium nigrum s. I., Diplostyla concolor, Drassyllus pusillus, Erigone atra, Erigone dentipalpis, Micrargus herbigradus, Oedothorax apicatus, Oedothorax fuscus, Oedothorax retusus, Pachygnatha clercki, Pachygnatha degeeri, Pardosa amentata, Pardosa lugubris s.I., Pardosa palustris, Pardosa prativaga, Pardosa pullata, Robertus lividus, Tenuiphantes tenuis, Trochosa ruricola, Trochosa terricola, Xysticus cristatus, Xysticus kochi
Cool temperate - Wetland	Alopecosa pulverulenta, Antistea elegans, Bathyphantes gracilis, Bathyphantes parvulus, Centromerus sylvaticus, Dicymbium nigrum s. l., Diplostyla concolor, Erigone atra, Erigone dentipalpis, Oedothorax gibbosus, Oedothorax retusus, Ozyptila trux, Pachygnatha clercki, Pachygnatha degeeri, Pardosa amentata, Pardosa prativaga, Pardosa pullata, Pirata piraticus, Piratula hygrophila, Piratula latitans, Pocadicnemis pumila, Tallusia experta, Tenuiphantes mengei, Tenuiphantes tenuis, Trochosa spinipalpis, Trochosa terricola, Walckenaeria atrotibialis
Warm temperate - Forest	Agroeca inopina, Alopecosa albofasciata, Alopecosa pulverulenta, Canariphantes zonatus, Episinus maculipes, Episinus truncatus, Eratigena feminea,

	Eratigena montigena, Eratigena picta, Hogna radiata, Liophrurillus flavitarsis, Mesiotelus mauritanicus, Pardosa hortensis, Pardosa proxima s.l., Phrurolithus minimus, Pulchellodromus bistigma, Scotina celans, Selamia reticulata, Tenuiphantes tenuis, Zelotes thorelli, Zodarion alacre, Zodarion italicum, Zodarion styliferum
Warm temperate - Open space	Agyneta rurestris, Alopecosa albofasciata, Asagena phalerata, Aulonia albimana, Diplostyla concolor, Drassodes lapidosus, Drassodes pubescens, Drassyllus praeficus, Erigone dentipalpis, Haplodrassus dalmatensis, Haplodrassus signifer, Hogna radiata, Nomisia exornata, Oedothorax apicatus, Pachygnatha degeeri, Pardosa hortensis, Pardosa prativaga, Pardosa proxima s.l., Phrurolithus festivus, Pisaura mirabilis, Scytodes thoracica, Tenuiphantes tenuis, Thanatus atratus, Trachyzelotes pedestris, Trochosa ruricola, Xysticus kochi

Terrestrial typical assemblages: Earthworms

Ecosystem type	Earthworm species	
Boreal - Open space	Aporrectodea caliginosa, Aporrectodea rosea, Dendrobaena octaedra, Lumbricus rubellus, Lumbricus terrestris	
Cool temperate - Forest	Aporrectodea caliginosa, Apporectodea chlorotica, Aporrectodea rosea, Dendrobaena octaedra, Denrodrilus rubidus, Lumbricus rubellus, Octolasion cyaneum	
Cool temperate - Open space	Aporrectodea caliginosa, Apporectodea chlorotica, Aporrectodea longa, Aporrectodea rosea, Lumbricus castaneus, Lumbricus rubellus, Lumbricus terrestris	
Cool temperate - Wetland	Aporrectodea caliginosa, Apporectodea chlorotica, Aporrectodea longa, Aporrectodea rosea, Eiseniella tetraedra, Lumbricus castaneus, Lumbricus rubellus, Lumbricus terrestris, Octolasion cyaneum, Octolasion tyrtaeum	
Warm temperate - Forest	Aporrectodea caliginosa, Aporrectodea rosea, Lumbricus friendi, Octolasion cyaneum	
Warm temperate - Open space	Aporrectodea caliginosa, Apporectodea chlorotica, Aporrectodea lissaensis, Aporrectodea rosea	

