Integrated risk assessment: from exposure through AOPs to ecosystem services

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PREFACE

The work presented in this PhD thesis was carried out at the section for Quantitative Sustainability Assessment (QSA) at the Department of Environmental and Resource Engineering (DTU Sustain) of the Technical University of Denmark (DTU) from November 2020 to October 2023. The PhD was conducted under the supervision of Professor Peter Fantke (QSA) and the co-supervision of Professor Michael Hauschild (QSA) and Professor Leo Posthuma from the National Institute for Public Health and the Environment (the RIVM) and Radboud University Nijmegen, Department of Environmental Science, The Netherlands. The PhD project was funded by the Prorisk "Best chemical risk assessment professionals for maximum Ecosystem Services benefit" project financed by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie, grant no. 859891.

This thesis presents the findings of the PhD project based on the four scientific articles listed below. These scientific articles are included as appendices, and throughout the text, they are referred to using roman numerals:

The thesis is organized in two parts: the first part puts into context the findings of the PhD in an introductive review; the second part consists of the papers listed below. These will be referred to in the text by their paper number written with the Roman numerals **I-IV**.

I Oginah, Susan A, Posthuma, L., Maltby, L., Hauschild, M., & Fantke, P. (2023). Linking freshwater ecotoxicity to damage on ecosystem services in life cycle assessment. *Environment International*, *171*, 107705. https://doi.org/10.1016/j.envint.2022.107705

II A curated aquatic ecotoxicity dataset for environmental protection, assessment, and management *(Manuscript in preparation)*.

III Oginah, Susan Anyango, Posthuma, L., Hauschild, M., Slootweg, J., Kosnik, M., & Fantke, P. (2023). To Split or Not to Split: Characterizing Chemical Pollution Impacts in Aquatic Ecosystems with Species Sensitivity Distributions for Specific Taxonomic Groups. *Environmental Science & Technology*, 1–13. https://doi.org/10.1021/acs.est.3c04968

IV Characterizing the link between the mixture toxic pressure and biodiversity loss in aquatic ecosystems *(Manuscript in preparation)*

In addition, the following scientific article, not included in this thesis, was also connected to this PhD project:

Douziech, M., Oginah, S. A., Owsianiak, M., Golsteijn, L., Zelm, R. V., Jolliet, O., Hauschild, M. Z., Posthuma, L. & Fantke. Ecotoxicity Impact Evaluation for Data Poor Chemicals under the Global Life Cycle Impact Assessment Method Framework (*Manuscript in preparation).*

ACKNOWLEDGEMENTS

I am filled with gratitude and a sense of fortune for having been surrounded by inspiring individuals throughout the past three years. I greatly appreciate my supervisors, Peter Fantke, Michael Hauschild, and Leo Posthuma, whose guidance has been invaluable.

Their constant support, valuable advice, and attention to detail have made a lasting impact, and I am immensely grateful for their guidance. They have been an invaluable companion, offering wisdom, feedback, and expertise that inspires me. Their insightful feedback has pushed me to improve my work; I am forever grateful.

I extend my thanks to all my co-authors for their knowledge sharing, and in particular, Jaap for the seamless collaboration. A special mention goes to my steadfast friends, family, and QSA colleagues for their unwavering encouragement and support over the years. Though often unspoken, your influence has empowered me to pursue my aspirations. This achievement would not have been attainable without every one of you.

Lastly, I thank Adrian for his boundless love and encouragement during my PhD.

SUMMARY

Chemical pollution exerts significant and far-reaching effects on biodiversity and the health of ecosystems. Over time, global product and technology consumption has increased the presence of human-made chemicals in the natural environment. Many of these chemicals persist in the environment and become pervasive over time, negatively affecting aquatic ecosystems globally. Aquatic ecosystems provide essential ecosystem services that benefit human society, such as the provision of food. Thus, to understand the link between chemical emissions from product and technology life cycles and their damage on ecosystem health, it is crucial to characterize damage on aquatic ecosystems associated with chemical emissions in life cycle assessment (relevant for product life cycle performance) and ecological risk assessment (relevant for water and ecosystem protection). This is pivotal in facilitating a worldwide shift towards a more sustainable application of chemicals across products and technologies and safeguarding the diversity of aquatic life.

The work presented in this PhD thesis addresses the link of the life cycle of chemical emissions to damage on aquatic ecosystem health by focusing on four research objectives: (i) to develop a consistent framework to link ecotoxicological effects on aquatic organisms to damage on species diversity, functional diversity, and ecosystem services that are fully in line with the boundary conditions of LCIA, (ii) to develop a systematic ecotoxicity test data curation approach to derive a transparent and high-quality dataset of effect test data for more than 10,000 chemicals, (iii) to improve ecotoxicity effects modeling by considering differences in sensitivity of species from different taxonomic groups toward chemical exposure, and (iv) to quantitatively characterize the relationship between mixture-toxicity pressure from chemicals and observed differences in aquatic intra- and inter-species occurrence.

After an introductory chapter, Chapter 2 summarizes possible methods to translate predicted ecotoxicity effects to species and functional diversity loss, culminating damage on ecosystem services damage in life cycle assessment (LCA). Section 1 of this chapter introduces a framework for linking freshwater ecotoxicity impacts to ecosystem services within LCA boundaries. Section 2 discusses approaches for linking ecotoxicity impacts to species loss, functional diversity loss, and ecosystem services damage from an LCA perspective. Section 3 explains the necessary biomonitoring methods for ES assessment, and Section 4 outlines how to link ecotoxicity effects to ecosystem service damage.

Chapter 3 outlines ecotoxicity datasets for different uses, including environmental standards, life cycle assessments, and water quality evaluation. Article II highlights data curation's importance for Articles III and IV. Sections 1 and 2 discuss current data and merging challenges to improve data quality, while Section 3 outlines a curation protocol, and Section 4 presents curated data for Article III and IV analysis.

Chapter 4 introduces splitting Species Sensitivity Distributions (SSDs) based on taxonomic grouping for better model fit and relevance in risk assessment unless data constraints exist. The chapter discusses the current use (Section 1) shortcomings of current SSD usage (Section 2) and introduces a conceptual framework for split SSDs (Section 3). It also covers the role of chemical use and mode of action in SSD derivation (Section 4) and highlights the importance of split SSDs in decision support (Section 5). Additionally, a case study for Life Cycle Assessment input is presented in Section 6.

Chapter 5 provides a stepwise approach to link ecotoxicity impacts with species loss, making it helpful in translating model-predicted species-level effects to damage on biodiversity and ecosystem services in decision-making, like Life Cycle Impact Assessment. The chapter consists of eight sections: Section 1 outlines the fundamental steps to derive the PAF to PDF relationship from monitoring datasets, Section 2 presents chemical concentration data and msPAF(EC10) calculation, Section 3 introduces other abiotic factors that can influence aquatic biodiversity, Section 4 presents species biomonitoring data, Section 5 explores covariation between abiotic pressures including the calculated msPAF, Section 6 discusses trends in species abundance and species richness against msPAF, Section 7 present sensitivity analysis, i.e., a robustness check using a subset of data from one region (water authority) in Netherland, and Section 8 presents the derived PAF to PDF relationship.

The PhD research suggests that the developed damage modeling approach fits well into the LCA framework, offering initial steps to translating ecotoxicity effects to ecosystem services damage. Furthermore, splitting species sensitivity distributions enhances the interpretation of assessment outputs, enabling a quantitative understanding of the link between a mixture toxic pressure and biodiversity loss.

In conclusion, the work conducted in this PhD project contributes to research within the field of life cycle impact assessment and ecological risk assessment by advancing the understanding of the impact of chemical pollution on

biodiversity and ecosystem health. It supports environmental protection, LCA, and global freshwater ecosystem management decisions.

RESUMÉ (DANISH)

Kemisk forurening har betydelige og vidtrækkende virkninger på biodiversiteten og økosystemernes sundhed. Over tid har det globale produkt- og teknologiforbrug øget tilstedeværelsen af menneskeskabte kemikalier i det naturlige miljø. Mange af disse kemikalier forbliver i miljøet og bliver gennemtrængende over tid, hvilket påvirker akvatiske økosystemer negativt globalt. Akvatiske økosystemer leverer væsentlige økosystemtjenester, der gavner det menneskelige samfund, såsom levering af mad. For at forstå sammenhængen mellem kemiske emissioner fra produkt- og teknologilivscyklusser og deres skader på økosystemernes sundhed, er det således afgørende at karakterisere skader på akvatiske økosystemer forbundet med kemiske emissioner i livscyklusvurdering (relevant for produktets livscykluspræstation) og økologiske risici. vurdering (relevant for vand- og økosystembeskyttelse). Dette er afgørende for at lette et verdensomspændende skift i retning af en mere bæredygtig anvendelse af kemikalier på tværs af produkter og teknologier og for at sikre mangfoldigheden af akvatisk liv.

Det arbejde, der præsenteres i denne ph.d.-afhandling, adresserer sammenhængen mellem kemiske emissioners livscyklus og skader på akvatiske økosystemers sundhed ved at fokusere på fire forskningsmål: (i) at udvikle en konsekvent ramme til at forbinde økotoksikologiske effekter på akvatiske organismer med skader på artsdiversiteten , funktionel mangfoldighed og økosystemtjenester, der er fuldt ud i overensstemmelse med grænsebetingelserne for LCIA, (ii) at udvikle en systematisk metode til kurering af økotoksicitetstestdata til at udlede et gennemsigtigt og højkvalitetsdatasæt af effekttestdata for mere end 10.000 kemikalier, (iii) at forbedre modellering af økotoksicitetseffekter ved at overveje forskelle i følsomhed af arter fra forskellige taksonomiske grupper over for kemisk eksponering, og (iv) at kvantitativt karakterisere forholdet mellem blandingstoksicitetstryk fra kemikalier og observerede forskelle i akvatiske intra- og inter-arter Hændelse.

Efter et indledende kapitel opsummerer kapitel 2 mulige metoder til at oversætte forudsagte økotoksicitetseffekter til arter og tab af funktionel diversitet, hvilket kulminerer skade på økosystemtjenesters skader i livscyklusvurdering (LCA). Afsnit 1 i dette kapitel introducerer en ramme for at forbinde ferskvands økotoksicitetspåvirkninger til økosystemtjenester inden for LCA-grænser. Afsnit 2 diskuterer tilgange til at forbinde økotoksicitetspåvirkninger med artstab, tab af funktionel diversitet og skade på økosystemtjenester fra et LCA-perspektiv. Afsnit 3 forklarer de nødvendige biomonitoreringsmetoder til ES-

vurdering, og afsnit 4 beskriver, hvordan økotoksicitetseffekter kan kobles til skader på økosystemtjenester.

Kapitel 3 skitserer økotoksicitetsdatasæt til forskellige anvendelser, herunder miljøstandarder, livscyklusvurderinger og vandkvalitetsevaluering. Artikel II fremhæver datakurations betydning for artikel III og IV. Afsnit 1 og 2 diskuterer aktuelle data og sammenlægningsudfordringer for at forbedre datakvaliteten, mens afsnit 3 skitserer en kurationsprotokol, og sektion 4 præsenterer kurerede data til artikel III og IV-analyse.

Kapitel 4 introducerer opdeling af artsfølsomhedsfordelinger (SSD'er) baseret på taksonomisk gruppering for bedre modeltilpasning og relevans i risikovurdering, medmindre der findes databegrænsninger. Kapitlet diskuterer den nuværende brug (afsnit 1) mangler ved den nuværende brug af SSD (afsnit 2) og introducerer en konceptuel ramme for opdelte SSD'er (afsnit 3). Den dækker også rollen af kemisk brug og virkemåde i SSD-afledning (afsnit 4) og fremhæver vigtigheden af opdelte SSD'er i beslutningsstøtte (afsnit 5). Derudover præsenteres et casestudie for input til livscyklusvurdering i afsnit 6.

Kapitel 5 giver en trinvis tilgang til at forbinde økotoksicitetspåvirkninger med artstab, hvilket gør det nyttigt at oversætte modelforudsagte effekter på artsniveau til skader på biodiversitet og økosystemtjenester i beslutningstagning, såsom livscykluspåvirkningsvurdering. Kapitlet består af otte sektioner: Afsnit 1 skitserer de grundlæggende trin til at udlede PAF til PDF-forholdet fra overvågningsdatasæt, Afsnit 2 præsenterer kemiske koncentrationsdata og msPAF(EC10)-beregning, Afsnit 3 introducerer andre abiotiske faktorer, der kan påvirke akvatisk biodiversitet, Afsnit 4 præsenterer arters biomonitoreringsdata, sektion 5 udforsker samvariation mellem abiotiske tryk inklusive den beregnede msPAF, sektion 6 diskuterer tendenser i artsoverflod og artsrigdom mod msPAF, sektion 7 præsenterer følsomhedsanalyse, dvs. en robusthedskontrol ved hjælp af en delmængde af data fra én region (vandmyndigheden) i Nederlandene, og afsnit 8 præsenterer det afledte PAF til PDF-forhold.

Ph.d.-forskningen tyder på, at den udviklede tilgang til skadesmodellering passer godt ind i LCA-rammen og tilbyder indledende trin til at oversætte økotoksicitetseffekter til skader på økosystemtjenester. Desuden forbedrer opsplitning af artsfølsomhedsfordelinger fortolkningen af vurderingsresultater, hvilket muliggør en kvantitativ forståelse af sammenhængen mellem en blandings toksiske tryk og tab af biodiversitet.

Afslutningsvis bidrager arbejdet i dette ph.d.-projekt til forskning inden for livscykluskonsekvensvurdering og økologisk risikovurdering ved at fremme forståelsen af kemisk forurenings indvirkning på biodiversitet og økosystemers sundhed. Det understøtter miljøbeskyttelse, LCA og globale beslutninger om forvaltning af ferskvandsøkosystemer.

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1 INTRODUCTION

1.1 BACKGROUND

The extensive use of chemicals in modern life, driven by global population growth and increasing per-capita consumption, has led to alarming chemical emissions throughout product life cycles (Kosnik et al., 2022; Syberg et al., 2017; Persson et al., 2022), posing a severe threat to aquatic ecosystems worldwide (Millennium Ecosystem Assessment, 2005). The adverse consequences of chemical pollution on freshwater ecosystems are manifold, ranging from the depletion of species diversity to the disruption of ecosystem functioning and the flow of crucial ecosystem services (ES)—affecting ES from water purification to recreational opportunities (Maltby et al., 2017). Such consequences ultimately, directly and indirectly, affect human society, making it imperative to comprehensively understand and assess the damage inflicted by chemical emissions on freshwater ecosystems—which is also the goal of decision-support tools like life cycle assessment (LCA) and ecological risk assessment (ERA).

However, evaluating the impact of chemical pollution and damage caused on ecosystem functioning and ES remains complex due to various factors, including the diversity of chemical compounds, the need to extrapolate from laboratory data to real-world conditions, the lack of methods or frameworks to systematically link the damage on ES, lack of high-quality data and the uncertainty associated with aggregating LCA indicator scores across different ES (Othoniel et al., 2015; Maia de Souza et al., 2018).

Challenge also arises from using ecotoxicity assessment methods like species sensitivity distributions (SSDs) to establish environmental protective standards or expected chemical impact levels where species assemblages are lumped together without accounting for differences in species sensitivity (Posthuma et al., 2002; Fox et al., 2021). This approach diverges from applied ecology principles, which treat species taxonomic groups differently (Schäfer et al., 2023). Thus, to enhance the accuracy and reliability of SSDs, it is crucial to address limitations related to data quality, statistical robustness, and variations in species sensitivity (Aldenberg et al., 2001; Fox et al., 2021).

Furthermore, the use of SSDs has extended in characterizing the role of mixture toxic pressure on biodiversity loss (Sigmund et al., 2023; Lemm et al., 2021; Schneeweiss et al., 2023). This is important as aquatic species are exposed to measurable levels of more than one chemical (i.e., unintended mixtures) and

non-chemical stressors (Schäfer et al., 2023), which is often not captured in various decision contexts. When species are exposed to increased toxic pressure in the field, they respond with varying changes in abundance due to their differences in sensitivities. Previous research reported a 1:1 relationship[1] between predicted ecological impacts of mixture toxicants and observed impacts, where increased predicted mixture toxic pressure (measured as msPAF; multi-substance Potentially Affected Fraction of species) corresponds to field impacts like species loss (Posthuma & de Zwart, 2012). The relationship between predicted impacts (msPAF) and observed impacts can take various forms influenced by species sensitivities and indirect effects; for example, opportunistic species may thrive under moderate PAF conditions.

Thus, considering the varying trends in species abundance influenced by direct and indirect effects in addition to aggregate metrics like species richness, which may mask important insights, is crucial for establishing a Potentially Affected Fraction-to-Potentially Disappeared Fraction (PAF-to-PDF) relationship, which is significant in various decision contexts, such as protection measures, Life Cycle Impact Assessment (LCIA), water quality restoration, protection standards (e.g., HC5), ecological risk assessment, and Chemical Safety Assessments (e.g., REACH). These assessments are essential for proactively ensuring 'safe use,' as seen with metrics like msPAF-EC10eq in LCIA and msPAF values for prioritizing remediation actions in polluted ecosystems.

While the pathway from chemical emissions to ecotoxicity effects expressed as Potentially Affected Fraction of exposed species (PAF) is well established in LCA (Fantke et al., 2021; Jolliet et al., 2003; Rosenbaum et al., 2008), the generic proxy link between the often-used Potentially Disappeared Fraction of species (PDF) based on associations with PAF requires further research to relate to species loss in the field. The current LCA assessments of ecosystem services have primarily concentrated on land use and land change with commonly no consideration of ecotoxicity impacts, even though approximately 25% of biodiversity impacts in aquatic ecosystems are currently attributed to chemical pollution (Lemm et al., 2021). Furthermore, translating predicted

[1] PAF to PDF 1:1 is based on acute EC50 test

ecotoxicity impacts into damage on freshwater species diversity, functional diversity, and ecosystem services is not yet considered at all in LCA (Maia de Souza et al., 2018; Liu et al., 2020; Othoniel et al., 2015; Rugani et al., 2019). Methodological challenges and data gaps hence currently impede progress in addressing damage from chemical emissions on ecosystem structure, functioning, and ecosystem services in LCA, constituting an important research gap.

This PhD project titled "Integrated risk assessment: from exposure through AOPs to ecosystem services" was defined to address this research gap, i.e., addressing the lack of suitable methods or frameworks to link ecotoxicity impacts to damage on freshwater ecosystems comprehensively, absence of high-quality data with essential information such as chemical mode of action, the necessity to account for species sensitivity variations in constructing SSDs, and the importance of assessing the impact of mixture toxic pressure on biodiversity loss to characterize the relationship between predicted impacts (msPAF) and observed impacts (species loss).

In this PhD project, the identified challenges were addressed by creating a comprehensive framework to link chemical effects on aquatic organisms to species diversity, functional diversity, and ecosystem services within the context of LCA, curating and harmonizing a high-quality dataset (255K data points), enhancing the assessment of chemical impacts using splitting SSDs accounting for sensitivity differences across taxonomic groups, and developing a tiered approach to quantify the impact of chemical mixture toxicity on aquatic species occurrence and its contribution to species loss.

Overall, this PhD project bridges the gap between predictive models regarding the damage caused by chemical pollution in freshwater ecosystems and real-world ecological consequences. It offers a valuable contribution to protecting and managing freshwater ecosystems and their critical ecosystem services in the face of growing chemical pollution threats originating from product and technology life cycles worldwide.

1.2 RESEARCH OBJECTIVES & THESIS OUTLINE

The PhD project's overarching goal was to improve the assessment of ecotoxicity-related damage on biodiversity in freshwater ecosystems within the LCA framework. In particular, the work conducted focused on the following four research objectives:

1. To develop a consistent framework to link ecotoxicological effects on aquatic organisms to damage on species diversity, functional diversity, and ecosystem services that are fully in line with the boundary conditions of LCIA
2. To develop a systematic ecotoxicity test data curation approach to derive a transparent and high-quality dataset of effect test data for more than 10,000 chemicals
3. To improve ecotoxicity effects modeling by considering differences in sensitivity of species from different taxonomic groups toward chemical exposure
4. To quantitatively characterize the relationship between mixture-toxicity pressure from chemicals and observed differences in aquatic intra- and inter-species occurrence.

2 IDENTIFYING METHODS TO LINK PREDICTED ECOTOXICITY EFFECT TO DAMAGE ON ECOSYSTEM SERVICES IN LCA

Chapter 2 summarizes the approaches for linking predicted ecotoxicity effects to species and functional diversity loss and ultimately to ecosystem services damage in life cycle assessment. The chapter is structured in four main sections. Section 1 outlines an overall framework for linking predicted freshwater ecotoxicity impacts up to damage on related ES within the boundary conditions of LCA. Section 2 presents possible approaches for linking predicted ecotoxicity impacts to species loss, functional diversity loss, and finally to damage on ES in LCA. Section 3 presents the biomonitoring method needed to assess and manage ES. Finally, Section 4 outlines how to link the ecotoxicity effect to damage on ES. All contents of this chapter are based on **Article I**. The parts taken directly from the Article are marked with "…".

2.1 FRAMEWORK FOR LINKING PREDICTED ECOTOXICITY EFFECTS TO DAMAGE ON ES

Article I illustrates the broader complexity of evaluating damage on ES, which is not straightforward. The primary step often involves translating predicted effects at the species level to structural biodiversity damage (species diversity or species loss in the context of LCA). This is followed by extending the damage to encompass losses in functional diversity and, ultimately, damage to relevant ES (Maltby et al., 2021; Truchy et al., 2015). Alternatively, it is possible to establish a direct link between species loss and damage to ES, bypassing the intermediary step of assessing impacts on ecological functions (Maltby et al., 2021). However, changes in ecosystem functioning can transpire without species loss (for example, due to behavioral shifts), leading to damage to ES directly resulting from such changes. See **Figure 1** below.

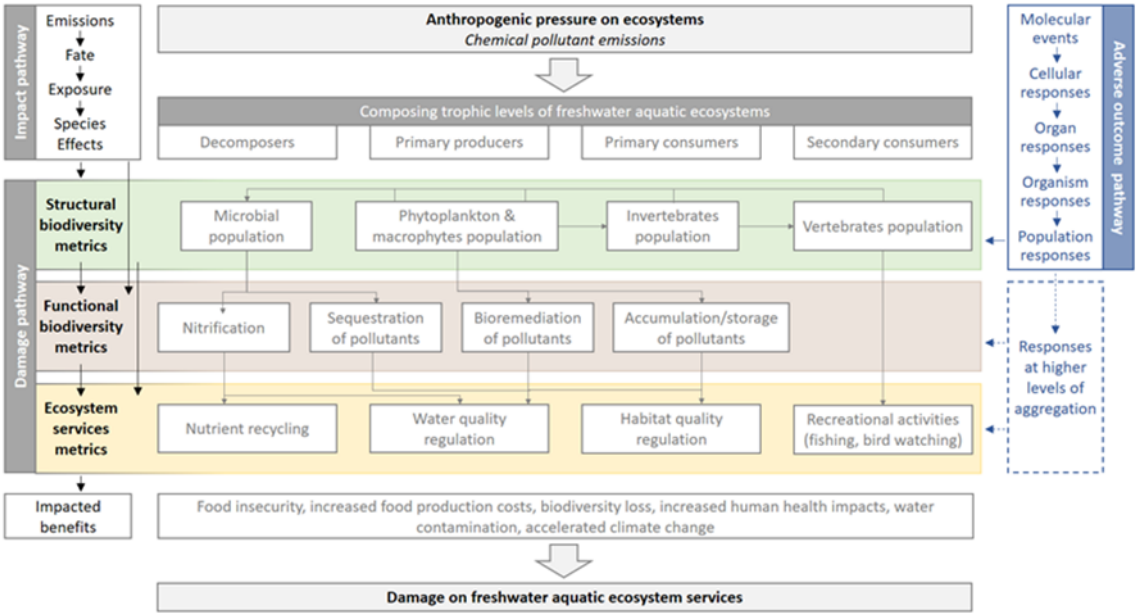

Figure 1. The proposed conceptual approach for translating the damage caused to various aspects of biodiversity (like the diversity of species and their functions) and the services provided by freshwater ecosystems due to the release of chemicals into the environment. The figure is taken from **Article I**.

Article I adapted the Adverse Ecosystem Service Pathway (AESP) conceptual framework (Awuah et al., 2020), drawing on information about ecotoxicity effects from species interactions and ES to build the impact pathway to damage on ES (Maltby et al., 2021). This approach builds on principles shared with other frameworks like life cycle assessment (LCA) and the Adverse Outcome Pathway (AOP) concept, which utilize causal effect chain approaches.

Figure 1 starts with chemical emissions in different environmental compartments, leading to freshwater ES damage. The initial step in the pathway involves predicting ecotoxicity effects at the species level, often quantified as the potentially affected fraction of species (PAF) exposed to a specific stressor, such as a chemical or mixture, typically derived from laboratory test data on sensitivity differences among species. Functional diversity level further links damage on structural biodiversity to damage in freshwater ecosystem functions, resulting in reduced performance and characteristics of affected species traits. Finally, the ES diversity level translates losses in species and functional diversity into damage on ES flow, affecting humans' benefits from a well-functioning freshwater ecosystem.

In practical terms, the protection of ecosystem biodiversity still heavily relies on extrapolating the effects of ecotoxicity at the individual organism level to damage at a higher level of biological organization from ecotoxicity laboratory

test data, which comes with uncertainties (Forbes et al., 2017). However, the field is progressing due to advancements in mechanistic models and quantitative adverse outcome pathways (AOPs). AOPs describe molecular initiating events, followed by subsequent events impairing an organism's functions. Relationships within the AESP concept offer valuable insights into predicting and quantifying impacts up to the community level (Schmid et al., 2021).

The AESP outlined in **Figure 1** by **Article I** aligns with the overall structure of the life cycle assessment (LCA) impact pathway, allowing for the accurate assessment of multiple exposure pathways that lead to net exposures and eventual damage (Escher et al., 2017; Clewell et al., 2020). To enhance the use of AOPs in ecological assessments at a higher biological level, Murphy et al. (2018) proposed a conceptual model that links population models (such as the dynamic energy budget model) with quantitative AOPs, using AOP key events to measure damage to DEB variables and process rates. However, it remains uncertain which elements of the AOP concept can be adapted as inputs for quantifying the link between ecotoxicity effects and ES damage.

In the LCA framework, "ecosystem quality" is one protection area, assessing damage through reduced biomass and species richness (Woods et al., 2018; Verones et al., 2017). Ecosystem services (ES) are evaluated alongside ecosystem quality and other protection areas (Verones et al., 2017). Initial ES damage assessments in LCA have mainly focused on land use and land change, with limited consideration of ecotoxicity impacts, despite the significant role of chemical pollution in aquatic ecosystem impacts (Lemm et al., 2021).

The pathway from emissions to ecotoxicity impacts is well-established in LCA, with predicted impacts often represented as the Potentially Disappeared Fraction of species (PDF) based on associations with the Potentially Affected Fraction of species (PAF) (Fantke et al., 2021; Jolliet et al., 2003; Rosenbaum et al., 2008). However, this proxy link needs further refinement to address variations in effects on species loss. Currently, translating predicted ecotoxicity impacts into damage on freshwater species diversity, functional diversity, and ecosystem services is not operational in LCA (Maia de Souza et al., 2018; Liu et al., 2020; Othoniel et al., 2015; Rugani et al., 2019).

2.2 SOURCE TO DAMAGE MODELLING APPROACH

As highlighted in **Article I**, the pathway from chemical emissions to ecotoxicity impacts is well addressed in LCA, with models like USEtox integrating factors for environmental fate, ecological exposure, and ecotoxicological effects (Owsianiak et al., 2023; Fantke et al., 2018). Environmental fate factors describe how emissions alter toxicant concentrations in various compartments like freshwater, while ecological exposure factors convert these concentrations into the bioavailable fraction for organisms. Effect factors link the bioavailable fraction to effects on species physiology, behavior, and populations, quantifying the Potentially Disappeared Fraction of species (PDF). Refinement is needed to reflect species disappearance under field conditions better.

Article I outlines possible approaches that can serve as a starting point for translating ecotoxicity impacts into damage on species diversity, functional diversity, and ecosystem services of freshwater ecosystems in the context of LCA, as detailed below.

2.2.1 APPROACHES TO LINK FRESHWATER ECOTOXICITY TO DAMAGE ON STRUCTURAL SPECIES DIVERSITY

As mentioned in **Article I**, linking freshwater ecotoxicity to damage on ecosystem services flow is a stepwise process. The initial step involves establishing a link between the ecotoxicity effects and their impact on species diversity (species loss) within an ecosystem. **Article I** provides an overview of approaches developed to consistently translate data on ecotoxicity effects from chemical exposure into eventual damage to species diversity within the product life cycle assessment (LCA) framework.

Initial approaches such as mean extinction and media recovery were proposed to link the ecotoxicity effect to species diversity and are now rarely used due to their inherent complexity and limited data availability (Larsen & Hauschild, 2007). An alternative method highlighted in **Article I** is environmental DNA (eDNA) analysis, where species DNA is extracted from sources like soil or water combined with gene sequencing, which offers a way to measure species diversity (Birrer et al., 2021). However, it is essential to note that this approach is limited in detecting only recently present species due to DNA degradation (Goldberg et al., 2016; Rees et al., 2014).

In **Article I**, an alternative approach involves using mechanistic models like energy budget and food web models to extrapolate individual-level effects to the potential community or population-level damage. Dynamic Energy Budget

(DEB) models simulate energy allocation in species, but they are specific to certain compounds and species (Dong et al., 2022; Forbes et al., 2017). Food web models like AQUATOX consider chemical flow in ecosystems and their impact on food webs. However, they are not widely used due to modeling challenges, i.e., AQUATOX primarily applies to organic chemicals and the lack of standardized impact indicators limiting practical use in LCA (Faber et al., 2019; Maltby et al., 2021).

On the other hand, population models incorporate species-specific life history traits like growth and reproduction, providing biological realism when predicting population damage based on different endpoints (Forbes et al., 2017; Maltby et al., 2021). However, they are limited to a few species, requiring broader coverage (EFSA et al., 2018; Maltby et al., 2021).

The Threshold Indicator Taxa Analysis (TITAN) method, highlighted in **Article I**, helps identify individual species or community breakpoints or thresholds in response to stressors. TITAN aids in recognizing differences in species sensitivity, distinguishing between opportunistic and sensitive species. Unlike the Principal Response Curve (PRC) approach, which analyzes multiple species responses in controlled experiments, TITAN utilizes field monitoring data (possibly multiple stressors) to derive species-specific thresholds based on pressure gradients and the overall community response pattern (Baker & King, 2010). This approach reflects the biological realism of species exposed to multiple stressors or mixtures in the field. Additionally, considering individual species response patterns plays a role in translating the effects of ecotoxicity into species loss, as discussed in **Chapter 5** (**Article IV)**.

2.2.2 APPROACHES TO LINK SPECIES LOSS TO DAMAGE ON FUNCTIONAL DIVERSITY

Functional diversity relates to variations in species traits that influence responses to environmental stressors like chemical emissions. Ecosystem functioning, which sustains an ecosystem through biological activities, is greatly affected by stressors impacting species traits and interactions within the food web (Truchy et al., 2015; Faber et al., 2019; Maltby et al., 2017). When species diversity is low, ecosystem functioning declines rapidly, but other biodiversity measures like genotype composition and functional groups also matter. Also, functional group redundancy is crucial for maintaining ecosystem functioning, depending on these groups' presence, composition, and traits (Faber et al., 2019; Haines-Young & Potschin, 2010; Rumschlag et al., 2020).

The function sensitivity distribution (FSD) proposed by **Article I** quantifies toxic chemicals' impact on ecosystem functioning by considering function-related endpoints (Posthuma et al., 2001). Developing and employing FSDs can facilitate a direct assessment of functional damage, analogous to establishing the PAF-PDF relationship through TITAN analysis, as described in section 2.2.1, which remains underutilized due to limited data availability (Posthuma & de Zwart, 2014).

Article I recommends the Trait Probability Density (TPD) framework for quantifying functional diversity components, integrating species abundance and intraspecific trait variability to estimate richness, evenness, and divergence. Functional evenness measures the extent of trait distribution within a single dimension, functional richness assesses species' spatial occupation within an ecological unit, and functional divergence quantifies trait-based abundance distribution. TPD can predict the functional structure of species populations and communities along chemical gradients but requires substantial trait data (Carmona et al., 2016).

Article I also recommends the phenotypic diversity method to translate changes in species diversity into damage to ecosystem functioning. This model directly links species diversity to phenotypic variance in ecosystem functioning, as represented by alterations in biomass production in response to toxic pressures. This approach emphasizes species functional groups as the fundamental units of the ecosystem, accounting for species sensitivity (Larsen & Hauschild, 2007).

2.2.3 APPROACHES TO LINK FUNCTIONAL LOSS TO DAMAGE ON ECOSYSTEM SERVICES DAMAGE

Based on the fact that damage on functional diversity loss can be linked to damage on related ES as an intermediate step of the main pathway in linking ecotoxicity effects to damage on ES or directly link species loss and damage on ecosystem services flow without necessarily considering the intermediate step of evaluating functional effects as pointed out in **Article I.** The role of biodiversity is thus crucial as it positively influences ecosystem functioning, and its loss reduces the efficiency of various ecological processes, including resource capture, biomass production, and nutrient cycling. Thus, high biodiversity is essential to maintain the flow of ecosystem services at different spatial and temporal scales.

However, understanding the consequences of biodiversity on ecosystem services requires considering functional traits (response traits) that influence the probability of extinction or establishment and how these traits drive ecosystem functioning (effect traits), as detailed in **Article I**. Emphasis is put on the need for mechanistic models to quantify the linkage between ecosystem functions and ecosystem services. However, challenges arise when incorporating ecosystem services regulated by multiple functions, which may respond differently to changes in biodiversity (**Article I)**.

Researchers have proposed quantitative models like Ecological Production Functions (EPFs) to link ecosystem functional diversity loss to damage to ecosystem service flows (Faber et al., 2019). Some online models, like the US Environmental Protection Agency EcoService, are based on EPFs but face limitations regarding standardized tests and exposure dose-response relationships (US EPA, 2018; Faber et al., 2021).

The cascade model is also proposed by **Article I** to provide a way to link changes in ecosystem structure and functions to ES in LCA as the model complements the LCA impact pathway framework. However, the cascade framework does not address ecotoxicity-related aspects and their influence on freshwater ES and the dynamics of ecosystem services (Maia de Souza et al., 2018; Rugani et al., 2019). Considering the developed conceptual Frameworks like InVEST, NESCS, and FEGS-CS[2] mentioned in **Article I,** the impacts of land use change or climate change on ecosystem services are assessed, but their applications in response to chemical stressors are less studied.

Article I also introduces a method by Syberg et al. (2017) that directly links ecotoxicity impacts to damage on ES by summing the calculated hazard quotients (HQ) across different chemicals, and it offers a practical way to set upper limits for chemical exposure in relation to human health, maintaining chemical exposure within safe limits and prompting remediation if those limits are surpassed.

[2] Valuation of Ecosystem Devices and Tradeoffs' (InVEST), 'Common International Classification of Ecosystem Services' (CICES) or the 'National Ecosystem Services Classification System' (NESCS) or the 'Final Ecosystem Goods and Services Classification System' (FEGS-CS)

2.3 MONITORING-BASED FRAMEWORK FOR ASSESSING AND MANAGING ECOSYSTEM SERVICES

As mentioned in **Article I,** practical assessment and management of ecosystem services (ES) depend on data-driven insights obtained from (bio) monitoring data and statistical analyses, with monitoring covering numerous sites relative to pressure metrics to avoid the 'curse of dimensionality.' An integrated approach, such as mixture toxic pressure quantification, overcomes issues related to the study of chemical pollution, emphasizing the importance of diagnosing relationships between pressure variables and impact variables using comprehensive training data gradients.

Global initiatives like the Group on Earth Observations Biodiversity Network (GEO BON ES) aim to monitor biodiversity and ecosystems (Vaz et al., 2021), but challenges persist, such as harmonizing ES metrics and incorporating social-cultural values into monitoring. Monitoring ES may focus on various aspects, including effect-based monitoring [3] and multiple stressor analysis (Chapman, 2012). Using biological quality elements instead of raw field monitoring data has been explored for European ecological status assessment (Posthuma et al., 2020).

Article I stressed that to understand how man-made pressures influence ES, there is a need to develop frameworks that combine ES monitoring with stressor status and trends, particularly when addressing chemical pollution alongside other pressures, which comes with challenges, requiring more comprehensive data and analytical tools to bridge the gap between applied ecology and ecotoxicology. This seems to be the challenge in ecotoxicity assessments as applied ecology relies on field data, while applied ecotoxicology often uses lab toxicity data.

[3] Focussing on the service providing unit (SPU): A collection of individual species necessary to deliver an ES.

2.4 WAY FORWARD IN LINKING PREDICTED ECOTOXICITY EFFECT TO DAMAGE ON ECOSYSTEM SERVICES

Article I emphasizes the importance of incorporating field-based monitoring data for biological realism when linking ecotoxicity impacts to ecosystem services (ES) damage. While models that encompass multiple populations or entire food webs can link ecotoxicity effects to species loss at the species diversity level, they introduce uncertainty due to extrapolation. The TITAN approach, relying on field-based monitoring data, presents a promising alternative, albeit with substantial data requirements.

A trait probability density framework can link species loss to ecosystem functioning loss, but additional data with functional diversity endpoints are needed. Quantitative ecological production functions can translate species diversity damage into functional and ES damage while accounting for extrapolation uncertainties, demanding robust models such as species sensitivity distributions (**Article I**). Although the EPF-based approach can link functional endpoints to ES changes, finding suitable endpoints for ecosystem assessment remains challenging. On a global scale, there is a need for frameworks or tools that integrate ES monitoring with the status and trends of chemical and other stressors at various spatial and temporal scales (**Article I**).

3 A CURATED AQUATIC ECOTOXICITY DATASET FOR ENVIRONMENTAL PROTECTION, ASSESSMENT, AND MANAGEMENT

Chapter 3 provides an overview of various ecotoxicity datasets that have been combined to serve different purposes, such as establishing environmental quality standards, conducting life cycle impact assessments, and assessing the environmental quality of surface waters. **Article II** also emphasizes the importance of data curation and harmonization to generate high-quality data for subsequent use in Articles III and IV. Section 1 discusses the current status of ecotoxicity data available for various applications. Section 2 addresses the challenges associated with merging databases, giving an example with DosReach1 and PubMass1 databases to enhance data quality for final usage. Section 3 outlines steps for the curation and harmonization process that will be applied in Article III. Section 4 presents the curated and harmonized high-quality data utilized in subsequent **Article III** and **Article IV** analyses.

3.1 STATE OF THE ART

Article II highlights that various programs and policy-driven initiatives, such as the European Green Deal and the Chemical Strategy for Sustainability in the EU, aim to reduce chemical pollution and its environmental consequences. However, effective responses are needed to achieve environmental goals, including setting environmental quality standards, monitoring chemicals in the environment, identifying and removing high-risk chemicals, and screening new chemicals for potential hazards, which comes with the challenge of accessing comprehensive ecotoxicological data due to data fragmentation and limited accessibility. Databases like the NORMAN Ecotoxicology Database, US EPA Ecotoxicology Knowledgebase, and EnviroTox provide valuable information but are discreet and not fully utilized. Therefore, there is a strong need for a curated and integrated global resource of aquatic ecotoxicity data to enhance environmental protection, assessment, and chemical management.

