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Protection of biodiversity as the ultimate goal of environmental safety assessment: how does chemical pollution affect biodiversity?

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Contents

Abstract

Biodiversity is unequivocally declining and chemical pollution is a major driver of its loss. Ecotoxicological studies report various effects of chemicals at different levels of biological organization, mostly individual and sub-organismal levels, while biodiversity is typically measured by taxonomic richness and abundance. This misalignment of metrics hampers building a causal link between chemical pollution and its effects on biodiversity. This review addresses the existing evidence (the obvious and the subtle ones) of the impact of chemicals on each sub-class of descriptors proposed by the list of Essential Biodiversity Variables (EBVs). For each biological level of organization, examples of ecotoxicological studies are reported that highlight strengths and weaknesses in describing the effects of chemical pollution on the specific biodiversity identifiers. In the last part, modelling is presented as one of the most powerful approaches to answer such a complex issue. Different modelling approaches are described according to their potential, for instance mechanistic models can simulate the effects of chemicals on organisms and populations while Bayesian network models and Threshold Indicator Taxa Analysis (TITAN) can be used to predict the risk of chemicals to communities and ecosystems. Finally, biodiversity in the regulatory context and future perspectives are discussed, highlighting how crucial the collaboration between ecologists and ecotoxicologists is, as well as the integration of data from field and laboratory studies, and the development of new specific indicators to track progress towards biodiversity conservation.

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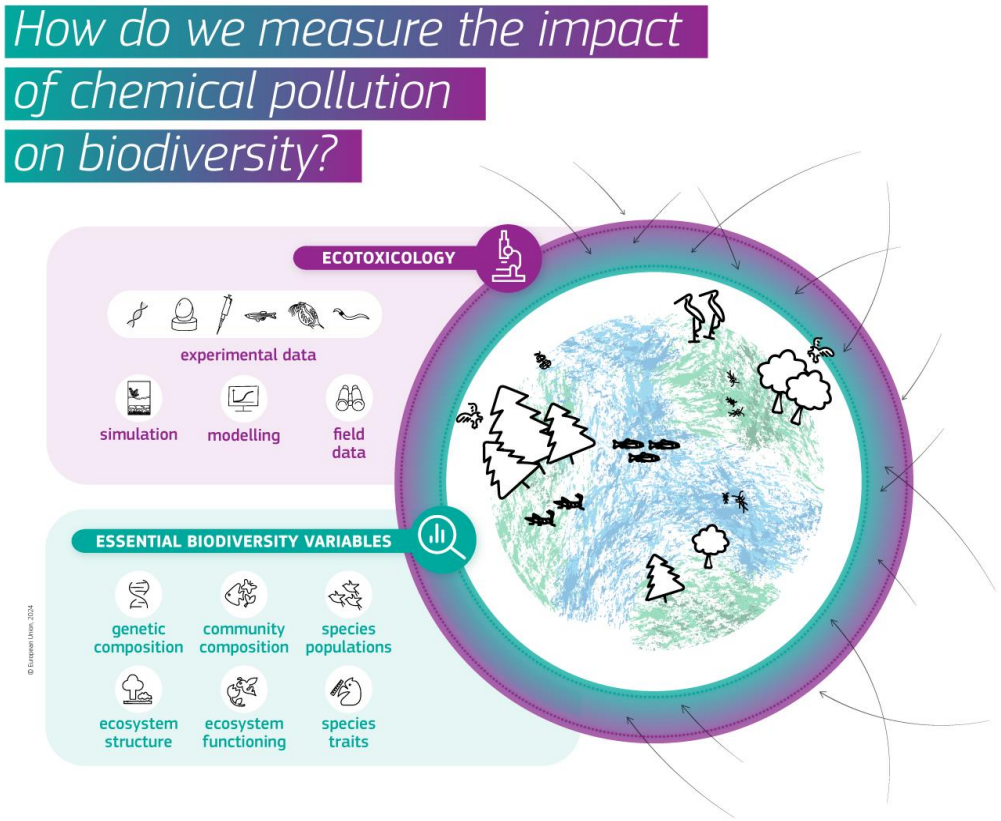
1. Introduction

1.1. Aim of the report

Chemical pollution is one of the main drivers of biodiversity loss. Therefore, there is an urgent need for tools and approaches that quantify the impact of chemicals on biodiversity in order to enable regulators to establish an improved framework for chemical management. However, establishing the evidence of the impact of chemical contamination on biodiversity in a scientifically sound manner and quantifying its effects comes with many challenges. Metrics of biodiversity loss and measurements of ecotoxicological effects are mostly not aligned and connected, and ecotoxicologists face the difficulty of predicting the effects of low concentrations of chemical mixtures on a long term in a changing environment and on many species by testing a few chemicals in few model species in controlled conditions (figure 1). This complex issue requires a transdisciplinary effort between ecologists and ecotoxicologists. After a short introduction on the definition of biodiversity and its global importance, this review addresses the existing evidence (the obvious and the subtle ones) of the impact of chemicals on each sub-class of descriptors proposed by the list of Essential Biodiversity Variables (EBVs). For each biological level of organization, examples of ecotoxicological studies are reported that highlight strengths and weaknesses in describing the effects of chemical pollution on the specific biodiversity identifiers. In the last part, modelling is presented as one of the most powerful approaches to address such a complex issue and different modelling approaches are described according to their potential. Finally, biodiversity in the regulatory context and future perspectives are discussed.

Figure 1 Graphical abstract illustrating metrics of biodiversity loss and type of measurements of ecotoxicological effects



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1.2. What is biodiversity and how to measure it?

Biodiversity or biological diversity is the variety of natural life, and its protection is crucial for maintaining the global ecosystem balance. It has a profound impact on human well-being in many ways, including regulation of water and air quality, soil fertility, pest control, pollination, food and timber production, disease prevention, and overall physical, mental, and cultural health (Harrison et al., 2014; Prakash et al., 2023). The definition of biodiversity provided by the Convention on Biological Diversity (United Nations Environment) includes “the diversity of species, the diversity within species and of ecosystems” regardless their direct contributions to human. However, identifying, measuring and quantifying those entities in their multifaceted complexity in a scientifically sound and harmonized way is challenging. To help standardise and coordinate the collection and monitoring of biodiversity data based on the environmental compartments, Pereira et al. (Pereira et al., 2013) proposed a set of Essential Biodiversity Variables (EBVs)(Pereira et al., 2013) derived from 84 biodiversity descriptors. Specifically, biodiversity descriptors are grouped under six classes, which are genetic composition, species populations, species traits, community composition, ecosystem structure, and ecosystem function. In general, richness of species is the metric most associated with the term biodiversity and one of the more frequently measured in ecology (Roswell et al., 2021). However, while

species richness expresses the number of species in a particular geographical area or ecosystem, it does not account for their abundance. Similarly, a change of species richness and abundance does not give any information on the functional diversity of their ecosystem. For instance, an increase of soil pathogens in cropland can be translated into an increase of soil microbial species richness compared to woodland, however the functional diversity in the agricultural fields is lower than the one in the wood (Labouyrie et al., 2023). The variety of EBVs descriptors highlights the large spectrum of characteristics falling under the definition of biodiversity. For instance, EBVs contain species abundances of selected terrestrial bird species, terrestrial mammals, butterflies, but also community biomass of selected functional groups of terrestrial arthropods (e.g. predators, decomposers), phenology of migration of highly migratory marine fish, or genetic diversity of selected freshwater taxa. Some additional examples of EBVs descriptors are reported in figure 2. 1. Identifying and harmonizing biodiversity metrics is crucial not only to monitor biodiversity but also to avoid mismeasurements and misinterpretations. Indeed, despite the global decline of biodiversity, there are some studies that reported apparent contradictory findings on biodiversity trends (Crossley et al., 2020; Haase et al., 2023; Pilotto et al., 2020). These results may come from the difficulty of dealing with different temporal and spatial scales and with many different measures of biodiversity (IPBES, 2019; Kuczynski et al., 2023; Ritter et al., 2019). Arguably, an essential step for more effective biodiversity conservation are harmonized metrics to properly assess pressures, state end responses to threats.