Aquatic typical assemblages: Diatoms

Ecosystem type	Diatom species		
RT2 Lowland, siliceous, medium-large streams	Achnanthidium minutissimum, Amphora pediculus inariensis Complex, Cocconeis placentula var placen- tula, Encyonema silesicacum minutum lange bertalotii, Eolimna minima seminulum atomoides, Eunotia arcus mucophila bilunaris Complex, Eunotia exigua elegans Complex, Eunotia implicata Complex, Fragi- laria capucina complex, Fragilaria tenera complex, Frustulia rhomboides Complex, Gomphonema parvulum Complex, Karayevia amoena nitidiformis, Meridion circulare, Navicula, Navicula cryptocephala, Navicula radiosa, Nitzschia dissipata recta Complex, Nitzschia palea paleacea, Nitzschia perminuta Complex, Planothidium lanceolatum, Psammothidium subatomoides, Tabellaria flocculosa Complex, Ulnaria ulna complex		
RT6/8 Lowland and mid- altitude, organic and siliceous streams	Achnanthidium minutissimum, Encyonema silesicacum minutum lange bertalotii, Eunotia arcus mucophila bilunaris Complex, Eunotia exigua elegans Complex, Eunotia implicata Complex, Eunotia incisa Complex, Eunotia pectinalis Complex, Fragilaria capucina complex, Fragilaria tenera complex, Frustulia rhomboides Complex, Gomphonema parvulum Complex, Navicula cryptotenella cryptotenelloides, Psammothidium helveticum chlidanos daonense, Tabellaria flocculosa Complex		
RT9 Mid-altitude, siliceous, very small-small streams	Achnanthidium minutissimum, Diatoma mesodon, Encyonema silesicacum minutum lange bertalotii, Eolimna minima seminulum atomoides, Eunotia, Eunotia exigua elegans Complex, Eunotia incisa Complex, Fragilaria capucina complex, Fragilaria tenera complex, Gomphonema, Gomphonema parvulum Complex, Meridion circulare, Planothidium lanceolatum, Psammothidium helveticum chlidanos daonense, Ulnaria ulna complex		
RT10 Mid-altitude, calcareous or mixed, medium-large streams	Achnanthidium minutissimum, Amphora pediculus inariensis Complex, Cocconeis pediculus, Denticula tenuis, Encyonema silesicacum minutum lange bertalotii, Encyonema ventricosum, Fragilaria capucina complex, Gomphonema, Gomphonema olivaceum olivaceoides, Gomphonema parvulum Complex, Gomphonema pumilum complex, Navicula cryptotenella cryptotenelloides, Navicula margalithii tripunctata, Nitzschia dissipata recta Complex, Nitzschia fonticola		
RT14_15_16 Highland and glacial streams	Achnanthidium minutissimum, Diatoma ehrenbergii, Encyonema silesicacum minutum lange bertalotii, Frag-		

	ilaria arcus, Fragilaria capucina complex, Gomphonema, Gomphonema olivaceum olivaceoides, Gomphonema parvulum Complex, Gomphonema pumilum complex, Reimeria sinuata
RT18 Mediterranean, mid altitude, medium-large streams	Achnanthidium minutissimum, Amphora pediculus inariensis Complex, Cocconeis pediculus, Cocconeis placentula var euglypta, Cymbella affinis, Cymbella excisa, Denticula tenuis, Encyonema silesicacum minutum lange bertalotii, Encyonopsis descripta falaisensis microcephala, Fragilaria capucina complex, Gomphonema, Gomphonema parvulum Complex, Gomphonema pumilum complex, Navicula cryptotenella cryptotenelloides, Reimeria sinuata, Ulnaria ulna complex

Aquatic typical assemblages: Macroinvertebrates

Ecosystem type	Macroinvertebrate species		
RT1 - Very large rivers	Chelicorophium curvispinum, Chelicorophium robustum, Dikerogammarus haemobaphes, Dikerogammarus villosus, Dreissena polymorpha, Echinogammarus ischnus, Jaera sarsi, Baetis, Caenis, Gammarus, Heptagenia, Hydropsyche, Hydroptila, Potamopyrgus, Chironomidae		
RT2 - Lowland, siliceous, medium-large streams	Ancylus fluviatilis, Baetis rhodani, Elmis aenea, Esolus parallelepipedus, Gammarus pulex, Hydropsyche pellucidula, Hydropsyche siltalai, Lepidostoma hirtum, Leuctra fusca, Limnius volckmari, Polycentropus flavomaculatus, Potamopyrgus antipodarum, Rhyacophila dorsalis, Serratella ignita, Stylodrilus heringianus, Chironomidae		
RT3 - Lowland, siliceous, small - very small streams	Physa fontinalis, Piscicola geometra, Ancylus fluviatilis Baetis rhodani, Elmis aenea, Gammarus pulex, Hydropsyche pellucidula, Hydropsyche siltalai, Limniu volckmari, Polycentropus flavomaculatus, Potamopyrgus antipodarum, Rhyacophila dorsalis, Sericostoma personatum, Serratella ignita, Simulium ornatum, Chironomidae		
RT4/5 Lowland calcareous streams	Potamothrix hammoniensis, Theodoxus fluviatilis, Ancylus fluviatilis, Baetis rhodani, Elmis aenea, Gammarus pulex, Glossiphonia complanata, Limnius		