3.2 COMBINING DIFFERENT DATA SOURCES

Article II presents three datasets: PubMass1, DosReach1, and Readacross1 (**Figure 2**). PubMass1 represents data collected from various peer- and non-peer-reviewed, publicly-available sources, whereas DosReach1 comprises ecotoxicity values supplied by industry to ECHA under EU REACH legislation not available to the public. Read-across1 is solely composed of predicted toxicity data from other measured data. These subsets vary in terms of data sources, curation steps, and qualitative aspects, such as taxonomical representation.

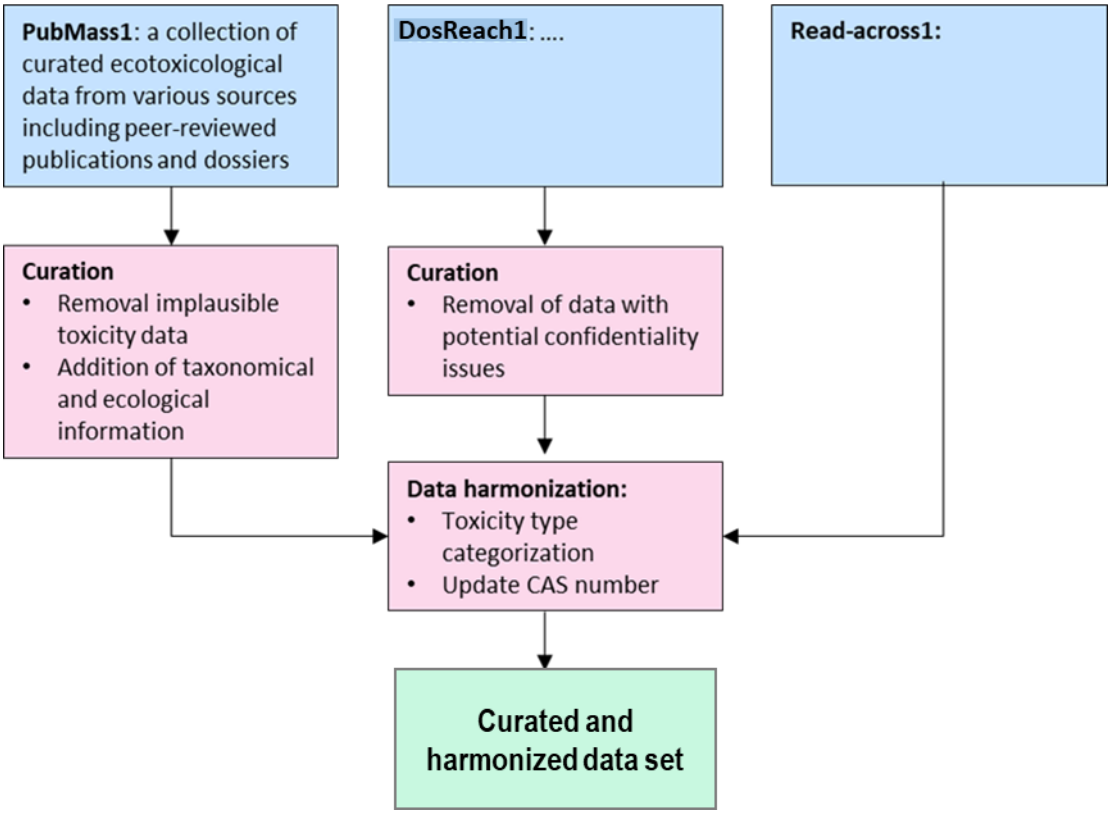

Figure 2. A flowchart depicting the various data subsets in blue and the curation and harmonization processes in pink, which ultimately lead to the curated and harmonized dataset described in **Article II**

Article II stresses that combining data from these subsets may introduce systematic differences in toxicity values due to test conditions, species choices, and other experimental design variations. Therefore, the decision to merge the data should be carefully considered, especially when systematic differences can impact the dataset's usability.

Furthermore, **Article II** examines potential systematic differences between PubMass1 and DosReach1 to assess the possibility of merging the two datasets, looking at sample means and distributions of species group-chemical pairs and exploring the overlaps and unique occurrences in the datasets for various endpoints. The most notable dataset overlap was in acute LC50 values (907 pairs), followed by acute NOEC (109 pairs), with Chronic NOEC showing 274 pairs and Chronic LC50 values having minimal overlap (8 pairs).

The density plot in **Figure 3a** by **Article II** indicates a substantial overlap between the density functions of DosReach1 and PubMass1 for acute logLC50 values. An example (**Figure 3 b**) highlighting the ecotoxicity data for a specific 'Species group - Chemical pair,' such as Fish x CAS 7758-98-7 (copper (II) sulfate), reveals variability in multiple toxicity values within the same pair, reflecting inter-test variability. To test for systematic differences in toxicity values between the two datasets, sample means for overlapping 'Species group - Chemical pairs' found in both datasets were compared.

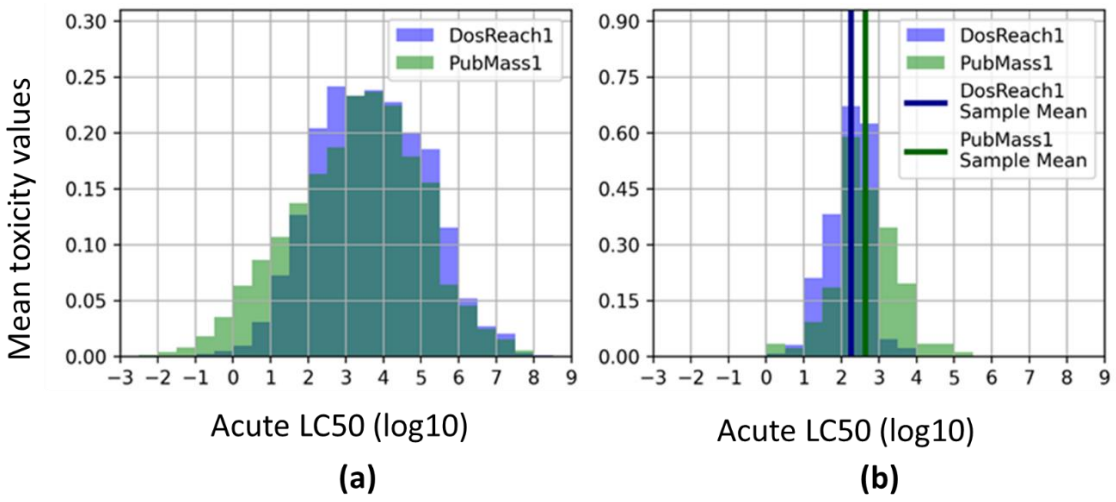

Figure 3. (a) Density plot of acute LC50 values (log10) in the comparable datasets, (b) density plot for the species group x chemical pair (Fish x CAS 7758-98-7 (copper (II) sulfate), with the most toxicity measurements and the sample mean of the repeated experiments where the residual variance is the inter-test variability. PubMass1 compiles publicly available data from diverse sources, while DosReach1 contains ecotoxicity values provided by industry to ECHA under non-public EU REACH legislation.

Findings by **Article II** suggest that differences in mean toxicity values between PubMass1 and DosReach1 exist, possibly due to random variations in experimental design like test species or exposure duration in a limited number of

studies underlying the data. This means that potential differences in toxicity values between the two subsets should be considered in subsequent analyses for specific chemicals.

3.3 DATA CURATION AND HARMONISATION

Table 1 outlines the data curation and harmonization steps for a subset of PubMass1, a laboratory-measured dataset selected for further analysis consisting of 255109 data points (**Figure 4**).

Table 1: Process flow for freshwater aquatic species HC20 development in LCIA. The table is modified from **Article III**

#	Step	Description/Explanation
1	***Pre-processing***	
1a	A unique list of **chemicals**	• Identified a unique list of chemicals from the database and updated the Chemical Abstracts Service Registry Number (CAS RN) by searching in the CompTox Dashboard
1b	Mapping of chemical classification on the unique list of chemicals	• Systematically classified chemicals using the ClassyFire taxonomy approach (Feunang et al., 2016)
1c	A unique list of toxic **modes of action** and mapping on the unique list of chemicals	• Mapped the toxic modes of action on the unique chemicals list using information from Pesticide Resistance Networks (FRAC, HRAC, IRAC) and the Verhaar scheme
1d	A unique list of **species**	• Identified the unique list of species from the database and updated the species name based on Global Names Resolver, IUCN, and GBIF databases
1e	Mapping of taxonomic classification on the unique list of species	• Assigned species' main taxonomic group (in line with "biological quality elements" defined under the EU Water Framework Directive), taxonomy levels, and habitat type based on IUCN, GBIF, and EnviroTox lists or manually searched.
1f	A unique list of **effect types** and **exposure test duration** and mapping on curated raw data	• Aggregated effect types into NOEC, EC10, and EC50 equivalents, remove ambiguous endpoints, and assign exposure test duration types based on the taxonomic group
2	***Dataset preparation***	
2a	**Extrapolation** to chronic EC10 equivalents (**$EC10^{eq}$**)	• Derived regression statistics based on patterns recognized by Aurisano et al. (2019), e.g., $chronic\ EC10 = alpha\ x\ acute\ NOEC + beta$ see **Table 2**
2b	**Aggregation** of data points per species	• Calculated average effect test values for each species-chemical combination
2c	**Criteria** definition for a minimum number of data points	• Classified chemicals as “data-rich” (chemicals with ≥3 distinct species from the ≥3 taxonomic groups) or as ″data-poor″ (<3 distinct species per taxonomic group and/or <3 taxonomic groups)

#	Step	Description/Explanation
3	***Effect calculation***	
3a	Deriving statistics for species sensitivity distribution (SSD): **single-SSD** and **split-SSD** per chemical	• Converted all chronic $EC10^{eq}$ into logarithmic (log_{10}) scale, i.e., derive a set of $log_{10}(EC10^{eq})$ and derive per chemical (single SSD) and per chemical-main taxonomic group combination (split-SSD per chemical) arithmetic mean and standard deviation
3b	Plotting **$EC10^{eq}$ data points on SSD** graphs	• Calculated the response probability (PAF) for each log10(EC10eq) of a chemical on a scatter plot using the formula 'PAF = (log10(EC10eq) rank - 0.5) / log10(EC10eq) count.'
3c	Plotting **fitted SSD** graphs	• Defined the range of log10(EC10eq) for each chemical and chemical-main taxonomic group combination and then derived a fitted cumulative normal distribution of log10(EC10eq) values over that range using the NORM.DIST function
3d	Deriving **HC20** per SSD	• Derived the HC20 as 'HC20 = 10^log(HC20)', the hazard concentration at which 20% of species show a probable effect above their log10(EC10eq)
3e	Comparison of HC20	• Used the criteria of non-crossing SSD curves, non-overlapping confidence intervals, label split-SSDs as significantly different or not, merge non-significant split-SSDs into a combined SSD, and label chemicals with at least one statistically significant split-SSD for specific targeting to identify SSD to split
3f	Statistical tests for evaluating the potential of splitting SSD	• Evaluated whether to split or not based on statistical analysis, e.g., mean comparison tests, toxic mode of action information, category use, and visual inspection of the SSD 95% confidence interval
4	***Uncertainty assessment***	
5a	Preliminary steps	• Determined standard deviation for each combination (chemical-species-effect type) to analyze how standard deviation varies to set up a fixed standard deviation in case of taxonomic group combination with a low number of data points (fixed-shaped SSD)
5b	Total uncertainty around the derived HC20	Quantified uncertainty around the derived HC20 values by combining two types of uncertainty: GSD^2_{inter} reflecting inter-species variability (i.e., variability across available effect values) and GSD^2_{intra} reflecting intra-species variability (i.e., variability around the effect values).

Table 2. An overview of regression equations derived from a comprehensive dataset of 9,868 chemicals used to extrapolate laboratory-derived species sensitivity endpoints to chronic EC10 equivalents for the subsequent building of SSD-EC10eq in **Article III**

Endpoints	Extrapolation equation
Acute NOEC	$\log \mathrm{EC10}_{\mathrm{chronic}} = 0.816 \times \log \mathrm{NOEC}_{\mathrm{acute}} + 0.021$
Chronic NOEC	$\log \mathrm{EC10}_{\mathrm{chronic}} = 0.965 \times \log \mathrm{NOEC}_{\mathrm{chronic}} - 0.144$
Acute EC50	$\log \mathrm{EC10}_{\mathrm{chronic}} = 0.869 \times \log \mathrm{EC50}_{\mathrm{acute}} - 0.508$
Chronic EC50	$\log \mathrm{EC10}_{\mathrm{chronic}} = 0.872 \times \log \mathrm{EC50}_{\mathrm{chronic}} + 0.733$
Acute EC10	$\log \mathrm{EC10}_{\mathrm{chronic}} = 0.813 \times \log \mathrm{EC10}_{\mathrm{acute}} + 0.967$

Figure 4 illustrates the distribution of the 255109 data points from the selected laboratory-measured dataset. Freshwater habitats constituted the majority at 91%, and acute toxicity data is the most prevalent endpoint, accounting for 48% across all habitats. This primarily includes acute EC50 values for freshwater, with 108,524 acute EC50s and 69,635 acute NOECs. In contrast, chronic EC50 data is less common, with only 8,376 records, and there are only 87 records available for terrestrial habitats. The limited ecotoxicity data for terrestrial habitats, specifically just 1,099 records, indicates limited research on terrestrial species.

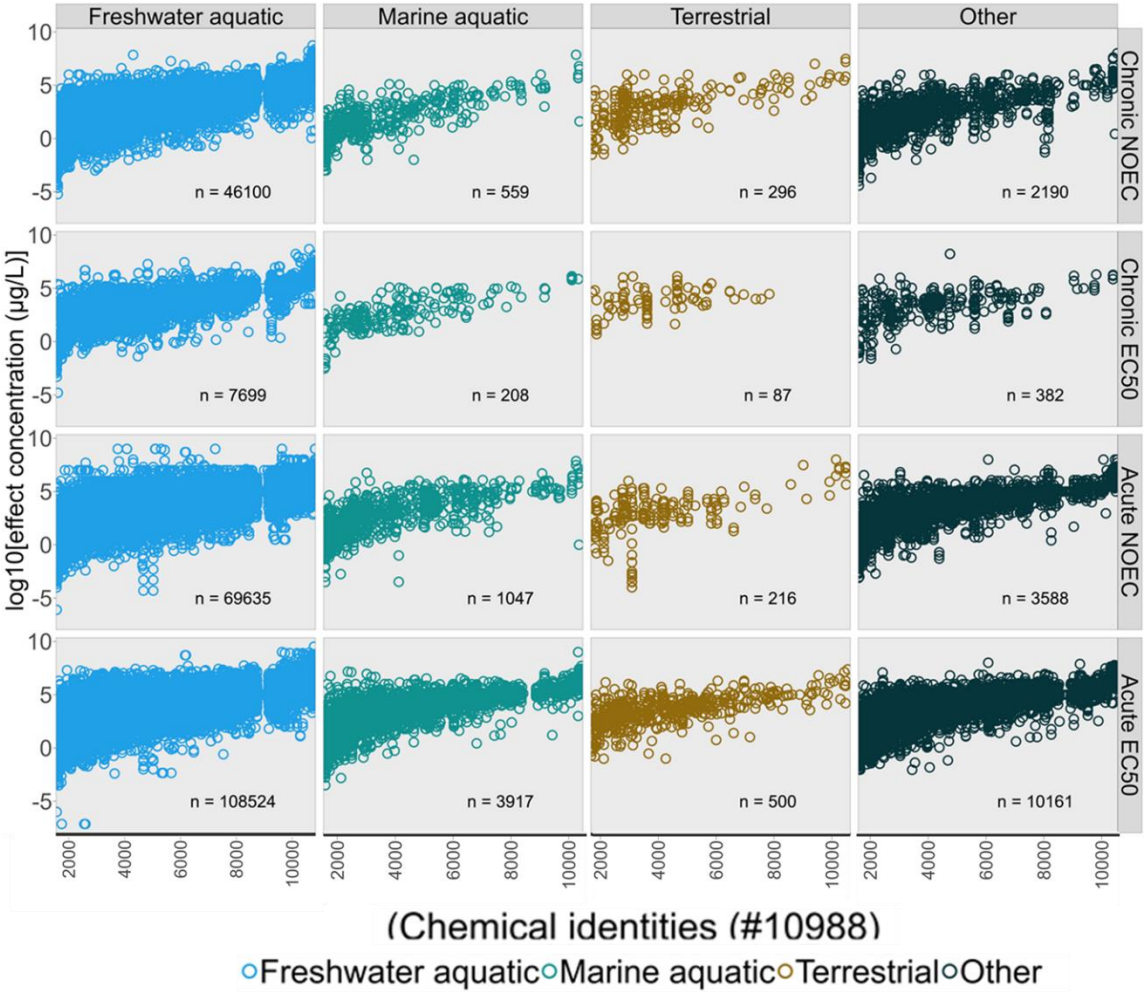

Figure 4. The distribution of 255109 effect concentrations (Y) for 10988 chemicals (X), categorized by taxonomic groups and endpoint types, with ranking based on mean chronic EC50 values per chemical for each column. Gaps on the x-axis represent missing data for specific Habitat*endpoint combinations. Figure taken from **Article II.**

3.4 HARMONIZED FRESHWATER ECOTOXICITY TEST DATA FOR SPLIT SSDs

Article III shows that after data curation and harmonization, the freshwater ecosystem dataset comprises 120,835 species-specific toxicity test data, 9,868 chemicals, 1,123 species, and 234 test endpoints, as illustrated in **Figure 5**. However, the dataset exhibits uneven representation across taxonomic groups, test durations, and endpoint types due to inconsistent global testing practices. Invertebrates constitute the majority (78%) of the primary taxonomic group in the dataset, with acute toxicity data being the dominant data type (71% across all taxonomic groups), primarily acute EC50s for invertebrates, including 44,077 acute EC50s and 20,000 acute NOECs. In contrast, chronic EC50 data

is less common (n=5,555), with a limited number (n=82) available for vertebrates.

"Species sensitivities span many orders of magnitude for short-term peak- and longer-term chronic exposures (**Figure 5**). For example, acute EC50s range between 7.3×10^{-8} and 3.3×10^{9} µg/L for invertebrates and chronic EC50s range from 1.6×10^{-5} to 1.0×10^{8} µg/L for invertebrates. Likewise, acute NOECs range between 8.0×10^{-7} and 1.0×10^{9} µg/L and chronic NOECs range between 6.0×10^{-6} and 2.5×10^{8} µg/L."

After curating the data, 180 chemicals were identified as data-rich, meeting the criterion of having data available for at least three distinct species from three or more taxonomic groups. This selection yielded 5,217 test endpoint data for developing and testing the SSD-splitting framework. The final data-rich subset, from which chronic EC10 values were derived, was characterized by a predominant presence of specific taxa, with invertebrates making up 47%, vertebrates 33%, and algae, cyanobacteria, and aquatic plants comprising 20% of the subset. Only 1.81% of the chemicals possessed sufficient data to potentially facilitate a complete split into three taxonomic group-specific SSDs, as presented in **Article III.**

The available database (120,835 values) reveals a greater focus on testing and evaluating invertebrates (Berger et al., 2016, 2018; Khamis et al., 2014; Lagadic & Caquet, 1998) and a notable scarcity of data for bacteria and fungi, hindering the assessment of risks for these groups and their vital roles in the ecosystem, e.g., nutrient cycling, highlighting the necessity for conducting additional tests on microorganisms within freshwater ecosystems.

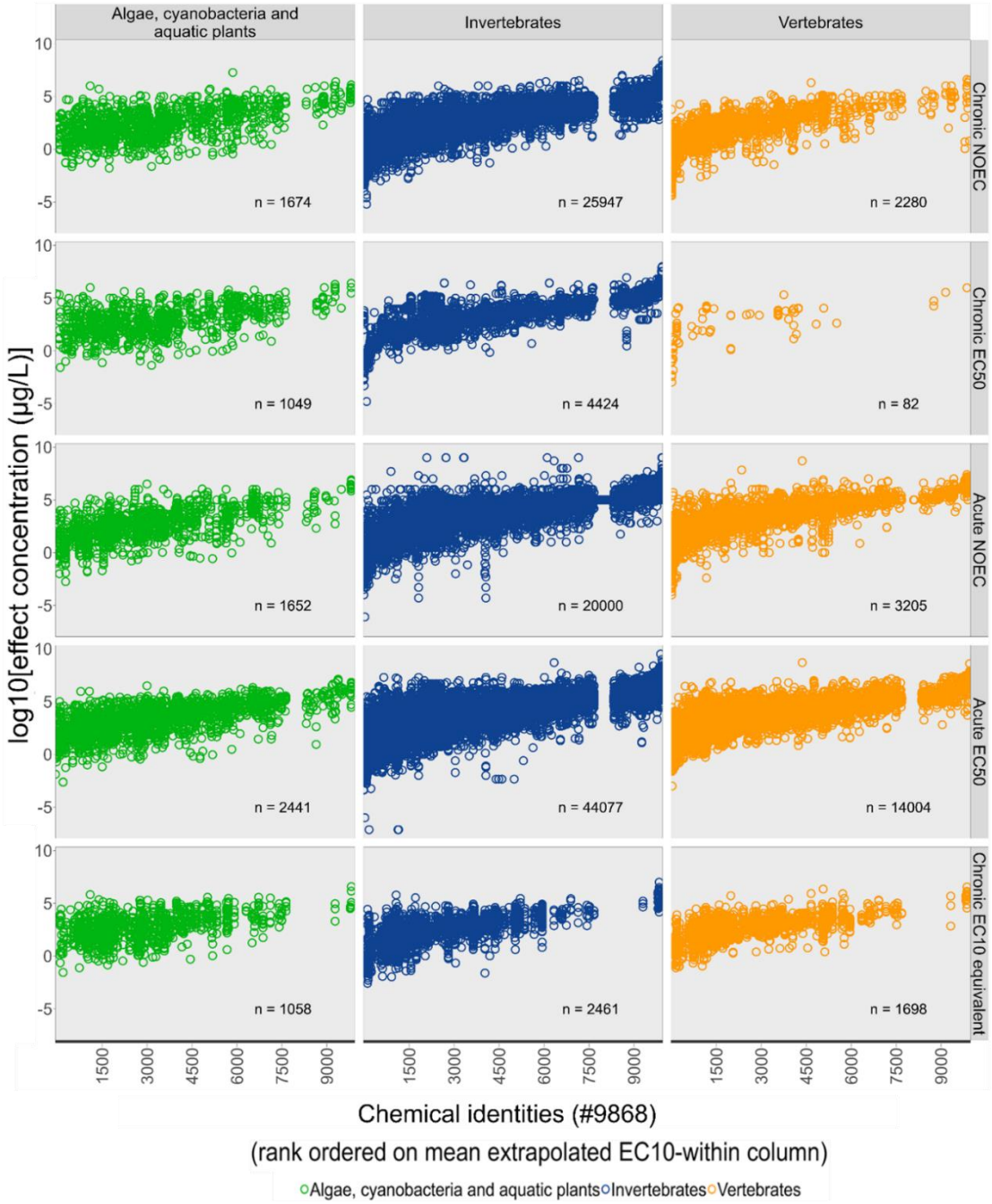

Figure 5. The distribution of 120,835 species sensitivity endpoints (Y) for 9,868 chemicals (X), categorized by taxonomic groups and endpoint types, with ranking based on mean extrapolated chronic EC10-equivalent values per chemical for each column. Gaps on the x-axis represent missing data for specific taxonomic group*endpoint combinations. Figure taken from **Article III.**

4 CHARACTERIZING CHEMICAL POLLUTION IMPACTS IN AQUATIC ECOSYSTEMS WITH SPECIES SENSITIVITY DISTRIBUTIONS FOR SPECIFIC TAXONOMIC GROUPS

Chapter 4 presents a scientific response to whether and how or not to split Species Sensitivity Distributions (SSDs). It is scientifically preferable (in terms of how well a model fits the data and its relevance to evaluating risks in exposed ecosystems) to separate SSDs based on taxonomic grouping unless there are constraints due to the quantity and quality of available test data. **Article III** discusses the splitting SSDs approach based on three taxonomic groups to overcome the challenge of not accounting for differences in sensitivity across species groups, which may be relevant when, for example, linking chemical effects to ecosystem functioning damage since different species groups may have very different roles in an ecosystem and its functioning. Although methods and results represent pragmatic choices on input data, statistical models and outputs, and a decision context, the study results have generic implications regarding the issue of splitting or not splitting SSDs. Section 1 outlines the current use of SSDs, which has shortcomings (Section 2). Section 3 presents the proposed conceptual framework for split SSD. Section 4 outlines the role of chemical use and mode of action in SSDs derivation. Section 5 presents the role of splitting the SSDs framework in decision support. Finally, section 6 presents a case study for use in LCIA. All contents of this chapter are based on **Article III**. The parts taken directly from the Article are marked with "…".

4.1 CURRENT USE OF SSDs

SSD-based outputs vary from the most classical and regulatory-adopted use of setting protective standards to analyzing the potentially affected fraction of species (PAF) and predicted mixture toxic pressures (expressed as msPAF, multi-substance Potentially Affected Fraction of species) in globally applied environmental quality assessments and life cycle assessment of products and technologies (Posthuma & de Zwart, 2014; Posthuma & de Zwart, 2006).

SSD approach employed by different jurisdictions worldwide has remained similar, e.g., the use of HC5 value for developing water quality benchmarks and environment protection standards, i.e., predicted no-effect concentration (PNEC). However, there is variation in the type of models fitted to SSDs. For

example, countries like Canada and North America have employed the use of a model-averaging approach (where the HCx is estimated using a weighted average of several individual SSDs), while in Europe, the SSD approach permits the use of any parametric distributions, e.g., log-normal, log-logistic, Burr type III (ECETOX, 2014). **Article III** illustrates the use of split SSDs using log-normal distribution methods, which has generic implications regarding the issue of splitting or not splitting regardless of pragmatic choices on input data, statistical models and outputs, and a decision context.

4.2 SHORTCOMINGS OF THE CURRENT SSD APPROACH

The SSD concept is not without shortcomings for all environmental problems with chemicals. The current approach to constructing SSDs assumes they describe the exposure-impact relationship for the entire species assemblages. This assumption may not always hold, particularly when considering specific modes of action of chemicals and differences in taxonomic groups' sensitivity toward chemical exposure. Non-split SSDs (current approach) may not fit well with data across taxonomic groups, potentially leading to inaccurate assessments (Fox et al., 2021). Different taxonomic groups have different functions within an ecosystem—this is important for understanding differences in sensitivities that may propagate differently when later linking effects to damage on ecosystem functioning as much as there is an interaction of species in the food web and functional redundancy ensuring the stability of the ecosystem (Haines-Young & Potschin, 2010; Baumgärtner, 2007).

In addition, limited test data leads to uncertainties in assessing ecological impacts. The quality of the underlying toxicity test data used to construct SSDs is crucial. If the data used in the analysis are of poor quality or have limitations, it can negatively reduce the accuracy and reliability of the SSDs. **Article IV** addresses another challenge of considering a mixture toxic pressure in the SSDs approach**.**

4.3 SYSTEMATIC DECISION TREE TO EVALUATE THE SPLITTING OF SSDs

Article III illustrates the process flow for deriving split SSDs for 180 selected chemicals after curating a high-quality dataset in **Figure 6**. We selected three

biological quality elements (inspired by the EU-Water Framework Directive, representing approaches from applied ecology) and a minimum of three data points per group to start the research. After that, we evaluated the variation of chemical impacts on different taxonomic groups (i.e., vertebrates, invertebrates, and algae, cyanobacteria, and aquatic plants) using a series of statistical tests, as well as the calculated HC20 (Hazardous Concentration, consensus metric) for use in LCIA and its confidence interval which includes the level of uncertainty. Because of poor robustness, we explored the split and re-merged data for many chemicals. Setting a criteria, which included "only SSDs with a squared geometric standard deviation $GSD^2 \leq 5$ around the log-mean as a cut-off point."

After the final step of verifying the robustness of SSDs of the selected 180 chemicals, Article **III** highlights that partial split SSDs (e.g., Algae, cyanobacteria, and aquatic plants versus Invertebrates and Vertebrates together) were found for many chemicals (n=75) than full split (n=3), and the rest of the chemicals (n=102) fall back in non-robust split SSDs (the classical no-split SSD).

The results suggest that 'always-splitting' is warranted as a starting point, but the available data characteristics may limit splitting. For example, few data points for the microorganism (e.g., bacteria and fungi) made it impossible to derive a split SSD for this group, which calls for the inclusion of more microorganisms in the laboratory test to allow for the assessment of risks given their role in the freshwater ecosystem, e.g., nutrient cycling.

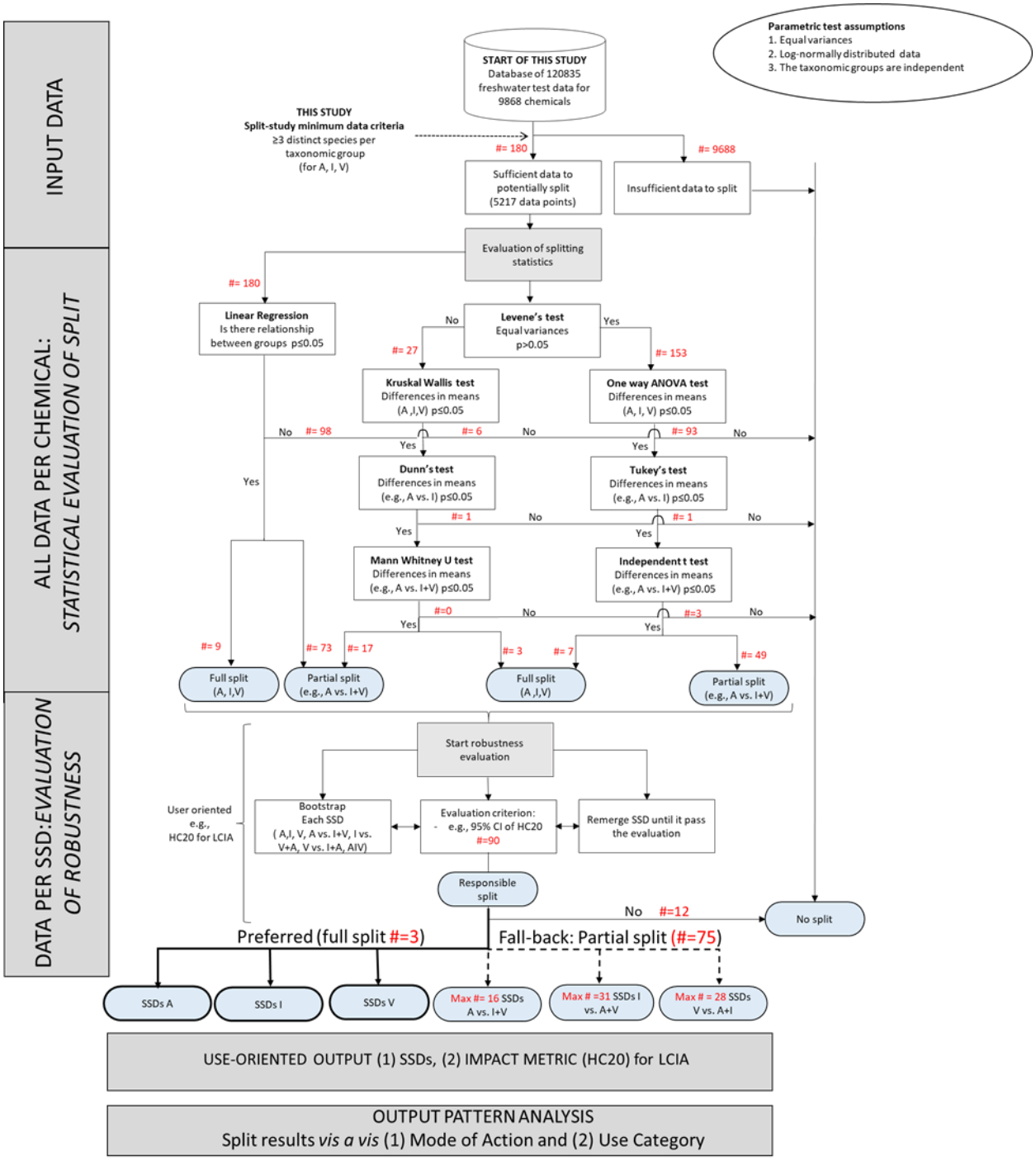

Figure 6. A decision tree for splitting SSDs based on three specific Water Framework Directive-defined Biological Quality Elements: algae, cyanobacteria, and aquatic plants (A), invertebrates (I), and vertebrates (V). We start from input data, where we select 180 chemicals to evaluate statistical motives to split using all available data per chemical (fully or partially). At the bottom, summarize results for the practical Life Cycle Impact Assessment

(20th percentile values), assessing if the statistical split aligns with the mode of action and the use of chemicals. Figure taken from **Article III**.

4.4 THE ROLE OF CHEMICAL MODE OF ACTION & USE CATEGORY INFORMATION IN BUILDING SSDs

Splitting is primarily justified in applied ecology, where bioassessment methods often focus on taxonomic groups. However, the rationale for splitting becomes even more compelling when considering chemicals with specific modes of action (MoA) or broadly defined chemical use categories. The relationship between taxon sensitivity and chemical mode of action (MoA is important as some chemicals show significant taxon-specific differences in toxicity, such as herbicide sensitivity of plants versus vertebrates and acetylcholinesterase inhibitor toxicity to invertebrates versus vertebrates. Thus, information about a chemical's MoA helps to identify taxa (or perhaps traits) that are likely to be sensitive—the idea of split-SSD was already discussed as future optimization in the Outlook Chapter of the SSD book of 2002 (Posthuma et al., 2002).

Article III outlines the outcomes of considering mechanistic MoA and chemical use categories, as shown in **Figure 7 and Figure 8**. For example, invertebrates exhibited lower sensitivity (lowest SSD mean) for chemicals designed as insecticides, which operate via AChE inhibition, while primary producers appeared at the lower end of the distributions for herbicides acting as photosynthesis inhibitors. These findings underscore the importance of incorporating MoA and using category information for chemicals when considering more appropriate models for individual taxonomic groups, as suggested by previous researchers (Maltby et al., 2005; Van Den Brink et al., 2006).

Article III supports previous research and theoretical findings, which indicate that chemicals with a non-specific Mode of Action (MoA), such as narcosis (as depicted in **Figure 7**, the vertical spread), tend to exhibit the least variation in sensitivity across taxonomic groups. This supports the notion that even among species that are not closely related, toxicity resulting from nonpolar narcosis is associated with relatively minor differences in sensitivity between species.

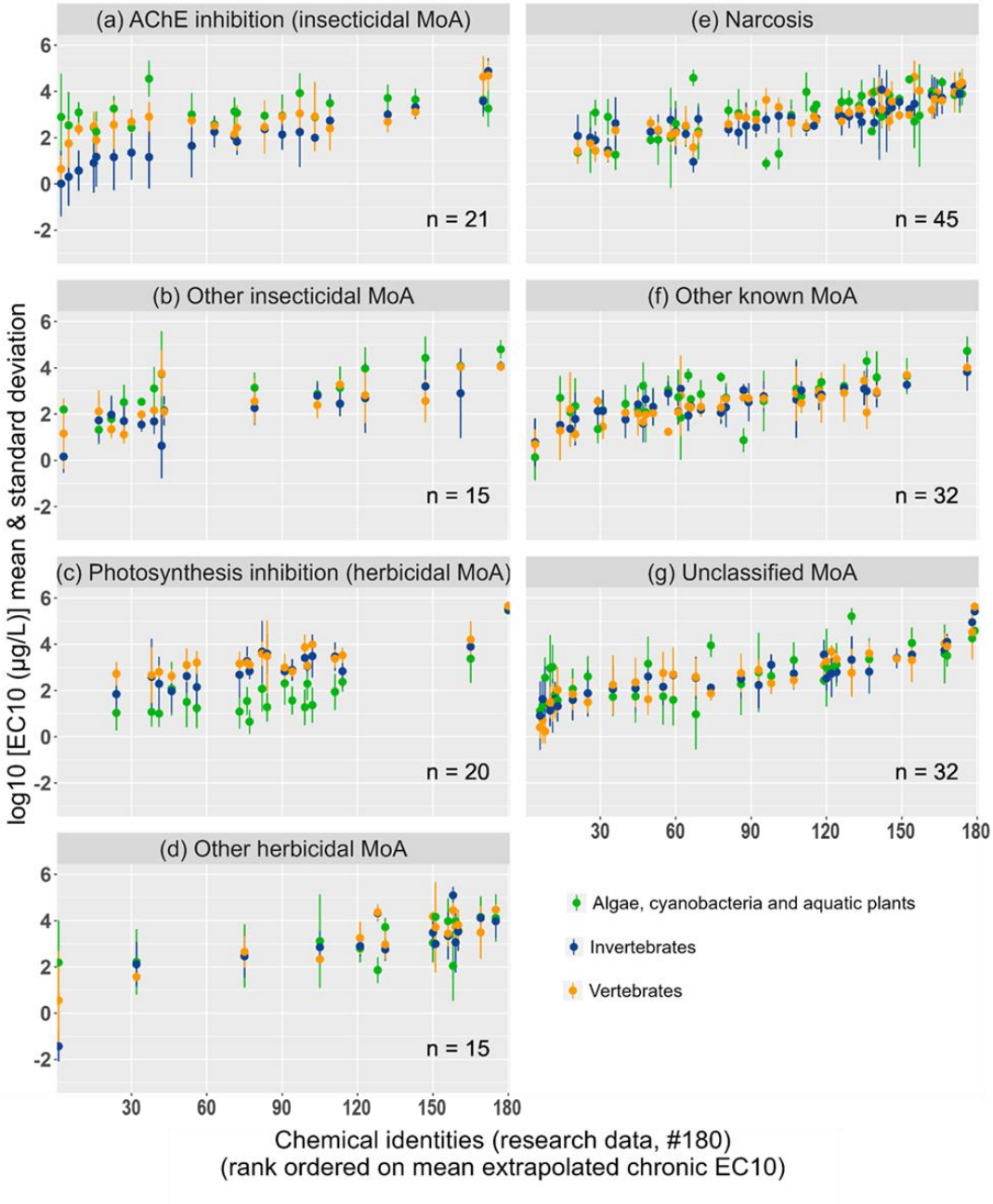

Figure 7. Species sensitivity impact metric (Y, chronic EC10 equivalents) distributions for 180 ranked chemicals (X). Chemicals within each panel are arranged by mean impact metric values computed from all available data for each chemical. When examining the split in SSDs, it broadly aligns visually with distinct dots and standard deviations for various taxonomic groups (represented by different colors). Each panel represents the grouped MoA targeting different taxonomic groups: (a) and (b) for chemicals targeting invertebrates; (c) and (d) for chemicals targeting algae, cyanobacteria, and aquatic plants as primary producers; (e) for chemicals with baseline toxicity and (f) includes chemicals for which MoA was provided but no target specified. Figure taken from **Article III.**

While there might be both applied ecological and statistical reasons to contemplate splitting data-rich chemicals with a narcotic MoA, the enhanced fit suggests improved outputs from SSDs for chemicals with specific MoAs. Hence, the emphasis is placed on splitting SSDs mainly for chemicals with specific MoAs in practical applications. However, certain outcomes cannot be predicted based on chemical MoA or the use category information. Many of the observed lower sensitivities in our SSDs indicate unintended side effects. Despite limited test data and scarcity of chemicals designed to target vertebrates, these side effects are evident.