1.3. Biodiversity loss and chemical pollution

The Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) in 2019 (IPBES, 2019) presented an overall detailed picture of a global decline of biodiversity. Since then, a high number of studies have been published in which estimations of both the global and the regional trend of biodiversity were found to be in line with the IPBES report: biodiversity of insects (Sánchez-Bayo & Wyckhuys, 2019; Wagner, 2020), birds (Burns et al., 2021; Gorta et al., 2019; Ogada et al., 2022), amphibians (Luedtke et al., 2023), plants (Daru et al., 2021; Jandt et al., 2022), mammals (Brodie et al., 2021) is globally declining. Amongst others, pollution has been identified as one of the five main global threats to biodiversity (IPBES, 2019; Isbell et al., 2023; Jaureguiberry et al., 2022). Still, the main concern of society regarding the negative effects of chemical pollution seems to be on human health, while climate change seems to lead the apprehension for biodiversity loss (Groh et al., 2022). The analysis of the impacts of chemical pollution on the ecological status is often perceived as separate and less relevant compared to that of other pressures and their impacts. However, this is probably due to imperfect and incomprehensive chemical impact assessments, not least because of delayed and sometimes subtle effects that might not be taken into account (Posthuma et al., 2020). The International Union for Conservation of Nature's Red List of threatened species (IUCN, 2024) places pollution amongst the major threats for species health, including different classes of pollution and an indication of their severity. From the IUCN red list database, within the European region, 118 endemic and critically endangered species resulted to be threatened by pollution linked with urban wastewater and industrial, military, agricultural, forestry effluents (accessed in June 2024). However, a clear causal link or a quantitative relationship between pollution and its effect on the species is not reported. For instance, the decrease of the population of the Canary long-eared bat is reported to occur because of pesticides, amongst other stressors (Russo & Cistrone, 2023). In particular, pollution is reported to impact the species via mortality which lead to a slow but significant population decline. In the case of Bythinella markovi, an endemic

freshwater gastropod, pollution originating from domestic and urban wastewater is reported as the main cause of decline, however the report does not provide the source of those data. It is likely that although both studies are based on field observations and measurements well documented, those data are unable to provide a causal relationship between the chemical exposure and the impacts. However, in order to limit the negative effects of chemical exposure on biodiversity and to be able to quantify the potential impact before a chemical is released in the environment, we should be able to understand and predict the toxic effects at the different biological levels describing biodiversity. In other words, we should be able to predict the alteration of biodiversity metrics due to exposure to contaminants. Therefore, in parallel it is of vital importance to harmonize measures of biodiversity. Biodiversity loss is driven by five main factors (invasive alien species, changes in land and sea use, climate change, overharvesting of natural resources, pollution) that may act together in a direct or indirect way. However, in this paper we deliberately focus on the direct impact of chemical pollution only, where chemical pollution occurs when not naturally present substance is introduced into the environment or when the a substance concentration exceed natural levels.

1.4. Biodiversity from an ecotoxicological perspective

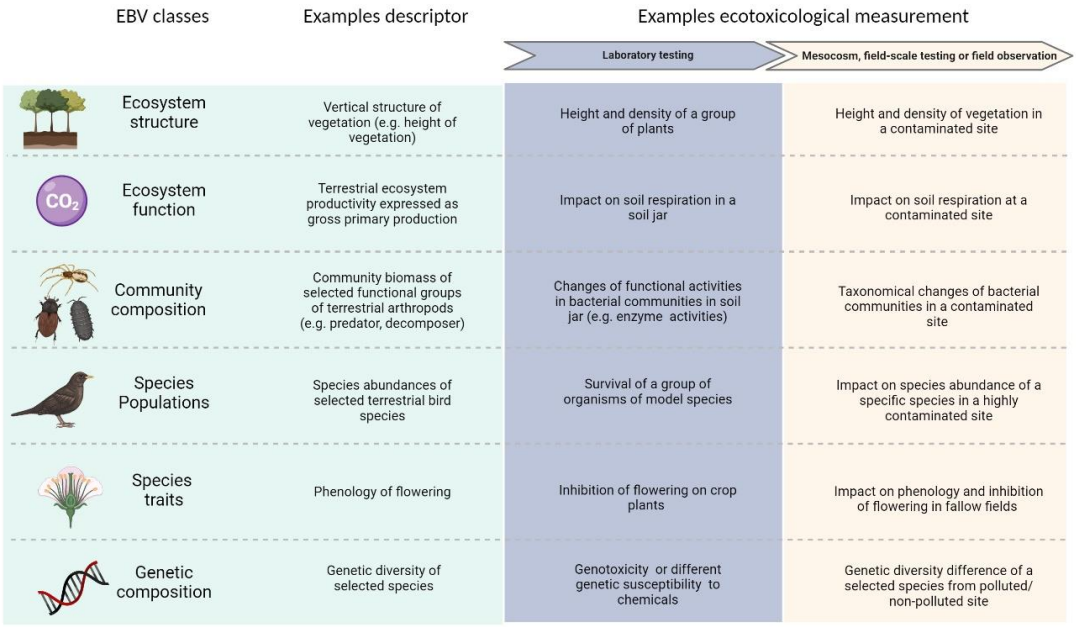
Considering the necessity to quantify and predict the impact of chemicals on biodiversity in a prospective manner, we should look for measurable toxic effects (endpoints) that can be considered “early warnings” of population impact and be able to extrapolate those impacts to ecological relevant scenarios. The current chemicals regulation approach faces some challenges such as the need to: extrapolate data produced by few model test species to ideally predict impacts on all species; include the ecosystem-specific ecological interactions amongst different species and with the abiotic factors; make optimal use of (bio)monitoring field data; predict toxicity of unintentional chemical mixtures; and finally to scale up data on effects at individual and sub-organism level to higher levels of biological organization. Biodiversity could be defined as an overarching biological level, which includes and requires the healthy functioning of all the other biological (sub)organizations (e.g. cell, organism, population, community, and ecosystem) as they are strictly interdependent. Under this point of view, potentially all ecotoxicological endpoints are useful to assess the impact of chemical exposure on biodiversity. The health of a population for instance will be affected by mortality of its individuals when this mortality is at such a rate that the population is no longer able to sustain itself. In a similar way, the population dynamic will be altered if mating success rate is changed by an altered secondary sexual characteristic. Several studies reported examples of how to link traditional ecotoxicological endpoints (e.g. growth, mortality, number of offspring) to population levels, giving data produced in a laboratory a meaningful ecological perspective (Martin et al., 2013; Salice et al., 2014; Theodorakis, 2001; Weir & Salice, 2021). For instance, in the European Food Safety Authority (EFSA) guidance for risk assessment of bees (EFSA, 2023a), in the lower tier risk assessment based on lethal effects on the individuals, the extrapolation step assumes a conservative one-to-one translation of individual to colony level effects for all experiments. Interestingly, a recent paper explored the predictable sequence of changes that drives populations to collapse such as altered behaviour of individuals, changes in fitness-related morphological traits, shifts in the population dynamics and eventually abundance declines (Cerini et al., 2023). While the study does not focus on chemical pollution, the transposition of those principles to ecotoxicology show that individual-level toxicological endpoints could be predictive of population collapse. Therefore, if exposure to a chemical leads to some adversity, there is a chance that the exposure is somehow causing a direct or indirect impact on biodiversity at the moment in which a specific effect threshold is reached. However, a lack of

knowledge about the complexity of factors affecting biodiversity makes it difficult to clearly identify the threshold at which an initiating event (at a low level of biological organization) triggers a cascade of events resulting in a shift of biodiversity. It is even harder to build a quantitative relationship and therefore to estimate any environmental exposure dose as point of departure of this cascade of events. Conceptually, we could apply the approach proposed by van Straalen (Van Straalen, 1993) to untangle the complexity of the impact of chemical on biodiversity. The model proposed by van Straalen and re-proposed by Rubach et al. (Rubach et al., 2011b) was developed for population vulnerability, however the approach can capture the impact on biodiversity only if we consider biodiversity as the highest level of biological organization. The ecotoxicological effects of a contaminants on biodiversity can be divided in three categories, 1) ecological sustainability: chemicals can affect resilience, adaptation, competition with other populations, changes in the trophic chain which have consequences on demography and recolonization; 2) toxicity, chemicals can cause direct toxicity which include toxicokinetic and toxicodynamic (TK-TD) processes; 3) exposure, chemicals can affect the habitat and food choice of the organism which is in turn influenced by ecological and behavioural factors; this category includes the exposure to mixtures of contaminants and its time-course. However, exposure and ecological sustainability are typically measured in field by recording observations and measurements with no controlled conditions, in semi-field systems following an experimental design without control of all the variables, or in mesocosm systems which simulate complex ecosystems under controlled conditions. Toxicity is usually assessed under laboratory conditions. This leads to a situation in which highly diverse types of data (data produced in the laboratory, in the field, by model estimation) are produced, which successfully answer different relevant research questions but are insufficiently complementary when we need to assess all three categories of effects to evaluate the impact on biodiversity.