	volckmari, Potamopyrgus antipodarum, Serratella ignita, Caenis
RT8/10/11/18 Large, mid- altitude streams	Serratella ignita, Baetis, Caenis, Ecdyonurus, Elmis, Esolus, Gammarus, Hydropsyche, Hydroptila, Leuctra, Limnius, Rhyacophila, Riolus, Chironomidae, Limoniidae, Oligochaeta, Simuliidae
RT9 - Small, mid-altitude siliceous streams	Agapetus ochripes, Heptagenia dalecarlica, Ithytrichia lamellaris, Potamophylax cingulatus, Alainites muticus, Ancylus fluviatilis, Baetis rhodani, Elmis aenea, Hydropsyche siltalai, Limnius volckmari, Polycentropus flavomaculatus, Hydraena, Isoperla, Leuctra, Protonemura, Rhyacophila, Sericostoma, Chironomidae
RT14/15/16 - High altitude streams	Baetis, Ecdyonurus, Elmis, Hydropsyche, Leuctra, Protonemura, Rhithrogena, Rhyacophila, Chironomidae, Empididae, Limnephilidae, Limoniidae, Oligochaeta, Simuliidae

Appendix 2: Terrestrial species sensitivities

Table A2.1: Source of earthworm	species used in ecotox	cicological assessment v	with their 14-
day LC ₅₀ (mg a.i./kg d.w.)			

Pesticide	ID	Species	Source	Coordinates (WGS84)	Natural soil pH	LC ₅₀ ±C.I.
Imidacloprid	1	A. longa	Grassland	49.193975, 8.096792	6.74±0.13	1.53±0.16
	2	A. caliginosa	Forest	49.223472, 8.187417	6.65±0.07	1.74±0.29
	3	A. rosea	Forest	49.223472, 8.187417	6.65±0.07	1.32±0.29
	4	A. caliginosa	Forest	49.211944, 8.188722	4.16±0.11	1.81±0.27
	5	A. caliginosa	Grassland	49.228772, 8.003750	6.38±0.04	1.97±0.30
	6	E. fetida	Compost	-	-	1.24±0.12
	7	A. rosea	Forest	49.200283, 8.092964	6.28±0.09	0.96±0.32
	8	A. rosea	Wetland	49.199667, 8.096469	6.33±0.05	0.91±0.27
	9	A. chlorotica	Forest	49.200547, 8.092903	6.18±0.21	0.72±0.28
	10	L. rubellus	Forest	49.200283, 8.092964	6.28±0.09	3.44±0.44
	11	L. rubellus	Forest	49.211944, 8.188722	4.16±0.11	2.88±0.44
	12	L. rubellus	Grassland	49.284167, 7.914136	4.24±0.09	3.53±0.46
	13	A. caliginosa	Grassland	49.284167, 7.914136	4.24±0.09	2.65±0.37
	14	E. fetida	Lab- culture	-	-	1.90±0.30
Copper	1	A. rosea	Forest	49.223472, 8.187417	6.65±0.07	220.29±97.34
	2	E. fetida	Compost	-	-	199.99±21.87
	3	A. caliginosa	Forest	49.211944, 8.188722	4.16±0.11	433.09±52.21
	4	A. chlorotica	Forest	49.200547, 8.092903	6.18±0.21	205.95±80.44
	5	A. rosea	Forest	49.200283, 8.092964	6.28±0.09	275.95±32.67
	6	A. caliginosa	Grassland	49.228772, 8.003750	6.38±0.04	259.09±98.62
	7	A. caliginosa	Grassland	49.284167, 7.914136	4.24±0.09	322.95±45.75
	8	A. caliginosa	Forest	49.223472, 8.187417	6.65±0.07	264.52±30.28
	9	A. caliginosa	Wetland	49.199667, 8.096469	6.33±0.05	302.84±45.53
	10	A. longa	Grassland	49.193975, 8.096792	6.74±0.13	263.97±34.79
	11	A. longa	Wetland	49.199667, 8.096469	6.33±0.05	220.94±41.19
	12	A. rosea	Wetland	49.199667, 8.096469	6.33±0.05	281.09±19.63
	13	L. rubellus	Forest	49.200283, 8.092964	6.28±0.09	249.66±20.03
	14	E. fetida	Lab- culture	-	-	326.07±36.26