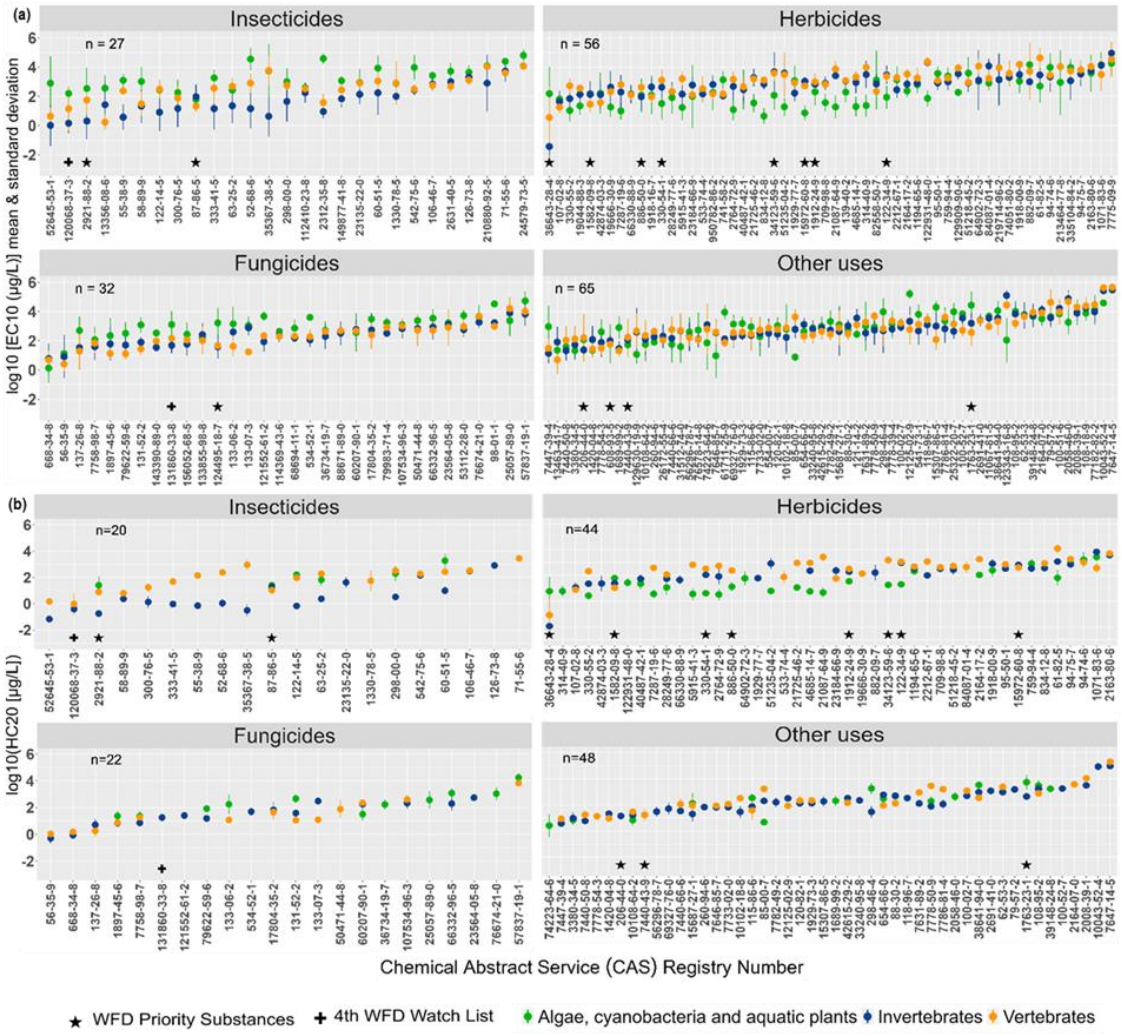

Figure 8. Illustrates the distributions of the species sensitivity impact metric (Y), represented by chronic EC10 equivalents. These distributions are organized into panels based on (a) different chemical use categories, e.g., insecticides, and (b) HC20-estimations, along with their standard deviations, are summarized for 134 chemicals with at least six data points (resulting in low uncertainty) which has matching patterns. Figure taken from **Article III.**

4.5 SPLIT SSDs FOR DECISION SUPPORT

Using split SSDs has multiple consequences for decision support. **Article III** shows that the developed splitting SSD method improves the interpretation of assessment outputs (hazardous concentrations used as protective environmental quality standards, the potentially affected fraction of species at measured or predicted environmental concentrations), which may have a noted impact on ecological risk assessments and regulatory decision-making if adopted in practice. For example, when SSDs are split, the HC5 for the most sensitive taxonomic group is lowered compared to the HC5 derived from a whole-assemblage SSD (**Figure 9**), and this is a highly relevant observation for this use of SSD outputs. Consequently, this has comparable consequences for the criteria adopted by regulators that rely on these calculated HC5 values, such as the Predicted No Effect Concentration (PNEC).

Article III confirms that chemicals with more datasets (e.g., Simazine) and a specific Mode of Action (MoA) offer a strong foundation for responsible SSD splitting. Thus, more significant data availability leads to more robust SSDs even after a partial split, even when considering narcotic chemicals (e.g., Sodium pentachlorophenate). However, species selection bias in laboratory testing, particularly for chemicals with specific MoAs, currently constrained our ability to establish fully split SSDs. For instance, Trichlorfon, an insecticide operating through AChE inhibition, statistically supports using a full-split.

Conversely, when only a limited number of data points are available for a non-target taxonomic group (specifically Algae, cyanobacteria, and aquatic plants with n=3), the SSD robustness check suggests that only partial splitting is advisable. In order to create a full split SSD, it is essential to accumulate sufficient data across all taxonomic groups. On the other end of the spectrum, avoiding compromising prediction accuracy with non-robust SSDs is crucial, which tends to occur for chemicals with limited data. For instance, while statistical tests on all available test data imply that it might be possible to separate primary producers from other groups in the case of Pyraflufen-ethyl, the broad and overlapping confidence intervals make the whole assemblage SSD statistically more robust compared to the partial-split alternative.

Thus, the decision to employ split SSDs should not be solely based on statistical assessments. It is equally important to evaluate whether splitting is more beneficial in practice, leading to improved decision support based on conceptual principles and trade-off effects.

Article III also demonstrates the practical applications of splitting SSD in various contexts, i.e., from the analyzed 15 chemicals designated as Water Framework Directive (WFD) Priority Substances (black stars), reflecting their ongoing concern in Europe and two chemicals from the 4th WFD Watch List (black crossed dots), highlighting their emerging concerns showing which taxonomic group a chemical affect most. Thus, splitting SSDs in decision-making becomes crucial when comparing insights from the traditional approach (without splitting) to our proposed (partial) split approach (**Figure 9)**.

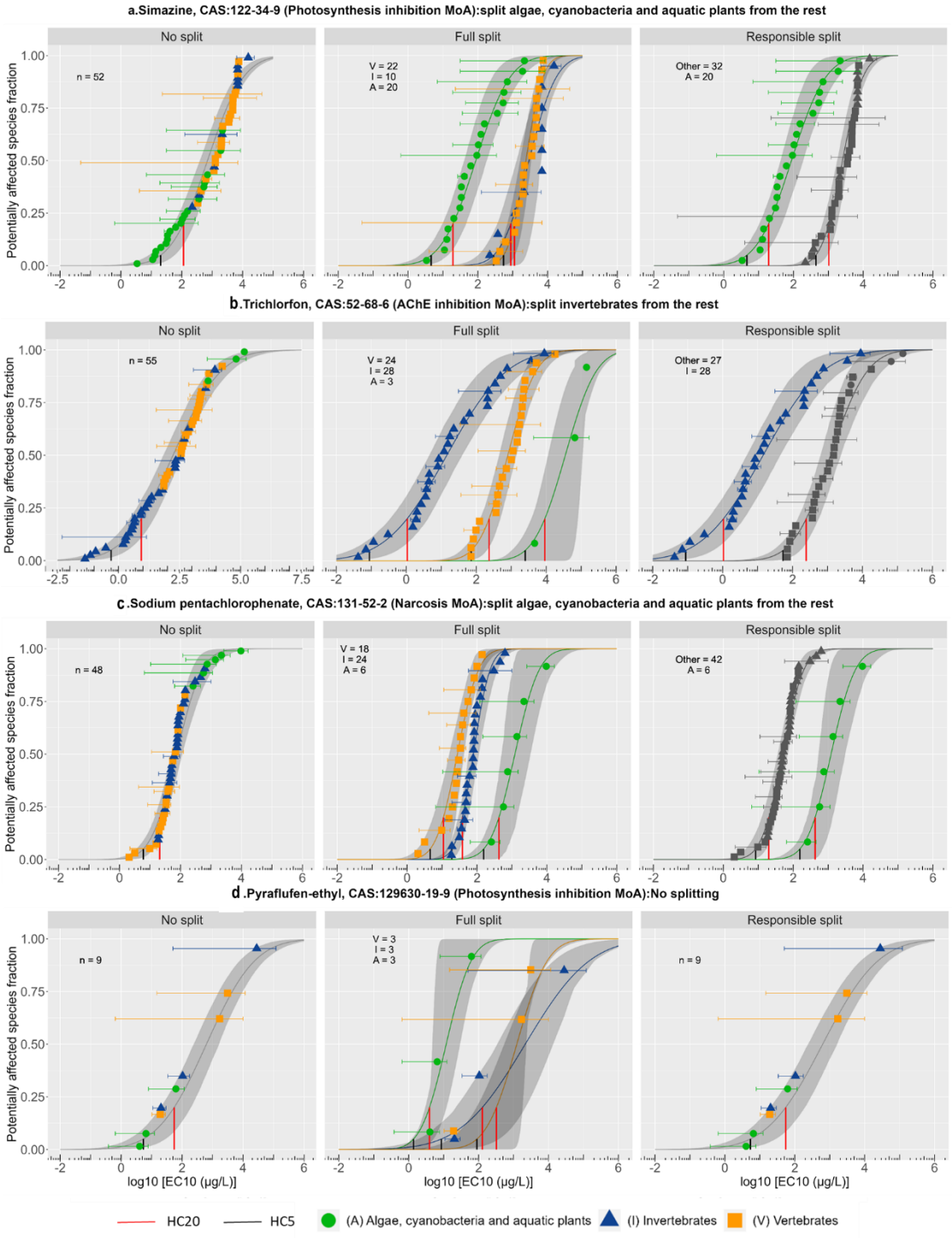

Figure 9. Illustrates the derived species sensitivity distributions (SSDs) following the SSD protocol; the columns represent three types of SSDs: no-split (the classical common approach, on the left), full split (in the middle), and responsible split (on the right). The rows, from top to bottom, illustrate the outcomes of various SSD splitting scenarios related to different Modes of Action (MoA) and chemicals with varying data richness. When comparing the black lines (HC5; protective standards) or red lines (HC20; used to derive impact

magnitudes in LCIA), differences of more than two orders of magnitude indicate consequences for these decision support applications and vice versa.
Figure modified from **Article III.**

From a scientific perspective, a study aiming to calibrate the predicted mixture toxic pressures (quantified as msPAF, multi-substance Potentially Affected Fraction of species) with observed effects on a specific species group would yield more meaningful results when based on split SSDs. This is due to the improved accuracy in impact assessment achieved through splitting, as depicted in **Figure 9**, which can help characterize the link between the Predicted Affected Fraction of species and the Potentially Disappeared Fraction (PDF) of species resulting from multiple chemical exposures (as discussed in **Article IV**).

4.6 RESULTING HC20s FOR LCIA USE

Article III followed the recommendations derived in the Global Life Cycle Impact Assessment Method (GLAM) effort under the auspices of the United Nations Environment Program to derive metrics for assessing ecotoxicity impacts (HC20-type criteria setting) in life cycle impact assessment (Owsianiak et al. 2023) as the concentration is closer to the environment concentration.

The associated uncertainty surrounding the derived HC20s specific to different taxonomic groups was calculated to account for the inter- and intraspecies variability (Emara et al., 2023), whose magnitude depended on the number of data points and test species.

Similar to the estimated protective environmental quality standard (which signifies no impact), splitting SSDs resulted in varying impact estimations for the impact metric employed in Life Cycle Impact Assessment (LCIA) at the 20th percentile (the red lines in **Figure 9**). These improved principles and model fits have significant implications for the practical application of SSDs. They are valuable for establishing protective standards and their utilization in environmental impact assessments and Life Cycle Impact Assessment (LCIA.

Article III highlights that "splitting is a better approach to deriving SSDs and using the models for decision support, provided the resulting SSDs are sufficiently robust. The relevance of decision support may be further increased when a split considers different service-providing units (SPU), a concept used

in the context of ecosystem services research. This is because it is key to protect and restore biodiversity in terms of structural characteristics of ecosystems (the present use) and functional characteristics and provided services. Assessments that would consider ecological information, such as functional groups or trait characteristics, may help to identify the SPU and ecosystem services that are both valuable and potentially impacted."

5 THE LINK BETWEEN THE MIXTURE TOXIC PRESSURE AND DAMAGE ON BIODIVERSITY IN AQUATIC ECOSYSTEMS

Chapter 5 presents a stepwise approach to characterize the link between ecotoxicity impacts and biodiversity loss (i.e., species richness and species abundance) in freshwater ecosystems, which is relevant for translating model-predicted organism level effects to damage on structural (bio)diversity and associated damage to ecosystem services, as the predicted impact metric in decision contexts, such as Life Cycle Impact Assessment of products and technologies.

Article IV utilizes monitoring data (abiotic variables and macrofauna) from the Netherlands regions to quantify the predicted impact of chemicals and their unintended mixtures as 'mixture toxic pressure' (unit: msPAF: multi-substance Potentially Affected Fraction of species, a measure of the proportion of species that are potentially affected by more than one chemicals) as species in the field are exposed to more than one chemicals in the field (Schäfer et al., 2023). Moreover, the actual disappearance of taxa under field conditions can now be determined by relating the chemical pollution msPAF metric and species abundance or richness, providing ecologically meaningful estimates of how the environment limits or changes species distribution and alters biodiversity metrics (Posthuma et al., 2020b).

Section 1 outlines the fundamental steps in characterizing the relationship between PAF and PDF. Section 2 presents data on monitored chemical concentrations and the computed msPAF for further analysis**.** Section 3 introduces six additional abiotic factors that play a role in biodiversity loss. Section 4 presents data on species monitoring across the Netherlands region. Section 5 details an analyzed covariation between msPAF and other pressures that could potentially bias the associations between toxic pressure and biological response metrics. Section 6 illustrates trends in species abundance and richness distribution in relation to a mixture toxic pressure. Section 7 presents the conducted robustness check of the biodiversity metrics patterns using a subset of data from one water authority region in the Netherlands. Finally, Section 8 presents the derived PAF to PDAF relationship. This chapter's contents are derived from **Article IV**. The parts taken directly from the Article are marked with "…".

5.1 WORKFLOW TO CHARACTERISE A LINK BETWEEN MIXTURE TOXIC PRESSURE AND IMPACTS ON SPECIES IN AQUATIC ECOSYSTEMS

Article IV illustrates the process flow to derive the relationship between the mixture toxic pressure and species loss in the field condition, as depicted in **Figure 10**. In order to describe the association between the mixture toxic pressure (quantified as predicted fraction of species chronically affected, msPAF; x) and two types of response variables, species abundance[4] and richness[5]. We started with the Netherlands' (NL) extensive (bio) monitoring data on invertebrates (collected from 1983 to 2014), chemical contaminants (collected from 1983 to 2015), and data on six other pressure site characteristics.

The first step involved selecting a monitoring dataset collected from 2000 to 2014. In the second step, the mixture toxic pressure of the samples at different sites was quantified using the SSD information from **Article III** and Posthuma et al. (2019).

In the third step, the chemical contaminant concentrations of separate chemicals were replaced with chronic msPAF-EC10 as a metric for the local mixtures that caused the impact, and the correlation between all the predictor variables, including toxic pressure, was determined using the variance inflation factor method to explore possible biases that might influence the PAF to PDF relationship.

The fourth step involved studying the variation in msPAF to the macrofauna abundance and richness distribution. Upon demonstrating that mixture exposure is a limiting factor affecting different taxa and biodiversity metrics, we categorized the msPAF into five groups[6] to compare the statistical significance difference in the species count, or per-species abundance values, between different groups and how many species (or abundance numbers) are lost when the

[4] Species abundance: taxon-specific; total of individuals from a given species within a given site

[5] Species richness: count of unique species per site

[6] Categorisation of msPAF into five groups with increasing toxic msPAF levels. Group 1 signifies a low-toxic sites with minimal effects, while Group 5 represents high toxicity sites with highest effect

protective standard criterion of 0.05 and 0.2 msPAF is exceeded. Lastly, we performed a robustness check by selecting data from one data-rich water authority and repeating the above steps to verify whether PAF-to-PDF findings are consistent **Figure 10**.

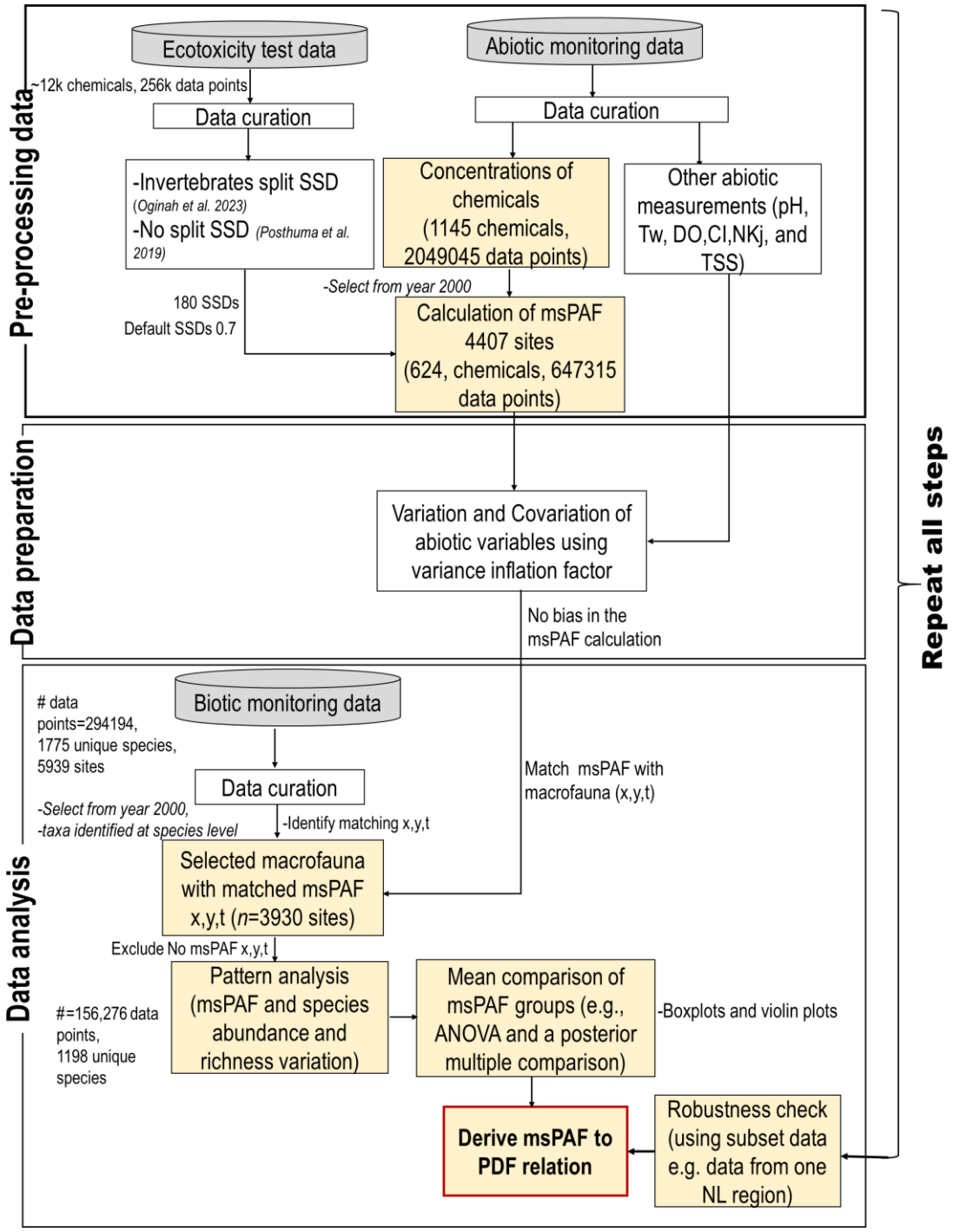

Figure 10. A step-by-step workflow of the data preparation and analysis procedures for deriving the msPAF to PDF relationship. The figure is taken from **Article IV.**

5.2 MONITORED DATA ON CHEMICALS AND A MIXTURE TOXIC PRESSURE IN THE NL REGION

Chemical concentrations were collected from 1983 to 2015 from 21 Dutch water authorities, out of which 18 water authorities were selected because of transparency in the reporting of the data collection results (**Article IV**). The dataset consisted of chemicals categorized as organic, inorganic, and heavy metals, which were included in the mixture toxic pressure calculation. All monitored chemicals were used in the assessments, such that msPAF-EC10 levels were considered to predict the fraction of species affected as the fraction of tested (i.e., non-adapted) species. In the field, some species may be adapted to the natural background concentration of non-synthetic chemicals such as heavy metals. In such cases, the observed effect in the field is smaller than the response of non-adapted species. In other words, the response (species loss or abundance change) would have been higher if none of the species could have adapted to the natural background concentrations. This phenomenon was not corrected because of a lack of site-specific information on natural background concentrations of non-synthetic chemicals.

Figure 11 shows that 2049045 data points from 1145 chemicals were initially measured across the NL region. Most measured chemicals in the field (n=238) had data points from 1-20. At the same time, 13 chemicals had more than 8,000 recorded data. After data curation, which included removing chemical concentration with a low detection limit and selecting sampling from 2000 to 2014, 647,315 data points remained for further analysis. To evaluate the combined toxic pressure of local unintended chemical mixtures, we calculated the combined toxic pressure of chemicals for each sampling site, considering the proportion available for uptake by species, utilizing transfer functions that estimate dissolved concentrations from measured values and water quality parameters (modifying factors). For specific compounds (PAF), we utilized individual chemical concentrations, SSD-shape parameters (σ and $m\mu$), and toxic mode of action (MoA). In cases where invertebrate SSDs were unavailable, an average sigma value of 0.7 was applied to account for species sensitivity variations.

The approach De Zwart & Posthuma (2005) proposed was used in **Article VI** to calculate PAF values per chemical per site before combining them into msPAF using a two-step approach. This approach considers concentration addition for chemicals with the same MoA and response addition for chemicals

with different MoA. Instead of compound concentrations, msPAF-EC10 values were used for subsequent analyses, enhancing the statistical power of the statistical pressure-response relationships by reducing the number of predictors.

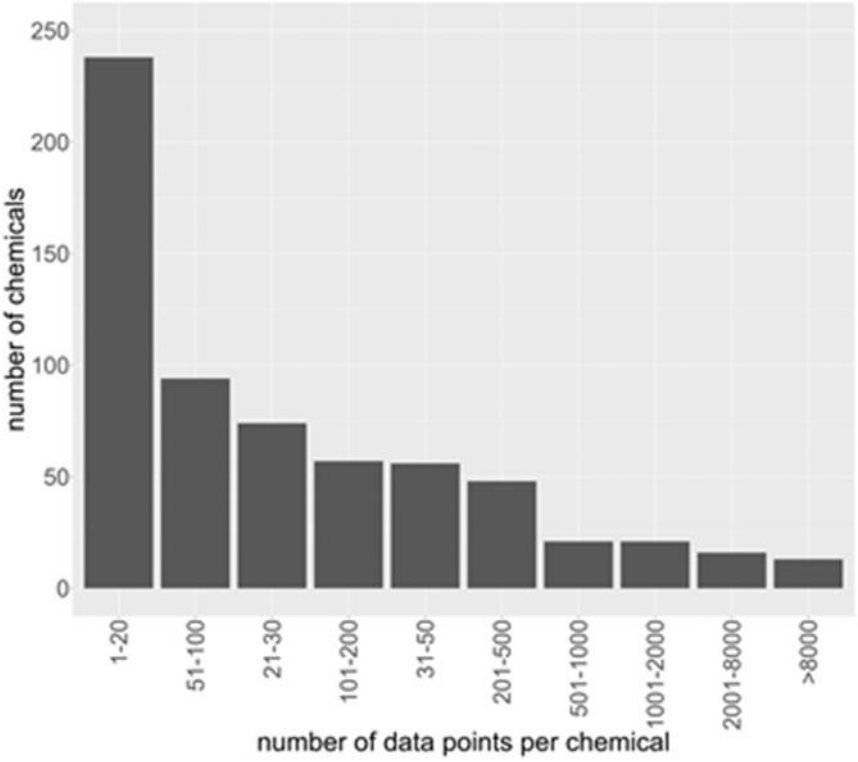

Figure 11. A summary of the total number of data points per chemical collected between 1983 and 2015 in the Netherlands' region (18 water authorities). Figure taken from **Article IV.**

Article VI highlights that the calculated msPAF-EC10eq ranged from 0 to 0.75 across 3632 sites, which has the predictive interpretation that the fraction of species that would be affected by ambient, unintended mixture exposures varies between none and 75% of the species showing an effect of 10% on a vital characteristic (such as growth and reproduction). Based on this, **Article IV** hypothesizes that this range of predicted effect level differences would relate to actual field effects, i.e., making it feasible to derive a PAF-to-PDF relationship. Note that the investigated relationship is, in fact, a msPAF-to-PDF relationship, as unintended mixture exposures characterize most sites. Note also that indirect effects may occur superimposed on the direct effects of chemical pollution, in the form of, e.g., altered predator-prey relationships or a limitation for sensitive species upon which opportunistic species may increase for part of the msPAF range.

Figure 12 suggests that the widest range of msPAF was recorded in the Delfland water authority, ranging between 0 to 75%, and lowest for the Schieland and Krimpenerwaard region <1%, indicating that the mixture toxic effect level also varies spatially.

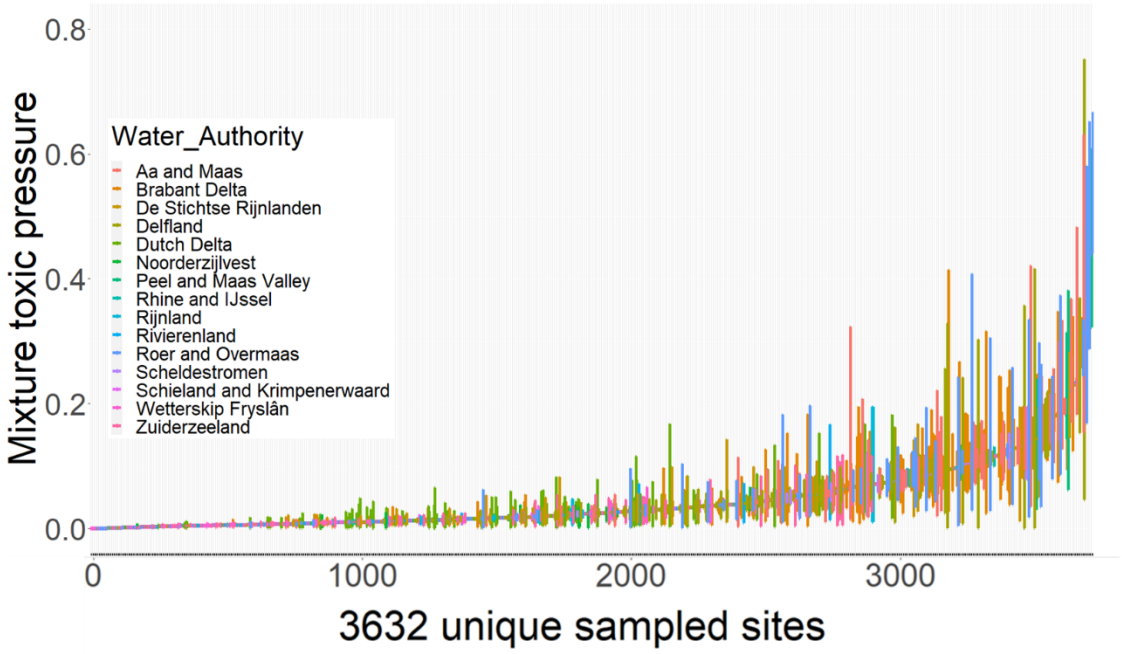

Figure 12. Shows the msPAF as a "dot" per XY-site (rank-ordered based on the msPAF values) and little to no variability of mixture toxic pressure in the data set (msPAF-EC10eq). The variation was based on the calculated msPAF values, averaging per chemical concentration measured across all years. Sampling per site occurred once on some sites and multiple times on some sites. The coloration corresponds to archetypes or data from different regional water authorities within the Netherlands. Figure taken from **Article IV.**

5.3 MONITORED DATA ON ABIOTIC PARAMETERS

In aquatic ecosystems, the impact of chemicals can be influenced by the concentration and toxicity mechanisms and surrounding environmental conditions, leading to multiple stressor effects or mixture toxicity effects (Posthuma, van Gils, et al., 2019).

Six abiotic predictors were analyzed to characterize the study sites (n= 231178) according to physical and chemical characteristics considered ecologically important in aquatic ecosystems (**Table 3**). The predictors were (1) Cl (chloride anions), a measure of water electrical conductivity, indicating the concentration of ions in the water; (2) DOC, a measure of the dissolved organic carbon; (3) NKj (Kjedahl nitrogen), refers to total nitrogen measured indicating the levels of the nutrient in the water, (4) TSS (total suspended solids); measures

the concentration of solid particles suspended in water influencing water clarity (e.g., sediment, organic matter), (5) Tw (temperature of the water); a measure of the temperature of the water and (6) pH; the level of acidity or alkalinity of the water. Mixture toxic pressure was added as the seventh predictor variable for further analysis.

Table 3 summarizes the physical and chemical water properties, which are possible explanatory variables on monitored species abundance and richness trends. The table is modified from **Article IV.**

#	Type	Predictor definition	Code	Units	Min	Max
1	Chemical	Conductivity index	Cl	µg/L	3.72	6.78
2	Chemical	Dissolved organic carbon	DOC	mg/L	0.43	1.74
3	Chemical	Total Nitrogen (N)	NKj	mg/L	0.13	5.15
4	Physical	Total suspended solids	TSS	mg/L	0.52	2.21
5	Physical	Temperature of the water	Tw	^{o}C	7.51	13.28
6	Chemical	pH(KCl extraction)	pH	-	4.78	9.38
7	Chemical	msPAFchronic-EC10	msPAF	% (0-100)	0	75

Studies have shown that abiotic factors, such as the water pH temperature, can synergistically interact with chemicals, increasing their toxicity, though the type of interaction varies depending on the specific chemical and species (Fischer et al., 2013; Posthuma et al., 2019).

5.4 MONITORED DATA ON SPECIES

Article IV highlights that 1,775 freshwater macroinvertebrates were initially collected in 1,754 unique monitoring locations in Netherlands surface water bodies (canals, rivers, brooks, ditches, ponds, lakes) from different regional water authorities (WA) in the Netherlands, known as water boards. Field experts collected samples from diverse local microhabitats to create a comprehensive sample, varying in vegetation, structure, soil properties, and water clarity. They employed a standard 20×30cm macrofauna net with a 0.5 mm mesh size to sample the water column and substrate. All captured macrofauna were systematically collected, sorted, preserved, and later identified at the finest taxonomic level in the laboratory (Beers et al., 2014; Hallmann &

Jongejans, 2021). However, only taxa identified at the species level were included in the analysis.

Specifically, as shown in **Figure 13**, 294194 data points on species information (rows of data) were collected between 1983 and 2014 by field experts from 18 water authorities in the NL region. Most species were from the Delfland water authority n= 45052; the lowest data was collected from the Rhine and IJssel water authority n= 1395. The total number of taxa collected in the entire region has generally decreased from 2010 onwards, with the most data collected in 2010, n=23577, and the least in 1991 (n=993). After data curation, 156,276 data points and 1198 species were included in the analysis.

Figure 13. The monitored macrofauna in 18 different regional water authorities in the Netherlands. Each subfigure illustrates a data pattern from a specific regional water authority

covering 1983 to 2014. The "n" values indicate the total number of available data records for each water authority throughout the years. Figure taken from **Article IV.**

5.5 VARIATION AND COVARIATION OF ABIOTIC FACTORS

The effects of abiotic factors on chemical mixture toxicity vary depending on the type of chemical, the specific organisms involved, and the environmental conditions (Sigmund et al., 2023). For example, pH changes can directly affect aquatic organisms' physiology and behavior, such that extreme pH levels make organisms more susceptible to the toxic effects of chemicals in their environment at the same time because chemicals exist in different ionized forms (e.g., as acids or bases) the ionized form may be more bioavailable or more toxic than the non-ionized form (Sigmund et al., 2023).

Table 4 summarizes the multiple linear regression outcomes, revealing that pH, TSS, Cl, and msPAF(EC10) substantially impact species richness. Moreover, a positive correlation is observed between pH, dissolved organic carbon, and water temperature, while an inverse association is evident between TSS, Cl, NKj, and msPAF concerning species richness.

Table 4. A summary of multiple linear regression analysis for abiotic factors (pH, Cl, DOC, TSS, NKj, Tw, and msPAF, representing water acidity/alkalinity, water electrical conductivity, dissolved organic carbon, total suspended solid particles, total nitrogen, water temperature, and msPAFchronic-EC10, respectively), influencing the richness biodiversity metric. The table is taken from **Article IV.**

Abiotic factors	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	29.2942	6.5871	4.447	0.0000
pH	7.7687	0.7768	10	0.0000
TSS	-20.7335	1.8044	-11.49	0.0000
Cl	-9.7388	0.7438	-13.092	0.0000
DOC	7.3168	1.994	3.669	0.0002
Tw	1.8949	0.5138	3.688	0.0002
NKj	-1.9299	0.5094	-3.789	0.0002
msPAF(EC10)	-31.0008	3.6988	-8.381	0.0000

Article IV indicates that covariation between msPAF and other pressures, if present, may bias the interpretation of the potential associations between toxic pressure and biological response metrics. Therefore, we evaluated collinearity

to exclude such bias in our conclusions. Variance Inflation Factors were quantified by correlating msPAF with the six abiotic factors to ascertain that the msPAF did not covary across the study area with other pressure factors because such covariation would imply a potential bias of the PAF to PDF relationship, i.e., PAF-to-PDF for other stressors. This was done by fitting a multiple regression model using species richness as the response variable and pH, TSS, Cl, DOC, Tw, NKj, and msPAF(EC10) as the predictor variables before calculating the VIF values as discussed by (Posthuma & de Zwart, 2012).

Article IV shows that the field data contained msPAF metrics sufficiently independent of other predictors, as all—the variation inflation factors were below 5. The variables displayed varying degrees of multicollinearity, with pH exhibiting the highest VIF at 1.91, CI at 1.61, DOC at 1.43, TSS at 1.35, NKj at 1.34, Tw 1.30, and msPAF (EC10) having the lowest VIF at 1.06 (**Figure 14**). Thus, **Article IV** concludes that the Variance Inflation Factor, as in earlier studies, indicated that msPAF remained independent of other stressors when considering toxic pressure and chemical/physical water properties (Posthuma & de Zwart, 2006; Posthuma & de Zwart, 2012). Therefore, the conclusions drawn from PAF-to-PDF patterns are not affected by the presence of the other stressors that were part of the research.

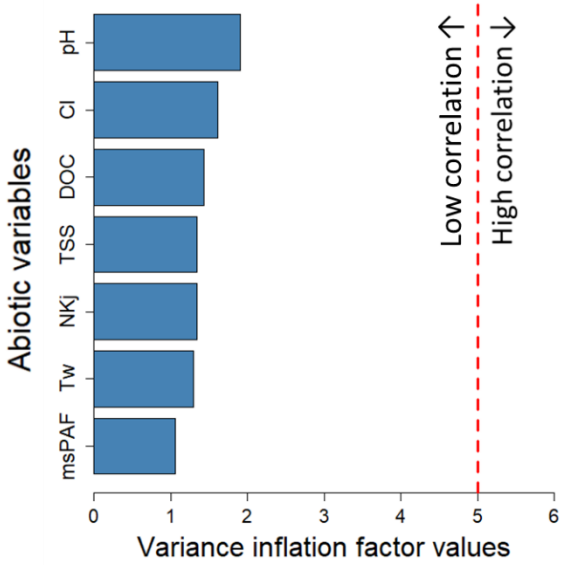

Figure 14. Shows the variation inflation values (VIF) between the msPAF and the six abiotic factors (pH, Cl, DOC, TSS, NKj, Tw, and msPAF, representing water acidity/alkalinity, water electrical conductivity, dissolved organic carbon, total suspended solid particles, total nitrogen, water temperature, and msPAFchronic-EC10, respectively), demonstrating that the influence of msPAF on species richness is uncorrelated. A VIF of 1 suggests no correlation

between the msPAF variable and other predictor variables, whereas a VIF exceeding 5 signifies a significant correlation among the predictor variables. Figure taken from **Article IV.**

5.6 TRENDS IN SPECIES DIVERSITY AGAINST msPAF GRADIENT

Considering the range of predicted msPAF values illustrated in **Figure 12**, we anticipated that unintentional combinations of chemicals would influence species abundance (varying by species) and overall species richness (as a combined metric) in distinct ways. **Article IV** highlights that patterns of macroinvertebrate distribution against the mixture toxic pressure in aquatic ecosystems effectively characterize the presence of impacts, which can be used to characterize the PAF-to-PDF relationship (Silva et al., 2022).

5.6.1 Species abundance distribution patterns

Article IV illustrates species-specific distribution patterns in **Figure 15** for 16 out of the 844 species selected with the most extensive data points. These subfigures reveal a general pattern in which the density of observed occurrences (represented by dots) decreases as msPAF values increase, indicating that toxic pressure is a limiting factor for species abundance. However, it is worth noting that some species exhibit a high abundance at moderate or higher values of X, suggesting that the working hypothesis does not consistently lead to a decline or neutral effect; there can be species where an increase in mixture toxic pressure leads to a neutral effect followed by a decline.

Species abundance distribution patterns summarize meaningful information on the impacts of pressures on ecosystems as they provide insights into alterations in the structure and dynamics of species communities. A more even distribution, where many species have similar abundances, may indicate a more stable community because it is less vulnerable to the loss of a single dominant species, and the species may be more resilient to environmental stressors, thus providing more stable ecosystem services and vice versa.