The use of laboratory ecotoxicological endpoints to assess impacts on biodiversity comes with huge challenges. While laboratory testing provides controlled conditions that facilitate the establishment of a causal relationship between the chemical and specific effect/s on the organism, laboratory testing cannot resemble all the environmental conditions relevant to explore impacts on biodiversity. Laboratory tests often ignore multiple species interactions and abiotic factors under conditions changing through time and across space such as seasonality, habitat degradation, temperature, extreme weather events, pressure through predation or feed availability, or multiple chemical exposures. However, laboratory testing allows the calculation of dose-response functions, promotes a rich collection of data and, most importantly, makes it possible to test the chemical before the use and the consequent release into the environment. (Bio)monitoring field-collected data remain the most valuable type of data for measuring the effect of chemicals on biodiversity because they represent a realistic picture of the local environment. To corroborate the importance of the use of field studies, the concept of bioindicators serves as a reminder. This is certainly not a new concept: the record of occurrence and/or abundance of habitat-specific sensitive species (indicator species) has been widely used to answer environmental quality assessment for decades (Holt & Miller, 2011; Parmar et al., 2016). Indicator species play a crucial role in assessing the ecological status of water bodies under the Water Framework Directive (EU, 2000) and a recent study reported that about 26% of the deviance in ecological status in 12 European rivers was explained by the presence of toxic substances (Lemm et al., 2021). Similarly, the diversity of lichen species relates to air quality and to contaminant concentrations (Abas et al., 2022), and the diversity of soil microorganisms relates to soil pollution. Is not the assessment of the impact of local chemical concentrations on sensitive species a proxy of the effects on biodiversity? However, being related to a specific area makes field-collected data not representatives for an overall

assessment. They are also considered weaker than laboratory testing when establishing the basis of causality and they allow a retrospective assessment only. In figure 2, for each EBV class, we report an example of a biodiversity descriptor, along with an example of an ecotoxicological test performed in the lab and in a more complex system, which can currently represent surrogates of the assessment of chemical impact on that specific descriptor.

Figure 2 Examples of a biodiversity descriptor, an ecotoxicological test performed in the lab and in a more complex system for each EBV class. Created in BioRender



Source: Own elaboration

Overall, there is a need to link laboratory tests, typically resulting in data of individual and sub-individual measurements (e.g. biochemical alterations) to higher biological organization (e.g. population level), and to include ecological factors (e.g. multiple-species interactions). In parallel, there is also a need to link field data (typically based on trait/population/taxonomic observations) to mechanistic processes and provide prospective information, as proposed by the concept of Trait-based Ecological Risk Assessment (TERA) approach (Culp et al., 2011; Van den Brink et al., 2011). Ideally, the integration of multiple type of data would lead to an Ecological Adverse Outcome Pathway (eco-AOP), which is an AOP that includes endpoints at the ecosystem level or at the biodiversity level. Although the first definition of AOP was created to support ecotoxicological research and risk assessment (Ankley et al., 2010), computational strategies (e.g. text mining by artificial intelligence) are more developed in the human health context. There is an urgent need of transposition of integrated in silico methodologies to an ecotoxicological perspective (Baudiffier et al., 2024). More broadly in silico methodologies such as mechanistic modelling are crucial tools in allowing scaling adverse outcomes along biological organizations (up and down)(Schmolke et al., 2010), in reducing animal testing (Larras et al., 2022), and in reaching higher environmental relevance (Forbes & Galic, 2016). In the following paragraphs, the impact of chemicals on biodiversity by ecotoxicological effects

(measurable endpoints) from different types of data (field, laboratory, modelling data) are presented following the classes of the Essential Biodiversity Variables defined by EUROPABON. Afterwards, in the paragraph “Modelling approaches enable “biodiversity toxicology””, a list of relevant examples is provided in view of their ability to extrapolate ecotoxicological endpoints to higher biological levels or to more ecologically relevant results.

2. The link between chemical impacts at different levels of biological organization to biodiversity

2.1. Genetic level

A global loss of genetic diversity in wild populations was estimated to be around 6% since the industrial revolution (Leigh et al., 2019). Genetic diversity is the difference among individuals within a population or species that is determined by genotype variation (Freeman & Herron, 2007). Its conservation is a central issue of the Kunming-Montreal Biodiversity Framework (https://www.cbd.int/gbf), and is reported to be crucial for maintenance of the viability and the adaptive potential of wild animal and plant populations and species (Kardos et al., 2021).

A chemical may alter the genetic variability of a population by 1) insulting the DNA structure, causing mutation to the DNA, or affecting DNA repair processes, 2) favouring genotypes that are more tolerant (Abdullahi et al., 2022), 3) reducing the size of the population through other toxic endpoints, 4) affecting the behaviour of the individuals altering specific habits that decrease the gene flow and the number of individual such as migration, reproduction or feeding habits (Ribeiro & Lopes, 2013; van Straalen & Timmermans, 2002). In particular, points 3 and 4 represent indirect ways by which a chemical can reduce genetic diversity of a population. Indeed, a genetic drift is expected because of the occurrence of a population bottleneck. A bottleneck is a drastic reduction of the size of a population due to external events, including direct lethality or indirect effects of the exposure to contaminants.

Genetic variability can be altered by a myriad of other environmental factors other than exposure to chemical contaminants. Amongst all those other factors, there is a variety of natural processes, which is at the basis of evolution. Therefore, it is difficult to distinguish between a natural genetic variation and a genetic change due to the exposure to chemicals. A common method to tackle this issue is to compare different values expressing a specific characteristic of the genetic diversity measured in specimens from a polluted site to the ones of a non-polluted site (Meng & Wang, 2023; Švara et al., 2022; Theodorakis et al., 2024; Yang et al., 2020) or specimens sampled following a strategy based on different levels of contamination (Bourret et al., 2008). However, care should be taken when designing experiments to assess the impact of specific stressors on the genetic variability in the field. To this end, Belfiore and Anderson suggest important steps to follow which are to estimate the proportions of covariance caused by different biological and environmental factors, to evaluate the probability of mutation based on the taxon and genetic locus, and to incorporate historical evidence into understanding background genetic structure (Belfiore & Anderson, 1998). Theodorakis reported an extensive list of causal criteria to be used when looking for evidence of pollution causing population genetic alteration, like for instance the use of multiple reference and contaminated populations and the establishment of biological plausibility (Theodorakis, 2003). It is important to note that the exposure to a genotoxic compound may also result in an increased nucleotide diversity due to mutations, which could be translated into an increased genetic diversity. Indeed, it is common to find reports of increased genetic diversity in polluted sites (DiBattista, 2008; Eeva et al., 2006).

Metrics to assess the different facets of genetic diversity were proposed (Hoban et al., 2020). Yet, there is an ongoing debate about the best way to describe genetic health of a population, particularly concerning neutral genetic diversity, which is not linked to phenotype. Metrics that focus on adaptive genetic diversity, which is subject to natural selection, have been suggested as potentially more effective in capturing the nuances of diversity (García-Dorado & Caballero, 2021; Teixeira & Huber, 2021). Anyhow, the above-mentioned appraisals have been shown to effectively estimate unnoticed

biodiversity levels and provide valuable insights for research in ecology and evolution (Mastretta-Yanes et al., 2024). However, they cannot be directly applicable to an ecotoxicological assessment. Coming the other way around, when a compound is tested as mutagenic or genotoxic in the lab, it is difficult to evaluate the extent to which it can cause a genetic change in a population. To help fill this gap, the integration of genetic ecotoxicological endpoints in biomonitoring campaigns would be desirable to cover the genetic impact at population level (Theodorakis, 2001). In the laboratory, genetic susceptibility to chemical exposure could be assessed by using AOPs that are already employed to identify functionally genetic variants (Kosnik et al., 2024). Another approach could be to form new, putative chemical–genetic variant–outcome linkages to describe the pool of genetic variants potentially implicated in different chemically induced adverse outcomes (Kosnik et al., 2024).

Taking into account the importance of genetic diversity as well as the difficulties in quantifying it, a discussion is ongoing if and how evolutionary toxicology should be included in regulations and ERA (Oziolor et al., 2020; Straub et al., 2020). Overall, to address the chemical impact on genetic biodiversity, multiple studies at various levels of biological organization and multiple metrics should be evaluated within a weight of evidence approach (Kanaka et al., 2023) (Theodorakis, 2003). .