Pesticide	Species	LC ₅₀	Soil type (Organic matter %)	Source
Imidacloprid	A. longa	1.53	LUFA 2.2 (2.77)	Present study
	A. caliginosa	2.01		
	A. chlorotica	0.72		
	A. rosea	1.05		
	E. fetida	1.54		
	L. rubellus	3.27		
	A. nocturna	3.74	Natural soil (2.8)	Literature
	A. icterica	2.80	Natural soil (2.8)	
Copper	A. longa	241.22	LUFA 2.2 (2.77)	Present study
	A. caliginosa	310.86		
	A. chlorotica	205.95		
	A. rosea	257.57		
	E. fetida	255.37		
	L. rubellus	249.66		
	L. terrestris	218.00	Natural soil (1.7)	Literature

Table A2.2: Earthworms 14-day LC₅₀ (mg a.i./kg d.w.) values used for the species sensitivity distributions for imidacloprid and copper

ID	Species	Coordinates	Hunting	HLZ	Habitat	LC ₅₀ (ng a.i./cm ²)	
1	P. saltans	49.2813, 8.2819	Free	Cool temperate	Forest	15.83 ± 10.33	
2	P. amentata	49.2535, 7.9620	Free	Cool temperate	Wetland	12.81 ± 7.98	
3	P. latitans	49.2535, 7.9620	Free	Cool temperate	Wetland	123.23 ± 75.94	
4	P. hygrophila	49.0401, 8.2551	Free	Cool temperate	Forest	108.78 ± 44.63	
5	P. hygrophila	49.2813, 8.2819	Free	Cool temperate	Forest	126.13 ± 35.72	
6	E. ovata	49.3537, 8.1983	Web	Cool temperate	Forest	67.64 ± 35.32	
7	P. agrestis	49.2048, 8.0927	Free	Cool temperate	Open space	14.75 ± 5.10	
8	D. graecus	38.0762, 12.7304	Web	Warm temperate	Open space	29.56 ± 8.51	
9	P. proxima	45.1502, 7.6076	Free	Warm temperate	Open space	16.79 ± 7.80	
10	P. tenuipes	45.1502, 7.6076	Free	Warm temperate	Open space	13.26 ± 8.74	
11	P. wagleri	45.1548, 7.5563	Free	Warm temperate	Wetland	35.97 ± 13.03	
12	P. mirabilis	49.2014, 8.1381	Free	Cool temperate	Open space	191.22 ± 51.76	
13	G. dentatum	45.9939, 8.9252	Web	Warm temperate	Wetland	91.44 ± 28.74	
14	P. hortensis	49.1900, 8.0997	Free	Cool temperate	Open space	14.80 ± 7.48	
15	T. hispanica	45.1490, 7.5947	Free	Warm temperate	Wetland	71.10 ± 29.11	
16	P. hortensis	42.4943, 3.0144	Free	Warm temperate	Forest	12.43 ± 30.22	
17	P. morosa	42.4707, 3.1187	Free	Warm temperate	Wetland	16.79 ± 14.80	
18	P. saltans	49.0389, 8.2165	Free	Cool temperate	Forest	22.22 ± 10.99	
19	P. saltans	49.2339, 8.3253	Free	Cool temperate	Forest	16.93 ± 13.37	
20	P. amentata	46.5406, 8.7132	Free	Boreal & Polar	Wetland	6.65 ± 2.09	
21	O. fuscus	57.2659, 10.3063	Free	Cool temperate	Open space	30.07 ± 19.22	
22	E. dentipalpis	57.2659, 10.3063	Web	Cool temperate	Open space	22.24 ± 12.93	
23	P. amentata	46.5737, 8.5584	Free	Boreal & Polar	Wetland	6.72 ± 1.92	
24	P. oreophila	46.5594, 8.5585	Free	Boreal & Polar	Open space	9.55 ± 5.61	
25	C. alpicola	46.5580, 8.5699	Free	Boreal & Polar	Open space	9.00 ± 3.24	
26	T. tenuis	57.4782, 10.4071	Web	Cool temperate	Open space	48.19 ± 14.23	
27	E. atra	57.2659, 10.3063	Web	Cool temperate	Open space	35.93 ± 10.49	
28	P. riparia	46.5408, 8.7109	Free	Boreal & Polar	Forest	11.99 ± 3.96	
29	X. desidiosus	46.5491, 8.7381	Free	Boreal & Polar	Open space	21.42 ± 9.50	
30	D. heeri	46.5491, 8.7381	Free	Boreal & Polar	Open space	7.60 ± 4.35	
31	H. signifer	46.5491, 8.7381	Free	Boreal & Polar	Open space	10.75 ± 5.52	
32	Z. apricorum	46.5580, 8.5699	Free	Boreal & Polar	Open space	16.37 ± 10.99	
33	M. trilobatus	57.4782, 10.4071	Web	Cool temperate	Open space	75.99 ± 27.39	
34	Z. spinimana	49.1933, 8.1164	Free	Warm temperate	Open space	14.64 ± 4.06	