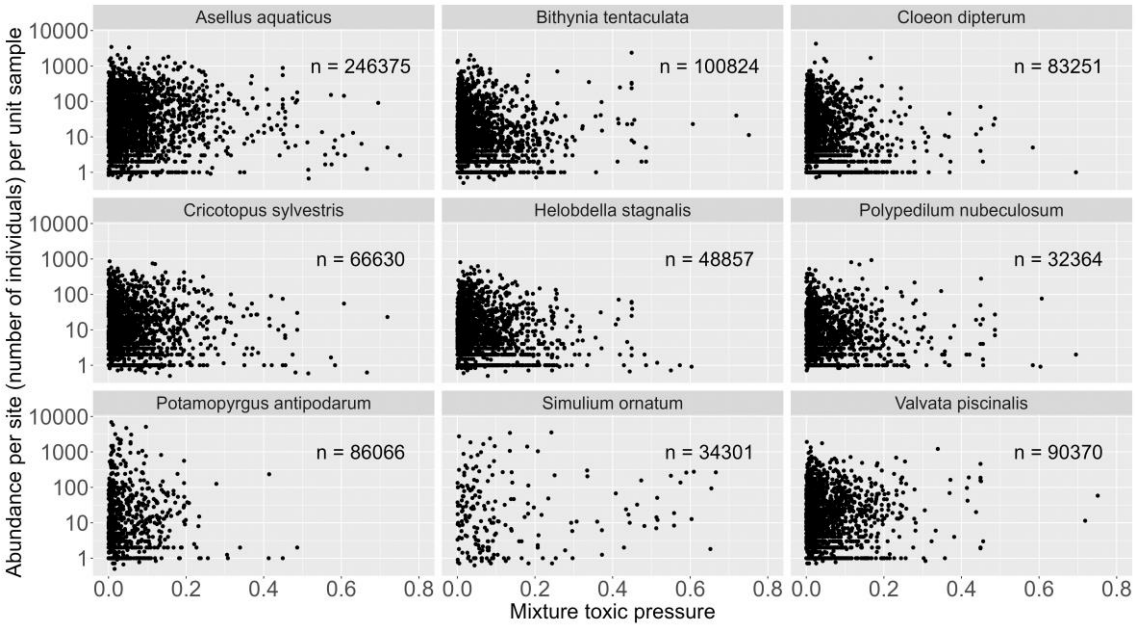

Figure 15. Illustrates the patterns of species-specific abundance observations (Y-axis represents a biodiversity metric) in relation to the increasing levels of msPAF-EC10eq (X-axis) for 16 selected species with the most data; "n" indicates the total number of individual species collected. Areas of the graph with high XY with less dense to no data points indicate toxic pressures as a limiting pressure to biodiversity. Each dot indicates a particular site where individual species were sampled. Figure taken from **Article IV.**

5.6.2 Species richness distribution patterns

Figure 16 compiles all the species abundance distribution data (as in **Figure 15**, but for all species) into a representation of the count of unique species found at each specific site. Similar to **Figure 15**, **Figure 16** lacks instances of high values for both High-X and High-Y, showing a reduced number of observed occurrences of species at increased mixture toxic pressure. However, no other discernible patterns were identified, except for the decrease in taxa diversity. This contrasts the various trends observed in the separate panels of species-specific abundance changes of the kind illustrated in **Figure 15**, where for some species, neutral or opportunistic responses were found (not shown in **Figure 16**). These findings emphasize the importance of examining abundance on a per-species basis because assessing richness alone as an aggregate response variable may obscure the three major abundance-change response types (decrease, neutral, opportunistic) to toxicity.

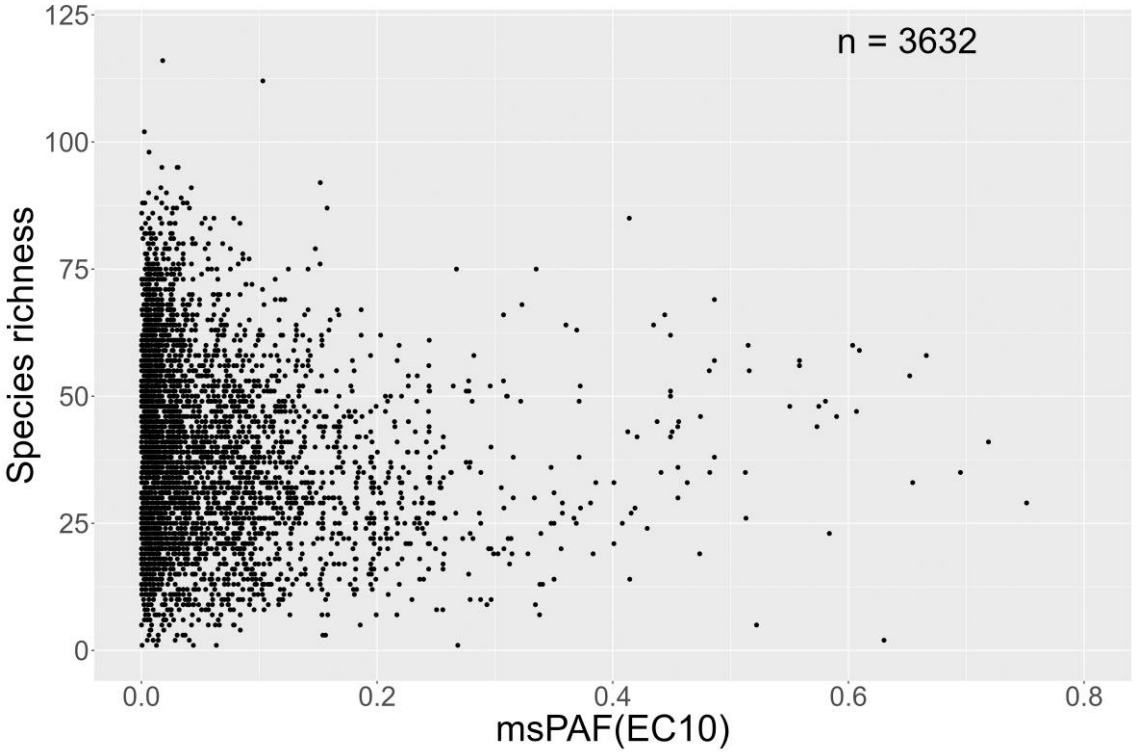

Figure 16. Illustration of the trend in species richness as msPAF levels increase. Similar to **Figure 15**, there is a notable absence of highX-highY values, highlighting that an increase in msPAF is a limiting factor for species richness. Each dot on the graph represents the number of unique species observed at a specific site (n=3632 sites). Figure taken from **Article IV.**

5.6.3 Species abundance pattern analysis statistical output

Species abundance data exhibited covariance with msPAF, resulting in some species increasing abundance, some remaining on average unaffected, and others showing sensitivity to changes for the range of pollution levels in the present study. In **Article IV,** species response patterns were categorized into three groups[7]. **Table 5** shows that out of the total 1098 species, 14 % of species (n= 151) did not occur beyond the protective criterion and were labeled "#1 most

[7] Three msPAF groups: Group 1 sites with msPAF 0 to 0.05, Group 2 sites with msPAF > 0.05-0.20 and Group 3 sites with msPAF > 0.2

sensitive"[8], while 40 % of species (n=437)[9] did not occur beyond 0.2 msPAF both potentially indicating sensitivity to the mixture toxic pressures and rarity. Conversely, 21 % of species (n=234)[10] showed abundance increased species abundance beyond 0.20 msPAF, with some increasing by over 50% (n=52). Only five species displayed a neutral (with abundance variability but no net decline) to mixture pressure. These patterns illustrate the varying influence of mixture pressure on different species, which is not visible with species richness as an aggregate response metric.

Table 5. Species abundance response pattern considering the protective criterion 0.05 and LCA working point of 0.2 in three groups, counting the number of species in each response category (related to the msPAF=0.05 protective criterion). The table is taken directly from **Article IV.**

#	Response category	Description	Count	%
1	Very incidental (1 site), minimal msPAF only	Species present in only Group 1 <=0.05 msPAF with one sample	45	4.1
2	Very incidental (2 sites), minimal msPAF only	Species present in only Group 1 <=0.05 msPAF with two samples	86	7.8
3	Incidental (2-10 sites), minimal msPAF only	Species present in only Group 1 <=0.05 msPAF with a maximum of 10 samples	17	1.5
4	Highly sensitive (>10 sites)	species present in only Group 1 <=0.05 msPAF with more than ten samples	3	0.3
5	Sensitive	species present in Group 1 and Group 2 >0.05 & <=0.2 msPAF	286	26.0
6	Relatively sensitive	species present in all the three msPAF groups and has more than 70 % change between group 3 and 1	41	3.7

[8] #1 most sensitive: Species categorised as very incidental (1 site), minimal msPAF only, Very incidental (2 sites), minimal msPAF only, Incidental (2-10 sites), minimal msPAF only, and Highly sensitive (>10 sites)

[9] Sum of species not present in Group 3: Response category from Very incidental (1 site), minimal msPAF only to sensitive

[10]Sum of all opportunistic species category response

#	Response category	Description	Count	%
7	Moderately sensitive	species present in all the three msPAF groups and has less than 70 % change between group 3 and 1	381	34.7
8	Neutral or neutral/variable	Species present in either two or all the three groups with 0 % change	5	0.5
9	Moderately opportunistic	species are present in all three msPAF groups and has less than 50 % change between group 3 and 1	182	16.6
10	Highly opportunistic	species present in all the three msPAF groups and has more than 50 % change between group 3 and 1	52	4.7
	Grand Total		1098	

5.6.4 Species richness pattern analysis statistical output

Species richness pattern analysis for the entire NL region followed a three-stage process for species richness patterns: the first stage utilized data for all species. In the second stage, species not initially classified in Group 1 (considered opportunistic species) were excluded. The final stage concentrated on the level of the protection criterion 0.05 and the relevant LCA working point of 0.20.

An overall pattern analysis of species loss using boxplots, violin plots, and statistical output (**Figure 17**, **Figure 18**, and **Figure 19**)[11] indicated decreased species richness as msPAF levels increased. In all figures, Group 1 represented areas with low toxic effects and high species richness, consistent with expectations for ecosystems under minimal toxic pressure. In the statistical analyses, the group 1 data are used as reference points for interpretations of significance and direction of change. In contrast to Group 1, Groups 2, 3, 4, and 5 in **Figure 17** and **Figure 18** represent sites with increasing mixture toxic pressure levels and whether impacts between msPAF data groups are significant. The figure

[11] **In Figure 17**: all the species are included in the analysis. In **Figure 18** opportunistic species are excluded; only species that are originally found in Group1 are included in the analysis and in **Figure 19** the level of protection criterion of 0.05 and LCA working point of 0.20 were considered for only species that are present from Group1

exhibits an evident decline in species richness, confirming the detrimental effects of toxic stress, from the Group with msPAF-values (msPAF groups) of 0.020-0.046. This observation aligns with previous studies (Posthuma & de Zwart, 2006; Posthuma & de Zwart, 2012). The patterns observed, with a continuous decline of an average number of species per Group, strongly suggest that mixtures have discernible impacts on various attributes of aquatic ecosystems, whether the abundance of taxa or overall species richness. Thus, assessing the effects of the entire chemical mixture, rather than evaluating each chemical individually, helps mitigate the challenges associated with the increasing complexity of data (often referred to as the "curse of dimensionality"); as the chemical variables are added, there is a corresponding reduction in statistical power (Posthuma, de Zwart et al., 2019).

In addition, **Article IV** highlights significant field species declines beyond 0.020 to 0.046 msPAF (Group 3) in **Figure 17** and **Figure 18**, suggesting that a significant decline (from on average 43.2 to 40.7 species between Group 1 and 3, i.e., 2.5%) in species diversity is initiated just below exposure level that was set to represent a protective standard regarding biodiversity loss in current regulations (defined as msPAF-NOEC=0.05), indicating the relevance of not exceeding the effect threshold if an ecosystem has to be truly protected against biodiversity loss due to chemical pollution.

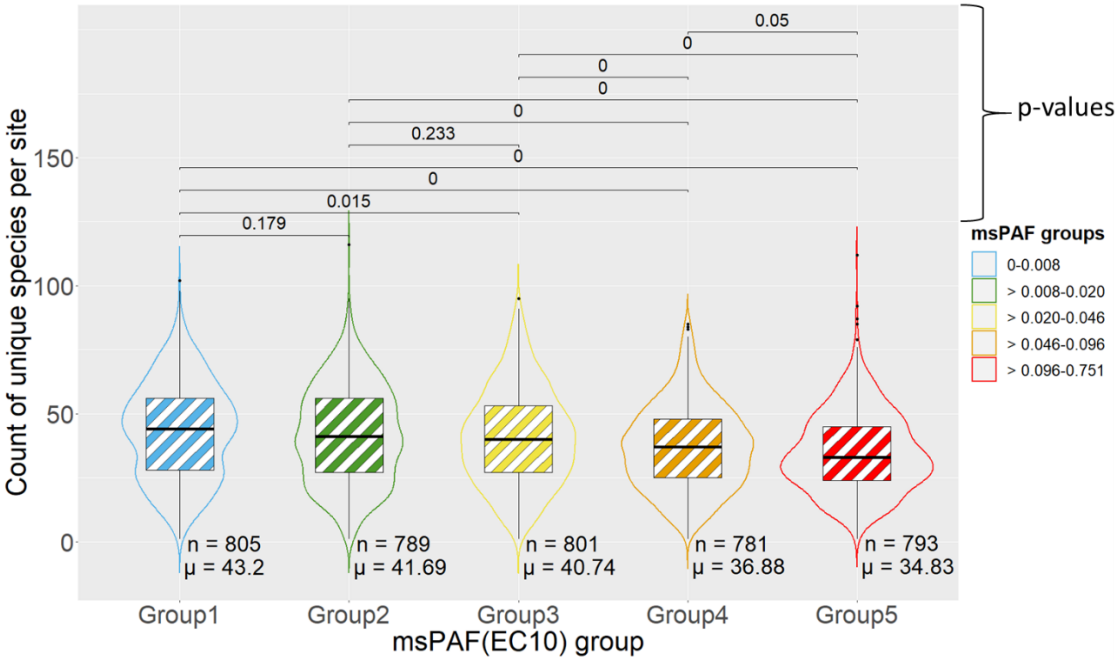

Figure 17. Box and violin plots illustrate species richness patterns for 1,198 species, msPAF categorized based on increasing toxic msPAF levels. Group 1 represents the low-toxic "pristine" region with minimal effects, while Groups 2, 3, 3, and 5 represent increasing and high

toxicity levels with significant species impacts, with p-values indicating the level of groups' significant differences in species numbers, "μ" represents the mean species count within each msPAF group, while "n" refers to the total number of sampled sites in each msPAF group. The legend shows the msPAF levels that are assigned to approx. equally-sized msPAF groups of data

In addition, **Article IV** identified 322 species absent from Group 1 but appearing at exposure conditions beyond "Group1" msPAF (sites with low toxic pressure), indicating the possibility of being opportunistic species. Such opportunistic species in Groups 2, 3, 4, and/or 5 can mask the effects of ecological disturbances or pollution (compared to Group 1) by thriving in altered environments. Thus, excluding opportunistic species provides a clearer picture of the true ecological changes when focusing on damage to the original (Group 1) fauna. A slight change in test outcomes was observed by **Article IV** in the average number of species for different groups when opportunistic species were removed (compare **Figure 17** and **Figure 18**). A significant effect again occurred onwards from Group 3 (in comparison to Group 1), with again a species loss from (on average) 43.3 to 40.4 species (i.e., 2.9%). On the other hand, changes observed for opportunistic species, regarding abundance or absence, provide additional and valuable insights into an ecosystem's health and quality; the appearance and abundance changes at higher levels of pollution pressure can serve as an early warning system for pollution (Olin et al., 2022).

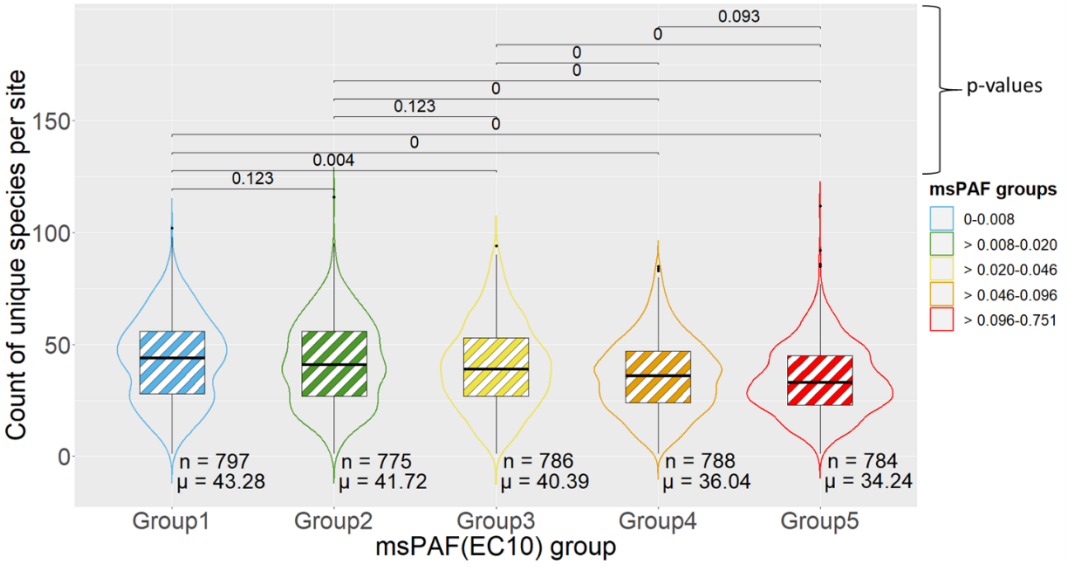

Figure 18. Box and violin plots depict species richness patterns for 876 selected species that were present initially from Group 1 (thus excluding opportunity species absent in Group 1), representing a low-toxicity region in the Netherlands, and Group 5, where high toxicity levels lead to pronounced species impacts. The plots reveal interquartile ranges and data spread,

while p-values at the top indicate significant group differences in species numbers. "μ" represents the mean species count within each msPAF group, while "n" refers to the total number of sampled sites in each msPAF group.

Findings from **Article IV** also resulted in data analyses whereby the raw data were interpreted in the context of two policy-relevant thresholds. These analyses indicate that in sites where the msPAF falls within the range of >0.05 to 0.20 (considering the regulatory protective criterion of 0.05 and the LCA working point to characterize damage of 0.20), the likelihood of finding approx. 41 species at a randomly selected clean ('protected') site drops to approx. 35 species at a site that is considered slightly polluted (0.05<msPAF<0.2), suggesting that even within this moderately affected range, approximately six species are lost, representing a 14% reduction in species richness in this exposure range (**Figure 19**). This finding is significant as it stresses the sensitivity of local ecosystems to variations in msPAF levels. Even at relatively low-to-moderate toxic stress levels, there is a measurable impact on species diversity. The relatively low mean further loss in the third group (msPAF >0.2) seems modest, but that is attributable to the relatively low number of observations in this group and the large spread in msPAF-values (the median msPAF of the third group is relatively close to 0.2).

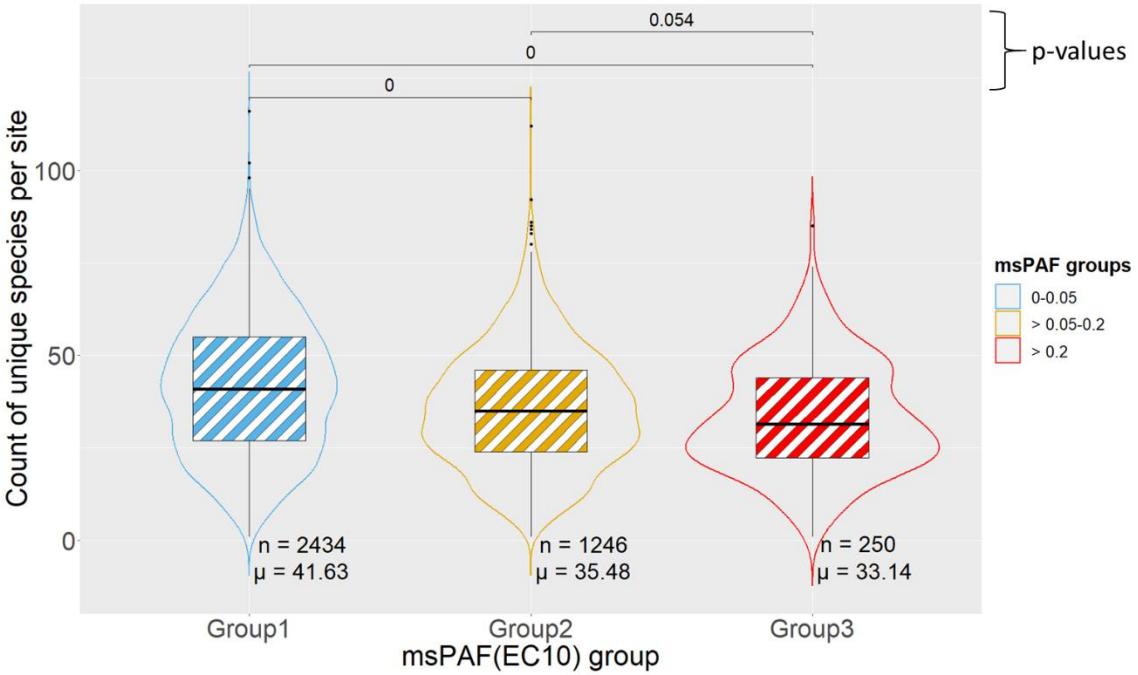

Figure 19. Box and violin plots for 876 species, based on a protective criterion 0.05 and an LCA working point of 0.20. Group 1 represents a pristine, low-toxicity NL region, while Group 3 represents areas with high toxicity, leading to noticeable species impacts. These plots show data distribution and significant group differences in species numbers indicated by p-values, "μ" represents the mean species count within each msPAF group, while "n" refers to the total number of sampled sites in each msPAF group.

5.7 ROBUSTNESS CHECK USING SUBSET DATA

5.7.1 Species abundance pattern analysis statistical output

Article IV presents findings from one region of the Netherlands (Delfland Water Authority) to check for consistency in the PAF-to-PDF relationship using data from 2002 to 2014. The species from the Delfland water authority displayed a range of species abundance response patterns, much like the broader NL region.

From the summary of the species response pattern (**Table 6**), 81 species classified as very incidental, incidental, or highly sensitive were not present beyond the protective threshold (0.05 msPAF)**.** In comparison, 54 % of species[12] declined beyond 0.2 msPAF from the Delfland region, suggesting sensitivity and potential rarity. Species that increased beyond 0.20 msPAF were classified as opportunistic, with some showing an increase exceeding 50% (n=16). Only six species exhibited resistance to mixture pressure. These patterns highlight the diverse impact of mixture pressure on different species.

Table 6. Species abundance response pattern considering the protective criterion 0.05 and LCA working point of 0.2 in three groups, counting the number of species in each response category (related to the msPAF=0.05 protective criterion). The table is taken directly from **Article IV.**

#	Response category	Description	Count	%
1	Very incidental (1 site), minimal msPAF only	Species present in only Group 1; <=0.05 msPAF	27	6.7
2	Very incidental (2 sites), minimal msPAF only	Species present in only Group 1; <=0.05 msPAF	43	10.6
3	Incidental (2-10 sites), minimal msPAF only	Species present in only Group 1 <=0.05 msPAF	9	2.2
4	Highly sensitive (>10 sites)	Species present in only Group 1 <=0.05 msPAF with more than ten samples	2	0.5
5	Sensitive	species present in Group 1 and Group 2; >0.05 & <=0.2 msPAF	138	34.2
6	Relatively sensitive	Species present in all three msPAF groups and has more than 70 % change between group 3 and 1	32	7.9
7	Moderately sensitive	Species present in all three msPAF groups and has less than 70 % change between group 3 and 1	94	23.3

[12] Species declined by 54%: This include species response categories not present beyond 0.2 msPAF (n=219)

#	Response category	Description	Count	%
8	Neutral	Species present in either two or all the three groups with 0 % change	6	1.5
9	Moderately opportunistic	Species present in all three msPAF groups and has less than 50 % change between group 3 and 1	37	9.2
10	Highly opportunistic	Species present in all three msPAF groups and has more than 50 % change between group 3 and 1	16	4.0
	Grand Total		404	

5.7.2 Species richness pattern analysis statistical output

Similarly to the entire NL species richness pattern analysis, the Delfland Water Authority's study followed a three-stage process for species richness patterns: the first stage utilized data for all species. In the second stage, species not initially classified in Group 1 (considered opportunistic species) were excluded. The final stage concentrated on the level of protection criterion of 0.05 and the relevant LCA working point of 0.20.

Box plots and violin plots depicted in **Figure 20** show species richness patterns among 434 species, categorized based on increasing toxic msPAF levels. This categorization provides valuable insights into the ecological effects of toxic stressors on the local invertebrate community in the specific Netherlands region. Note that the number of study sites in each msPAF group is substantially smaller than for the whole-NL data set, which implies that similar patterns will have a lower tendency to be also statistically significant (due to the effects of data numbers on statistical power).

Group 1, representing the areas with low toxic effects, exhibits relatively high species richness. This observation aligns with expectations that ecosystems with minimal toxic pressure tend to support a greater diversity of species. In contrast, Group 5, which signifies regions with high toxicity levels and substantial species impacts, shows a notable decline in species richness. This decline indicates the adverse effects of toxic stress on local biodiversity. The difference in species numbers among these groups is only statistically significant between Group 1 and Group 5, as shown by the p-values, and is on the border of significance for Group 4 ($p=0.06$), see **Figure 20**. The variation in species richness patterns across these five groups (again with a continuous de-

crease in the average number of species in each Group) underlines the importance of considering toxic msPAF levels when assessing ecological health and biodiversity in local ecosystems.

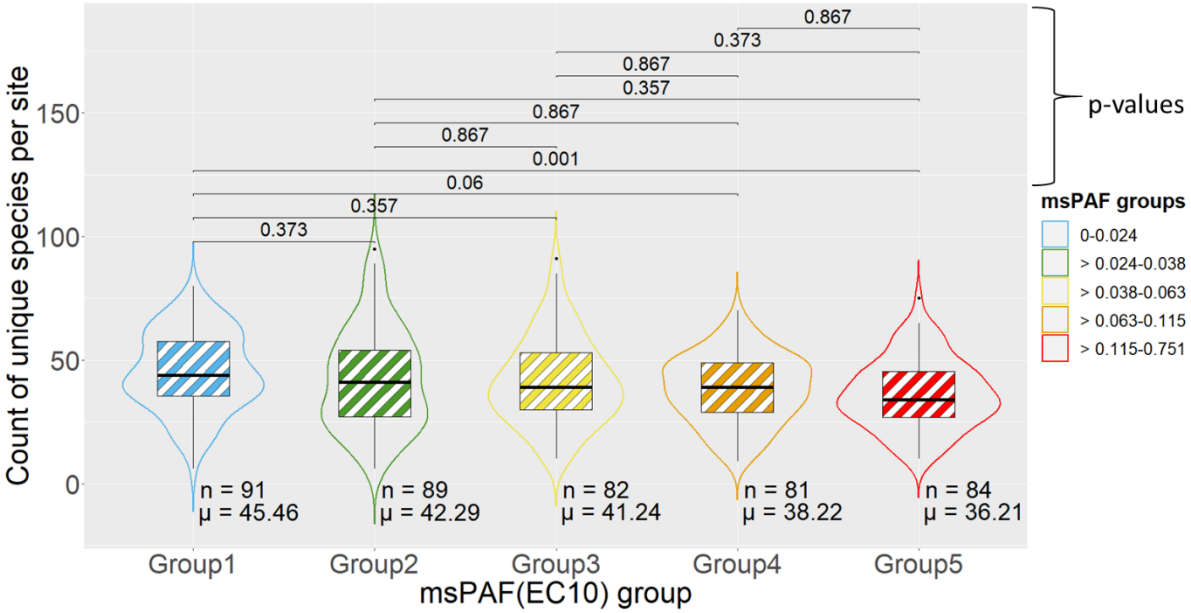

Figure 20. Box and violin plots illustrate species richness patterns among 434 species, msPAF categorized based on increasing toxic msPAF levels. Group 1 signifies a low-toxic “pristine" NL region with minimal effects, while Group 5 represents high toxicity levels with significant species impacts, with p-values indicating group differences in species numbers, "μ" represents the mean species count within each msPAF group, while "n" refers to the total number of sampled sites in each msPAF group.

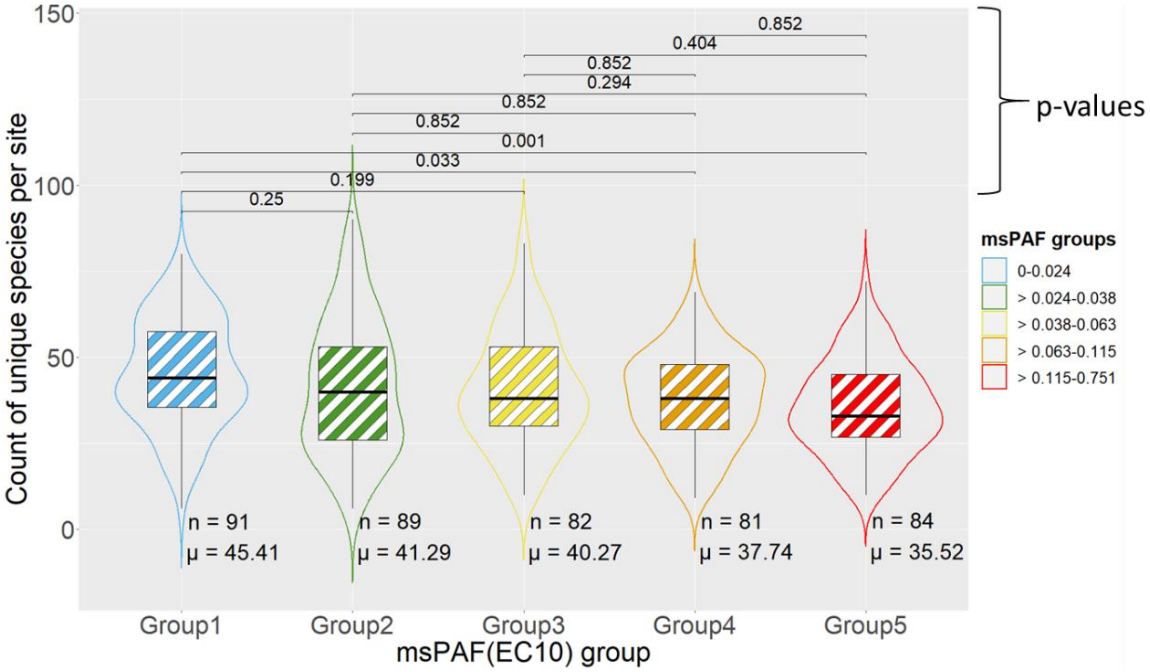

Figure 21. Box and violin plots depict species richness patterns for 404 selected species present initially from Group 1, representing a pristine, low-toxicity region in the Netherlands, and Group 5, where high toxicity levels lead to pronounced species impacts. The plots reveal interquartile ranges and data spread, while p-values at the top indicate significant group differences in species numbers, "μ" represents the mean species count within each msPAF group, while "n" refers to the total number of sampled sites in each msPAF group.

The finding from the selected NL region (Delfland region) by **Article IV** for the analyses that focus on the two regulatory thresholds (msPAF = 0.05 and msPAF=0.2) (**Figure 22**) resembles the findings of the similar analyses that were made for the entire NL region (**Figure 19**). On average, the study indicates that it is possible to randomly collect 37 out of the initial 43 species in the moderately polluted (0.05<msPAF,0.2) areas, implying that around six unique species are lost in this pollution msPAF group, constituting a 14% reduction in species richness when msPAF levels vary between 0.05 and 0. This 13.4% reduction in species richness indicates the ecological consequences of relatively low increasing msPAF levels. It further emphasizes the importance of adhering to established regulatory limits and implementing effective conservation strategies to safeguard ecosystems.

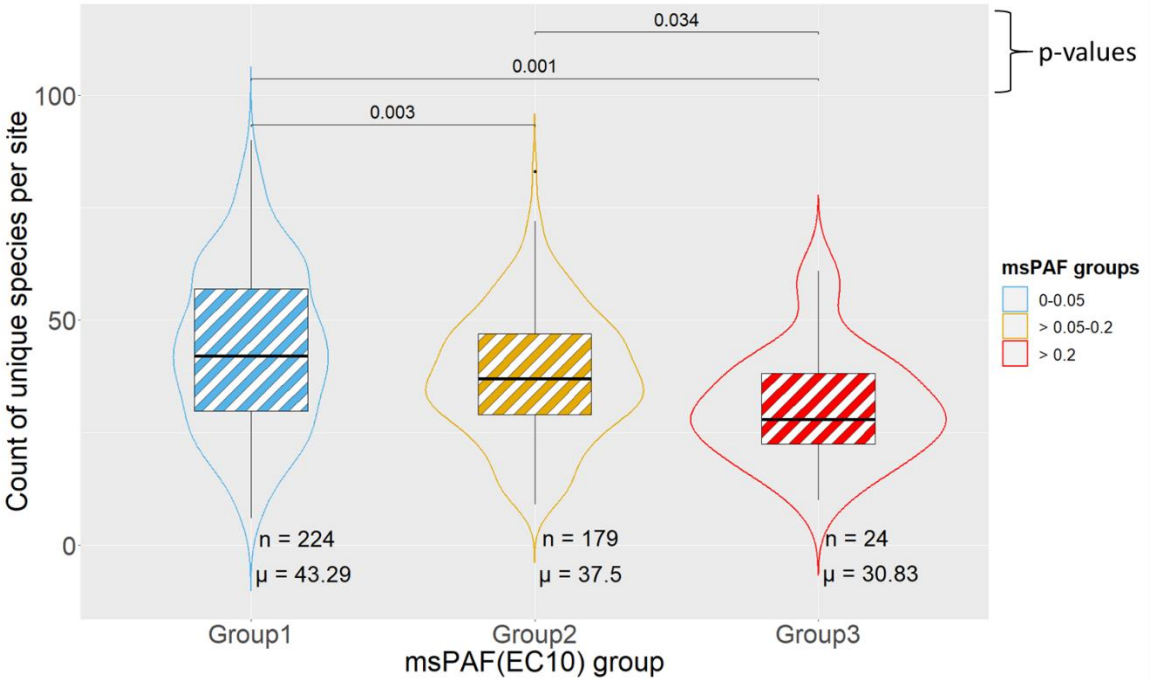

Figure 22. Box and violin plots for 404 species for only the Delfland region, based on a protective criterion of 0.05 and an LCA working point of 0.20. Group 1 represents a pristine, low-toxicity site, while Group 3 experiences high toxicity, leading to noticeable species impacts. These plots show data distribution and significant group differences in species numbers indicated by p-values, "μ" represents the mean species count within each msPAF group, while "n" refers to the total number of sampled sites in each msPAF group.

5.8 DERIVED msPAF TO PDF RELATIONSHIP

Article IV excluded species found in fewer than 10 locations from the analysis to establish a consistent relationship between PAF and PDF and to ensure that the analysis is based on a sufficiently representative sample of species, thus avoiding biases that might arise from very rare or localized species.

The statistical findings revealed that among the 735 species sampled in 10 or more sites across the entire NL region, 12% of these species (n=91) exhibited a significant change (sensitive and opportunistic responses aggregated) in abundance when the protective threshold of 0.05 msPAF was exceeded while absent in Group 3, a further 9% of the species showed such changes between Group 1 and Group 3. In contrast, 7% of the species showed significant changes when 0.2 msPAF was surpassed (change between Groups 2 and 3, as shown in **Table 7**). It is important to note that the statistical results are somewhat constrained by the limited number of samples available for each species, as the mean comparisons were conducted only for species within the three defined

groups. Indicating that a more definitive conclusion about the relationships between PAF and PDF can only be drawn from robust statistical results.

Table 7. Summarizes statistical results for species with significant abundance change in the NL region by comparing three groups that were categorized based on low msPAF (Group 1), 0.05<msPAF<0.2 (Group 2), and msPAF>0.2 (Group 3). It considers a protection criterion for msPAF of 0.05 and an LCA working point of 0.20 for species over 10 sites.

Species response groups	**Group1 vs Group2**	**Group1 vs Group3**	**Group2 vs Group3**
Very incidental (1 site), minimal msPAF only	0	0	0
Very incidental (2 sites), minimal msPAF only	0	0	0
Incidental (>2-10 sites), minimal msPAF only	0	0	0
Highly sensitive (>10 sites)	0	0	0
Sensitive	0	0	0
Relatively sensitive	3	8	3
Moderately sensitive	54	29	28
Neutral	0	0	0
Moderately opportunistic	28	21	15
Highly opportunistic	6	9	5
Total	91	67	51
Fraction significant from 735	12%	9%	7%

On the other hand, again focusing on the robustness check on one data-rich region, **Article IV** similarly showed that from the Delfland region, 9 % of species (n=19 Group 1→2). 5% (Group 1→Group 3) and 5% (Group 2→3) showed significant change when 0.05 msPAF and 0.20 msPAF were exceeded, respectively (**Table 8**).

Table 8. Summarizes statistical results for species responses with significant abundance change in the Delfland region, comparing three groups. It considers a protection criterion and an LCA working point of 0.20 for species over 10 sites.

Species response pattern groups	**Group1 vs Group2**	**Group1 vs Group3**	**Group2 vs Group3**
Very incidental (1 site), minimal msPAF only	0	0	0
Very incidental (2 sites), minimal msPAF only	0	0	0
Incidental (>2-10 sites), minimal msPAF only	0	0	0
Highly sensitive (>10 sites)	0	0	0
Sensitive	0	0	0
Relatively sensitive	6	4	0
Moderately sensitive	9	3	2
Neutral	0	0	0
Moderately opportunistic	3	3	4

Highly opportunistic	1	2	4
Total	19	12	10
Fraction significant from 221	9%	5%	5%

The species richness analysis for the entire NL region, focusing on species occurring in over 10 sites, demonstrated a statistically significant change. The results reveal that different msPAF thresholds notably impact species richness. When the threshold of 0.05 msPAF is exceeded, there is an average reduction of 15% in species. This means that, on average, 6 species are lost from the region under these conditions. When the threshold is more stringent at 0.2 msPAF, the reduction in species richness increases to 20%, resulting in an average loss of approximately 8 species. It is important to note that these reductions are described as statistically significant. This indicates that the observed changes in species richness are not likely due to random chance but are associated with the changes in mixture toxic pressure (**Figure 23**).