2.2. Trait level

Traits are well-defined, measurable properties of organisms, usually measured at the individual level and used comparatively within and across species. Functional traits are traits that strongly influence organismal performance (Violle et al., 2007). On the one hand, traits can shape the way a chemical interacts with the organisms. Morphology and physiology can influence the toxicokinetics of a chemical; for instance the presence of an exoskeleton limits the uptake of a chemical to mainly a dietary exposure or the amount of lipids influence the accumulation of lipophilic substances (Rubach et al., 2011a; van den Brink et al., 2019). On the other hand, chemicals can impact a high number of traits that could potentially have consequences on the vulnerability of populations.

EBV subclasses for species traits include different descriptors of phenology. However, there are a few more traits worth mentioning as proven to be affected by chemicals and linked with the health of populations in a more direct way. Behavioural traits are an important class of traits that has been shown to be highly sensitive to chemical exposure (Saaristo et al., 2018). Alterations of 1) foraging, 2) predation (Relyea & Edwards, 2010), 3) mating, 4) parental care, 5) [sexual] communication, 6) social behaviour, 7) spatial behaviour are directly linked to vital aspects such as survival and reproduction and therefore population health and fitness (Bro-Jørgensen et al., 2019; Ford et al., 2021) and, ultimately, to biodiversity. For example, in the laboratory, relevant concentrations of the insecticide endosulfan interfered with the pheromonal system of amphibians causing disrupted mate choice and lowered mating success (Park et al., 2001). From field observations, we know that the pheromonal system is crucial in the daily life of amphibians such as conspecific recognition, migration, and social behaviour. Exposure to environmentally relevant concentrations of 17α-ethynylestradiol (EE2) led to a change in attractiveness of male fish (secondary sexual trait expression) resulting in a stronger sexual selection from the female. This increased competition for mates could reduce the effective population size, resulting in a loss of variation due to genetic drift (Partridge et al., 2010). Phenology and abundance of flowering of non-target species (species that are not intentionally targeted by chemical control such as herbicides) was shown to be negatively affected by herbicides and pesticides (Carpenter et al., 2020; Dupont et al., 2018) and heavy metals (Ryser & Sauder, 2006). The phenology of mangrove was shown to be affected by trace elements in Mexico (Celis-Hernandez et al., 2022). Effects across metamorphosis of a pesticide on the fitness of damselfly females strongly depend on the phenology of egg hatching (Tuzun & Stoks, 2017). The chemical toxicity on

the biological rhythms has only recently caught the scientist's attention and although our knowledge is still limited, there is evidence that the circadian disruption due to chemical exposure affects the daily activity pattern and performance of fish (Tan et al., 2023) and mosquitos (Melvin et al., 2016) having therefore consequences on the functioning of the organism (Thoré et al., 2024). Energy metabolism can be altered by exposure to chemicals (Dutra et al., 2011; English et al., 2021). Changes in energy metabolism can have direct effects on more apical functioning such as survival, growth (Feijão et al., 2020). Reproduction can also be affected by energy availability and this is based on the principle that the organism will "prefer" to survive instead of producing offspring (Kooijman, 2010). A change in energy regulation can also result in alteration of other functionalities (Goodchild et al., 2019) such as moving and therefore ability to escape predation, foraging efficiency, migratory ability (Gerson et al., 2019), burrowing (Goodchild et al., 2016), and disease susceptibility, and leading to different impacts depending on the life stage (Fidder et al., 2016). Several morphometric variables have been shown to be directly altered by exposure to chemical contaminants. Examples are changes in wing length in D. melanogaster (Cvetkovic et al., 2020), ano-genital distance in wild male minks (Persson & Magnusson, 2015), size of sexual organs in polar bears (Sonne et al., 2006), thickness of tadpole tail (Relyea, 2012). Morphometrics are considered a tool for identification of species in natural populations which is an EBV (Dwivedi & De, 2023).

Another trait category concerns traits determining reproductive performance. Toxicity affecting reproduction is more intuitively linked with population vulnerability and biodiversity loss. Indeed, population stability depends on the health of new generations of offspring. Reproductive success decreased by chemical exposure may lead to population declines or even extinctions. Adverse effects such as number, survival, growth, general development and reproductive performance of offspring have been used for decades by ecotoxicologists as a proxy to assess the chronic effects of contaminants in different model species in a laboratory set-up. At sub-individual level, development of reproductive organs, examination of their tissue and cells, and sub-cellular markers are used to predict the reproductive toxicity of chemicals. A number of chemicals can interact with hormonal receptors, altering hormone concentrations in the blood, metabolism, and sexual development, which may lead to abnormalities such as altered sperm quality, penis anatomy, production of oocytes, etc. However, while lab tests assessing altered reproductive traits are performed as standard ecotoxicological test, field studies providing casual evidence of the pollution impact on reproductive performance are scarce. For instance, a 7-year, whole-lake in Canada showed that chronic exposure of fathead minnow to low concentrations of 17α-ethynylestradiol led to feminization of males through the production of vitellogenin mRNA and protein, impacts on gonadal development and, ultimately, a near extinction of this species from the lake (Kidd et al., 2007). However, lack of evidence of chemical exposure or endocrine activity or reproductive adversity in wildlife makes it very difficult to establish the link in a scientifically sound way.

If on the one hand, more and better-designed field studies are needed (Marlatt et al., 2022); on the other, the use of data from laboratory test experiments should be optimised and maximised because providing the unequivocal biological link with population fitness. To this end, several models have been proposed that extrapolate reproductive endpoints to the population level (Gabsi et al., 2014; Kuhn et al., 2000). Finally, trait-based tools in ecotoxicology have been discussed in recent decades as promising approaches to better incorporate ecology into the ecological risk assessment of chemicals (Van den Brink et al., 2013). Recently, there have been concrete attempts to apply such tools. For instance, predictive sensitivity models were developed to reveal spatial differences in the sensitivity of species assemblages towards different chemical modes of action, combining information on acute chemical sensitivity, traits and taxonomy of related species (Van den Berg et al., 2020). Based on the idea that traits are dependent on each other, the interspecific trait integration approach was used as a tool to explain plant species richness along a soil metal gradient. The study

reported that the new approach describes the loss of plant species richness better than functional diversity indices: this may be linked with the fact that the exposure to the pollutant led to a selection of increasingly coordinated sets of functional traits (Delhaye et al., 2020).

2.3. Species populations

Species population is an EBV class including different descriptors of species distribution and species abundance (e.g. species distributions of terrestrial birds, species abundances of butterflies). The ecological definition of species distribution is the way a species is spatially dispersed, which is influenced by the spatial scale at which it is assessed (Henderson et al., 2023; Mirmonsef et al., 2017). Because of the number of factors affecting species distribution, evidence of the effect of chemical exposure is difficult to establish unequivocally in big regions. However, multiple studies can be listed as examples of the effect of toxicants on the distribution of populations on a smaller spatial scale. Typically, the spatial scale and the metrics depend on the average body size and home range of the taxonomic group. When those studies focus on small body size and/or sessile organisms, e.g. bacteria, yeasts, invertebrates identifiable by eDNA techniques or plants identifiable by remote sensing, studies report species richness and community structure (or community composition) shift, rather than spatial distribution. In addition, it is very common to perform those studies along a gradient of a specific pollutant from a specific source (e.g. wastewater discharge, road with car traffic, mine). The reason is that the assessment of the impact of the chemical exposure on high levels of biological organization is easier on a small area having a wide range of contaminant concentrations. For instance, from a timber factory, a transect of 80 meters covering different orders of magnitude of copper concentrations in the soil was defined to measure the distribution of different species of earthworms (Mirmonsef et al., 2017). A similar study (Lévêque et al., 2015) reported earthworm density, diversity (expressed as richness) and ratio of adult/juvenile earthworms. In another study (Phillips et al., 2021), pollinator density was assessed within meters from road edges. Similarly, in the freshwater compartment, the impact of chemical pollution on community is more often assessed by measurement of richness of unicellular organisms, algae, macro-invertebrates, insects (Armitage et al., 2007; Cortelezzi et al., 2011; Iwasaki et al., 2011). Birds represent a well-studied group of wild animals. A snap-shot of the last twenty years of research of the effect of pollution on bird communities is presented by Richard and co-workers (Richard et al., 2021).