Table A2.3: Source of spider species used in ecotoxicological assessment with their 24-hour LC_{50} (ng a.i./cm²)
HLZ	Species	LC ₅₀	Habitat
Boreal & Polar	P. amentata	6.69	Wetland
	P. oreophila	9.55	Open space
	C. alpicola	9.00	Open space
	P. riparia	11.99	Forest
	X. desidiosus	21.42	Open space
	D. heeri	7.60	Open space
	H. signifer	10.75	Open space
	Z. apricorum	16.37	Open space
Cool temperate	P. saltans	18.13	Forest
	P. amentata	12.81	Wetland
	P. latitans	123.23	Wetland
	P. hygrophila	117.14	Forest
	E. ovata	67.64	Forest
	P. agrestis	14.75	Open space
	P. mirabilis	191.22	Open space
	P. hortensis	14.80	Open space
	O. fuscus	30.07	Open space
	E. dentipalpis	22.24	Open space
	T. tenuis	48.19	Open space
	E. atra	35.93	Open space
	M. trilobatus	75.99	Open space
Warm temperate	D. graecus	29.56	Open space
	P. proxima	16.79	Open space
	P. tenuipes	13.26	Open space
	P. wagleri	35.97	Wetland
	G. dentatum	91.44	Wetland
	T. hispanica	71.10	Wetland
	P. hortensis	12.43	Forest
	P. morosa	16.79	Wetland
	Z. spinimana	14.64	Open space

Table A2.4: Spiders 24-hour $LC_{\rm 50}$ (ng a.i./cm²) values used for the species sensitivity distributions

Appendix 3: Answers to the questions of the break-out groups

Presented by the rapporteurs (Mixed group: Frederic de Laender, Terrestrial group: Vanessa Roeben, Aquatic group: Karel Viane)

How important is it to consider spatial variation when predicting chemical sensitivity?

Mixed group (online):

- Seems not that important for data set compared to other factors
- discussion that it is not an artifact of extrapolation in hSSD models
- Species composition way more important than habitats aggregating larger spatial scales

Terrestrial group:

- Results do not indicate major variation in chemical sensitivity in assemblages for the two components (spider to a certain extent, but not for earthworms)
- For earthworms the soil properties are potentially more relevant than the species (*E. fetida* sensitivity)
- Many factors affect the species assemblages and distribution, e.g., soil type, soil composition, pH, temperature, moisture, food availability —> are important to a different extend at different sites and different interactions with sites/management practice —> multiple scale —> modelling needs data
- Differences regarding environmental factors and differences in the communities that need to be considered (both sides)
- Scale is important when talking about spatial
- Assemblages can not only change from N-C-S but also on small scale, e.g., in-field off-field

Aquatic group:

- Vulnerability or sensitivity?
- Vulnerability: takes into account recovery
- Spatial variation
- This project: spatial variation in composition (different assemblages)
- but all lab based toxicity data
- Spatial can also refer to landscape (eg bioavailability, recovery)
- Different assemblages do not necessarily have different sensitivities
- Variation between mesocosms e.g., imidacloprid > 2 orders of magnitude
- Other experiences: same thresholds between different mesocosms
- Copper REACH dossier: 12-14 ecoregions similar ideas
- Bioavailability AND sensitivity (related to endemic species)
- Based on local measurements (bioavailability)
- Leaving out endemic species more adapted to local conditions

- The project focuses on inherent sensitivities of core species
- Importance of spatial dimension will be compound specific
- Role of generic vs. specific mode of action

Under which circumstances would spatial variation be important?