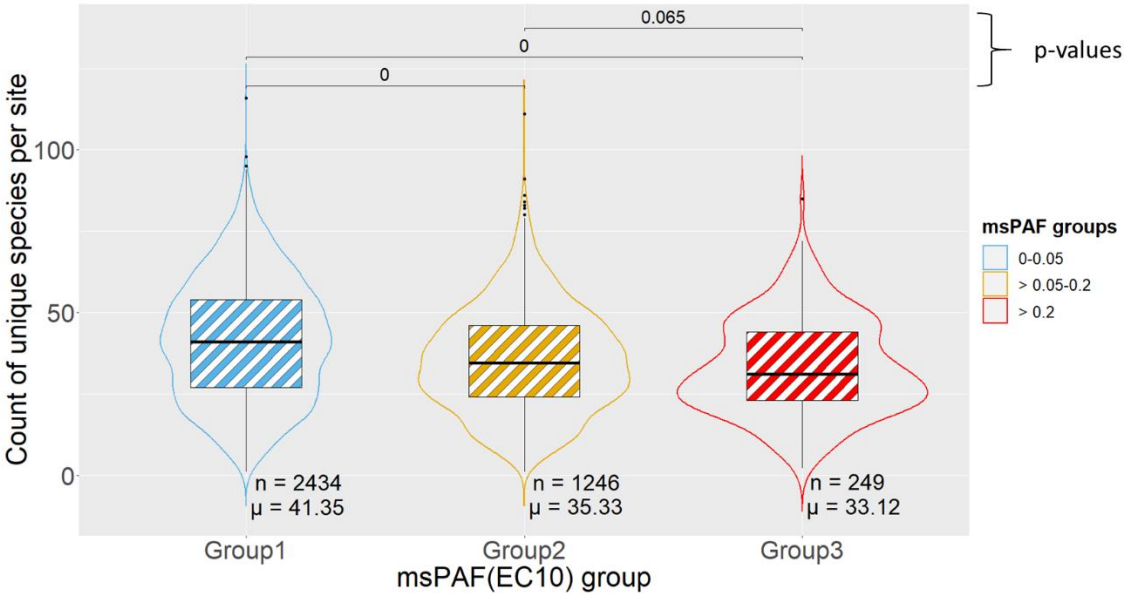

Figure 23. Box and violin plots for 735 species occurring in 10 or more sites across the Netherlands, based on a protective criterion 0.05 and an LCA working point of 0.20. Group 1 represents a pristine, low-toxicity NL region, while Group 3 experiences high toxicity, leading to noticeable species impacts. These plots show data distribution and significant group differences in species numbers indicated by p-values, "μ" represents the mean species count within each msPAF group, while "n" refers to the total number of sampled sites in each msPAF group.

In the Delfland region, the results indicate a statistically significant change in species richness. When PAF ranges from >0.05 to 0.20 msPAF, there is an

average reduction of 11% in species richness, resulting in an average loss of about 5 species. Furthermore, when the threshold of 0.2 msPAF is exceeded, the reduction in species richness increases to 17%[13], leading to an average loss of approximately 7 species (**Figure 24**).

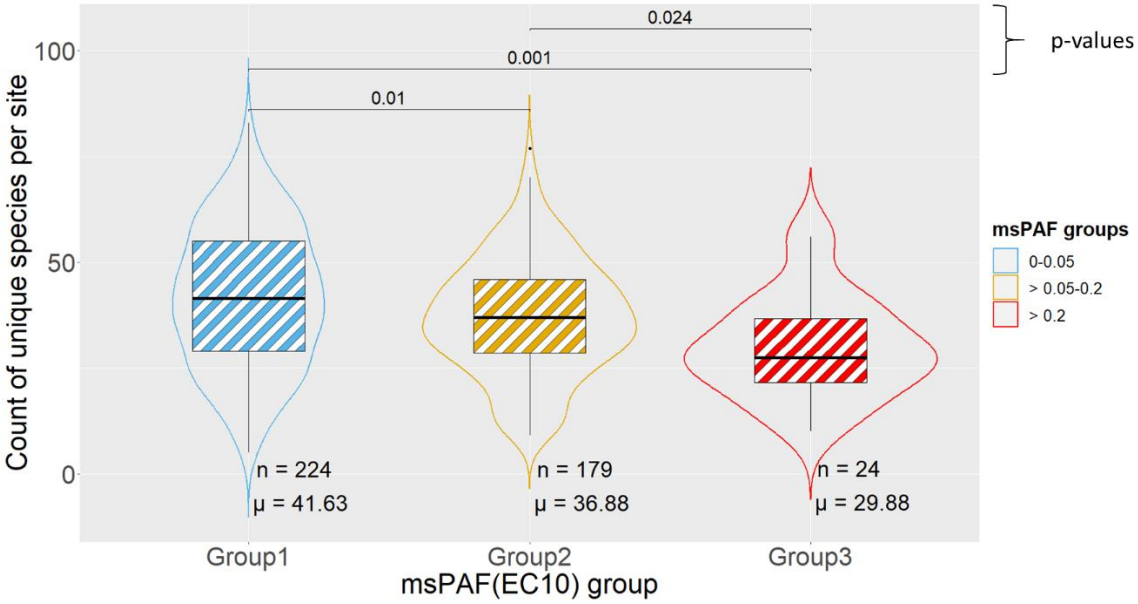

Figure 24. Box and violin plots for 221 species occurring in 10 or more sites for the Delfland region only, based on a protective criterion 0.05 and an LCA working point of 0.20. Group 1 represents a pristine, low toxicity, while Group 3 experiences high toxicity, leading to noticeable species impacts. These plots show data distribution and significant group differences in species numbers indicated by p-values, "μ" represents the mean species count within each msPAF group, while "n" refers to the total number of sampled sites in each msPAF group.

Article IV derived the final msPAF-to-PDF link using the count of unique species having a minimum of 10 data points (**Figure 23**), applying the equation below, which involved deriving the quantification of the observed fraction of species lost per magnitude of change in msPAF, which can be determined now for two violin-plot based observations and subsequently (by interpolation) to

[13] 17 % reduction: Percentage differences between group 3 and 1

derive the Potentially Disappeared Fraction of species at a site as a consequence of emissions of chemicals, if the latter is predicted in LCA at a working point of 0.2.

The equation to derive the change in PDF (delta PDF) per change in msPAF (delta msPAF) is as follows:

$$\Delta\text{PDF} = 1 - (\text{PDF}_{\text{final}}/\text{PDF}_{\text{initial}}) \quad \text{(eq. 1)}$$

$$\frac{\Delta\text{PDF}}{\Delta\text{msPAF}} = \frac{\text{PDF}_{\text{final}} - \text{PDF}_{\text{initial}}}{\text{msPAF}_{\text{final}} - \text{msPAF}_{\text{initial}}} \quad \text{(eq. 2)}$$

Below, we illustrate how we derived PDF/msPAF for the entire Netherlands region using the average species count in each msPAF group (**Figure 23**), with the msPAF median of 0.013, 0.089, and 0.267 at Group 1 (<=0.05 msPAF), Group 2 (>0.05 to 0.20 msPAF) and Group 3 (>0.2 msPAF) respectively.

Point #1 (group 1 to group 2):

$$\frac{41.35}{0.013} - \frac{35.33}{0.089} = \frac{0.145}{0.076} = 1.92 \text{ PDF/msPAF}$$

Point #2 (group 1 to group 3):

$$\frac{41.35}{0.013} - \frac{33.12}{0.267} = \frac{0.199}{0.254} = 0.783 \text{ PDF/msPAF}$$

Next, using the two PDF/msPAF points (1.92 and 0.783) as the y-axis with their respective delta msPAF (0.076 and 0.254) as the x-axis, an interpolation was done using linear regression to predict the PDF change at 0.2 msPAF. The change in PDF/msPAF at 0.2 was interpolated to 1.127 (**Figure 25**). Following the same procedure, we interpolate the change in PDF/msPAF at 0.2 using **Figure 24**, using data from the Delfland region as a robustness check; the interpolated PAF-to-PDF value was found to be 1.298 (**Figure 25**).

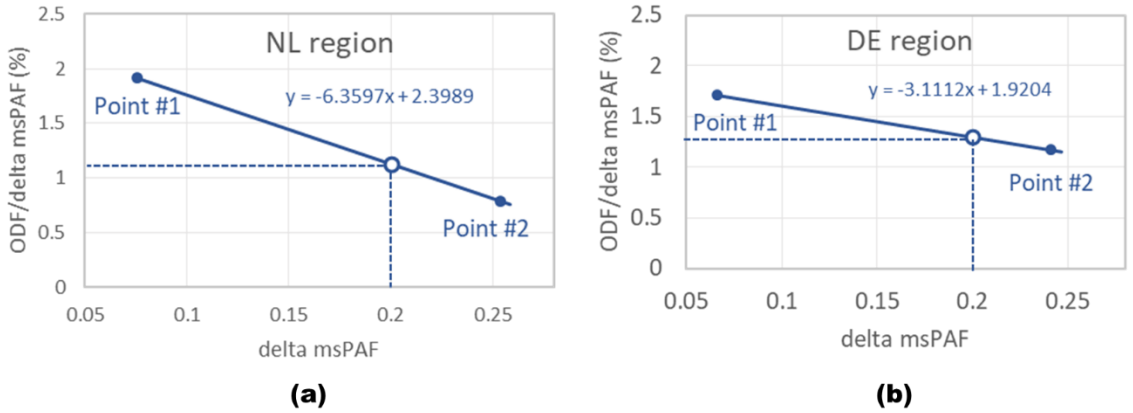

Figure 25. (a) Illustration of the observed fraction of disappeared (ODF) species in field-exposed species assemblages of aquatic invertebrates lost (Y) per unit change of mixture toxic pressure (X), based on two comparisons (as in **Figure 23**), namely between Group 1 and 3, and Group 1 and 2. The Potentially Disappeared Fraction per unit of PAF (and msPAF) is interpolated from the observations at the working point X=0.2. (a) data for the whole of NL: PAF-to-PDF is 1.127, and (b) data for the DE region (Defland region) PAF-to-PDF is 1.298. Figure taken from **Article IV.**

The comprehensive monitoring data in the Netherlands sheds light on the impact of chemical mixture toxic pressure on invertebrates across various locations. **Article IV** emphasizes the pivotal role of a mixture toxic pressure in shaping invertebrate distribution independently of other environmental factors, ensuring unbiased msPAF-to-PDF patterns. Pattern analysis by **Article IV** also provides valuable insights into characterizing predicted impacts and species disappearances. Given the data of **Figure 23** and **Figure 24**, the PAF-to-PDF association is characterized by interpolation as in **Figure 25** and interpolated as 1.127 (one unit change of msPAF implies 1.127% species loss).

In the past, scholars conducted studies on the PAF-to-PDF relationship, where they observed that the ratio of HC50-EC50 to PDF was approximately 1:1 (Posthuma & de Zwart, 2012), similar to this study. At first glance, this may seem strange, but it is not surprising upon closer examination, even though we initially expected some variation. This similarity can be explained as follows: In the previous studies on HC50-EC50, the impact on the environment was assessed by counting net species loss without distinguishing "group 1 species." This means species present in group-clean were counted in all groups and scored when lost, regardless of the group in which the loss occurred. This method focused on assessing the loss of "typical species present in reference conditions and lost due to pressure toxicity." It did not consider the total change

in all species across different groups compared to the total number of species in group 1. For instance, if 10 species were lost and 8 new species appeared, the "old" way of counting species loss resulted in a count of 2 (in the HC50-EC50 case). This approach led to an underestimate of the actual species loss. In the present case, we now count the species loss as "10" in this example. The nature of the effects being examined causes the current 1:1 relationship to resemble the old one.

6 CONCLUSIONS & RECOMMENDATIONS

6.1 PHD PROJECT OUTCOMES

This PhD project contributed to the field of life cycle impact assessment and ecological risk assessment. The proposed frameworks and results advance the understanding of the role of chemical pollution on biodiversity loss and enhance decision-support in environmental protection, LCA, and freshwater ecosystem management on a global scale. This thesis addressed the PhD's four defined research objectives and summarized the results obtained in the preceding chapters.

By addressing the first objective[14], the present thesis develops an overall consistent framework for linking chemical effects on aquatic organisms to damage on species diversity, functional diversity, and ecosystem services (ES) within the boundary conditions of LCA. **Article I** emphasizes the transition from early, complex methods to more practical and biologically realistic models, such as TITAN, to assess the effects of ecotoxicity on species diversity and combine various functional diversity components to translate species loss into damage to functional diversity. In addition, **Article I** suggests various models and frameworks, such as Ecological Production Functions and the cascade model, as tools to assess the consequences of alterations in the structure and functioning of ecosystems on ecosystem services. **Article I** stresses the significance of data-driven insights and integrated approaches in assessing and managing ecosystem services.

Article II developed an extensive ecotoxicity database (>250k entries) using semi-automated methods to harmonize the taxonomy and chemical information to support the need for comprehensive data by addressing the second research objective[15] of this PhD project. A high-quality freshwater ecotoxicity test data

[14] First research objective: To develop a consistent framework to link ecotoxicological effects on aquatic organisms to damage on species diversity, functional diversity, and ecosystem services that are fully in line with the boundary conditions of LCIA

[15] The second objective of this study: To develop a systematic ecotoxicity test data curation approach to derive a transparent and high-quality dataset of effect test data for more than 10,000 chemicals

was further selected in a case study to improve ecotoxicological impact assessment methods based on Species Sensitivity Distribution modeling, addressing the third objective[16] of this PhD project. **Article III** provides insights into the creation of split SSDs for 180 selected chemicals through the developed decision tree, showing the importance of high-quality data, chemical modes of action, and robust statistical analysis to assess chemical impacts on different taxonomic groups (i.e., vertebrates, invertebrates, and algae, cyanobacteria, and aquatic plants) except for microorganisms due to limited data. In addition, **Article III** demonstrates that employing split SSDs enhances the interpretability of assessment outputs, notably by lowering the Hazardous Concentrations, i.e., HC5 of the most sensitive taxonomic group, a vital consideration for setting regulatory criteria like the Predicted No Effect Concentration (PNEC) and HC20 the impact metric employed in the Life Cycle Impact Assessment (LCIA).

Using the gained data and insights in validating predicted impacts, **Article IV** addresses the fourth objective[17] of this study by developing a tiered approach for quantitatively relating chemical mixture toxic pressure to monitoring-based aquatic species occurrence to derive insights into chemical pollution's contribution to damage on species loss. Field observations by **Article IV** demonstrate that exceeding protective standards leads to abundance and biodiversity decline, emphasizing the importance of staying below the effect threshold for ecosystem protection. This stresses the need to balance environmental protection and human activities to ensure the long-term sustainability of natural systems. The data assessed in **Article IV** offer valuable insights into the alignment between predicted impacts using the toxic pressure metric and actual taxa loss in field conditions at 0.2 msPAF (affecting 20% of species).

The extensive analysis of Dutch monitoring data conducted by **Article IV** revealed significant patterns in species assemblages exposed to pollutants. Many

[16] The third objective of this study: To improve ecotoxicity effects modelling by considering differences in sensitivity of species from different taxonomic groups toward chemical exposure

[17] The fourth objective of this study: To quantitatively characterize the relationship between mixture-toxicity pressure from chemicals and observed differences in aquatic intra- and inter-species occurrence

species exhibit changes in their abundance, summarized using aggregated species count metrics. These metrics consider both species occurring in Group 1 and those responding negatively. The data analysis has led to observing a wide range of response patterns. These patterns can be transformed into a PAF-to-PDF association through interpolation, which is essential for Life Cycle Assessment (LCA) ecotoxicity assessments. At the specific LCA working point selected, there is a practical relationship where the fraction of species lost per unit increase in msPAF can be approximately rounded to a 1:1 ratio. This means that for every unit change in msPAF, one species has an associated loss (both on a fractional scale).

7 LIMITATIONS & FUTURE PERSPECTIVES

This PhD project highlighted several limitations:

- **Article I** highlights limitations in the proposed conceptual framework for translating organism-level effects to ES damage, including data constraints, model uncertainty, and the need for robust models that consider sensitivity in species differences. For instance, TITAN requires extensive data available for only specific areas, pressure sets, and taxonomic groups, limiting its broader applicability. Additionally, insufficient data for functional diversity endpoints hinders the development of a comprehensive framework. Models considering multiple populations or food webs introduce uncertainty due to extrapolation to higher biological organizations, potentially impacting result reliability. Developing reliable models to link chemical-induced changes in key service-providing units to damage on ES remains challenging.
- In order to build one global aquatic database merging all available databases, **Article II** highlights the limitations arising from differences in toxicity values, test conditions, species choices, and other experimental design variations that can impact the database data usability.
- **Article III** highlights that building full split sensitivity distributions for taxonomic groups to gain insights into differences in species sensitivity requires comprehensive measured data for all relevant taxonomic groups, including currently limited microorganisms test data. Most importantly, low-quality or limited data can significantly reduce SSDs' accuracy and reliability.
- Ideally, to establish a msPAF-to-PDF relationship using biomonitoring data, comprehensive data that covers changes over time for all relevant species and chemical concentrations in an aquatic ecosystem, measured at the same time and place, is required. However, such comprehensive data is currently lacking. Thus, many data points were excluded as they did not have matching geospatial coordinates and timestamps. This departure from uniform spatiotemporal attributes is not common in many monitoring datasets, as they were not originally designed to meet the specific requirements of this study. Another limitation highlighted by **Article IV** is the lack of distinction between natural and human-caused sources of contaminants, which is essential for a more precise evaluation of environmental risks from chemical mixtures.

Based on the knowledge and experience gained during this PhD project, future research should focus on the following recommended aspects:

- While the developed framework in **Article I** offers valuable insights and approaches for addressing ES in Life Cycle Assessment, the practical implementation and standardization of the models, such as the cascade model, require further development. A notable constraint in the complete adoption of the ecosystem services (ES) framework within Life Cycle Assessment (LCA) lies in the insufficient availability of data to effectively translate ecotoxicity effects to damage on freshwater ecosystem services, encompassing both structural and functional diversity while also addressing the complexities of multiple causal chains and the impact of distinct taxonomic groups and functional endpoints. Additionally, there is a need to develop comprehensive frameworks and tools that combine knowledge of ecosystem service monitoring and chemical stressor assessment at different spatial and temporal scales, which remains a challenge in Life Cycle Assessment.
- Data completeness and accuracy are crucial for deriving meaningful conclusions about chemical impacts on various taxonomic groups. The developed decision tree to improve ecotoxicity assessment output by splitting SSDs by **Article III** needs to cover other taxonomic groups like microorganisms for holistic assessment of chemical impacts. Thus, efforts should be made to include more data on microorganisms in laboratory tests. This expansion would provide a more comprehensive understanding of chemical effects on these critical components of freshwater ecosystems.
- As much as **Article III** focused on species-level response, focusing on species' traits that govern their susceptibility and resilience to stressors provides additional information in identifying impacts on biodiversity loss.
- **Article IV** assumed that metal concentrations in the NL region had shown a consistent, unchanging pattern with negligible sources of variation and that the local species have adapted to the inherent background levels of metals. The primary objective of the research was to explore the potential consequences of chemical mixtures, which included metals, without delving into explicitly addressing contaminants sources. Thus, future studies need to differentiate between natural and anthropogenic sources of contaminants to obtain a more accurate assessment of potential environmental risks and impacts of chemical mixtures.

- The dynamics of biotic factors, such as predation, can also strongly influence the effects of anthropogenic chemical contaminants in natural systems and abiotic factors that **Article IV** did not consider**.**

Despite the limitations in the developed approaches, such as data availability while translating ecotoxicity effects into ecosystem service damage, this thesis serves as a valuable starting point for linking ecotoxicity effects to ecosystem damage on a global scale. We curated and harmonized a large aquatic ecotoxicity dataset with 255k data points to address the lack of comprehensive data. We also developed a splitting species sensitivity distribution approach with broad implications regardless of data, models, or decision context, which addresses the challenge of having robust models that recognize differences in species sensitivity while quantifying species-level ecotoxicity effects. Splitting species sensitivity distribution approach improved the interpretation of assessment outputs, in which the outcome was used to quantitatively characterize the link between the mixture toxic pressure and biodiversity loss, revealing that for every unit change in msPAF, there is an associated more or less equal loss of species fraction. Thus, the PAF-to-PDF relationship from this PhD study can be summarised as a rounded 1:1 association.

The next step to translate structural biodiversity loss (species richness) into ecosystem functioning and ecosystem service damage will entail using insights from this PhD study, which established that a unit increase in msPAF corresponds to a 1.127% species loss. This requires identifying the functions of lost species in aquatic ecosystems, understanding their impact on ecosystem functioning using proposed approaches such as trait probability density framework, and further linking this to the damage on the flow of ecosystem services using methods like Ecological Production Functions.

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APPENDIX: ARTICLES

I Oginah, Susan A, Posthuma, L., Maltby, L., Hauschild, M., & Fantke, P. (2023). Linking freshwater ecotoxicity to damage on ecosystem services in life cycle assessment. Environment International, 171, 107705. https://doi.org/10.1016/j.envint.2022.107705

III Oginah, Susan Anyango, Posthuma, L., Hauschild, M., Slootweg, J., Kosnik, M., & Fantke, P. (2023). To Split or Not to Split: Characterizing Chemical Pollution Impacts in Aquatic Ecosystems with Species Sensitivity Distributions for Specific Taxonomic Groups. Environmental Science & Technology, 1–13. https://doi.org/10.1021/acs.est.3c04968

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Review article

Linking freshwater ecotoxicity to damage on ecosystem services in life cycle assessment

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ABSTRACT

Freshwater ecosystems provide major benefits to human wellbeing—so-called ecosystem services (ES)—but are currently threatened among others by ecotoxicological pressure from chemicals reaching the environment. There is an increased motivation to incorporate ES in quantification tools that support decision-making, such as life cycle assessment (LCA). However, mechanistic models and frameworks that can systematically translate ecotoxicity effect data from chemical tests into eventual damage on species diversity, functional diversity, and ES in the field are still missing. While current approaches focus on translating predicted ecotoxicity impacts to damage in terms of species loss, no approaches are available in LCA and other comparative assessment frameworks for linking ecotoxicity to damage on ecosystem functioning or ES.

To overcome this challenge, we propose a way forward based on evaluating available approaches to characterize damage of chemical pollution on freshwater ES. We first outline an overall framework for linking freshwater ecotoxicity effects to damage on related ES in compliance with the boundary conditions of quantitative, comparative assessments. Second, within the proposed framework, we present possible approaches for stepwise linking ecotoxicity effects to species loss, functional diversity loss, and damage on ES. Finally, we discuss strengths, limitations, and data availability of possible approaches for each step.

Although most approaches for directly deriving damage on ES from either species loss or damage to functional diversity have not been operationalized, there are some promising ways forward. The Threshold Indicator Taxa ANalysis (TITAN) seems suitable to translate predicted ecotoxicity effects to a metric of quantitative damage on species diversity. A Trait Probability Density Framework (TPD) approach that incorporates various functional diversity components and functional groups could be adapted to link species loss to functional diversity loss. An Ecological Production Function (EPF) approach seems most promising for further linking functional diversity loss to damage on ES flows for human wellbeing. However, in order to integrate the entire pathway from predicted freshwater ecotoxicity to damage on ES into LCA and other comparative frameworks, the approaches adopted for each step need to be harmonized in terms of assumptions, boundary conditions and consistent interfaces with each other.

1. Introduction

Aquatic ecosystems provide essential benefits to our global society and human wellbeing (UNEP, 2017). These benefits are collectively known as ecosystem services (ES) (Awuah et al., 2020; Faber et al., 2019). Obvious ES that are provided by freshwater ecosystems mainly relate to the provisioning of food and drinking water, cultural services, recreational fishing, and ecotourism (Banerjee et al., 2013; Syberg et al., 2017; UNEP, 2017). Other benefits, such as maintaining habitat quality, water quality regulation through organic matter degradation and toxicant removal, and nutrient recycling, are less obvious yet essential for a sustainable development (UNEP, 2017).

Despite these benefits, freshwater ecosystems face continuously increasing pressures from human activities, such as pollution from

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chemicals emitted along product life cycles (Syberg et al., 2017; Carney Almroth et al. 2022; Kosnik et al., 2022; Persson et al., 2022), which interfere with species diversity and the ecosystem functions depending on those (Awuah et al., 2020), both of which are essential for providing ES. More specifically, chemical pollution from human activities and its pressure on aquatic ecosystems has been listed as a driving factor limiting maintenance of the desired ecological and chemical status of freshwater ecosystems worldwide (Posthuma et al., 2020; Millennium Ecosystem Assessment, 2005). Such pressure mainly occurs through interference with ecosystem structure (i.e. species abundances and species assemblage composition) and functions (e.g. dynamic food webs) (Maltby et al., 2017a, 2017b). Chemical pollution pressure on freshwater ecosystems does not only have a direct impact on aquatic species (referred to as services providing units (SPU) in the context of ES) but also reduces their capacity to generate ES in ways that negatively impact human wellbeing, thus constituting a threat to sustainable ES production (Awuah et al., 2020; Liu et al., 2020).

Several authors have considered how to incorporate protecting or restoring ES in decision-making (Daily et al., 2009; Faber et al., 2019; Faber et al., 2021; Maltby et al., 2021), which requires knowledge of the characteristics and interlinkages of ES as well as tools that enable quantifying and evaluating ES (Maia de Souza et al., 2018). This requires an assessment along the source-to-damage pathway from evaluating the pressures, relating pressures to impacts on aquatic ecosystems (fate-exposure-effect chains), and translating these impacts into damage (referred to as damage on a defined environmental area of protection, such as ecosystem quality) caused to ecosystem structure (species abundance change/species loss/species diversity loss), damage on ecosystem functioning (functional diversity loss), and finally damage on relevant, interconnected ES.

Quantitative decision support tools, such as life cycle assessment (LCA), chemical substitution or chemical footprinting, have been developed in support of assessing and increasing environmental sustainability of products and technologies (Koellner and Geyer, 2013; Fantke & Illner, 2019; Liu et al., 2020; Othoniel et al., 2015). Such tools are generally designed to quantify the pathways from pressures to damages on ecosystems (Woods et al., 2018), which also includes the ecotoxicity impact pathway associated with chemical emissions along product life cycles (Fantke et al., 2018; Henderson et al., 2011; Westh et al., 2015). Ecotoxicity impact characterization is part of the life cycle impact assessment (LCIA) phase of LCA, and a recognized element of e.g. the European Product Environmental Footprint (PEF) approach for comparative evaluation of product-related footprints (Fantke et al., 2018; Saouter et al. 2017a, b).

The translation of predicted ecotoxicity impacts into aquatic species loss as LCA-metric for damage on ecosystem quality remains challenging, given the large diversity of chemical compounds, the required step to extrapolate from ecotoxicity test data to predicted toxic pressure, the largely unresolved association between the predicted toxic pressure and structural or functional damage in terms of, for example, species loss and altered food web function in the field, and the location-dependent variation in many parameters that influence the outcome of the impact pathway from emissions to change in ecosystem services. For the purpose of comparative LCA, the mechanistic or empirical association between insights from laboratory test data and eventual damage in the field is of interest, given the principle that other impact pathways also aim to characterize damage in the same units. Considering this pathway for chemical pollution highlights various challenges related to disconnects between current approaches and final damage aspects. There may be, for example, within-ecosystem species shifts as function of chemical pressure that would not lead to net species loss or significant functional damage (Liess et al., 2021).

Damage on ecosystem functioning or even further on ES associated with ecotoxicity impacts are currently not addressed in LCA. This is despite the fact that inclusion of ES in LCA to assess the importance and magnitude of different stressors on ecosystems and their respective services is the focus of several ongoing research efforts (Liu et al., 2020; Maia de Souza et al., 2018; Othoniel et al., 2015; Rugani et al., 2019). Among these efforts, Othoniel et al. (2015) identified challenges in emerging approaches for addressing ES in LCA, which include insufficient knowledge on spatiotemporal aspects and uncertainty in aggregating LCA indicator scores, and which does not reflect differences in damage levels across ES. They suggested that LCIA modelling of ES could benefit when harmonized with existing, integrated multiscale dynamic ES approaches (Othoniel et al., 2015; Maia de Souza et al., 2018).

In another study, Maia de Souza et al. (2018) discuss gaps and potential solutions for integrating ES assessment more broadly into the LCA framework. They propose that tools relying on extrapolation of ecosystems' functional production to their ES, such as the 'Integrated Valuation of Ecosystem Services and Tradeoffs' (InVEST) or the 'Multiscale Integrated Model of Ecosystem Services' (MIMES), might be useful to address the nonlinear nature of ES responses to pressures. Furthermore, they propose that applying ecosystem classification frameworks, such as the 'Common International Classification of Ecosystem Services' (CICES) or the 'National Ecosystem Services Classification System' (NESCS) or the 'Final Ecosystem Goods and Services Classification System' (FEGS-CS), can be relevant starting points to evaluate impacts from an ecosystem functional level up to damage on human wellbeing via ES. While such tools and classification systems seem to be useful for generally addressing ES in LCA, their applicability to ecotoxicity-related damage on ES is currently unclear. Rugani et al. (2019) and Liu et al. (2020) propose a cascade framework that generally links changes in ecosystem structure and functions to changes in human wellbeing, and that aligns with the LCA cause effect chain model. This cascade framework is based on earlier work by Haines-Young and Potschin (2013), which links the flow of different ES from the source to their value for human wellbeing (Maia de Souza et al., 2018). In this cascade framework, again, ecotoxicity-related aspects and their influence on aquatic ES are not currently considered.

An approach that was discussed for overcoming the complexity of assessing ES, which could also be potentially useful in the context of LCA, is the use of ecological production functions (EPFs) to quantify and predict changes between specific ecosystem functions and ES (Bruins et al., 2017; Faber et al., 2021; Othoniel et al., 2015), by linking to changes in the characteristics and performance of service providing units (SPU), such as biomass, species richness or functional traits. However, various links from ecotoxicity impacts to damage on ES remain unaddressed or face significant data gaps.

In all, despite some emerging concepts to generally evaluate ES in LCA, challenges for including freshwater ES associated with ecotoxicity impacts from chemical life cycle emissions would be valuable for decision support, though remain largely unresolved. In order to quantify ecotoxicity-related damage on services provided by freshwater ecosystems, the main human-valued ES need to be first defined, including their underlying pathways from pressures to species and functional diversity loss in freshwater ecosystems, and finally to damage on ES. The present study aims at addressing this knowledge gap and proposes a way forward to characterize damage of chemical pollution on ES of freshwater ecosystems in LCA. This is done by focusing on three specific objectives: (a) to outline an overall framework for linking predicted freshwater ecotoxicity impacts to damage on related ES in compliance with the boundary conditions of LCA; (b) to present possible approaches for linking predicted ecotoxicity impacts to species loss and functional diversity loss, and finally to damage on ES in LCA; and (c) to discuss strengths, limitations and data availability of possible approaches for each step from ecotoxicity impacts to damage on ES.

2. Conceptual framework to link chemical emissions to damage on ecosystem services

Linking chemical emissions via predicted ecotoxicity impacts to

damage on ES is not straightforward. When developing the pathway from ecotoxicity impacts to damage on ES, the main link is often from predicted species-level effects to damage on structural biodiversity (in the context of LCA typically referred to as species diversity or species loss), further to damage on functional (bio-)diversity loss, and finally to damage on related ES (Truchy et al., 2015; Maltby et al., 2021). Alternatively, there is the option to derive a direct link from species loss to damage on ES, without considering the intermediate step of evaluating impacts on any ecological function (Maltby et al., 2021). Further, ecosystem functioning can change without species loss (i.e., due to behavioural change), so that damage to ES may follow directly from such ecotoxicity effects (Truchy et al., 2015).

In the present study, we illustrate the broader complexity of the impact pathway for freshwater ecosystems and its connections between ecotoxicity, species loss, functional damage, and ES damage. As starting point, we adapted the Adverse Ecosystem Service Pathway (AESP) conceptual framework (Awuah et al., 2020) based on information on the ecotoxicity effects of species food web interactions and ES from Maltby et al.(2021). The principles of that links to other frameworks, especially LCA, but also the Adverse Outcome Pathway (AOP) concept, as all are variants of a causal chain approach, developed and utilized from different perspectives for different practical purposes. Fig. 1 illustrates the overall pathway starting from chemical emissions in different environmental compartments to damage on freshwater ES, whilst relating the various frameworks. The initial step of the pathway, from emission to predicted species-level ecotoxicity effects, commonly yields the Potentially Affected Fraction of species (PAF) exposed by a particular stressor (e.g., a chemical or mixture), as metric of expected impacts resulting from a particular pressure level; for chemical pollutants, this metric is commonly derived from data on across-species differences in sensitivity obtained from laboratory test data for separately tested chemicals. As the thus-predicted impacts empirically relate to effect magnitudes in the field (Posthuma et al., 2020), this metric can empirically be translated into species loss. The functional diversity level further relates ecotoxicity impacts and species loss to damage on freshwater ecosystem functions due to reduction in the performance and characteristics of affected species traits. Finally, species and functional diversity loss is then translated into damage on ES, impacting benefits that humans receive from a well-functioning freshwater ecosystem.

In practice, protection of biodiversity at the ecosystem level still relies primarily on extrapolating ecotoxicity effects at the level of the individual organism. This is based on data from ecotoxicity tests, extrapolated to structural ecosystem properties (i.e., populations and communities) and some ES of importance for human wellbeing, whereby current uncertainties in this assessment process are reflected in the magnitude of uncertainty factors utilized in the derivation of environmental quality standards that aim to ascertain sufficient protection even under data uncertainties (Forbes et al., 2017). However, with the current advancement in mechanistic models and quantitative adverse outcome pathways (AOPs), predictive ecotoxicology is continuously advancing (Forbes et al., 2017; Schmid et al., 2021), such that impacts that may occur when those standards are exceeded are increasingly quantified (e.g., Posthuma et al., 2019).

As shown in Fig. 1, AOPs complement the AESP framework and can be useful for linking ecotoxicity effects to damage on species and functional diversity loss. AOP describes initiating key effects, followed by series of subsequent events, eventually leading to impaired functions in an organism, thereby defining relationships within the AESP concept and providing helpful information in predicting and quantifying impacts up to the community level (Schmid et al., 2021). Although emphasis in the present review is on expanding towards eventual ES damage, the framework in Fig. 1 can be further combined with the Aggregated Exposure Pathway (AEP) concept, which aligns with the overall LCA impact-pathway structure, by allowing to correctly address multiple pathways of exposure that lead to eventual net exposures and eventual damage (Clewell et al., 2020; Escher et al., 2017). To improve the use of AOPs in ecological assessments at a higher level of biological organization, Murphy et al. (2018) proposed a conceptual model linking

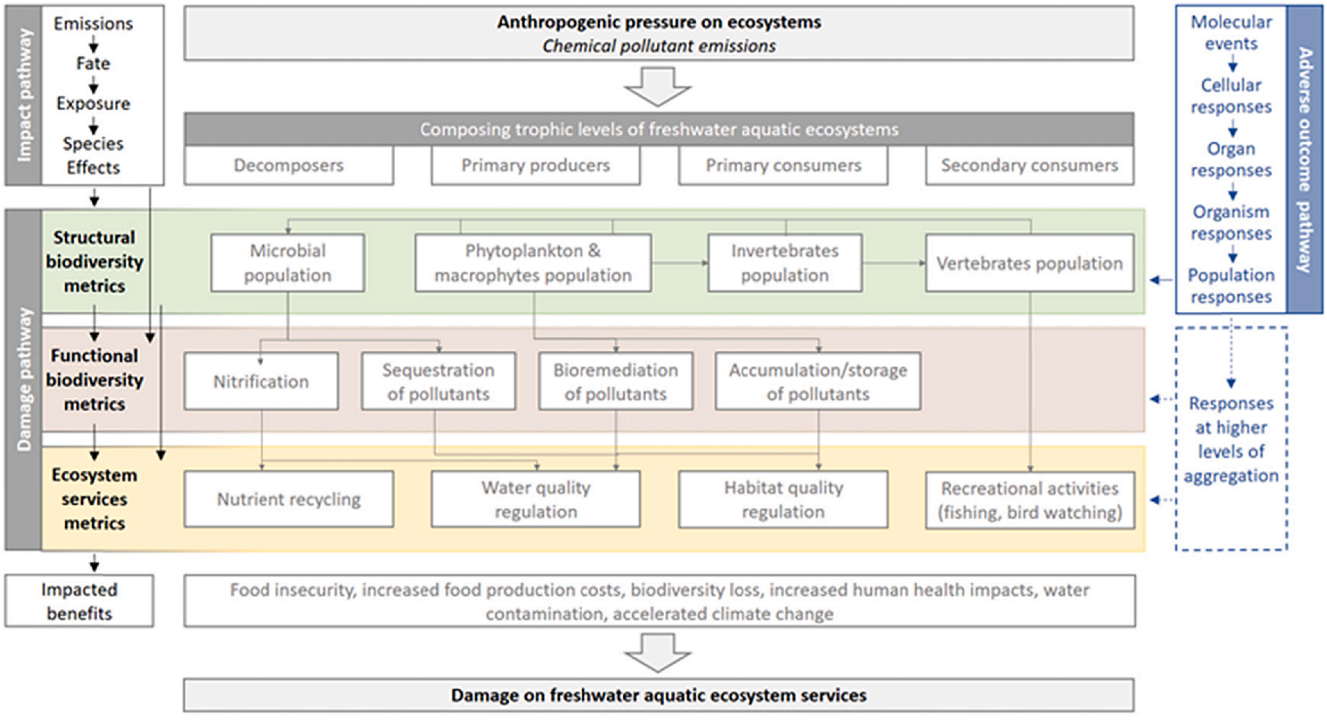

Fig. 1. Conceptual framework for translating ecotoxicity impacts into damage on structural biodiversity (e.g. species diversity), functional biodiversity, and freshwater ecosystem services from chemical emissions into the environment. Shown are the steps of a cause-effect chain (left), the current mechanistic reflection of those in Adverse Outcome Pathway approaches (right), and the operational steps utilized in applied ecotoxicology (in the forms of chemical safety assessment and environmental quality assessment). The framework illustrates that various parts are well-developed, whereas other parts are still lacking (dotted box).

population models, i.e., the dynamic energy budget (DEB) model and quantitative AOPs, utilizing AOP key events as a measure inducing damage in the DEB variables and processes rates. However, it is still unclear which elements of the AOP concept can be used or adapted as input for quantifying any link from ecotoxicity impacts to damage on ES. More broadly, whilst conceptual approaches and frameworks may be linked as in Fig. 1, their current or future use for decision support also depends on available data.

In the LCA framework, "ecosystem quality" is one of the main defined areas of protection (Verones et al., 2017), with reduced biomass and loss of species richness used to currently indicate damage on ecosystem structure and functioning (Woods et al., 2018). ES are currently addressed at the same level as ecosystem quality and other areas of protection in LCA (Verones et al., 2017). Initial approaches for evaluating damage on ES in the LCA context so far only address land use and land change impact drivers (Liu et al., 2020; Rugani et al., 2019), while linking ecotoxicity impacts to ES damage is currently missing.