Under the EBV species population class, the second variable is population abundance, which is the number of individuals in the population. However, most of time the abundance is measured in a relative way and expressed as density (population abundance of a taxon in a unit of surface/volume) (Callaghan et al., 2024). The ability to collect data as absolute abundance is hampered by the number of specimens and the mobility of the specific taxa, in particular taxa that have low density and are sessile are more likely data-rich. The abundance of taxa with very high density are typically measured in much smaller area (e.g. bacteria community in soil). Beside this, the variety of sampling methodologies and spatial scopes for each population makes it intractable to compare absolute abundance among species (Leung et al., 2022). Because of all these reasons, the assessment of the impact of toxicity of contaminants on population is directly quantified rarely. As already mentioned related to the species distribution, field studies are conducted to measure the chemical impact on population abundance but the objectives of the studies often follow practical reasoning. More frequently those studies are related to extreme contaminant concentrations, occurring for instance due to an incident (e.g. oil spill) and the effect is detectable at the population level (Zhou et al., 2019). It can happen that even in the case of high contaminant concentration the study failed to identify any contaminant-induced alteration population-level endpoints in the wild because the endpoints chosen relevant for demography impact assessment are not sensitive for that given species. For instance, a

study failed to detect changes of population parameters of aquatic turtles across a gradient of PCB contamination because turtles are long-lived and largely sedentary predators, which are attributes that may make them poor indicators of contaminant effects on populations (Gibbs et al., 2017). For long-lived species, detecting adverse contaminant effects could prove challenging because response can be delayed and population rate decreases very slowly. This is even more important if the exposure is continuous or contaminants are persistent (Salice et al., 2014). Nevertheless, in the same study the effects of PCBs at population level of turtles were proven by using an integrative demographic matrix model starting from life history data survival and growth endpoints (Salice et al., 2014). The model used in the study showed that effects of PCBs on juvenile survival, growth and size at hatching could lead to an impact at the population level highlighting an effect that the field observations could not catch alone. In fact, more often simulations are performed which estimate a potential impact on population starting from individual effects. Bergek et al. studied the effect of pollution on natural eelpout populations by combining the outputs from population matrices (Leslie, 1945) with data from laboratory and field studies. The results showed that survival of larvae is most important for population growth (Bergek et al., 2012). Another approach is represented by the calculation of the multi-substance potentially affected fraction of species (msPAF) which estimates the potential loss of species within a group of species studied due to combined effects from exposure to multiple chemicals (de Zwart & Posthuma, 2005). Similarly to the concept of species sensitivity distribution (SSD) (Posthuma et al., 2001), the msPAF allows prediction of the percentage of species potentially affected by the presence of environmental contaminants by comparing environmental concentrations of one or more pollutants with reference toxicity values (Posthuma & de Zwart, 2006; Rämö et al., 2018). Although initially developed to estimate toxic risk of pollutants on multiple species, the PAF approach has also been used to visualize the expected effects on a single taxon, and particularly for the fish species (Bellier et al., 2024). Although there are distinct ecological definitions for population abundance, species distribution, community composition (Kissling et al., 2018), the choices of how the chemical impact on those entities is measured are dictated by the available techniques, by the interest on a specific ecological function, by the mobility and home range of species, and by the sensitivity of the toxicological endpoints.

2.4. Community

Based on the EBV list, community composition is defined by four types of descriptors: community abundance, taxonomic/phylogenetic diversity, trait diversity, and interaction diversity. The community abundance is the number or biomass of all individuals (belonging to one or more species) in a given community, measured (or modelled) over contiguous spatial and temporal units. Under an ecotoxicological point of view, the impact of chemicals on community abundance could be affected by any changes in population demography of the species belonging to the community. Therefore, the methods for measuring the effects of chemicals on the species population are applicable to community abundances. However, when considering the community as a whole, a shift in the community structure can occur as a consequence of the change of tolerance of a species to a contaminant. This can lead to the replacement of sensitive species by tolerant species (Tlili et al., 2016). Pollution-induced community tolerance (PICT) is a concept that evaluates whether pollutants have exerted a selection pressure on natural communities (Blanck et al., 1988) and eliminated sensitive species from a community and thereby increased the general tolerance. Toxicological endpoints reflecting tolerance changes in a community are typically functional descriptors, such as photosynthetic activity and DNA synthesis measured in algae or bacteria, respectively. However, those endpoints are sensitive to specific mode of actions of chemical classes and may not reflect the overall chemical pressure. Changes in community structure are also assessed by taxonomic analysis of algal or diatom communities, or by denaturing gradient gel electrophoresis (DGGE) or DNA sequencing

technologies of biofilms (Tlili et al., 2020). Taxonomic diversity is the number of species present in the community. Beside the above-mentioned microorganism communities, the community taxonomic diversity is typically measured within a geographical area which is delimited based on different criteria (e.g. land use, geomorphology). For instance, the impact of atrazine and tribenuron-methyl on the plant community was studied in fallow fields. After three years of annual, sublethal exposure, the herbicides altered the species composition, decreased the number of plant species, and affected the relative frequencies of some plants (Qi et al., 2020). In a laboratory, to a certain extent it is possible to test chemical effects on small communities. Co-culture of representative microalgae was used as proxy of a freshwater phytoplankton community. The exposure to disinfectants disrupted the original community structure, changing the dominant species into a cyanobacteria potentially causing algal blooms (Cui et al., 2022). Chemical effects on soil microbiota enzymatic activities are also studies in a laboratory set-up (Sim et al., 2023). However, such tests alone ignore additional abiotic or biotic factors that may affect these results. Mesocosm studies are a well established way to study community level toxicity. Field-collected zooplankton communities have been exposed in a mesocosm test to anti-fouling substances and PAHs on functional diversity (Hjorth et al., 2006; Hjorth et al., 2007). Pereira et al. combined different types of data to predict the planktonic community shift due to exposure to chemicals. The authors used field data to build a plankton ecological network and ecotoxicological data from literature to simulate disturbance, then a fuzzy model was developed and the impacted network via an ecological network analysis was predicted (Pereira et al., 2019). Trait diversity of a community refers to the range of traits present among organisms within an ecological assemblage. This is measured or modelled across continuous spatial and temporal scales. Generally, for each trait, a complete measurement of the community is required to obtain a distribution of trait values. This trait distribution is often condensed into a single measure, such as functional divergence or functional richness. Toxicity of chemical contaminants on species can cause a decrease in morphological and physiological performance which in turn may generate a noticeable effect on the competitiveness and invasion process by other species (Sun et al., 2023).

Interaction diversity is an EBV subclass that describes the diversity and structure of multi-trophic interactions between organisms within ecological assemblages, measured (or modelled) over contiguous spatial and temporal units. Measurements of interaction diversity could include those derived from ecological networks and food web analyses. Interactions include competition, predation, mutualism, commensalism, and parasitism, which together create a network on which the food-chain structure builds. Contaminants can alter these interactions and this is one of the response of the community to chemical pollution (Clements & Rohr, 2009). Effects of chemical pollution on the food web can be subtle and very gradual or completely inert until a sudden change brings the food chain to be drastically altered with big consequences for the whole ecosystem and its services. Effects in the food-web, “trophic cascades”, can be caused by a change in the abundance of the highest trophic level (i.e., top-down cascade) or by a change in the primary producers level (i.e., bottom-up cascade)(Heath et al., 2014). Changes in predator populations can affect the availability of their prey, leading to shifts in prey population density and pressure on resources. Similarly, modifications in prey resources can impact the number of prey, which in turn affects predator populations. How the contaminants impact the cascading effects, such as changes in resources or shifts in predator populations, remains poorly understood. In this area, modelling appears to be the methodology best able to build a comprehensive framework for understanding impacts of toxins on multi-trophic community dynamics. A two-species food chain is more commonly evaluated, e.g. Daphnia spp. and phytoplankton (Kooi et al., 2008; Prosnier et al., 2015). Multiple species representing two food chain levels are also studied: for example rainbow trout and its preys (Huang et al., 2015) or barn owl feeding on several prey distinct by their trophic positions (Baudrot et al., 2018). The presence of multiple species per trophic level (horizontal diversity, while vertical diversity is represented by

multiple trophic levels) has been shown to be important for the resilience of the food web itself. Therefore, when assessing the impact of chemicals in community interactions it would be good to include competition and not only predation (Zhao et al., 2019).