Mixed group (online):

- becomes important, where conservation status is linked to habitat type
- theoretical question: why does a species occur in a site and not in another site
- can be important for individual species: Example of court case of vole in Germany

Terrestrial group:

See answers above

Aquatic group:

- Specific acting chemical most relevant
- Habitat type e.g., landscape with agriculture (pressure)
- importance of local level of protection
- Food web perspective?
- Different sensitivities between different freshwater compositions (modeling)
- Trait composition of community: similar vulnerability across different countries/continents
- Depends on bioavailability (+ background) + possibility to control exposure / mitigation

What are the challenges of quantifying spatial variation in sensitivity and which approaches should be considered in future studies?

Mixed group (online):

- toxicity data gap: more data needed on species-level
- large scale study on toxicant effects in ecosystems comparing the level of sensitivity. However, such a study would provide not only spatial signal (and focus on inherent sensitivity) but also be associated with all kinds of other environmental gradients (exposure, additional stressors)
- meta-analysis of mesocosm and field studies from different regions
- relative importance of different factors determining vulnerability to chemicals in field

Terrestrial group:

- Challenge: high complexity and multiple influencing aspects, e.g., soil type, soil composition, pH, temperature, moisture, food availability
- Technical Challenges:
 - Collect and maintain spiders in the lab

- Species selection
- How to quantify the sensitivity (which soil, which conditions)
- Future studies:
 - Include impacted areas
 - Broader taxonomic coverage would be desirable but species richness does not necessarily relate to species abundance
 - Multiple stressors
 - Field or (semi-)field studies in different sites, e.g., TMEs

Aquatic group:

- Information on local community => Improved biomonitoring to species level
- Lack of toxicity data \rightarrow NAMS to better understand (TK) mechanisms
- Lab-to-field => use existing knowledge on Bioavailability (organics)
 - Bioavailability: Abiotic variables
 - Differences in inherent sensitivities: adaptation at individual/population/community
 - Multiple stressors e.g. food availability
 - Science (precise) & regulatory (protective) challenge → Empirical will take a lot of work, Mechanistic approach (TK) more promising (TD/AOP more conserved)
- Ecological scenarios:
 - Can be different for chemical groups
 - e.g. Down the drain vs pesticides
 - Not necessarily limited to one chemical, also other factors can be accounted for
 - Mechanistic modelling to reduce uncertainty,
 - better capture variability + can take other local factors/stressors into account

Should we have a zonal (i.e. spatially separated) risk assessment for current chemical risk assessment focusing on structural endpoints?

Mixed group (online):

- Generally: the more ecological the RA becomes, the more relevant does spatial variation become. It is, for example, more important in higher than in lower tiers of the current pesticide RA. Or: robustness against stressors only relevant when taking recovery into account
- the more ecological RA becomes, the more factors are considered that capture spatial signal (e.g., temperature)
- this data does not suggest we should, but the question depends on the context
- physicochemical properties may determine relevance of spatial variation
- Clear link between environmental factors and zones

Terrestrial group:

• Based on the data we cannot give a clear answer as more information is needed (see answers to question 3)

• The Birds & Mammals Guidance could be helpful, e.g., using focal species, traits, feeding types etc.

Aquatic group:

- Spatial aspect is implemented at different levels between regulations (REACH, PPP, down the drain) YET some chemicals fall under multiple regulations
- SSDs
- Assemblage specific vs general HC5
- Streams vs ponds vs ...
- Distribution of HC5s: more confidence in current assessment factors
- Identify which assemblages are most sensitive
- Community/food web aspect
- Unclear how to include spatial aspect in current regulations (e.g., ZPAP: no harm)
- Broad use vs. specific use chemicals: clear where chemical is being used
- Potential benefit of spatial in RA ("zones")
- Spatial most clear for pesticides
- Can we be overprotective?
- Intention of tier I methods: implicit because you want to protect all systems + identify areas of concern
- Spatial dimension provides more options to risk assessors

Would the conclusion change for a chemical risk assessment using traits to estimate ecosystem services?

Mixed group (online):

- Structural endpoints are generally more sensitive than functional endpoints. This suggests that spatial variation might be less relevant for trait-based or Ecosystem Services-based RA
- Question is difficult to answer without knowing how such a RA looks like

Terrestrial group:

no response

Aquatic group:

- ES currently not really considered in regulations, mostly ecosystem structure
- Unclear question: Is the importance of spatial dimension different for ES? No
- But by definition ES = spatially explicit so it might be even more important to be spatially explicit