The impact pathway from emissions to ecotoxicity impacts expressed at the level of affected species fractions is already covered well in LCA, whereby predicted impacts – expressed as Potentially Affected Fraction of species (PAF) – are extrapolated to damage – expressed as Potentially Disappeared Fraction of species (PDF) on the basis of empirical PAF-PDF associations (Jolliet et al., 2003; Rosenbaum et al., 2008; Fantke et al., 2021). This proxy link, however, requires further refinement to consider relevant differences in impacts when translating those into species loss among species, environments and locations. With that, despite initial attempts, translating predicted ecotoxicity impacts into damage on freshwater species diversity and further to damage on functional diversity and ES is currently not operational in LCA (Liu et al., 2020; Maia de Souza et al., 2018; Othoniel et al., 2015; Rugani et al., 2019; Verones et al., 2017). LCA aims at quantifying the pressure on ecosystems and other aspects attributable exclusively to one or more studied products or system life cycles. With the fact that ecosystems are in reality affected to a multitude of different stressors from all sorts of sources and products, one of the challenges in linking ecotoxicity impacts to damages on ES is to identify which fraction of the damages can be allocated to chemical emissions of a given life cycle. There are, however, motives to develop ES-damage frameworks in LCA, given that currently approx. one-fourth of biodiversity impacts in aquatic ecosystems is attributed to chemical pollution effects (Lemm et al., 2021). In the following, we hence outline a proposal for translating ecotoxicity impacts to species loss, functional diversity loss and finally to damage on ES for consistent inclusion into the LCA framework.

3. Source-to-damage modelling approach

Assessing ecotoxicity impacts on freshwater ecosystems requires looking at the source of damage and the overall framework from emission to ecotoxicity damage on species diversity, functional diversity, and ES. The pathway from emissions to ecotoxicity effects is already covered, for instance, in the global scientific consensus model USEtox, where ecotoxicity effects of a chemical emitted into the environment are assessed by combining factors characterizing environmental fate, ecological exposure, and ecotoxicological effects (Fantke et al., 2018; Henderson et al., 2011; Owsianiak et al., 2023; Rosenbaum et al., 2008). Environmental fate factors relate emissions to changes in concentration of a toxicant in the different environmental compartments, including freshwater. Ecological exposure factors then translate the resulting chemical concentrations into the bioavailable fraction of chemicals in the relevant exposure compartments. Effect factors finally link the bioavailable fraction of a chemical in the exposed freshwater environment to impacts on the physiology, behaviour, life history, and ultimately the population of an exposed species (Spurgeon et al., 2020) via different effect mechanisms. This impact pathway commonly ends currently with the quantification of the PDF. Although the potentially disappeared species likely all have their functions in an exposed ecosystem (Faber et al., 2021), the step to damage to functional diversity and ultimately damage on ES delivery still needs to be made.

The scientific literature provides some opportunities that could serve as a starting point for translating ecotoxicity impacts into damage on species diversity, functional diversity, and ecosystem services of freshwater ecosystems in the context of LCA and similar frameworks (see Fig. 1). The opportunities and their features are provided in Table 1. All elements are further elaborated in the subsequent sections.

3.1. From freshwater ecotoxicity to damage on structural species diversity

Effects of chemicals on freshwater ecosystem species range from direct acute and chronic toxicity in organisms to many sub-lethal or indirect impacts on behaviour, functional roles, predator–prey relationships, and food web dynamics (Chagnon et al., 2015). If considered mechanistically, assessments would require quantification and understanding of the full set of linkages between direct ecotoxicity effects and their consequential damage if they should be translated into species loss and associated changes in food webs, functions and services. Various elements of this 'full approach' have received attention, to be potentially developed into practicable approaches.

Three approaches were initially developed to be potentially used as a starting point to translate ecotoxicity impacts into damage on species diversity expressed as species loss. These approaches include the media recovery approach that is based on species richness (the number of individuals or biomass) recovery after exposure to a toxicant, the mean extinction approach that quantifies the expected survival rate of different species when exposed to a stressor, and the genetic diversity approach that is based on changes in species genetic diversity (Larsen & Hauschild, 2007). The genetic diversity approach could help solve problems with addressing diversity within species versus diversity between species (the latter is what we refer to as 'species diversity'), focusing on within species and between population variations.

Genetic and species diversity are fundamental components of assessing impacts on biodiversity (Hoban et al. 2022). Both are influenced by the same ecological processes: species selection, migration, drift, and speciation/mutation (Vellend, 2010). Genetic diversity, that is variation in the genetic make-up of species, enables populations to adapt to changing environments and offers 'insurance' against stressor impacts (Vellend & Geber, 2005), such that individuals with desirable traits (i.e., alleles) in a population can survive to produce offspring and allow for the continuation of generations. In contrast, species diversity focuses on variation between species, i.e., the number of species within a community (Vellend & Geber, 2005).

The possibility that genetic and species diversity influence each other has been acknowledged for decades (Bolin & Lau, 2022). A positive relationship between species diversity and genetic diversity has been observed in communities exposed to certain stressors (Vellend & Geber, 2005; Blum et al., 2012). This positive relationship can be linked to the genotypes of a focal species having a competitive advantage against different species within the community, and other species having a competitive advantage against the genotype of common focal species (Bolin & Lau, 2022; Vellend, 2006; Vellend, 2008). However, high genetic diversity can also negatively influence species diversity if it reduces available niche spaces for heterospecific species (Bolin & Lau, 2022; Vellend & Geber, 2005). In some cases, genetic diversity may change without a change in species abundance (Hoban et al., 2022), while changes in species diversity may alter the positive species interactions resulting in changes in the ecosystem processes (Cardinale et al., 2002). However, these approaches are currently rarely used, mainly due to their intrinsic complexity and low availability of data, especially for the mean extinction and genetic diversity approaches (Larsen & Hauschild, 2007). Environmental DNA (eDNA) describes the use of species DNA extracted from soil, water, or ice. Combined with gene sequencing, eDNA provides a way of measuring species diversity, assigning functionality, and consequently gaining an insight into food

Table 1
Overview of approaches, and their features, that are potentially useful for translating ecotoxicity impacts into damage on species diversity, further relating to damage on functional diversity, and finally linking to damage on ecosystem services of freshwater ecosystems in the context of LCA.

Step	Approach	Description	Data needs/ availability	Spatial scope	Assumptions
Ecotoxicity impacts to species diversity damage	The Dynamic Energy Budget (DEB) model models [1]	DEB models explore and predict the effect of a toxicant on both plants and animals growth and reproduction over time and over the entire species lifecycle	Limited data availability	Landscape and regional	Species size is a proxy for species maturity. Processes influencing internal exposure are different from those causing damage
	Food web models e.g., AQUATOX [2]	It represents a full effect on the aquatic food web	Limited data availability	Local and regional	Toxic effect is additive when many organic chemicals are simulated simultaneously
	Population models [3,4,5]	Provide insight into how a toxicant causes stress on individual species population fitness characteristics	Limited data availability	Local and regional	The population is closed demographically and females drive population dynamics
	Mean extinction time [6]	Quantifies the expected survival rate of different species when exposed to a stressor	Limited data availability	Local	No interactions between subpopulations
	Media recovery model [6]	Based on recovering of species richness after exposure to a toxicant	High data availability	Local	The species are assumed to disappear when the toxicant reaches threshold and reappear when the toxicant disappears. The assumption doesn't not hold for a large scale where population reduction would lead to genetic drift and therefore reduction in genetic diversity
	Genetic diversity [6]	Indicates the number of genetically different individuals within the same species	Limited data availability	Local	More genetic variation suggests capacity of the population of organisms to survive stress
	The Principal Response Curve (PRC) approach [7,8]	PRC display effects of a stressor in the course of time	Limited data availability	Local and regional	Follows linearity assumptions but is capable of showing nonlinear treatment effects
	Threshold Indicator Taxa Analysis (TITAN) [9,10]	TITAN approach links field data, to measured environmental concentrations in predicting effects	Limited data availability	Local and regional	Quantitative indices and individual taxon output represent the general nature of community response to a chemical
	Environmental DNA (eDNA) combined with RNA sequencing [11]	Gives an insight into the community composition using the RNA gene expression patterns and the quantity of the DNA	High data availability	Local	A shift in species community composition suggests altered community function
Species diversity damage to functional diversity damage	Trait probability density framework (TPD) [12]	TPD describes the nature of trait distribution within a multidimensional hyper volumes	Limited data availability	Regional	Interspecific variability is considered more significant than intraspecific trait variability
	Functional sensitivity distribution (FSD) [13]	FSD describes the sensitivity of multiple species exposed to a hazardous compound affecting their ecological function	Functional endpoints. Limited data availability	Local	FSD of tested species resembles the FSD of species assemblage in the field
	Phenotypic diversity model [6]	Links directly phenotypic variation to ecosystem functioning	Limited data availability	Local	Reduction in phenotypic variance from toxic pressure affects ecosystem functioning
Functional diversity damage to ecosystem services damage	Common International Classification of Ecosystem Services (CICES) [14,15]	Hierarchical classification system which is tailored to accounting i.e., the value of ecosystems and the cost of their depletion taking into account abiotic resources	High data needs	Local and regional	Focuses on identification of the final ES directly linked to values valued by human beings
	National Ecosystem Services Classification System (NESCS) [14,15]	Hierarchical classification system which identifies pathway through which changes in the ecosystems impact ES flow to humans	High data needs	National	There is a clear division between natural systems and human systems
	Final Ecosystem Goods and Services Classification System (FEGS-CS) [14,16]	Hierarchical ES classification framework that provides distinction between intermediate and final ES and linkage between ES flow and human well being	High data needs	Local and regional	There is a fine separation of the intermediate and final ES
	Cascade model [17]	Represents the flow of ES in a logical scheme of chains from their generation to their value to humans well-being	High data needs	National	ES flow in a linear, logical scheme of chains
	Ecological Production Functions (EPFs) [18]	Quantifies connection between ecosystem structure and processes to ecosystem function and ES importance for human wellbeing based on function –related descriptors	High data needs	Local	EPFs represent outcomes of ecological processes

[1: EFSA et al., 2018], [2: Park et al., 2008], [3: Earl, 2019, 4: Forbes et al., 2017, 5: Maltby et al., 2021], [6: Larsen & Hauschild, 2007], [7: Van Den Brink et al., 2000, 8: Moser et al., 2007], [9: Berger et al., 2016, 10: Baker & King, 2010], [11: Birrer et al., 2021], [12: Carmona et al., 2016], [13: Posthuma & de Zwart, 2014], [14: Maia de Souza et al., 2018, 15: US-EPA, 2018], [16: Landers & Nahlik, 2013], [17: Rugani et al., 2019], [18: Faber et al., 2021].

webs without species observation or trapping (Birrer et al., 2021). However, it is difficult to accurately quantify species diversity from eDNA, since different species shed DNA at different rates, which is also influenced by environmental factors such as UV light and microbial activity (Goldberg et al., 2016). Thus, due to DNA degradation, only the recent presence of species can be accurately detected (Goldberg et al., 2016; Rees et al., 2014).

Another type of approaches in linking ecotoxicity effect to species loss (i.e. loss in species diversity) consists of the idea to develop and use mechanistic models such as dynamic energy budget models (DEB), population models, and food web models to extrapolate effects at individual species levels to damage at the population level or community

level (Faber et al., 2019; Forbes & Galic, 2016; Forbes et al., 2017). DEB models simulate how species assimilate and allocate energy for physiological processes (e.g., growth, development, and reproduction) while also reflecting how changes in the environmental conditions (e.g., exposure to chemicals, resource availability, and temperature) change those energy flows (Dong et al., 2022; Forbes et al., 2017). DEB models facilitate extrapolation of chemical effects across species and service providing units (Forbes et al., 2017). DEB models are also flexible, allowing for incorporation of chemical modes of action depending on the processes affected by the toxicant. Thus, they provide a potential to mechanistically explore toxicity beyond mere dose effect descriptions for separate ecotoxicity endpoints (EFSA et al., 2018). However, DEB models are compound- and species-specific, with currently only a very limited array of species and chemicals covered (EFSA et al., 2018).

Population models are another opportunity, which utilizes information on individual species' life history characteristics (such as juvenile period, growth rate, reproductive output), thus bringing additional biological realism when predicting damage to populations from data on various endpoints (Forbes et al., 2017; Maltby et al., 2021). However, population models extrapolate changes in specific individual species performance to impacts on population dynamics and structure, with a need to cover a broader range of species (i.e. limited number of possible species for which models are readily available) and flexibility in predicting ecotoxicity effect under different conditions and habitats (EFSA et al., 2018; Maltby et al., 2021).

Food web models, such as AQUATOX (Park et al., 2008), consider the flow of toxic substances through the food web (i.e., species interactions) and ecotoxicity impacts on the food web structure (Faber et al., 2019; Maltby et al., 2021). Thus, food web models would provide the damage information aimed at, when it is known which species are threatened by the presence of a toxic substance and how that affects the food web structure and/or function (Jørgensen, 2016). Food web models can provide information on the biomass of species, individuals, and populations with a possibility to further predict damage on ES (Galic et al., 2019). However, food web models have not yet been widely used because of the difficulty of modelling the flow and fate of toxic substances in complex and highly spatiotemporally varying food webs (Jørgensen, 2016). Food web models like AQUATOX can currently model effects associated only with organic chemicals (Park et al., 2008). Furthermore, the lack of standardized impact indicators currently limits the applicability of food web models for use in practical LCA (Maltby et al., 2021).

Translating ecotoxicity impacts into species loss can also be achieved using the principal response curve (PRC) approach. This approach uses data on multiple species responses from controlled experiments, e.g., mesocosms. However, PRC statistics are only feasible for data with repeated measures over time (Van Den Brink et al., 2000; Van Den Brink & Braak, 1999). Unlike mechanistic models that allow for extrapolation of ecotoxicity effects to novel conditions, the PRC approach can usually not be extrapolated beyond experimental test conditions (Jager, 2016; Forbes et al., 2017). Furthermore, it is not possible to recognize sensitive species with a different response pattern with the PRC method (Moser et al., 2007).

In contrast to PRC derived from mesocosm-type test data series, the Threshold Indicator Taxa Analysis (TITAN) approach uses field monitoring data on multiple stressed system to derive species-specific differences in abundance response thresholds given pressure level gradients (Baker & King, 2010). TITAN's capacity to identify abrupt changes (so-called "breaking points") in occurrence and abundance of taxa along a chemical gradient makes it appropriate to identify sensitive taxa showing a clear response to a chemical gradient under field conditions (Berger et al., 2016). Given that TITAN analyses can be used to track changes in species abundance under chemical pollution pressure, in terms of fractions of species affected at given field exposures (Berger et al., 2016; Baker & King, 2010), there is latitude to use TITAN to characterize field effects across species, and relate that to the predicted impacts as generated with SSD models. With that, the TITAN approach is a promising empirical starting point for relating predicted ecotoxicity impacts (PAF) into damage in the field in terms of species loss (PDF). However, the approach is constrained by limited data availability, i.e., to be operationally applied in the LCA framework, it requires large-scale monitoring data with species occurrences and abundance patterns at different sites along with measured chemicals or mixture concentrations. That is, the use of the TITAN approach provides insights in empirical PAF-PDF associations for particular study areas, particular chemical pollution pressures and particular species groups, so that LCA damage assessment would be best served by analysis of diverse, multiple field response data sets. As yet, available work consist of (Berger et al., 2016) analyses, and ongoing work focuses on establishing PAF-PDF relationships for Dutch surface water monitoring data.

The challenges of most mechanistic models and the empirical approaches are partly conceptual but mostly also related to available data, as highlighted above, including the need to cover a wider variety of species, currently limited coverage of chemicals and different organisms' specific endpoints, which still require attention. Using the SSD approach to cover a broader range of species can bridge part of the data-related gap and with that can help refining some of the models (EFSA et al., 2018). Furthermore, comparing the magnitude of different effect endpoints (e.g. reproduction vs growth) from SSDs would provide an option of deriving consistent metrics for translating ecotoxicity effects into damage at species diversity level while utilizing available data.

3.2. *From species loss to damage on functional diversity*

Functional diversity is the variation of traits between organisms (Carmona et al., 2016). Species' functional traits determine how they respond to environmental conditions and disturbances, such as emissions of chemical stressors. Characterization of functional diversity through various components such as functional richness, functional evenness, and functional divergence has great potential to answer different ecological questions, including impacts of any disturbance on the assembly of biological communities. Functional evenness is the amount of functional volume occupied by a trait density distribution indicating a range in a single trait case. Functional richness is the amount of space occupied by species in an ecological unit. In contrast, functional divergence is an indicator of the degree of the distribution of abundance within the functional trait volume (Carmona et al., 2016).

At the community level, estimating functional diversity within a community of species is often determined as a function of differences in individual species traits (Carmona et al., 2016). That is, any stressor that has a strong influence on the composition and diversity of species traits and interaction in the food web is having an influence on an ecosystem function based on those traits (Truchy et al., 2015; Faber et al., 2019; Maltby et al., 2017a, 2017b).

Ecosystem functioning relates to the sum of all processes that sustain an ecosystem through biological activities (Reiss et al., 2009; Truchy et al., 2015). Processes at the ecosystem level emerge from species' interaction with each other in their food web and with the environment, which often involves transformation of nutrients and energy, generation of the species habitat structures, and maintenance of the species populations (Truchy et al., 2015; Faber et al., 2019; Maltby et al., 2017a, 2017b). Dominant processes associated with freshwater ecosystem functioning are nutrient cycling, organic matter transformation, primary productivity, secondary productivity, and ecosystem metabolism (Harrison et al., 2022). A specific process consists of the option of sequestration or detoxification of pollutants influencing water quality in the ecosystem (Maltby et al., 2021). As discussed in Haines-Young & Potschin (2010), ecosystem functioning is highly associated with species biodiversity, such that a decrease in ecosystem functioning occurs more rapidly when there is low species diversity. Apart from the number of different species (i.e., species diversity), other measures of biodiversity essential for ecosystem functioning include species abundance, the

composition of the genotypes in the ecosystem population, and functional groups (Haines-Young & Potschin, 2010). As much as an ecosystem can reduce species diversity without impacting its functioning due to redundancy in species' functional traits, the redundancy of functional groups ensures a continuous functioning of an ecosystem (Baumgärtner, 2007). Such redundancy largely depends on the presence and composition of species functional groups and traits (Faber et al., 2019; Haines-Young & Potschin, 2010; Rumschlag et al., 2020).

Chemical pollution may have a specific impact in ecosystems and their functional characteristics. That is, differences in the match, or mismatch, of chemical modes of action and species traits (e.g., insecticides and insect traits presence or absent) determine how chemical exposures affect species and which consequences on ecosystem functioning or to be expected (Chagnon et al., 2015). Chemical modes of action can also help identify the most sensitive species. That is, such a species or set of species traits may form the food web, so that the entire functioning of the ecosystem would be compromised if the sensitive species are affected, much more than when the sensitive species are at the end of the food web. For example, exposure of phytoplankton to herbicides decreases community composition before a decline in ecosystem functioning, i.e., reduced community respiration and primary productivity (Rumschlag et al., 2020). In contrast, insecticides reduce zooplankton composition before impacting community respiration and the primary productivity of phytoplankton (Rumschlag et al., 2020).

According to Sodré & Bozelli (2019), chemical stressors can decrease organisms' body size, thus affecting many physiological functions. The magnitude of a biotic ecosystem function is a consequence of the rate of ecosystem processes and related change in producing biomass (e.g. photosynthetic rate and primary producers' biomass). Considering ecosystem functions takes into account the number of species (richness), identity (composition), and abundance of species in a community that contribute to a specific function.

The function sensitivity distribution (FSD) approach has been proposed to quantify the impact of a toxic chemical on the functioning of an ecosystem by considering function-related endpoints (Posthuma et al., 2001). Its application would be based on the empirical observation that – similar to differences across species in sensitivity to chemical exposures – the functional endpoints follow a bell-shaped distribution. Development and application of FSDs would enable direct evaluation of a functional damage assessment, similar to the establishment of the PAF-PDF relationship which can be determined utilizing TITAN analysis, as described above. However, this approach is currently rarely used due to its limited data availability (Posthuma & de Zwart, 2014).

Given various concepts and components in estimating functional diversity, Carmona et al. (2016) proposed a trait probability density (TPD) framework that unifies existing quantification approaches for functional diversity components. TPD considers species abundance and intraspecific trait variability to derive estimates for different functional diversity components, i.e., functional richness, functional evenness, and functional divergence. With available data, using TPD would, allow predictions of functional impacts across various spatial scales, given that it is assumed that values of the TPD framework of an ecological unit are directly proportional to the relative abundance of their trait values (Carmona et al., 2016). TPD functions may be directly applied to predict the functional structure of species populations and communities along chemical gradients. The method requires substantial trait data (Carmona et al., 2016).

The phenotypic diversity model (i.e., genetic relationship between different groups of species) could also provide a way to translate changes in species diversity into damage on ecosystem functioning. Species diversity directly links phenotypic variance to ecosystem functioning, represented as a change in biomass production in an ecosystem from a toxic pressure. With a focus on species functional groups as the basic unit of the ecosystem, species sensitivity is taken into consideration in this approach (Larsen & Hauschild, 2007).

Functional indicators that measure functional effect traits or rates or attributes of processes have been proposed. Such indicators have been proposed, since it is considered difficult to measure ecosystem functions or predict them from underlying structural impacts. On this relationship, it can be reasoned that highly aggregated functional metrics (such as primary productivity) are relatively insensitive as compared to underlying structural impacts. Exploiting the relationship between potential functional indicators that are more directly connected to mechanistic processes can help link species loss to ecosystem function loss by assessing how a change in the state related to processes impact rates of processes within the food web. However, changes in multiple interacting functions at the food web level and across different trophic levels are indicated by processes measured at the food web level, such as the flow of energy through the food web (Harrison et al., 2022).

Combining different functional diversity components, FSD, and functional indicators (Posthuma & de Zwart, 2014; Carmona et al., 2016; Harrison et al., 2022) can hence provide a possible starting point in translating species loss to damage on functional diversity. Furthermore, eDNA and sRNA measurements may provide a direct way of measuring species diversity, in addition to getting an insight into the community function dynamics from direct observation of species (biomonitoring data).

An overview of the features of different approaches that could potentially serve as a starting point for translating damage on species diversity into damage on functional diversity of freshwater ecosystems in the context of LCA is provided in Table 1. Different functional indicators with related taxa and processes are provided in Table 2, for metrics representing rather high levels of aggregation.

3.3. From functional loss to damage on ecosystem services

Damage on functional diversity loss can be linked to damage on related ES as an intermediate step of the main pathway in linking ecotoxicity effects to damage on ES (Truchy et al., 2015; Maltby et al., 2021). However, there is also a direct link from species loss to damage on ES, without explicitly considering the intermediate step of evaluating affecting any function (Maltby et al., 2021).

Freshwater ES are dependent on freshwater organism interactions and processes (Chagnon et al., 2015). For example, microbial decomposers and invertebrate detritivores degrade leaf litter, which in turn aids in nutrient cycling. However, when microbial decomposers and invertebrate detritivores are exposed to toxic chemicals, it may cause feeding inhibition and mortality. This, in turn, might damage ecosystem services such as leaf litter breakdown, decomposition, and primary productivity rate and flow of ES, e.g., nutrient cycling and support for other freshwater organisms (Peters et al., 2013; Chagnon et al., 2015).

Biodiversity is the variety of life forms, including the variation of genes, species, and functional traits. Biodiversity and ecosystem functioning relationships (BEF) have been studied for several decades (Cardinale et al., 2012; van der Plas, 2019), with researchers often reporting the BEF relationship as nonlinear. Diversity of the community positively influences ecosystem functioning (van der Plas, 2019). While biodiversity loss reduces the number of genes, species, and functional groups, it consequently decreases the efficiency by which species communities capture essential resources, produce biomass, decompose and recycle nutrients (Cardinale et al., 2012).

Some studies have shown that environmental change may damage ecosystem functioning without affecting species richness by affecting population density and community composition as the community competes for limited resources at one trophic level (Spaak et al., 2017). However, biodiversity loss across trophic levels can influence ecosystem functioning more strongly than diversity loss within a trophic level, since food web interactions are key mediators of ecosystem functioning (Cardinale et al., 2012). Hence, high biodiversity is required to maintain the multifunctionality of ecosystems across spatial and temporal scales (Cardinale et al., 2012).

Table 2
Functional indicators possible for translating species loss to damage on ecosystem functioning with related taxa and processes dominant for freshwater ecosystem (Harrison et al., 2022).

Ecosystem function	Processes	State related to processes	Freshwater taxa	Food web metrics
Ecosystem metabolism	Respiration, extracellular enzyme activity, amino acid uptake in biofilm, microbial electron transport system activity	Dissolved oxygen concentration	Microbes	Substrate use metabolic profile
Organic matter transformation	Leaf litter decomposition, detritivores feeding rate	Biomass of fungi	Fungi, invertebrates detritivores, heterotrophic microbes	Detritivores feeding preference
Nutrient cycling	Denitrification, Nitrogen dioxide flux	Total P or C or N; Organic C or N; Nitrites or Nitrates	Microbes	Functional composition and traits of taxa
Primary productivity	Rates of biomass production, oxygen production or carbon dioxide consumption	Biomass or abundance or density of algae, biofilm, phytoplankton, or macrophytes Chlorophyll-a concentration, amount of glutamine sythetase	Macrophytes, algae, phytoplankton, autotrophic microbes	Fish functional composition, invertebrates feeding groups
Secondary productivity	Growth rates or rates of biomass production	Biomass or abundance or density of heterotrophic microbes, invertebrates, or fish	Vertebrates, invertebrates	Phytoplankton functional composition

BEF has often been measured without extending to known ES. Likewise, biodiversity and ecosystem services relationships (BES) have often been described without understanding the underlying ecosystem functions (Cardinale et al., 2012). Predicting biodiversity-related consequences on ES also requires understanding of which functional traits place biodiversity at a higher probability of extinction or establishment, i.e., response traits, and how response traits drive ecosystem functioning, i.e., effect traits (Cardinale et al., 2012; Suding et al., 2008).

For example, diverse communities are more productive because they contain key species that greatly influence productivity, and differences in functional traits increase the total resource capture (Cardinale et al., 2012). Furthermore, functional traits influence the extent to which ecosystem functioning changes after the extinction of biological traits (Cardinale et al., 2012).

Many ES ultimately depend on the variety of life forms (Scherer-Lorenzen et al., 2022). Therefore, successfully understanding the linkages between biodiversity, ecosystem functioning and ES requires quantifying the networks of mechanistic links between ecosystem functions and ES using e.g. mechanistic models (Cardinale et al., 2012). However, challenges still exist when incorporating ES regulated by multiple functions in the BEF relationship, which does not necessarily respond to changes in biodiversity in the same way. Mismatch in how organisms interact at different spatial and temporal scales also complicates integrating food webs into BEF and BES (Cardinale et al., 2012).

According to van der Plas (2019), functional diversity is a stronger predictor of ecosystem functioning than biodiversity, partly because of the presence of a particular functional group (i.e., keystone species) that drives ecosystem processes or abiotic conditions that outweigh the biodiversity effect, such that environment variation and biodiversity jointly drive ecosystem functioning.

Studies directly assessing ecotoxicity impacts on freshwater ecosystem functioning, which could facilitate further translation of functional loss to damage on ES, are rare due to little understanding of biodiversity-ecosystem-function/services relationships and the availability of mechanistic models (e.g., ecological production functions, EPFs) to link chemical-induced effects on individual species to ES delivery (Faber et al., 2019).

The quantitative ecological production functions (EPFs) approach provides quantifiable links from ecosystem functional diversity loss to damage on ES flows (Faber et al., 2019) or a direct link of ecosystem characteristics (i.e., SPU) to final ES (Bruins et al., 2017; Forbes et al., 2017), which can be used as a starting point for translating species loss into damage on ES. Online models, such as U.S. Environmental Protection Agency EcoService, have been developed based on the EPFs approach to quantify damage on ES (US EPA, 2018). However, no standardized test exists for most taxa in EPFs (Faber et al., 2021). Also, existing quantitative models incorporating ecological production functions have limited chemical exposure dose–response relationships (Faber et al., 2019), which are essential as they can be further extrapolated to damage on related ES.

Syberg et al. (2017) proposed to create a 'direct' link from ecotoxicity impacts (using PAF as predicted impact metric) to damage on ES until the full pathway from ecotoxicity impacts via damage on genetic and function diversity to damage on ES is better understood. In the approach proposed by Syberg et al. (2017), damage on ES from ecotoxicity impacts is derived from the sum of hazard quotients (*HQ*) across chemicals *i* that is derived as ratio of measured chemical concentrations in freshwater environments (C_i, mg/l) and the related threshold ($C_{\mathrm{ref},i}$, mg/l) set to indicate an upper-limit safe chemical level for human consumption for each chemical as $HQ = \sum_i \left(C_i / C_{\mathrm{ref},i}\right)$. This approach can be considered a pragmatic approach which sets a human health related upper boundary on chemical exposure, such that exposure of man through ecosystems is not affected by separate chemicals or unintended mixtures, whilst exceedance of that boundary would warrant remediation to safeguard human health.

ES conceptual frameworks also offer ways of linking ecosystem functioning loss to damage on the ES. From earlier reviews conducted on ES methods and applications to freshwater ecosystems (Bagstad et al., 2013; Maia de Souza et al., 2018), most established methods, such as the InVEST approach, help assess risk from land use change or climate change, but applications in response to chemical stressors have not been studied. Maia de Souza et al. (2018) suggest applying NESCS and FEGS-CS, ES classification frameworks to understand the impacts between ecosystem functions and final ES provided for humans, which could also serve as a starting point for application in the LCA framework. FEGS-CS and NESCS frameworks can translate damage on the functional level of an ecosystem to damage on ES and offer a distinction between intermediate and final ES (Maia de Souza et al., 2018). Intermediate ES are not directly used or consumed by humans but are considered necessary for producing final ES delivery.

The cascade model proposed by Rugani et al. (2019) and Liu et al. (2020) links changes in ecosystem structure and functions to human wellbeing changes in a cause-effect chain model in soil ecosystems. With that, this model complements the LCIA impact-pathway framework by providing information about trade-offs (i.e., costs and benefits) of a particular stressor on ES flows (Rugani et al., 2019). However, ecotoxicity-related aspects and their influence on freshwater ES are not currently addressed in the cascade framework. In addition, this model is currently not able to address the dynamics and nonlinear nature of ES (Maia de Souza et al., 2018).

Overall, numerous knowledge gaps remain for successfully translating ecotoxicity impacts into damage on freshwater ES, either directly from species loss or through functional diversity loss. This includes (a) the lack of comprehensive and integrated approaches to assess impacts of chemicals and other stressors while taking into account different routes of chemical exposures, (b) the overestimation or underestimation of potential chemical risk on SPUs, which reduces the accuracy of ES assessment, (c) the complexity in analysing ES trade-offs, i.e. protecting one ES resulting in downstream effects on other ES (Syberg et al., 2017).

The challenge of overestimation or underestimation of the risk on SPUs may be addressed in part by generating separate SSDs for different species groups, which uses ecological information on species communities such as functional groups or trait characteristics (Van den Brink et al., 2021). This may help identify SPUs, i.e. ES that are potentially at risk (Faber et al., 2021; Oginah et al., 2021).

Current methods that link individual elements along the pathway from ecotoxicity impacts to damage on ES delivery (Fig. 1) are still in their infancy, and possible adaptations are in the early stages. Current methods or frameworks do not systematically link ecosystem functions loss to damage on ES from chemical impacts. However, applying the ES frameworks and cascade model, which incorporates EPFs, provides a possible way forward to translate functional loss to damage on ES and with that to include damage on ES associated with ecotoxicity impacts on freshwater ecosystems into LCA. The aggregated ES consequences resemble the aggregated life cycle impacts in terms of species losses modeled at damage level in current state of the art LCIA methods. It is not the intention, however, to predict concrete ES consequences in any specific ecosystem but rather to estimate an overall consequence of a given product or system life cycle.

4. Monitoring-based framework for ES assessment and management

One of the key problems of ecotoxicity assessments and assessing damage is the need for laboratory-to-field extrapolation, given that stressors studied in applied ecology (such as nutrient enrichment) are addressed based on ecological concepts and field data, whilst stressors studied in applied ecotoxicology are most often relaying on laboratory toxicity data. Whilst there are mechanism-based approaches which could be applied in PAF-PDF characterization of damage, it is key to highlight the final issue that the predicted damage should relate to true damage, that is: that the lab-field extrapolation for chemical pollution impacts is correct. The latter can be judged by analyses of landscape-level ecosystem data. Assessment and management of ES eventually require data-driven insights to recognize ES deterioration upon adding more man-made pressures and improvement upon less man-made pressures. Data-driven insights can be obtained from (bio-)monitoring data, combined with appropriate statistical analyses. The latter should be able to characterize the relative roles of different pressures on ecological metrics, be it species abundance data, aggregated structural biodiversity metrics, or aggregated ES metrics. In an ideal case, the damage predicted by any of the mechanistic models should relate to damage in the field.

Generally, the (bio-)monitoring data should cover a number of sites that vastly exceed the number of pressure metrics to avoid the so-called 'curse of dimensionality'. Few sites mean that each added pressure parameter reduces the power of statistical analyses unless sufficient increases in the number of study sites are substantiated. One of the key problems in this respect is the study of chemical pollution through separate exposure or risk metrics for each chemical. The problem was solved by summarizing all chemicals, or mode-of-action subgroups, via mixture toxic pressure quantification (Posthuma et al., 2019).

The statistical diagnostic assessments also need to take into account that there are different types of ecosystems (e.g., a lake, a river, a brook), such that the natural conditions are represented in a multitude of non– or minimally disturbed ecosystem types, whereby damage should be considered relative to those different reference states.

Regarding the statistical analyses aimed at diagnosing relationships between pressure variables and impact variables, the best 'training' data need to consist of the longest possible data gradients for all pressures (e.g., very low to very high pH, ibidem toxic pressure), where the covariance amongst the pressures is below a critical level. This can be checked by calculating, e.g., the Variance Inflation Factor, which should be below a threshold above which interpretation bias (in diagnosing probable causes of impacts) occurs (Lemm et al., 2021).

Monitoring-based approaches involve repetitive data collection to determine trends in parameters or endpoints that comprise ES (Chapman, 2012). Characterization of spatial and temporal relationships and trends in (bio-)monitoring data, aimed at relating multiple pressures to variation and changes in biotic parameters, can assist in predicting the future status of ES under alternative management strategies. At the global level, the Group on Earth Observations Biodiversity Network (GEO BON ES) was established to promote the monitoring of biodiversity and ecosystems for the scientific community and decision-making (Vaz et al., 2021). With satellite sensors, aspects of ecosystem functioning, such as the primary production, can be quantified (Vaz et al., 2021).

Multiple stress analyses have been made for various pressure combinations, areas, species groups, and practical aims. Examples are Grizzetti et al. (2019) and Lemm et al. (2021), focusing on characterizing water quality as a function of a suite of pressures, including unintended complex mixtures. The examples are suitable for exploring and prioritizing alternative management scenarios' potential effects. Similar studies exploring such matters for ES are scarce.

There are global monitoring platform for ES and biodiversity inspired initiatives, such as the Global Biodiversity Information Facility (GBIF) and the Ocean Biodiversity Information System (OBIS) (Vaz et al., 2021). These approaches still face challenges, such as the lack of methods to combine ES monitoring observations and data across different scales, harmonized ES metrics that link interactions between people and ecosystems, and difficulty in incorporating diverse social-cultural values and knowledge into monitoring activities (Vaz et al., 2021). All those problems have been recognized in the diagnostic studies of non-ES impact metrics, confirming that successful studies require a combination of sufficient site numbers (given pressure numbers), good handling of natural variability of non– or minimally disturbed ecosystem types, and a sufficiently wide range of non– or limitedly co-varying pressure metrics, whilst recognizing the specific situation for chemical pollution (and the laboratory-field extrapolation issue) as pressure factor.

In the ES field, monitoring can have a different focus. For instance, for recreation fishing ES, monitoring can either focus on the effect of a stressor on the fishery SPU values, on the ways of preserving fishery SPU values, and on the state of the ecosystem in terms of the SPU, i.e., effect-based monitoring (Chapman, 2012). Because the effects may be incorrectly attributed to the measured chemicals when focusing on those separately from the other pressures, multiple stressor analysis is recommended as a better way of monitoring damage on ES (Chapman, 2012). An example is monitoring toxic pressure across the Netherlands on water quality (KIWK, 2022). This study calculates the key toxicity factor from previous water quality information, such as contaminant locations, causes and measures taken. Water quality managers use the key toxicity factor as a decision-support tool to identify locations and substance groups that most threaten the water quality (KIWK, 2022).

An attempt was also made earlier to monitor the ecological status of the aquatic ecosystem in Europe as an indicator of water quality, which involved using ecological status metrics from biological quality elements information instead of raw field monitoring data (Posthuma et al., 2020). Using the biological quality elements was a key step that solved the issue of natural differences in non– or minimally disturbed reference status across ecosystems. Because current knowledge on monitoring freshwater ES and stressors is usually stored on separate data platforms,

without spatial alignment, it is currently not straightforward to execute a diagnostic analysis of ES data at any geographical scale, apart from some early studies such as (Grizzetti et al., 2019).

For a holistic understanding of how ES can be influenced by one or multiple man-made pressures, efforts are still needed to further develop the data, statistical analysis frameworks, and tools that combine knowledge of ES monitoring with the status and trends of stressors at different spatial and temporal scales. This is particularly challenging when there is interest in chemical pollution as a spatio-temporally variable pressure next to various other pressures, given that applied ecology and applied ecotoxicology need to be bridged by summary concepts such as 'mixture toxic pressure.'.

5. Conclusions and outlook

To address damage on freshwater ES in LCA associated with toxic chemical emissions along product and technology life cycles, related ecotoxicity impacts need to be linked to damage on species (i.e. structural) and functional diversity and finally to damage on ES. This needs to consider approaches that utilize field-based monitoring data with biological realism and align with LCA boundary conditions.

For a holistic assessment of the entire ecosystem rather than individual species population, models that consider multiple populations or entire food webs (Jørgensen, 2016) can help translating ecotoxicity effects into species loss, expressing damage on an ecosystem's species diversity. However, because such models depend on extrapolation of effects to higher biological organizations, leading to higher uncertainty in the output, a novel approach such as TITAN is a promising way forward, which instead builds on field-based monitoring data. TITAN approach, however, has high data needs that are currently available for a few study areas, specific pressure sets and specific taxonomic groups under study.

A trait probability density framework incorporating various functional diversity components can subsequently link species loss to functional diversity loss. However, more data with functional diversity endpoints are still needed before this framework can be operationalized.

Quantitative ecological production functions could finally translate damage on species diversity to functional loss and damage on ES, if uncertainty in extrapolating from the relevant SPUs and functions to ES is considered (Maltby et al., 2021). The challenge of multiple chains of effects can be potentially addressed by applying population or food web models to identify the structural changes in the food web due to the direct or indirect impact of a chemical or other stressor (Maltby et al., 2021). However, there is a need to develop robust models that extrapolate chemical-induced changes in key SPU attributes to changes in ES delivery by incorporating knowledge on how SSDs can be reliably used to address effects on specific species groups associated with certain ES over other species groups that are less affected i.e. split-SSDs (Maltby et al., 2005; Van Den Brink et al., 2006; Maltby et al., 2009) and including EPF that integrate multiple ES and their potential interactions.

The advantage of using EPF-based approaches is that they allow for measured functional endpoints to be further linked to changes in ES delivery. However, identifying endpoints suitable for ecosystem assessment remains a challenge (Syberg et al., 2017), where for example additional functional endpoints should be considered that are particularly relevant for freshwater ecosystems (Maltby et al., 2017a, 2017b; Faber et al., 2021). At the global levels, frameworks or tools that may combine knowledge of ES monitoring and status and trends of chemical and other stressor at different spatial and temporal scales are still needed. The ideal-world expectation for decision support would provide the assessor with specific damage insights per region; however, LCA is an approach founded in the emitter-perspective, which delivers generic potentials to cause harm also useful for decision support purposes. The outputs of LCA are useful as they allow for generically selecting the least-harmful, functionally equivalent product systems.