2.5. Ecosystem functioning and structure

Following the EBV classification, variables under ecosystem structure class are variables related to habitats such as free river flow or river continuity, ecosystem distribution of freshwater habitats, structural complexity of riparian habitats, and ecosystem distribution of terrestrial habitats. The fact that the diversity of habitats, their connectivity, and their general status are all crucial aspects for different biodiversity variables is easily understood. Less obvious may be the fact that species biodiversity (presence of specific species) can affect the habitat type or status as well. The best example of this interplay of variables are the activity of the so called “ecosystem engineers”(Hastings et al., 2007). Ecosystem engineers are species that are particularly impactful at changing their environment (Alper, 1998). Those species create microhabitats and alter biological and chemical conditions that influence other species by modifying niche and resource availability, which drive ecosystem functioning. Therefore, toxic effects due to chemical pollution on those species are directly affecting the ecosystem structure. Examples of these species are corals (van Dam et al., 2011), beavers (Peterson & Schulte, 2016), cushion plants (Richir et al., 2013), mangroves (Celis-Hernandez et al., 2022) and different microorganisms (Alper, 1998). Organisms allow the ecosystem functioning, which is all the biotic and abiotic processes that occur within an ecosystem and may contribute to ecosystem services either directly or indirectly. Although ecosystem function is an EBV class itself containing descriptors of the diversity of the ecosystem functions, typically studies report on how biodiversity promotes ecosystem functioning, rather than considering ecosystem functioning as a descriptor of biodiversity. Instead, biodiversity is intended as species richness (Hong et al., 2022) or multiple other facets of biodiversity (e.g. trait diversity) that provide ecosystem functioning. It is important to emphasize that although many species may act as functionally redundant species for explaining a specific ecosystem function and service under specific conditions, many species are still needed to maintain multi ecosystem functionality at multiple spatial and temporal scales in a changing world.

Therefore, it seems naïve to focus the protection on few key species based on the current knowledge and under conditions at present (Ali, 2023; Isbell et al., 2011). Many ecotoxicological studies are conducted in which different ecosystem functions are measured such as primary productivity and metabolic function (Rumschlag et al., 2020; Vonk & Kraak, 2020), organic matter decomposition (Artigas et al., 2012), elemental cycling (Morin & Artigas, 2023). However, there is wide variation in the use of the term "ecosystem function” and in the way a specific function is conceived in the experimental design and then measured. This high variation between studies hampered the derivation of concentration–effect relationships (Peters et al., 2013). For instance, measures such as detritivore feeding rates and chlorophyll-α concentration are related to organic matter decomposition and primary productivity. Yet, they are not a direct assessment of those functions but rather measures of different processes that make up a particular function. Since measures are often interconnected and nested within each other, it is of great importance to define the metrics of the ecosystem function for the evaluation of the impact of chemical exposure (Harrison et al., 2022).

3. Modelling approaches enable “biodiversity toxicology”

Modelling has proven to be a valuable tool for ecotoxicologists, ecologists and environmental risk assessors facing the challenges of analysing complex systems. Models can come from different domains, and mechanistic and statistical / empirical models show large differences in this context. Statistical models use empirical methods to identify relations between explanatory variables and responses in datasets. This can have great value, and allow significant relations in large data sets to be detected. Limitations of statistical models include an incomplete understanding of the underlying mechanisms, and the inability to extrapolate to new, untested conditions. Recently, progress in machine learning techniques indicates new possibilities in the use of empirical modelling for the prediction of complex systems. In contrast, mechanistic models aim at describing the internal structure of systems based on available knowledge, capturing the causal relationship between influencing factors and the dynamics of the system as it emerges from the simulation of biological, chemical, and physical processes. In addition to understanding causal relationships, they also have the potential to extrapolate observations to new and untested conditions (assuming the same causal relationships hold). Mechanistic models can deliver estimations as potential answers to very complex problems such as the assessment of the chemical impact on biodiversity. As described above, there is an urgent need to assess the effects of pollution at different levels of biological organisation and under realistic environmental conditions. However, at the same time there is a need to reduce animal testing, and to make better use of existing data, irrespective whether this was collected under standardised laboratory conditions or in field studies. Many of these aims can be achieved by using mathematical and simulation models for integrating data and for extrapolating chemical effects across levels of biological organisation, that is, molecular toxicological effects to apical outcomes at the individual level, individual level effects of chemical exposure to the population level, from there to the community or ecosystem level, and so ultimately to the different biodiversity endpoints. To this end, mechanistic models can be used to:

— Simulate the toxic effects in organisms exposed to single or multiple chemicals in a time-dependent manner. TK-TD models estimate the internal concentration based on a given exposure concentration and the toxic effect that is caused. In particular, TK models can estimate uptake and elimination rates of the chemical in the organism based on measurements of the internal concentration of the chemical within the organism over time (Ardestani & van Gestel, 2013; Dalhoff et al., 2020). More complex TK models also include distribution and actual availability of the chemical within the organisms in the different organs and tissues (multiple-compartment toxicokinetic or physiologically based kinetic models, PBK models). Generalized TK models are available for example for invertebrates, birds, fish and murine species (Baier et al., 2022; Wang et al., 2022). TD accounts for the internal processes of damage and organism recovery and can capture lethal and sublethal effects. Concerning lethal effects, the General Unified Threshold model of Survival (GUTS) framework (Jager & Ashauer, 2018) allows the time course of processes leading to the death of organisms to be simulated. It accounts in a generic way for bioaccumulation, distribution, biotransformation, and elimination of the chemicals in the organisms, and for the consecutive accrual of, and recovery from, damage, which leads to an increased hazard, and eventually to an increased probability of death (Jager et al., 2011). There are a plenty of applications of GUTS in the scientific literature, but the most obvious application of GUTS models is the estimation of effects of chemicals under other than tested exposure profiles in laboratory conditions (Brock et al., 2021). In addition, GUTS models have been applied to check if acute and chronic effects could be explained by the same mode of action (Focks et al., 2018; Gergs et al., 2021). When more than a single exposure route is relevant, as in a recent example for terrestrial habitats, different exposure routes were assessed with a TKTD model for honey bees, where the BeeGUTS model was calibrated on contact and oral tests together (Baas et al., 2022). The

consideration of toxicity as a dynamic process allowed implement of a check for time-reinforced toxicity in the recently published EFSA guidance on the risk assessment of Plant Protection Products (PPPs) on bees, which means that the effect of a chemical increases for longer exposure more than Haber's law would predict (EFSA, 2023a). The simulation of TK and TD processes can account for multiple chemicals in parallel, so that the formulation of the principles of concentration addition and response addition in a GUTS context allows for the analysis of dynamic mixtures over time, even without having mixture toxicity data available for model calibration (Bart et al., 2021). TD processes, however, do not always lead to mortality, but can also lead to other, sublethal impacts on exposed individuals, for example on growth or reproduction. Most of TK-TD models used for the analysis of sublethal effects are based on dynamic energy budget (DEB) theory. DEB models describe how individuals acquire and utilize energy, and can serve as a link between different levels of biological organization (Kooijman & Metz, 1984). They are based on the theory, which describes the acquisition of energy by an individual organism, and its utilization for growth, reproduction and survival. It is a universal law and species differ only in their parameter values (Nisbet et al., 2000). It has to be noted that the data that are required to parameterise those DEB models are not routinely collected during lab ecotoxicological testing. In particular, observations of growth and reproduction are not routinely recorded over time and are sometimes difficult to obtain, for example for long-lived species or in-soil species. More recently, the DEBkiss model was published as a simplified version of the DEB model, where the exclusion of the energy reserve block considers that organisms are never in lack of food (Jager & Zimmer, 2012). Successful applications of DEB modelling include mammals (Desforges et al., 2017), fish (Jager et al., 2018), krill (Jager & Ravagnan, 2015), and many other species. The DEBtox model constitutes an application of the DEB theory to understand toxic effects as dynamic process and was first developed to address incorporation of different endpoints as the results of ecotoxicological testing (Kooijman & Bedaux, 1996). More recently, the term DEB-TKTD was proposed as an overarching category for the combination of DEB theory and TKTD modelling (Jager et al., 2023; Sherborne et al., 2020). Example applications of DEB-TKTD models include the analysis of toxicant effects on cyprinid fish species (Accolla et al., 2022), in the copepod Nitocra spinipes (Koch & De Schamphelaere, 2021), and in Gammarus pulex at different temperatures (Huang et al., 2024). A DEB-TKTD approach was used to analyse and predict the joint effects of multiple stressors, including chemical, temperature, and food availability (Goussen et al., 2020). Interestingly, DEB theory was applied to make quantitative predictions of chemical exposure to individuals, starting from suborganismal data, connecting AOP key events in early-stage fish to DEB processes through the damage that is produced at a rate proportional to the internal toxicant concentration (Stevenson et al., 2023).

— Extrapolate individual-level toxicological effects to population levels under consideration of changeable biotic and abiotic variables. Population models can be used to translate lethal and sublethal impacts on organisms into changes at the population level, and to estimate long-term impacts to a species from exposure to chemicals, both of which are challenging to assess in field studies. In particular, individual-based modelling (IBM) allows prediction of population dynamics directly from the properties and behaviour of the individual organism. IBMs were successfully used to predict population dynamics starting from individuals toxic effects in a number of model species but also in coastal birds (Stillman & Goss-Custard, 2010) or in stream salmonids (Railsback & Harvey, 2002). IBMs provide a unique approach for the extrapolation of toxicological effects from controlled laboratory conditions to changeable biotic and abiotic variables, including the consideration of spatio-temporal variability, including also animal behaviour in terms of movements. Examples of IBMs being used as a tool for the propagation of sublethal effects on individuals to population levels under consideration of environmental variables include small mammals (Dalkvist et al., 2009; Topping et al., 2009; Wang, 2013) or skylarks (Topping & Odderskær, 2004). ALMaSS (Animal, Land and Man Symulation System) is a flexible system for implementing IBMs of selected species (Topping et al., 2009) within a representation of agricultural activities in a landscape based on dynamic modelling on geographical information system. In an aquatic context, effects of pulsed-exposure to pesticide on populations of three different aquatic species was estimated with a combination of models. The GUTS model was linked to three different IBMs, translating individual survival into population-level effects. The impact of pulsed insecticide exposures on populations were modelled using the spatially explicit IBM metapopulation model for assessing spatial and temporal effects of pesticides (MASTEP) for Gammarus pulex, the Chaoborus IBM for populations of Chaoborus crystallinus, and the “IdamP” model for populations of Daphnia magna (Dohmen et al., 2016). However, IBMs often require a significant species-specific set of life-history data that is difficult to obtain for all species. Integral projection models (IPM) were shown to link chemical and nonchemical effects to growth, survival and reproduction (population dynamics) of fathead minnows (Pollesch et al., 2022). Another approach which allows extrapolation to the population including spatial variables is represented by matrix models (Charles et al., 2009). A multi-region matrix population model was developed to explore how the demography of brown trout population living in a river network varies in response to different spatial scenarios of cadmium contamination. Age structure, spatial distribution, and demographic and migration processes are taken into account in the model (Chaumot et al., 2003). The combination of DEB-TKTD model and matrix population model was used to extrapolate effects of the toxic compound on the individual Daphnia magna (reduced fecundity, growth and survival) to the population level. All the effects were integrated into a single parameter, the population growth rate, which is calculated continuously against exposure concentration (Billoir et al., 2007).

— Extrapolate individual based-toxicological effects to ecosystem or community levels. A spatially explicit and individual-based plant community model IBC-grass was used for different grassland communities and field boundary community (Reeg et al., 2017). The model accounting for different variables (inter- and intraspecific competition for space and resources, growth, mortality and disturbances like grazing, trampling, mowing and herbicide impact) was tested against empirical data based on species-specific dose responses and shown to be able to realistically predict short –term herbicide impact on the community (Reeg et al., 2018), also with a user-friendly and open source graphical user interface (GUI) (Reeg et al., 2020). Bayesian network models are increasingly used as tools to support probabilistic environmental risk assessments. Mentzel et al. applied Bayesian model to link various sources of information in a probabilistic framework, to predict the risk of pesticides to aquatic communities (Mentzel et al., 2024). Threshold Indicator Taxa Analysis (TITAN) is a method for interpreting taxon contributions to community change along novel environmental gradients (Baker & King, 2010) and was able to translate predicted ecotoxicity effects to a metric of quantitative damage on species diversity (Costas et al., 2018; Simonin et al., 2021). TITAN allowed the calculation of the chemical concentrations that marked a decrease of taxon occurrences and abundances for individual invertebrate taxa (Berger et al., 2016). The AQUATOX model (Galic et al., 2019; Park et al., 2008) integrates environmental fate of chemicals and their impacts on food webs in aquatic environments. It has been used to simulate the propagation of a pesticide-induced predatory fish mortality through the overall food chain leading eventually to decreased water quality. A spatially explicit model for estimating risk of pesticide exposure to bird population was built upon existing models: the Terrestrial Investigation Model (TIM), the Markov Chain Nest Productivity Model (MCnest) that considers both acute and chronic effects resulting from insecticide exposure during the northern temperate breeding season, and HexSim to simulate spatially explicit population dynamics (Etterson et al., 2021).

— Extrapolate across species. Existing theory suggests that TD parameters are constant across species or compounds if receptors and target sites are shared (Jager & Ashauer, 2018). Based on this, Singer et al. applied GUTS to jointly model survival for multiple species with shared receptors and pathways by incorporating the relations among TD parameters (Singer et al., 2023). This would allow the reduction of animal testing and potential reduction complexity of ecosystem models because of their shared TDs. Conservation of a molecular target across species can be used as a line-of-evidence to predict the likelihood of chemical susceptibility and this is the basis of the SeqAPASS web-based application (LaLone et al., 2016). This tool assesses available molecular target information to describe protein sequence similarity at the primary amino acid sequence, conserved domain, and individual amino acid residue levels. Since its publication, several studies have demonstrated its applicability (Cheng et al., 2021; Dufourcq Sekatcheff et al.; Schumann et al., 2024). The conservation of a molecular target and toxicokinetic factors are important factors in predicting susceptibility across species, but other factors must also be considered such as species morphological and physiological traits (such as inter-/intraspecies variations and life stages) and binding domain configurations. While these variables alone may not be definitive predictors, in silico analysis of available data can contribute to a more comprehensive understanding of species susceptibility. Rivetti et al. presented a novel pipeline (R package Genes-to-Pathways Species Conservation Analysis (G2P-SCAN)) to improve access to relevant data sources and structure the data in order to facilitate the understanding of the conservation of human biological processes and pathways across a broad range of species (mammalians, fish, invertebrates, and yeast)(Rivetti et al., 2023). Recently, Haigis et al. studied the molecular initiating events (MIEs) and adverse outcomes and evaluated both their plausible domain of applicability (taxa they are likely applicable to) and empirical domain of applicability (where evidence for applicability to various taxa exists) in relation to the thyroid hormone system disruption. There was evidence of structural conservation across vertebrate taxa and especially for fish and amphibians, which enables the applicability of impaired neurodevelopment, neurosensory development and reproduction across vertebrate taxa (Haigis et al., 2023).

These and other examples show that mechanistic mathematical and simulation models can play a crucial role in connecting chemical pollution with biodiversity loss, because they can solve the dilemma that chemical pollution interacts with the lowest levels of biological organisation, whereas the impact on biodiversity becomes often visible only at higher levels. Effect models can extrapolate lethal or sub-lethal toxicity information observed at (sub-)individual levels in laboratory studies across levels of biological organisation, considering population ecology, variable environmental conditions, under exposure to multiple chemicals or even to multiple stressors, and can finally estimate impacts on biodiversity at population, community or ecosystem levels. This potential of mechanistic effect modelling can be exploited, advancing model-based impact assessment beyond purely statistical analyses. It is a clear advantage of modelling approaches that they can make more use of existing data, and especially that they can integrate data from independent studies and different levels of biological organisation. For example, standard toxicity studies for a number of chemicals could be used to calibrate both GUTS and DEB-TKTD models. Those could be integrated into a DEB-IBM model for an indicator species, which has been developed based on physiological and ecological data and knowledge from the respective literature. The resulting TKTD-IBM model could be used to simulate population growth and viability under a range of environmental scenarios that include exposure to mixtures of chemicals. Results of such a simulation exercise could be compared with data from environmental monitoring studies, with focus on abundances of indicator species. Such a study would not only allow to statistically identify relations between chemicals and elements of biodiversity, but moreover to understand which factors, including specific chemicals, with their timing of peak

concentrations, or the co-occurrence of chemicals determine negative impacts with changes of other environmental factors such as temperature or food.