Overall, we highlighted key elements to develop a framework and associated potentially useful approaches for integration in LCA and similar assessment frameworks that link ecotoxicity impacts on aquatic freshwater species to damage on genetic and functional diversity at the ecosystem level, and further to damage on ES delivery. More attention needs to be paid to developing and refining mechanistic damage models with standardized functional endpoints and structures that align with cause-effect chain modelling, such as the cascade model. By providing an overall framework as well as an evaluation of potentially useful scientific and practical approaches, our study constitutes a useful starting point for addressing current challenges in linking ecotoxicity impacts to damage on freshwater ES, either directly from species loss or through functional diversity loss.

Declaration of Competing Interest

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

Data availability

No data was used for the research described in the article.

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Article III

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Article

To Split or Not to Split: Characterizing Chemical Pollution Impacts in Aquatic Ecosystems with Species Sensitivity Distributions for Specific Taxonomic Groups

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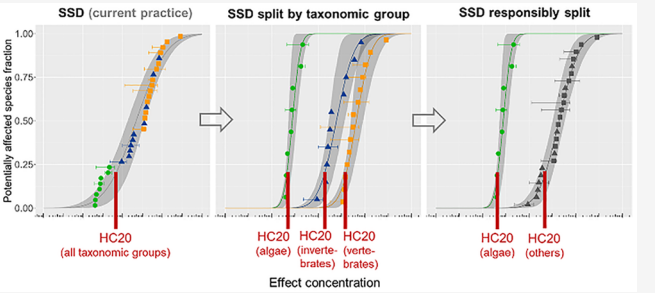
ABSTRACT: Bridging applied ecology and ecotoxicology is key to protect ecosystems. These disciplines show a mismatch, especially when evaluating pressures. Contrasting to applied ecology, ecotoxicological impacts are often characterized for whole species assemblages based on Species Sensitivity Distributions (SSDs). SSDs are statistical models describing per chemical across-species sensitivity variation based on laboratory toxicity tests. To assist in the aligning of the disciplines and improve decision-support uses of SSDs, we investigate taxonomic-group-specific SSDs for algae/cyanobacteria/aquatic plants, invertebrates, and vertebrates for 180 chemicals with sufficient test data. We show that splitting improves pollution impact assessments for chemicals with a specific mode of action and, surprisingly, for narcotic chemicals. We provide a framework for splitting SSDs that can be applied to serve in environmental protection, life cycle assessment, and management of freshwater ecosystems. We illustrate that using split SSDs has potentially large implications for the decision-support of SSD-based outputs around the globe.



KEYWORDS: *freshwater ecosystems, mode of action, ecotoxicity, Water Framework Directive, water quality, life cycle impact assessment*

INTRODUCTION

Characterizing ecotoxicity effects, whether as part of chemical safety assessment, evaluating the environmental performance of products and services in a life cycle perspective, or environmental quality characterization, requires addressing different chemicals' potential to cause harm on different species,[1] while bridging the disciplines of applied ecology and ecotoxicology.[2] This can be achieved using chemical-specific species sensitivity distributions (SSDs). SSDs are classically used to describe variations in sensitivity across multiple species and are commonly derived from collections of laboratory toxicity test endpoints, such as no-observed effect concentrations (NOECs) or the effect concentration causing a response in 50% of the exposed individuals (EC50s).[3,4] Field-based Species Sensitivity Distributions (fSSDs) have been proposed as they are considered more ecologically relevant. However, they present challenges, e.g., the isolation of the effect of a single chemical from combined effects of multiple stressors. Recognizing the regulatory and other practical uses of current laboratory-data-based SSDs, we focus on the "classical", laboratory-data-based SSDs in the present paper.

Laboratory-toxicity data-based SSDs are practically used for regulatory purposes and Life Cycle Impact Assessment (LCIA), e.g., to derive protective standards (threshold concentrations) or expected impact levels of ambient chemical pollution.[5,6] Recently, their use has expanded to the comprehensive diagnosis of the role of chemical pollution as a driver for biodiversity loss in polluted ecosystems by using SSD-based mixture toxic pressure information (expressed as msPAF, the multisubstance Potentially Affected Fraction of species) as pressure metric, as this resulted in reduced parameters numbers and thus improved statistical power in diagnostic analyses.[2,7,8] The choice of required input data and the statistical distribution methods vary among jurisdictions. Models commonly used to fit SSDs include log-normal, log−logistic, or other models that fit the available data well, and commonly, confidence intervals or other metrics of variability and uncertainty are reported.[3,9] Crucial to acknowledge is that SSDs are commonly fitted to all available test data per chemical—following the principles developed by the earliest users—where it is assumed that the SSD describes the exposure-impact relationship for whole field species

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assemblages. Today, two key motives support the derivation of SSDs for distinct taxonomic groups, i.e., split SSDs.

- First, as common in applied ecology, environmental assessment practices focus on separate taxonomic groups (rather than whole assemblages).[10]
- Second, as recognized in applied ecotoxicology, different compound groups can have different (specific) modes of action (MoAs) (such as insecticides affecting insects most), which implies that the currently used, nonsplit SSDs may show poor statistical fits to data across taxonomic groups.[3,11]

The latter argument was already given in an outlook on developments in SSDs in 2002, arguing that a split in taxonomically distinct SSDs and accounting for the mode of action knowledge would be beneficial statistically and conceptually, with improved interpretation for the decision-support uses of SSD-outputs,[12] with further discussions by Fox et al. 2021.[3] Commonly, the derived toxic thresholds, i.e., protective environmental concentrations (HC5) and Predicted No Effect Concentration (PNEC) for aquatic communities and regulatory applications, could be improved by splitting the SSDs regardless of the SSD distributions used.[3,13]

The optional splitting of SSDs comes with a potential trade-off. They become statistically less robust because split SSDs are based on data per taxonomic group. Decision support applications require robust SSDs, defined by their insensitivity to changes in the available collections of input data. Robustness can be characterized with a statistical approach, enabling categorization of optional splitting as "responsible splits" (robust resulting SSDs per taxonomic group) or not (nonrobust outcomes, statistical trade-offs).

In the context of the use of SSDs in setting environmental quality standards, for life cycle assessments, and for diagnostic assessments of causes of global change, the present study's main goal was to investigate whether and how the splitting of SSDs—here, according to taxonomic groups—can be systematically undertaken, that is for chemicals with and without a specific mode of action while accounting for statistical trade-offs and to evaluate whether such splitting yields an improved impact characterization of exposure to chemical contaminants. To achieve this goal, we defined four specific objectives: (i) To derive a harmonized ecotoxicity database from available, curated freshwater ecotoxicity test data and to enrich this database with taxonomic and mode of action information. (ii) To propose a generically applicable framework for deriving split SSDs and demonstrate the utility of the framework for a set of chemicals with sufficient available freshwater test data. (iii) To evaluate whether using the proposed framework would result in different decision-support outcomes, i.e., improved characterization of the expected ecological impacts of chemical pollution, given that many chemicals have limited available test data. (iv) To, finally, derive practical and broadly applicable rules describing when and how chemical-specific ecotoxicity data can be split responsibly. In order to illustrate the potential relevance of splitting SSDs, we apply these rules to a set of chemicals and illustrate the effects for the derivation of protective standards and for the derivation of chemical-specific ecotoxicity effect factors for use in LCIA.[14,15]

METHODS

Overview. The study on splitting SSDs was developed with a selection of test data, the choice for the log-normal model to create SSDs, and following a stepwise approach for broader applications when splitting SSDs would be considered, where example outputs focus on both protective criteria and LCIA. These steps are elaborated below. We emphasize that the findings on splitting SSDs are generic and can be applied to other data selection criteria, statistical models, and SSD decision-support outputs. We followed the recommendations derived in the Global Life Cycle Impact Assessment Method (GLAM) effort under the auspices of the United Nations Environment Programme to derive metrics for assessing ecotoxicity impacts in LCIA as discussed by Owsianiak et al. 2023.[15] Main reasons are that the derived effect threshold of 20th percentile is close to the domain of environmentally relevant concentrations and that this threshold requires only a minimum of 5 species to have 1 tested species falling within the range below the HC20.

Experimental Test Data Curation and Harmonization. We started from a database of experimental ecotoxicity effect test data for 9868 chemicals,[4] from which we selected experimental data (i.e., we removed read-across data) for freshwater species for data harmonization and curation. This process included harmonization of species names, classification of species into taxonomic groups, and calculation of average effect test values across data points per combination of species and chemical (see Table A1). We then selected chemicals with data for three or more distinct species and taxonomic groups. The taxonomic groups used in developing the split-SSD argument were pragmatically inspired by the European Union (EU)-Water Framework Directive, which discerns various Biological Quality Elements—from which we selected three groups (here: Algae, cyanobacteria, and aquatic plants (A), Invertebrates (I), and Vertebrates (V)). The resulting data set for 180 chemicals is provided in the Supporting Information (Excel file Table S1).

Chemicals were first classified according to a systematic taxonomy based on the ClassyFire approach and assigned a mode of action (MoA) based on classifications from different pesticide resistance action committees, the Verhaar scheme,[16,17] and reported MoA information,[18] before mapping the chemical MoA to taxonomic groups.[19] If MoA information was lacking, the MoA reported for the chemical class was used. Chemical use categories were derived from prescribed use information (pesticidecompendium.bcpc.org).

As a next step, recognizing that the available raw species sensitivity data are of diverse kinds (acute NOEC, chronic NOEC, acute EC50, etc.), we used a set of data-driven extrapolation formulas to "translate" the diverse raw data types into a set of harmonized endpoint data, to enable derivation of SSDs from those. Since we illustrate the methodology for the context of LCIA, we extrapolated the available test endpoint data (e.g., acute and chronic NOEC, EC50, and EC10) to chronic EC10 equiv (the recommended starting point for deriving ecotoxicity impacts in LCIA). This was done by applying the formulas of Table 1, based on data-driven patterns recognized and described by Aurisano et al. 2019.[20]

Chemicals were then classified as "data-rich" (chemicals with ≥3 distinct species from the ≥3 taxonomic groups) for the present study or as "data-poor" (<3 distinct species per taxonomic group and/or <3 taxonomic groups) in line with, e.g., Müller et al. 2017.[21] The set of "data-rich" chemicals was kept for further analysis.

Systematic Decision Tree to Evaluate the Splitting of SSDs. All SSDs in the present study were derived as log-normal

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Table 1. Overview of Regression Equations Derived Based on All Available Freshwater Test Data for 9868 Chemicals Used to Extrapolate Laboratory-Test Derived Species Sensitivity EndPoints (the Diverse Set of Reported Sensitivity Metrics: Column 1) to Chronic EC10 equiv, Used in the Present Study to Derive SSD-EC10eq Based on the Extrapolated EC10-Equivalent Data for the 180 Chemicals

Endpoints	Extrapolation equation
Acute NOEC	$\log EC10_{chronic} = 0.816 \times \log NOEC_{acute} + 0.021$
Chronic NOEC	$\log EC10_{chronic} = 0.965 \times \log NOEC_{chronic} - 0.144$
Acute EC50	$\log EC10_{chronic} = 0.869 \times \log EC50_{acute} - 0.508$
Chronic EC50	$\log EC10_{chronic} = 0.872 \times \log EC50_{chronic} + 0.733$
Acute EC10	$\log EC10_{chronic} = 0.813 \times \log EC10_{acute} + 0.967$

distribution of species sensitivity (here: chronic EC10 equiv) data. Note that other SSD models may be fitted to the data, and those can be split for taxonomic groups, and the reasoning below can be specifically adapted if needed for those models.

A log-normal distribution is characterized by its mean and standard deviation, which are also used as moments of the log-normal SSDs (as μ and σ, respectively). A systematic evaluation decision tree was designed to distinguish between alternative outcomes (full split into three SSDs, if not: partial split, if not: no split). Given the two main arguments for splitting the data in taxonomic-group-specific SSDs, the first decision point is a statistical test series to evaluate whether some taxonomic subsets of data differ significantly from other subsets. The statistical tests for inter-SSD comparisons can conveniently be based on generally applied statistical test methods given the underlying distribution model (log-normal). The systematic evaluation decision tree for splitting SSDs thus considers among other evaluations of (dis)similarities in slopes and means, as depicted in Figure 1.

The following tests were executed, starting from the raw data and the relevant descriptive statistics (μ and σ), as illustrated in Figure 1. Levene's test was run to check the homogeneity of variances (considering thus σ = slope differences among SSDs). If variances are not significantly different, a one-way analysis of variance (ANOVA) (parametric) was run to evaluate the (dis)similarity of means (μ, the position parameter of the SSDs) among subsets of the three taxonomically grouped test data. Nonhomogeneous variance is a signal of differences across subsets of the data; in this case, the Kruskal–Wallis test (nonparametric) was applied to evaluate differences in μ. *A posteriori* multiple comparisons tests followed if there were significant differences between compared taxonomic groups (that is, Tukey's HSD (parametric) and Dunn's (non-parametric) tests, with the p-value set at 0.05). If full split (A $\neq$ I $\neq$ V) is *not* supported, the independent t test (parametric) and Mann–Whitney U test (nonparametric) were used to compare the mean of one taxonomic group versus the other two groups merged, e.g., A $\neq$ I + V. For further confirmation, the conclusion drawn from these tests on splitting SSDs was evaluated by deriving a linear regression model, whereby one group is considered an "anchor" to test whether others differ from the anchor (see Table 2).

The set of results (on differences in σ's and/or μ's) were collated, together with the *a posteriori* test results, to draw a conclusion on statistical motives to employ a full or partial split based on the whole assemblage of test data for a chemical (minimum: 3 × 3 = 9). The resulting SSDs may be three SSDs based on (as a minimum) three data points each, which can thus produce nonrobust outcomes based on the identified subsets of the data. Therefore, the decision tree proceeds with an evaluation of the robustness of the resulting SSDs, whereby nonrobust outcomes are identified. Robust SSDs were defined by quantifying the confidence interval around the derived LCIA ecotoxicity impact metric (i.e., HC20). A robust SSD yields an HC20 with a narrow confidence interval, whereby we pragmatically applied 5 squared geometric standard deviation as the boundary below which we identify the HC20 of a split-, partially split, or no-split as robust. Where a split caused a thus-defined nonrobust SSD, it was investigated whether partial remerging (i.e., A+V, I+V, or A+I) or full-remerging (A+I+V) resulted in robust SSDs (following the same robustness test).

All the statistical analyses and the building of split SSDs were performed in R version 4.1.2,[22] modifying and expanding existing code to construct SSDs (edild.github.io/ssd). Figures were generated using the ggplot R package, version 3.4.1.[23]

Evaluating Mode of Action and Use Category Information. The results of the splitting procedure were evaluated *vis-a-vis* information on MoA and use categories, whereby it was expected that specific modes of action (e.g., insecticidal action) or use category (e.g., labeling as an insecticide) would imply splitting off (at least) the target taxonomic group as a separate SSD. Data were plotted in different subgroups and combinations to verify associations between these two aspects and the results of the splitting approach.

Derivation of User-Oriented Impact Metrics (HC20) Values for LCIA. The SSDs that resulted from the split-assessments are (in the case study) SSDs based on chronic EC10 equiv (SSD-EC10eq) of a compound, which are in turn used to define the ecotoxicological effect factor of that compound at the HC20-level (the 20th percentile of that SSD).[15,24] Thus, for sufficiently robust SSDs, we derived those values for all of the studied compounds. In addition to this, we also illustrate the impacts of splitting on protective regulatory standards (related to HC5, PNEC, and similar concepts) for some selected compounds. Finally, the uncertainty assessment around taxonomic group-specific HC20s was quantified by combining intraspecies and interspecies variability (see Supporting Information Uncertainty analysis section for details).

RESULTS

Harmonized Ecotoxicity Test Data for Freshwater Species. We start with 120,835 species-specific toxicity test data, totaling 9868 chemicals, 1123 species, and 234 test endpoints, distributed as shown in Figure 2. Because of nonsystematic global testing practices, the data set does not equally cover the taxonomic groups, test durations, and endpoint types. Invertebrates are the primary taxonomic group (78%) in the data set. Likewise, acute toxicity data dominate the data set (71% of the total data across taxonomic groups), mainly acute EC50s for invertebrates (44,077 acute EC50s and 20,000 acute NOECs), with fewer chronic EC50s (n = 5555), of which only very few (n = 82) data are for vertebrates.

Species sensitivities span many orders of magnitude for both short-term peak and longer-term chronic exposures (Figure 2). For example, acute EC50s range between 7.3×10^{-8} and 3.3×10^{-9} μg/L for invertebrates, and chronic EC50s range from 1.6×10^{-5} to 1.0×10^{-8} μg/L for invertebrates. Likewise, acute NOECs range between 8.0×10^{-7} and 1.0×10^{-9} μg/L, and chronic NOECs range between 6.0×10^{-6} and 2.5×10^{-8} μg/L.

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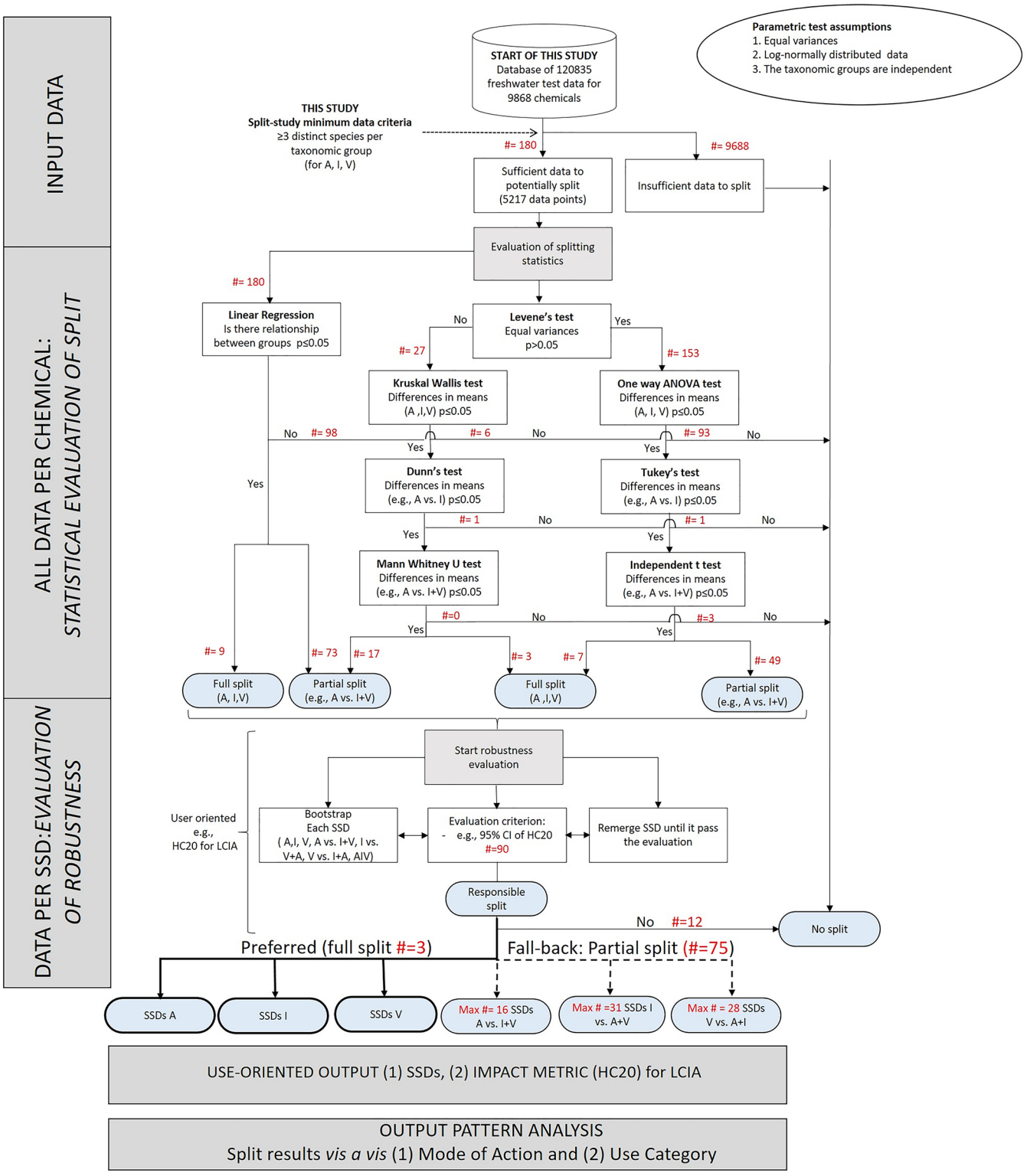

Figure 1. Schematic summary of a decision tree for evaluating whether the assemblage of available ecotoxicity data for a chemical can be subdivided into (here) three taxonomic groups based on three specific Water Framework Directive defined Biological Quality Elements: Algae, cyanobacteria, and aquatic plants (A), Invertebrates (I), and Vertebrates (V). Numbers of cases involved in each step (as result) are shown in red. (top) Description and selection of input data for the start of the splitting approach. (middle) Analysis steps to evaluate statistical motives to split using all available data per chemical (fully or partially). (bottom) Evaluating whether the split results in a trade-off of a nonrobust species sensitivity distribution (SSD) for one or more subgroups. (bottom gray blocks) Summarizing results for practical Life Cycle Impact Assessment (20th percentile values) and evaluating whether statistical split covaries with the mode of action and use category information. Note that the no-split approach is the historically best-known form and most frequently applied format of constructing SSDs and using those for environmental decision support purposes, prescribed in various policy guidance documents (details vary across jurisdictions).

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Table 2. List of Statistical Tests Used to Evaluate the Potential of Split SSDs for Different Taxonomic Groups: Algae, Cyanobacteria, and Aquatic Plants (A), Invertebrates (I), and Vertebrates (V)

Step	Statistical test	Description	Interpretation
1	Levene's test	-Tests the null hypothesis that the variances (related to the SSD σ-parameter) of different taxonomic groups (A, I, V) are equal	$p > 0.05$ $\sigma_1 \approx \sigma_2 \approx \sigma_3$
		-If $p > 0.05$, the test does not (fails to) reject the null hypothesis that the variances of different taxonomic groups are equal. If $p < 0.05$, then taxonomic groups have different slopes (SSDs are different)	
1.1	One way ANOVA test	-Tests the null hypothesis that the mean (related to the SSD μ-parameter) of different taxonomic groups is equal (A, I, V)	$p \leq 0.05$: $\mu_1 \neq \mu_2 \neq \mu_3$
		-If $p > 0.05$, then taxonomic groups have the same mean. If $p \leq 0.05$, then taxonomic groups have different means (SSDs have different position parameters)	
1.1.1	Tukey's test	-Test multiple pairwise comparisons between groups' means (μ) to identify which groups have a different mean (e.g., A vs I)	$p \leq 0.05$: $\mu_A \neq \mu_I$
		If $p > 0.05$, then taxonomic groups have the same mean	
		-If $p \leq 0.05$, then taxonomic groups have different means (specific split of SSDs).	
1.1.2	Independent t test	-Tests the null hypothesis that the mean (μ) of different taxonomic groups is equal (e.g., V vs I+A)	$p \leq 0.05$: $\mu_V \neq \mu_{I+A}$
		-If $p > 0.05$, then taxonomic groups have the same mean	
		-If $p \leq 0.05$, then taxonomic groups have different means. (specific split of SSDs)	
1.2	Kruskal–Wallis test	-Nonparametric equivalent of ANOVA test, tests the null hypothesis that the mean (μ) of different taxonomic groups is equal (A, I, V)	$p \leq 0.05$: $\mu_1 \neq \mu_2 \neq \mu_3$
		-If $p > 0.05$, then taxonomic groups have the same mean	
		-If $p \leq 0.05$, then taxonomic groups have different means.	
1.2.1	Dunn's test	-Nonparametric post hoc test similar to Tukey's test. Test multiple pairwise comparisons between groups' mean to identify which taxonomic groups have different means (e.g., A vs I)	$p \leq 0.05$: $\mu_A \neq \mu_I$
		-If $p > 0.05$, then taxonomic groups have the same mean	
		-If $p \leq 0.05$, then taxonomic groups have different means.	
1.2.2	Mann–Whitney U test	-Nonparametric equivalent to independent t test, test the null hypothesis that the mean (μ) is equal across different taxonomic groups (V vs I+A)	$p \leq 0.05$: $\mu_V \neq \mu_{I+A}$
		-If $p > 0.05$, then taxonomic groups have the same mean	
		-If $p \leq 0.05$, then taxonomic groups have different means.	
2.0	Linear Regression	-Linear model with categorical predictors.	$p \leq 0.05$: $\mu_A \neq \mu_I$
		-Test group-level differences between groups (e.g., A vs I). If $p \leq 0.05$: the test rejects the null hypothesis that the mean (μ) is equal across different taxonomic groups; thus, the taxonomic groups are not related	

From the curated data, 180 chemicals were selected as data-rich (chemicals with data for ≥3 distinct species from ≥3 taxonomic groups), yielding 5217 test end point data for developing and testing the SSD-splitting framework. Upon deriving the chronic-EC10-values from these data, some taxa dominate the final data-rich subset for further study steps, with 47% invertebrates, 33% vertebrates, and 20% algae, cyanobacteria, and aquatic plants. Note that only 1.81% of the chemicals have sufficient data for a potential full split into three taxonomic group-specific SSDs.

Split SSDs for Different Taxonomic Groups and Relation to Mode of Action. Statistically significant support for full or partial splitting into SSDs representing Algae, cyanobacteria, and aquatic plants (A), Invertebrates (I), and Vertebrates (V), or the combinations of AI, AV, or IV, was found for 3 (<2%) and 75 (42%) out of 180 data-rich chemicals, respectively, based on statistical tests comparing the mean and standard deviation of SSDs (procedure in Figure 1). Notably, the available data support the use of a split-SSD modeling approach for some narcotic chemicals (see Figure 3), which is visually indicated by nonoverlapping means (dots) and standard deviations for different taxonomic groups. This latter outcome was highly unexpected, given three decades of accepted no-split SSD practices and the expectation that split-SSDs would be found for only chemicals with a specific mode of action. However, a split can also be warranted for narcotic chemicals, provided that the available data set is sufficiently rich. The Supporting Information Excel file (Excel Table S2) presents all statistical details and characteristics of the resulting SSDs.

We found a clear and consistent pattern of higher sensitivity of the targeted taxonomic group for chemicals with a specific MoA (63% of the 180 chemicals; see Supporting Information Figure S1), with, as expected, the more sensitive taxonomic groups predominantly represented at the lower end of the shown sensitivity distribution patterns (See Figure 3). For example, I and A are the most sensitive groups in the panels shown on insecticides (especially AChE (Acetyl Choline Esterase) inhibition) and photosynthesis inhibition, respectively. The nontargeted groups are often not statistically different, resulting in a partial split, likely partly attributable to the absence of an MoA-related mechanism that would induce a split or possibly also due to lower numbers of test data for the nontargeted taxonomic groups.

We summarized the data further, using the "working point" on impacts that are used to derive LCIA Effect Factors based on global consensus recommendations (the Hazardous Concentration for 20% of the species, HC20, derived from chronic EC10 equiv). This yielded similar results, here illustrated for the gross chemical use categories. Again, there was a match between the expected and observed sensitive taxonomic groups, e.g., insecticides and I and herbicides and A; Figure 4a. Unexpectedly, one herbicide (tributyltin-cation, CAS 36643-28-4) with an endocrine-disrupting MoA affected invertebrates, which appeared as the most sensitive taxonomic group. Fungicides showed general toxicity, with the most sensitive

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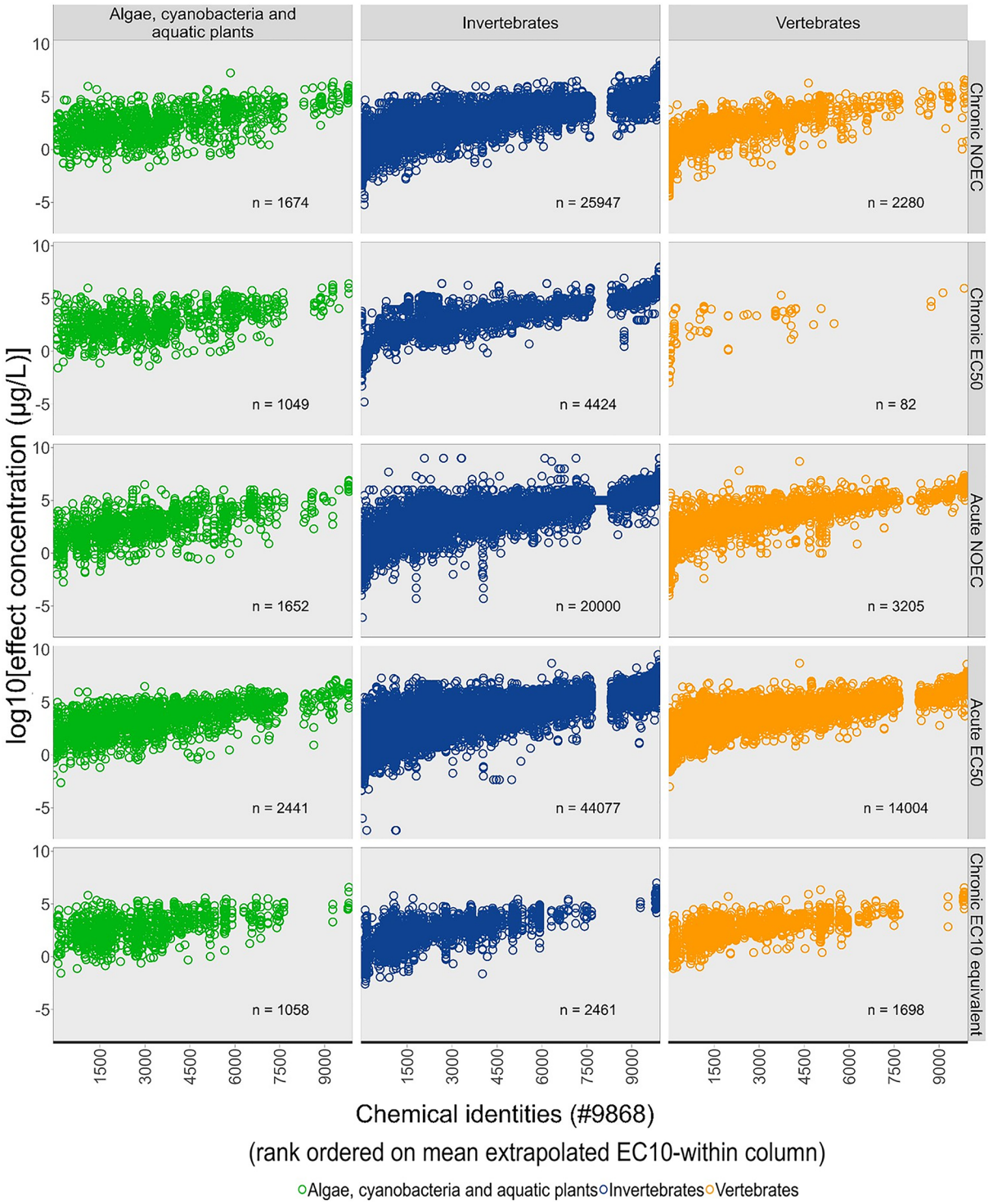

Figure 2. Distribution of 120,835 species sensitivity endpoints (Y) for 9868 rank-ordered chemicals (X), distinguishing taxonomic groups (colors) and endpoint types (measured: rows 1 to 4; extrapolated split-research data: row 5). Rank order was based on mean extrapolated chronic EC10-equivalent values per chemical for each column. On the *x*-axis, value gaps lack data for taxonomic group × endpoint combinations (e.g., vertebrates × NOEC) for the gap intervals.

taxonomic groups differing across chemicals, with algae, cyanobacteria, and aquatic plants tending to be less sensitive than other taxonomic groups. Moreover, chemicals with no specified use category ("Other uses") showed no clear pattern of any taxonomic group being affected most.

Specific Regulatory and Decision-Relevance Issues. The relevance of our findings for the various contemporary decision-support uses of SSDs is illustrated first by the fact that our analyses cover 15 Water Framework Directive (WFD) Priority Substances (marking chemicals of current Europe-wide concern, black stars in Figure 4) and two chemicals listed under the fourth WFD Watch List (black crossed dots in Figure 4, marking chemicals of emerging concern for water quality policies).[25] Second, the relevance of splitting for the outcomes of

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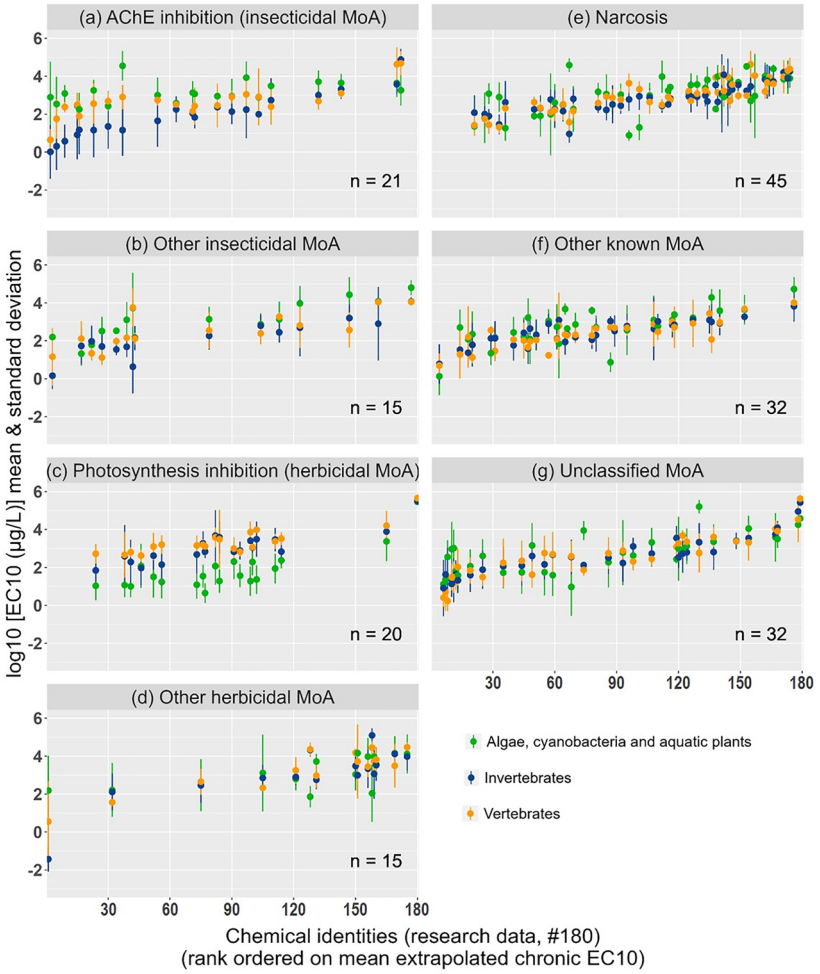

Figure 3. Distributions of the species sensitivity impact metric (Y, chronic EC10 equiv) for 180 rank-ordered chemicals (X). Taxonomic groups are colored. Chemicals within each panel are rank-ordered based on the mean impact metric values calculated across all data per chemical. MoA = Mode of Action, *n* = number of chemicals in each panel. SSD-splitting grossly coincides (visually) with nonoverlapping dots and standard deviations for different taxonomic groups (colors). Letters in each panel: For chemicals targeting invertebrates: (a) AChE inhibition (insecticidal MoA; chemical class with most data for this MoA); (b) other insecticidal MoA (lumped); for chemicals targeting algae, cyanobacteria, and aquatic plants as primary producers; (c) photosynthesis inhibition (herbicidal MoA with most data); (d) other herbicidal MoA (lumped); for chemicals with baseline toxicity: (e) Narcosis. (f) "Other known MoA" includes chemicals for which MoA was provided but for which a specific targeted taxonomic group is unknown. (g) "Unclassified MoA" includes chemicals for which MoA information was lacking.

using SSDs for decision making shows as substantial differences between SSD-based insights on protective criteria and/or impacts of chemicals generated without (classic approach) and with applying our proposed (partial) split, elaborated in the next section further.

Third, based on currently available data, the split concerns a relatively large proportion of the chemicals. The use category "herbicides" involved 56 chemicals, among which data for 29 chemicals (statistical output) supported a full or partial split, with a suggested partial splitting of algae, cyanobacteria, and aquatic plants from the other taxonomic groups for 25 chemicals (A vs I+V). The use category "insecticides" involved 27 chemicals, among which data for 20 chemicals (statistical output) supported a full or partial split, with a suggested splitting of I from the other groups for 14 chemicals. The "fungicide" category involved 32 chemicals. Data for 14 of these chemicals supported a partial split; data for 14 of these chemicals supported a partial split, whereby 12, 8, and 6 were separated from the rest of the group for primary producers, invertebrates, and vertebrates, respectively.

The "Other uses" category involved 65 chemicals; data for 27 (statistical output) chemicals supported partial splitting, with data for 15, 14, and 17 chemicals showing a splitting of A, I, and V from the rest, respectively. This indicates that there are certain chemicals in industrial or other (nonagricultural) uses to which particular taxonomic groups are more sensitive than others. For instance, algae, cyanobacteria, and aquatic plants appeared sensitive to 2,4-dinitrotoluene, and vertebrates appeared sensitive to phenol, both relevant in polymer and plastic production. Again, note that splitting SSDs is not limited to chemicals with a specific known MoA or specified use category.