However, mechanistic models require substantial efforts for setup and analysis, and significant amounts of data for parameterisation. When combining multiple levels of biological organisation, the complexity of such models can become overwhelming, and in addition, uncertainties that are associated with TK-TD processes, population ecology and ecological interactions between multiple species and within an ecosystem require careful consideration. It is important to handle properly the uncertainties, including for example uncertainty propagation along the levels of biological organisation. With respect to biodiversity impact assessment, probably the largest disadvantage is that mechanistic models usually do not simulate biodiversity as such. The simulation of sets of indicator species can be used as bridge, and the further development of community models would be beneficial for strengthening the assessment of chemical impacts on biodiversity. In this context, also methods such as TITAN, and the further development of linkages between methods such as SeqAPASS and other molecular-based tools and mechanistic models would provide a way forward.

4. Biodiversity in the current environmental risk assessment of chemicals and future perspectives

The European chemicals industry is subject to strict regulations, with environmental risk assessments (ERAs) being customized for various chemical classes based on their hazards, applications, and environmental exposure patterns. The European Commission is intensifying its efforts to protect and restore biodiversity, as highlighted in a series of strategic documents (EC, 2020; EC, 2021); on the other hand, the regulatory framework for chemicals does not often explicitly define biodiversity and how to assess the impact of chemicals on biodiversity. Biodiversity protection remains a very broad goal. For instance, in the regulation concerning the placing of PPP on the market, biodiversity protection is translated by general objectives such as preventing unacceptable effects on the environment (EU, 2009), in particular any long-term repercussions for the abundance and diversity of non-target species (e.g. birds, mammals, arthropods). Those repercussions are quantitatively not defined and non-target organisms are tested as single species representing entire taxonomic groups (e.g. bees, earthworms, microorganisms). Therefore, the results of these tests do not fully reflect the complexity and variability of responses to chemical exposure that exist across different species and communities. This point represents a big limitation of the current regulation: it appears clear that extrapolation of ecotoxicological responses across species is a crucial goal for both the scientific and the regulatory area. In the domain of PPPs, in order to make the general protection goal operational for use, in 2010 EFSA introduced the concept of specific protection goals (SPGs)(EFSA, 2010; Nienstedt et al., 2012), which have been further developed for biodiversity later in 2016 (EFSA, 2016a). The approach follows the view that biodiversity is the source of many ecosystem services and plays an essential role in sustaining ecosystem functioning. Therefore, ecosystem services are used to identify SPGs for biodiversity, either through conservation of the species providing the service or as underpinning ecosystem structures and processes relevant for the delivery of services, and often as both. Beside the huge challenge to define SPGs themselves, a practical issue of this approach is the evaluation of acceptable magnitude of effect based on the Normal Operating Range (NOR). NOR is defined as the acceptable range of values of a measured endpoint that is normally observed during a predefined period for a reference population, community, ecosystem or process (EFSA, 2016b). It is used to set the magnitude of effects tolerated by the population, at which the service is still provided (EFSA, 2023b). For instance to assess the risks of pesticides to honey bees, EFSA defined a maximum permitted level of 10% colony size reduction (EFSA, 2023a), which should still enable the population to pollinate and regulate biodiversity. This was also adopted by ECHA for the assessment of biocides (ECHA, 2024). This level allows a direct comparison between the predicted effects at colony level and the SPG defined by the trigger value of maximal 10% of colony size reduction. In particular, the guidance describes the extrapolation of the quantification of the effects at the individual level for each risk case (acute oral, acute contact, chronic, larvae) based on standard laboratory ecotoxicological studies to colony/population level effects by the BEEHAVE model (Becher et al., 2014). However, due to a lack of data, a quantitative magnitude of acceptable effects for bumblebees and solitary bees was not defined, therefore requiring more frequent higher-tier studies. The identification of such thresholds is a challenging and time- and resource- consuming activity, which appears to be not feasible for a large number of species. Another critical point of this approach is that the appropriate identification of focal species (perhaps multiple within the same taxonomic group because of their different ecological roles) presupposes an all-embracing knowledge of the ecosystems and of all the interactions, processes and other indirect effects on which the full resilience and sustainability of the service provided may depend. Beside the uncertainty linked with such lack of knowledge, ecological interactions amongst species at different biological organization levels (e.g. communities) are usually neglected. As a consequence of a tolerable magnitude of effects, an impact

could occur and be accepted as long as the population is somehow able to deliver the ecological service. However, this decision does not consider how this change, referred to as inconsequential, is actually affecting the ecological network, or is affecting its capability to face other stresses, and to be resilient (EFSA, 2016b). This is particularly true as we know that the same population is exposed to multiple chemicals, occurring in the environment as unintentional mixtures, also outside of a defined agricultural field. As one of the pillars of risk assessment, the assessment of the actual exposure concentrations and the consideration of realistic mixtures of chemicals is as crucial as the evaluation of the hazard.

For an improvement of the current ERA, there is a strong need for collaboration between the domains of ecology and ecotoxicology. It is a priority to make a better use of the available knowledge of ecological interactions with the scope to parameterise population dynamics and ecosystem processes, to establish predictive food-web models, to develop mechanistic effect models, and to optimize data collection during laboratory testing and biomonitoring in the field towards their use for modelling extrapolation. The EU is committed to uphold international biodiversity commitments, prioritizing the preservation, conservation, and restoration of the rich variety of life and the most recent document showing this commitment is the EU Biodiversity Strategy for 2030 (EC, 2020) containing 16 targets to be achieved by 2030. All the progress is reported in a public dashboard (EC, 2023) developed by the European Commission's Knowledge Centre for Biodiversity (KCBD), which makes use of a set of indicators to monitor each target and sub-targets. The dashboard is based on different knowledge sources such as the European Environment Agency (EEA), the statistical office of the EU, Eurostat, and other services of the European Commission (Marei Viti et al., 2024). Although the Biodiversity strategy states "Biodiversity is suffering from the release of nutrients, chemical pesticides, pharmaceuticals, hazardous chemicals, urban and industrial wastewater, and other waste including litter and plastics. All of these pressures must be reduced.", target 6 "the risk and use of chemical pesticides is reduced by 50%, and the use of more hazardous pesticides is reduced by 50%" is the single target that directly addresses the risk related to the use of one class of chemicals only. For target 6, indicators are under development. Other chemical groups of concern (e.g. heavy metals, industrial chemicals, pharmaceuticals) are not included in the target, and yet these can also have negative effects on different biological levels of biodiversity, as described in the present work. This has led to expressions of concern by the scientific community (Groh et al., 2022; Sylvester et al., 2023). In addition to the targets of the Biodiversity Strategy, the EEA reports three existing EU indicators that measure 1) hazardous substances in marine organisms in European seas, 2) industrial pollutant releases to water in Europe, and 3) ecological status of surface waters in Europe (EEA, 2024). The first indicator reports nine contaminant concentrations in marine species and the second indicator covers the concentrations of contaminants released from industries in waters. The third one instead shows the influence of pressures, including pollution on specific quality elements of surface water ecosystems. The classification of the ecological status is determined for each of the surface water bodies based on biological quality elements (e.g. taxonomic composition of phytoplankton and macrophytes, abundance of benthic invertebrates) and supported by physico chemical and hydromorphological quality elements, which include the Environmental Quality Standards (EQS) from the EU Water Framework Directive (EU, 2000) that is derived using ecotoxicological data (Kristensen et al., 2018). The "ecological status of surface waters in Europe" probably represents the indicator with the closest existing link between chemical exposure and biodiversity.

5. Conclusions

In summary, addressing pollution as driver of biodiversity loss and tracking progress of regulations for the use of chemicals in Europe requires strong links between ecotoxicology and ecology at both scientific and regulatory levels. Amongst others, mechanistic modelling tools may help building such a bridge because they are able to predict and estimate the response of organisms within complex systems. A big challenge is setting "trigger" values of quantifiable endpoints meaningful to translate monitoring data (chemical concentrations and biodiversity data) into information manageable for regulators. Then (new) biodiversity indicators should be defined to enable the evaluation of policy options and management strategies related to pollution and its impact on the environment.

Modernizing the current environmental risk assessment of chemicals should include an integration of multi-dimensional data originating from field and laboratory studies, by mapping existing data within evidence-based analysis and AOP frameworks, and incorporating new data generated by modelling.

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The portal data.europa.eu provides access to open datasets from the EU institutions, bodies and agencies. These can be downloaded and reused for free, for both commercial and non-commercial purposes. The portal also provides access to a wealth of datasets from European countries.

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