Quantitative Implications of Split SSDs for Decision Support. The relevance of the proposed split-SSD approach in decision support is shown quantitatively in Figure 5, illustrating all potential outcomes (no split, full split, and partial split). Vertical black and red lines show that critical concentrations (at the fifth and 20th percentile levels) derived with the SSDs differ substantially between the classical no-split approach and the full- or partial-split approaches. That is shown for the derivation of protective environmental standards (black vertical lines, where a

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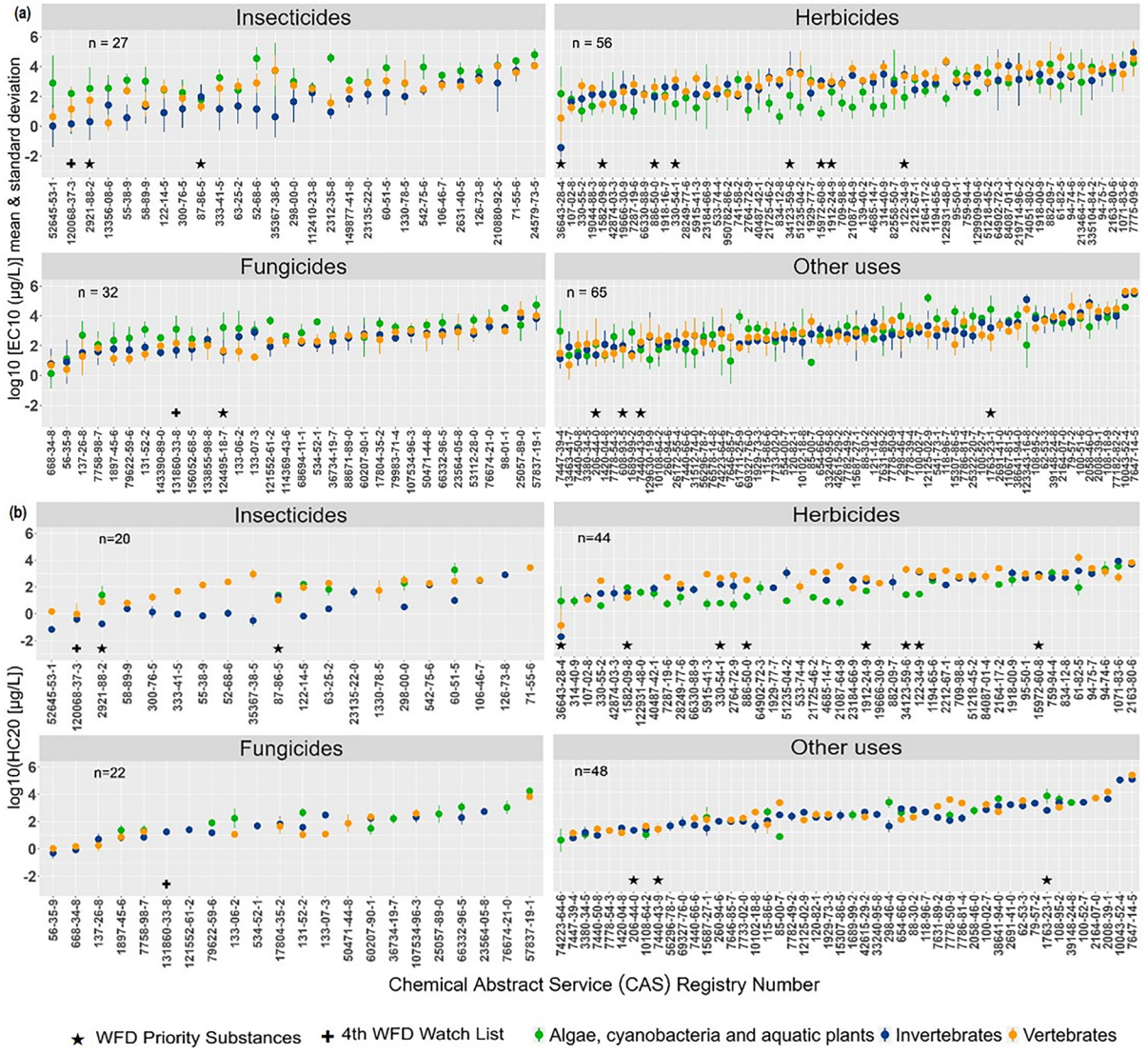

Figure 4. (a) Distributions of the species sensitivity impact metric (Y, chronic EC10 equiv) for chemical use categories (panels), rank ordered as in panel (b). Use categories: Insecticides are chemicals used for targeting invertebrates; Herbicides are chemicals used for targeting algae, cyanobacteria and aquatic plants as primary producers; Fungicides are chemicals targeting fungi. "Other uses" are chemicals for which the targeted taxonomic group is not specified (e.g., industrial chemicals). (b). Similar results present the HC20-metric that is practically used in Life Cycle Impact Assessment global consensus approaches (20th percentile of the distribution of the above impact metric across tested species) for only robust SSDs. Percentile values of SSDs are expressed in LCIA as HC = Hazardous Concentration. HC20-estimates (with standard deviations) are summarized for 134 chemicals with ≥6 data points (low uncertainty) for 261 chemical-taxonomic group combinations.

regulatory-defined low-end, fifth percentile value of a chronic-data SSD is used to express a critical protective concentration, X) and for the estimation of the impact of pollution, by quantifying the potentially affected fraction of species (Y) at an ambient exposure level (X), here at the 20th percentile of the SSD (red vertical lines). Figure 5 also illustrates the influence of splitting for confidence intervals around the SSDs (gray bands), which are wider for data-poor SSDs. In these examples, the HC5 or HC20 can shift by more than 1 order of magnitude as a consequence of splitting, as compared to the whole-assemblage (classical) SSD.

The first 4 rows of the plots (i.e., simazine, fenthion, trichlorfon, and sodium pentachlorophenate) illustrate SSD patterns for chemicals with nonoverlapping 95% confidence interval (CI) ranges of their split SSDs, supporting a partial or full split (supported by statistical tests, see Methods). In comparison, pyraflufen-ethyl showed overlapping 95% CI ranges, and statistical tests did not support splitting. In all cases where a responsible split was supported by statistical evaluation, the whole-species assemblage SSDs showed that the observed test data for one or more particular taxonomic groups were unevenly distributed over the SSD, with, e.g., the sensitive taxonomic group clustering toward the lower tail (Figure 5).

Decision Tree and Resulting HC20s. Figures 3−5 summarize and illustrate results and decision-supporting relevance of splitting to derive taxonomic group-specific SSDs. These results reflect outcomes of a systematic decision tree (Figure 1), in which test data, statistical testing to motivate a split, and user-required SSD-robustness considerations are combined. In our analysis, the assessment of all available data reveals that a full or partial split would be supported for 90 of the 180 chemicals. However, judgment of the trade-off effect on SSD robustness showed that three chemicals resulted in robust full split SSDs, 75 in partial split SSDs as a fallback option, and

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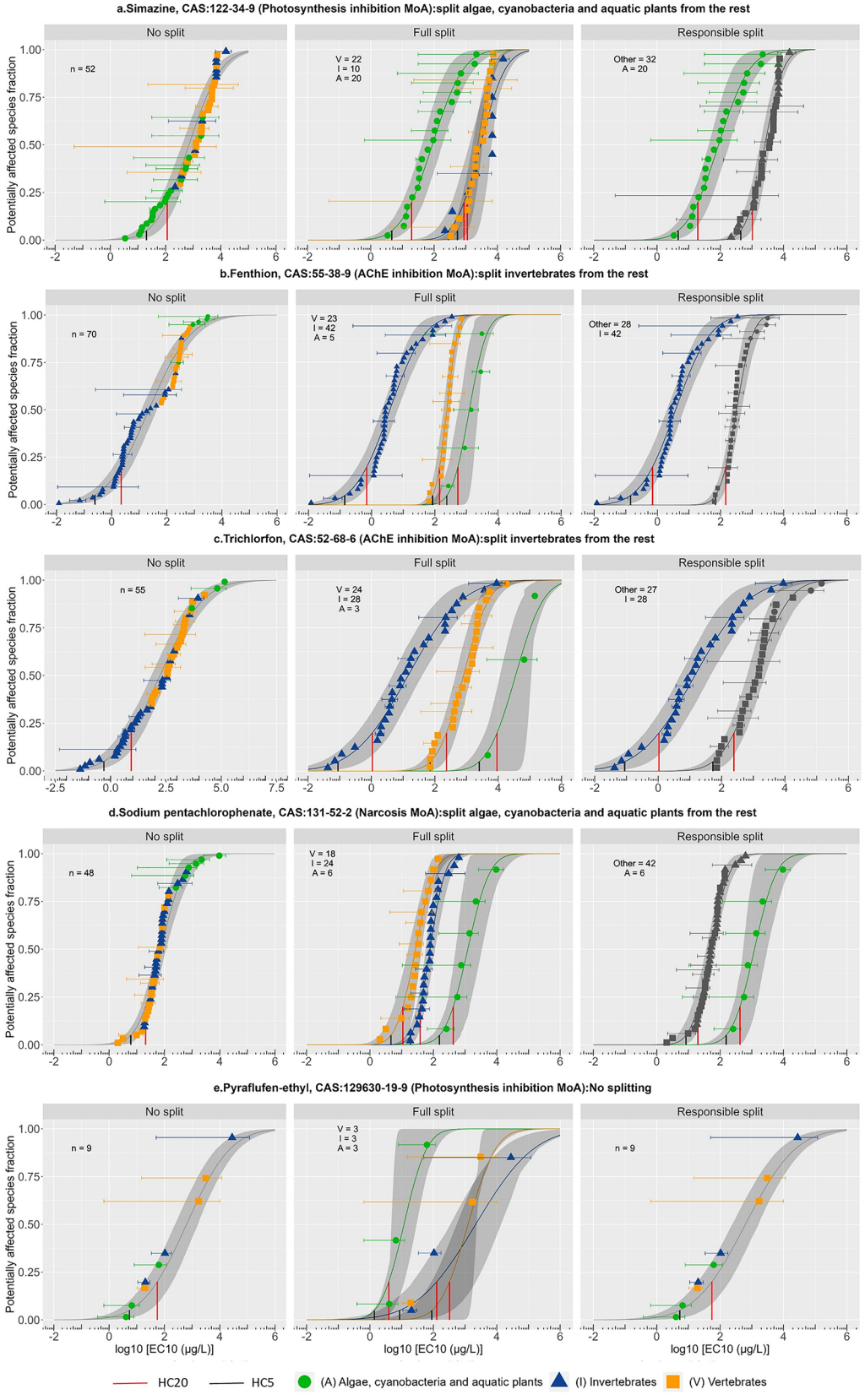

Figure 5. Illustration of deriving no-split, full-split, or partially split (taxonomic group-specific) species sensitivity distributions and its consequences for deriving protective environmental quality standards and for use in Life Cycle Impact Assessment (LCIA). There is no consequence for these decision support applications if vertical black or red lines, respectively, are similar for full- or partial-split SSDs compared to no-split SSDs. Black or red lines for a chemical show differences of up to more than 2 orders of magnitude. Columns: no-split (historically the common SSD use, left), full split (middle), and responsible split (right) SSDs. Rows are selected chemicals. Panels show sensitivity endpoints across species (dots: chronic EC10-equivalents), sigmoid curves (log-normal fitted SSDs), and 95% confidence intervals (shaded). Protective standards (maximum concentrations) are

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Figure 5. continued

derived from a uniform policy-chosen Y = 0.05, with protective threshold concentrations (Hazardous Concentration for 5% of the species, HC5) derived on X (black lines). Impact magnitudes in LCIA are derived from a uniform Y = 0.2 with similar consequences derived on X (red lines). Rows from top to bottom illustrate the results of different MoA-related splitting situations and results from data-richer to data-poorer chemicals. Row 1: data-rich herbicide (simazine); rows 2 and 3 data-rich insecticides (fenthion and trichlorfon); row 4 data-rich chemical with baseline toxicity (sodium pentachlorophenate); row 5: data-poor herbicide (pyraflufen-ethyl). The error lines of the data points show intraspecies variability in the test data set for each chemical. Interspecies variability is represented by 95% confidence intervals (gray bands) based on bootstrapping (1000 iterations). With sufficient data, SSD splitting is supported for chemicals with specific MoA (rows 1, 2, 3, and 5) but (surprisingly) also for baseline toxicity (rows 4).

102 in nonrobust split SSDs, of which the latter are therefore represented by the classical no-split SSD (Excel Table S2). The final result on SSDs robustness involved a pragmatic decision to include only SSDs with a squared geometric standard deviation (GSD^2) $\leq$ 5 around the log-mean as a cutoff point. The sequence of steps and analyses of the shown decision tree can be used to judge whether available test data on a chemical can be (partially) split SSDs into robust results for chemicals other than the 180 study chemicals included in this study (shown for the log-normal model, but applicable in a similar way to other data and model choices).

DISCUSSION

Triggered by a need to set regulatory, protective environmental quality benchmarks, the global regulatory use of SSDs started with recognizing that log-transformed ecotoxicity test data collected for various species appeared to follow a bell-shaped distribution.[4] Thereupon, the first broad use of SSDs was to derive protective environmental quality standards from all available data, based on the assumption that the distribution of sensitivities of tested species resembles that of the nontested field species assemblage.[6] This subsequently provided the basis for a wide array of uses of SSDs in environmental quality protection, assessment, and management. However, almost all of the (regulatory) applications still derive SSD from all data, aiming to represent the whole species assemblage following initial decisions. Various jurisdictions prescribe different minimum requirements to the underlying effect data, recognizing (i) that different taxonomic groups should be represented to fulfill the adopted assumption and (ii) that more test data generally imply more robust SSD models. The present study reconsidered all this and evaluated from "first-principles" (the two ecological and statistical motives) whether the derivation of a (partially) split SSD is warranted and, if so, how that would operate and work out for decision support uses. Illustrated by chemicals with a minimum of necessary test data, we demonstrate that an improved fit of SSDs to the underlying data can be found even for some chemicals with a narcotic (nonspecific) MoA, in contrast to common expectations and our own initial beliefs.

Although the results show how the splitting of data into taxonomic group-specific SSDs improves the fit of the models to the data and the final decision-support interpretations caused by that, it should be recognized that the use of SSDs derived from laboratory toxicity data is not a panacea for all environmental problems with chemicals. The complexity and diversity of the chemical pollution problem has, so far, resulted in additional useful methods to characterize hazards, among which we include the derivation and use of field SSDs and bioassays—which are both methods that do not require laboratory-to-field extrapolation (as in classical SSDs). Environmental policies and bridging applied ecology and ecotoxicology can be served by investigating multiple lines of evidence to disentangle the effects of multiple stressors and unintended ambient mixtures.

The present study provides the scientific answer on "to split or not to split"; it is scientifically better to split SSDs according to taxonomic grouping (better fit of the models to the data and associated decision-support implications) unless the number and quality of available test data limits that. Whether this is implemented in practice and how far this would provide improved environmental assessments and management depend on the jurisdiction, the available data per chemical, and the difference in SSDs between taxonomic groups. If implemented in practice, there would be consequences for data collection (seeking to add data that appear missing for specific taxonomic groups) as well as for derivation of protective standards (such as HC5, PNEC, and similar terms) for LCIA and environmental quality assessment (potentially affected fraction of species). The split-approach undoubtedly results in fewer data per (split-)SSD, which may have various consequences in practice. Such SSDs may be statistically robust (as in the present study results). However, the splitting also relates to the debate on the "minimum number of species (or taxonomic groups) per SSD" and using an optional Safety Factor on an HC5 derived from a split-SSD. The minimum-number debate would need to ascertain that a (likely lower) minimum number of tests should represent the sensitivity variation within a taxonomic group. The Safety Factor debate—which was triggered by uncertainties—would need to consider that the method likely lowers the HC5 because of accounting for the sensitive taxonomic group, addressing part of the uncertainties of the "classical" approach. Such debates can start upon adopting splitting based on the methodology laid out in the present study.

Our results suggest that "always-splitting" is warranted as a starting point for any assessment but that splitting may be limited, and has trade-offs, given the characteristics of the available data. Specifically, an exploration of the available base data (120,835 toxicity endpoint values) shows that invertebrates are frequently tested and evaluated,[26−29] which results in a higher likelihood of finding robust split SSDs for invertebrates. The lowest number of data was available for bacteria and fungi, which hampers assessment of risks for these groups as well as for important functions of microorganisms in ecosystems, even without the opportunity to derive a split SSD for this group, where relevant. This points to the need for more tests of microorganisms in freshwater ecosystems. Despite limitations due to data scarcity, it is evident that a partial split may result when assessing chemicals other than the 180 studied chemicals.

The Role of Mode of Action and Use Category Information. Splitting is principally warranted from the viewpoint of applied ecology, where distinctive bioassessment approaches focusing on taxonomic groups are common. However, the motives for splitting have always been expected to be stronger for chemicals with a specific MoA or more grossly defined chemical use categories. In general, the results of both

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mechanistic MoA and chemical use category considerations closely matched expectations on this, supported by patterns shown in Figure 3 and Figure 4. For instance, invertebrates appear at the lower level of the sensitivity distribution (lowest SSD mean) for chemicals designed to operate via the AChE-inhibition and labeled as insecticides, while primary producers fall into the lower tails of the distributions for photosynthesis inhibitors used as herbicides. Our results confirm that it is key to consider MoA and/or the use category (where this applies, such as for pesticides) information on chemicals to trigger, considering the use of better-fitting models for separate taxonomic groups.[30−32] The better principles and the better fit have implications for practical uses of SSDs, both for deriving protective standards and for use in environmental impact assessments and LCIA (Figure 5).

We made two additional notable observations. First, confirming previous studies and theory, the lowest variation in sensitivity across taxonomic groups was found in chemicals with nonspecific MoA, i.e., narcosis (Figure 3, vertical spread). This supports the hypothesis that even among not closely related species, toxicity through nonpolar narcosis is associated with relatively lower interspecies sensitivity differences.[33] Although there may be an applied ecology and a statistical reason to consider splitting data-rich chemicals with a narcotic MoA, the improved fit mainly implies improved SSD-based outputs for chemicals with specific MoA, making the latter the primary focus for aiming at splitting SSDs in practice.[3] Second, some outcomes are not predictable by MoA or use category information. For example, the herbicide tributyltin-cation (CAS 36643-28-4) is relevant in chemical formulations designed to control weeds, but there are potential side effects on invertebrates, more than for other herbicides. Most of the observed lower sensitivities in our SSDs are evidence of unwanted side effects, which are visible despite the diversity of the side effects, a low number of tests, and a few chemicals designed to control vertebrates.

The application of splitting or not has various implications for decision support. Scientifically, it is likely that a study on the calibration between data on the predicted msPAF values for an array of sampling sites and the observed effects on a particular taxonomic group at those sites is more meaningful when based on split SSDs, given the more accurate impact assessment upon splitting (Figure 5). For example, observed impacts on invertebrate species derived from ecological monitoring can be better calibrated to the msPAF derived from the SSD-Invertebrates than from the classical overall SSD, with a misfit of the SSD to the test data. This calibration (higher PAF implies higher risk, proven with calibration data or not) is often used as a basis for the decision-supporting uses of SSDs. Specific calibration work can now be undertaken to quantify the Potentially Disappearing Fraction (PDF) of species due to chemical exposure, given the possibility of quantifying impacts in terms of PAF from the split SSDs. PDF is a biodiversity damage metric used in LCIA for all impact categories related to ecosystem quality.[34,35]

In general, the decision-support uses of SSDs will be conceptually improved and numerically altered with split SSDs, provided that those are robust. This holds for data sets, model choices, or SSD-based output metrics that differ from the data, model, and metrics used in the present paper. The finding that splitting SSD is relevant in any case holds without prejudice to either of these matters. Upon splitting, conceptual numeric improvements may result in more accurate protective standards and better quantitative impact assessment of predicted or observed ambient pollution.[36] The black lines in Figure 5 (subfigures a, b, c, and d) illustrate that a protective (no impact) environmental quality standard, estimated as fifth percentile from an SSD of chronic data, is lower when a split SSD is employed. That is understandable, as HC5 for the most sensitive group now represents a protection of 95% of the species in that group. In turn, this has (similar) implications for regulatory-adopted criteria based on these estimated HC 5s, such as the PNEC. The red lines in Figure 5 (subfigures a, b, c, and d) show similar results for the LCIA-employed impact metric at the 20th percentile, leading to different impact estimates of the use of chemicals in products based on splitting.

After evaluation of the decision tree (Figure 1), our observation confirms that chemicals with more data (e.g., simazine and fenthion) and a specific MoA provide the strongest basis for responsible splitting. Thus, the more data, the more robust the SSDs after the (partial) split, even for narcotic chemicals (e.g., sodium pentachlorophenate). However, species selection bias during laboratory testing (i.e., for chemicals with specific MoA) currently limits to often find full-split SSDs. For example, trichlorfon, an insecticide operating via the AChE inhibition, statistically supports a full-split SSD. In contrast, few data points for a nontarget taxonomic group (i.e., Algae, cyanobacteria, and aquatic plants; $n = 3$) result only in partial splitting based on the SSD robustness check, indicating the need to include more tests for nontarget species to derive taxonomic group-specific split SSDs where appropriate. Thus, for one to have a full split SSD, all the taxonomic groups require sufficient data. At the other end of the spectrum, avoiding a negative trade-off for prediction accuracy with nonrobust SSDs that occur for data-poor chemicals is essential. For example, although the statistical tests on all available test data suggest that the primary producers could be split off from the rest of the groups for pyraflufen-ethyl, the broad and overlapping confidence intervals render the whole-assemblage SSD statistically more robust as compared to the partial-split alternative. Statistical assessments may be used to decide on split SSDs, or not, but not solely. It is also important to evaluate whether splitting is better in practice, yielding better decision support based on conceptual principles and trade-off effects. Relatively data-poor chemicals may result in split SSDs that are not robust for one or more taxonomic groups because the process of splitting counters the statistical rule that "more data result in more robust SSDs" (see Figure S2).

We selected 180 data-rich chemicals to develop a decision tree for splitting SSDs (Figure 1), which can be employed (or adapted) for all chemicals and taxonomic groups or other ways to group chemicals, species, or statistical models. In the exploration of data for a chemical, it is likely that a partial split (e.g., Algae, cyanobacteria, and aquatic plants versus Invertebrates and Vertebrates together) is found for many chemicals or other taxonomic groups than investigated in the present study. Applications to split according to specific traits or trophic positions would follow the same decision tree logic, resulting in risk information on chemical effects on specific traits or trophic positions.

Overall, our study highlights that splitting is a better approach in deriving SSDs and using the models for decision support, provided that the resulting SSDs are sufficiently robust. Robust split results improve the fit of the models to the data and, therefore, the interpretation of SSDs in the discussed uses. The relevance for decision support may potentially be further increased when a split would consider different service-providing units (SPUs), a concept used in the context of

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ecosystem services research.[34] This is because it is key not only to protect and restore biodiversity in terms of structural characteristics of ecosystems (the present use) but also in terms of functional characteristics and provided services.[37,38] Assessments that would consider ecological information, such as functional groups or trait characteristics, may help to identify the SPU and ecosystem services that are both valuable and potentially impacted.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c04968.

A protocol for deriving split SSDs, including an R code used for split SSDs statistical analysis based on the process flow in Table A1 and uncertainty analysis in Table A2 (PDF)

Data used to evaluate the splitting of SSDs (XLSX)

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Notes

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Supplemental Material

To split or not to split: Characterizing chemical pollution impacts in aquatic ecosystems with species sensitivity distributions for specific taxonomic groups

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The supplemental material consists of five sections with two supplemental figures and two supplemental tables.

Total number of pages: 33

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Experimental test data curation and harmonization

Table A1 Process flow for developing SSD-based hazard concentrations (HC20) for freshwater aquatic species for use in Life Cycle Impact Assessment (LCIA)

#	Step	Description/Explanation
1	***Pre-processing***	
1a	A unique list of **chemicals**	• Identify the unique list of chemicals from the list published in Posthuma et al. 2019, and map the list of chemicals for which experimental data were available in the curated raw data underlying Posthuma et al. 2019 • Removal of double entries based on CAS RN search in CompTox Dashboard • Manual identification of validity of CAS RN not listed in CompTox Dashboard • Search missing chemical descriptors from CompTox in chemidplus and pubchem databases
1b	Mapping of chemical classification on the unique list of chemicals	• Extract from ClassyFire chemical taxonomy kingdom, superclass, class, and subclass (where available) for all unique chemicals based on CAS and SMILES matches • Extract from Pesticide Compendium (earlier: Alan Wood) chemical class and target class (e.g., herbicides) for all unique chemicals based on CAS matches • Manual identification of chemical taxonomy for chemicals that were not identifiable in ClassyFire
1c	A unique list of toxic **modes of action** and mapping on the unique list of chemicals	• Identify from Pesticide Resistance Networks (FRAC, HRAC, IRAC) a unique list of specific modes of action, and extract related chemical groups and example chemicals listed • Extract from Verhaar scheme 4 generic modes of action classes for all unique chemicals based on CAS matches • Map the toxic modes of action on the unique chemicals list
1d	A unique list of **species**	• Identify the unique list of species from the list available in curated raw data underlying Posthuma et al. 2019, table "Selected_Tox_data_measured" • Correction of misspelled species names based on searching species names in Global Names Resolver • Remove double entries based on genus and species names • Remove varieties and sub-species names and merge those into data for the same species • Replace common species names with scientific species names based on IUCN species lists, and complement by manual searches for common names not listed in IUCN • Replace old and synonym species names with current species names according to IUCN and GBIF taxonomy, and in case of mismatches between IUCN and GBIF, take IUCN as a priority • Extract a list of habitats from the list in curated raw data underlying Posthuma et al. 2019, and assign either "freshwater," "marine," "terrestrial," or "other" as habitat type, and map habitat types on the list of species • Filter out all species that are not associated with "freshwater" as habitat type • Define default species for different taxonomic levels (e.g., family) based on common species used in risk assessment or else based on dominant species within a given level (e.g., genus)

#	Step	Description/Explanation
		• Assign scientific species name to all entries that are reported for higher level only (e.g., family or genus): for dominant species within a given level (e.g., genus), assign this species, else assign no species • Assign artificial species name for levels where no species name is reported, and no default species name can be assigned, based on test-ID in curated raw data underlying Posthuma et al. 2019 (to keep data points separate for supposedly distinct species)
1e	Mapping of taxonomic classification on the unique list of species	• Extract as background list of unique species and their taxonomy (i.e., kingdom, phylum, family, order, class, genus, species) from IUCN (based on web-search function) and from GBIF (based on code-based downloads) • Extract from IUCN, GBIF, or EnviroTox taxonomy databases habitat type (freshwater or saltwater) for all unique species names based on scientific species name matches • Define four main taxonomic groups (i.e., algae, cyanobacteria, and aquatic plants; vertebrates; invertebrates; other microorganisms) in line with "biological quality elements" defined under the EU Water Framework Directive, Annex V (phytoplankton, aquatic flora, benthic invertebrates, and fish fauna) as well as with the concept of trophic levels in aquatic ecosystems (primary producers/autotrophs, primary consumers/ herbivores, secondary consumers/carnivores, decomposers/detritivores): 1. Algae, cyanobacteria, and aquatic plants, 2. Invertebrates (crustaceans and non-crustaceans), 3. Vertebrates (including fish), 4. Other microorganisms (e.g., bacteria) • Extract from IUCN, GBIF, and EnviroTox lists the taxonomy, habitat type, and main taxonomic group for all unique species names in our dataset based on scientific species name matches • Manually assign taxonomy, habitat type, and main taxonomic group for species names not included in IUCN, GBIF, or EnviroTox based on additional sources (e.g., WoRMS)
1f	A unique list of **effect types** & **exposure test duration** and mapping on curated raw data	• Extract from column "TypeToxData" and column "HarmonizedExposureDuration.d." in curated raw data underlying Posthuma et al. 2019, table "Selected_Tox_data_measured," respectively a list of unique effect types and the exposure test durations (in days) • Aggregate effect types into equivalents of NOEC, EC10, and EC50, and remove ambiguous endpoints that cannot be aggregated into one of the given effect types • Assign aggregated effect type and exposure test duration type to all data points in curated raw data underlying Posthuma et al. 2019 • Extract from Table 1 of Aurisano et al. 2019 exposure test duration thresholds (separating test durations into exposure test duration types' acute' or 'chronic') per main taxonomic group • Assign exposure test duration types to reported harmonized exposure test durations of all data points in curated raw data underlying Posthuma et al. 2019, based on the main taxonomic group and exposure test duration matching
2	***Dataset preparation***	
2a	**Extrapolation** to chronic EC10 equivalents (**$EC10^{eq}$**)	**The approach used here for consistency** • Extract different endpoints test data (i.e., acute NOECs, chronic NOECs, acute EC10, acute EC50, and chronic EC50) • Compare in a separate regression equation against the chronic EC10 for the same species-chemical combination

#	Step	Description/Explanation
		• Use the full regression statistics (e.g., $chronic\ EC10 = alpha\ x\ acute\ NOEC + beta$) if the slope is not close to unity • Compare the resulting regression results with Aurisano et al. 2019 **Alternative approach** • Extract from Aurisano et al. 2019 extrapolation factors from effect types to $EC10^{eq}$ and from exposure test duration types to 'chronic,' separately for each main taxonomic group (Table 3) or, if not available, taxonomic group-generically (Table 4) • Apply the extrapolations factors from Aurisano et al. 2019 to all data points in curated raw data underlying Posthuma et al. 2019 to arrive at a full set of chronic $EC10^{eq}$
2b	**Aggregation** of data points per species	• Identify per chemical and exposure test duration type all data points in the full set of chronic $EC10^{eq}$ generated from curated raw data underlying Posthuma et al. 2019 that belong to the same harmonized species • Calculate the arithmetic mean, min-max range, and data point count across all chronic $EC10^{eq}$ data points per chemical-species combination for each exposure test duration type (i.e., once for chronic $EC10^{eq}$ derived only from chronic data and once for chronic $EC10^{eq}$ derived from chronic and acute data)
2c	**Criteria** definition for a minimum number of data points	• Count for each chemical the number of chronic $EC10^{eq}$ across species per main taxonomic group and the number of main taxonomic groups, separately for each exposure test duration type (i.e., once for chronic $EC10^{eq}$ derived only from chronic data, and once for chronic $EC10^{eq}$ derived from chronic and acute data) • Define as a threshold between data-poor and data-rich chemicals a minimum of 3 distinct species per main taxonomic group (or trophic level) and a minimum of 3 distinct main taxonomic groups (or trophic levels) based on recommendations by Rosenbaum et al. 2008 • Assign the thresholds for data-poor vs. data-rich chemicals to each chemical separately for each exposure test duration type (i.e., once for chronic $EC10^{eq}$ derived only from chronic data and once for chronic $EC10^{eq}$ derived from chronic and acute data) • Flag chemicals that are above the defined thresholds for data-poor vs. data-rich chemicals as 'data-rich,' separately for chemicals only based on chronic data vs. chemicals based on chronic and acute data
3	***Effect calculation***	
3a	Deriving statistics for **single-SSD** and **split-SSD** per chemical	• Convert all chronic $EC10^{eq}$ into logarithmic (log_{10}) scale, i.e. derive a set of $log_{10}(EC10^{eq})$ • Derive the number of $log_{10}(EC10^{eq})$ per chemical (single SSD) and per chemical-main taxonomic group combination (split-SSD per chemical) • Calculate per chemical (i.e., for single SSD) and per chemical-main taxonomic group combination (i.e., for split-SSD) the arithmetic mean and standard deviation of all $log_{10}(EC10^{eq})$, which yields the geometric mean and geometric standard deviation across respective $EC10^{eq}$
3b	Plotting **$EC10^{eq}$ data points on SSD** graphs	• Derive the number of $log_{10}(EC10^{eq})$ per chemical • Rank in ascending order each $log_{10}(EC10^{eq})$ per chemical against the count of all $log_{10}(EC10^{eq})$ for the same chemical, e.g. using in Microsoft Excel the RANK.EQ function as 'RANK.EQ($log_{10}(EC10^{eq})$ count, $log_{10}(EC10^{eq})$ value, 1)' • Derive per chemical for each $log_{10}(EC10^{eq})$ as x-value on a scatter plot the corresponding response probability (potentially-affected fraction

#	Step	Description/Explanation
		PAF of species) as y-axis, as 'PAF = ($\log_{10}(EC10^{eq})$ rank – H) / $\log_{10}(EC10^{eq})$ count', with the Hazen constant H = 0.5 (see Barnett 1975)
3c	Plotting **fitted SSD** graphs	• Define per chemical, and per chemical-main taxonomic group combination the range of $\log_{10}(EC10^{eq})$ for the x-axis of a scatter plot with smooth lines • Derive a fitted cumulative normal distribution of $\log_{10}(EC10^{eq})$ values (representing a log-normal distribution of $EC10^{eq}$) over the specified x-axis range, e.g., using in Microsoft Excel the NORM.DIST function as 'NORM.DIST($\log_{10}(EC10^{eq})$ value, arithmetic mean, standard deviation, 1)', as detailed in, e.g., Chapter 5 of Posthuma et al. 2002
3d	Deriving **HC20** per SSD	• Define on an SSD graph the value 0.2 as the percentile at which 20% of species per chemical or per chemical-main taxonomic group combination show a response (y-axis) at their respective $\log_{10}(EC10^{eq})$ values (x-axis) • Define this percentile as HC20 on each SSD, denoting the hazard concentration at which 20% of species show a probable effect above their $\log_{10}(EC10^{eq})$; see Owsianiak et al. 2019 • Calculate the log(HC20) from the fitted SSD as the inverse of the normal cumulative distribution, e.g., using Microsoft Excel the NORM.INV function as 'NORM.INV(0.2, arithmetic mean, standard deviation)' • Derive the final HC20 as 'HC20 = 10^log(HC20)', as detailed in, e.g., Chapter 5 of Posthuma et al. 2002
3e	Comparison of HC20	• Define for split-SSD a set of combined criteria for indicating significantly different SSDs across chemical-main taxonomic group combinations: 1. Fitted SSD curves are not crossing each other over the relevant $\log_{10}(EC10^{eq})$ range, 2. 95% confidence intervals of SSD curves are not overlapping over the relevant $\log_{10}(EC10^{eq})$ range, or SSD-related HC20 values show at least a factor of 10 difference 3. The number of data points is high enough to build a robust SSD (i.e., a minimum of 10 data points representing distinct species within one main taxonomic group or a minimum of 10 data points and at least 3 data points per main taxonomic group) • Label split-SSDs as either significantly different or not based on applying the set of combined criteria • Merge split-SSDs that are not significantly different into a combined SSD and redo the comparison with other split-SSDs, yielding ultimately a set of statistically significant split-SSDs per chemical, indicating main taxonomic groups that are specifically targeted (i.e., related species mainly showing effects at lower $EC10^{eq}$) as compared to main taxonomic groups that are not targeted (i.e., related species mainly showing effects at higher $EC10^{eq}$) • Label those chemicals for which at least one main taxonomic group fulfills the criteria for a statistically significant split-SSD
3f	Statistical tests for evaluating the potential of splitting SSD	• Follow the statistical tests steps in the systematic evaluation framework for spitting SSDs, as shown in Figure 1 • Evaluate whether to split SSD or not based on the statistical outcome with the MoA information, use category, visually check the SSD 95% confidence interval • Responsible split has low uncertainty around the HC20, no overlapping or crossing CI, and has a different level of sensitivity (HC20) from the rest of the group

#	Step	Description/Explanation
4	***Uncertainty assessment***	
5a	Preliminary steps	• Determine σ for each combination (chemical-species-effect type) to analyse how sd varies to set up a fixed sd in case of taxonomic group combination with a low number of data points (fixed shaped SSD)
5b	Total uncertainty around the derived HC20	• Quantifying uncertainty around the derived HC20 values by combining two types of uncertainty: GSD^2_{inter} reflecting inter-species variability (i.e., variability across available effect values), and GSD^2_{intra} reflecting intra-species variability (i.e., variability around the effect values). More details are in **Table A2**

Uncertainty analysis

Table A2 presents the specific steps to quantify the total uncertainty around the calculated HC20 values; $\mathbf{GSD^2_{total}}$ (squared geometric standard deviation) combining both the $\mathbf{GSD^2_{inter}}$ inter-species variability and $\mathbf{GSD^2_{intra}}$ intra-species variability per taxonomic group-chemical combination (for simplicity, from now on referred to as 'combination') is described in more detail in the following section.

Table A2 Overview of the approach for quantifying uncertainty around the derived HC20 values by combining two types of uncertainty: GSD^2_{inter} reflecting inter-species variability (i.e., variability across available effect values), and GSD^2_{intra} reflecting intra-species variability (i.e., variability around the effect values).

Inter-species variability
We calculated GSD^2_{inter}, which reflects the variability across available effect values for each taxonomic group per chemical combination, hereafter referred to as combination HC20. To estimate GSD^2_{inter} we started from the lognormal distribution fitted through the available effect values extrapolated chronic EC10 equivalent. When fitting the lognormal distribution, one of the two moments used is σ (standard deviation of the available for a chemical). We thus estimated the 95% CI (confidence interval) of σ via the function "fitdistr" in the R package MASS [1], and from this 95% CI we derived an upper and lower bound for $HC20_{\text{chronic EC10 equivalent}}$ ($HC20^{\text{inter,upper}}_{\text{chronic EC10 equivalent}}$ and $HC20^{\text{inter, lower}}_{\text{chronic EC10 equivalent}}$) by fitting two new lognormal distributions using instead of σ its 95% CI. GSD^2_{inter} is then calculated as:[2,3] $GSD^2_{inter} = \sqrt{HC20^{\text{inter, upper}}_{\text{chronic EC10 equivalent}} / HC20^{\text{inter, lower}}_{\text{chronic EC10 equivalent}}}$ We did not derive a chemical-specific group combination GSD^2_{inter} in the case of the data-poor taxonomic group (< 6 records available) because GSD^2_{inter} might be highly biased by the limited number of effect values available. For these chemicals, we applied a fixed GSD^2_{inter} calculated as 97.5 %-ile of the estimated GSD^2_{inter} across taxonomic groups with six records available.
Intra-species variability
We calculated GSD^2_{intra}, which reflects variability specific to the effect values for each combination $HC20_{\text{chronic EC10 equivalent}}$. To estimate GSD^2_{intra} we started from the record-specific distribution around the extrapolated effect value. This record-specific distribution is based on the uncertainty distributions assigned when extrapolating the

measured data to chronic EC10 equivalent from different endpoints (NOEC, EC50, and EC10). The record-specific uncertainty is propagated from the extrapolated chronic EC10 equivalent to the derived $HC20_{\text{chronic EC10 equivalent}}$ via a bootstrap method. Firstly, 1000 bootstrap samples were sampled from the estimated distributions around the extrapolated chronic EC10 equivalent for each combination. Secondly, 1000 lognormal distributions were fitted to the bootstrap samples using μ as the median of the resampled effect values and σ as the same σ used to derive $HC20_{\text{chronic EC10 equivalent}}$, based on the originally available effect values (in practice, only μ varies, and always the same shaped distribution is fitted to the resamples). Thirdly, from the 1000 fits, we derived an upper and lower bound for $HC20_{\text{chronic EC10 equivalent}}$ ($HC20^{\text{intra, upper}}_{\text{chronic EC10 equivalent}}$ and $HC20^{\text{intra, lower}}_{\text{chronic EC10 equivalent}}$). GSD^2_{intra} was then calculated as:[2,3]

$$GSD^2_{\text{intra}} = \sqrt{HC20^{\text{intra, upper}}_{\text{chronic EC10 equivalent}} / HC20^{\text{intra, lower}}_{\text{chronic EC10 equivalent}}}$$

We could calculate the upper and lower bounds for all the combinations since we had at least 3 unique species per taxonomic group.

Total Uncertainty

To determine the total uncertainty in $HC20_{\text{chronic EC10 equivalent}}$ we combined the resulting inter-species variability with that resulting from the intra-species variability:

$$GSD^2_{\text{total}} = 10^{\sqrt{\log10\left(\left(GSD^2_{\text{inter}}\right)\right) + \log10\left(\left(GSD^2_{\text{intra}}\right)\right)}}$$

The calculated HC20 effect threshold per taxonomic group and its 95% CI account for the inter- and intra-species variability.

SSDs robustness check from total uncertainty

To assess robust SSDs to determine "responsible split" SSDs, preferred full split, partial split as the fallback option, and the overall non-robust SSDs. We pragmatically set the GSD^2_{total} of 5 (i.e., less than 5 orders of magnitude) as the criterion to define sufficiently robust SSDs, where SSDs were considered robust when statistically shown to be significantly different from the rest and uncertainty is ≤ 5.

66

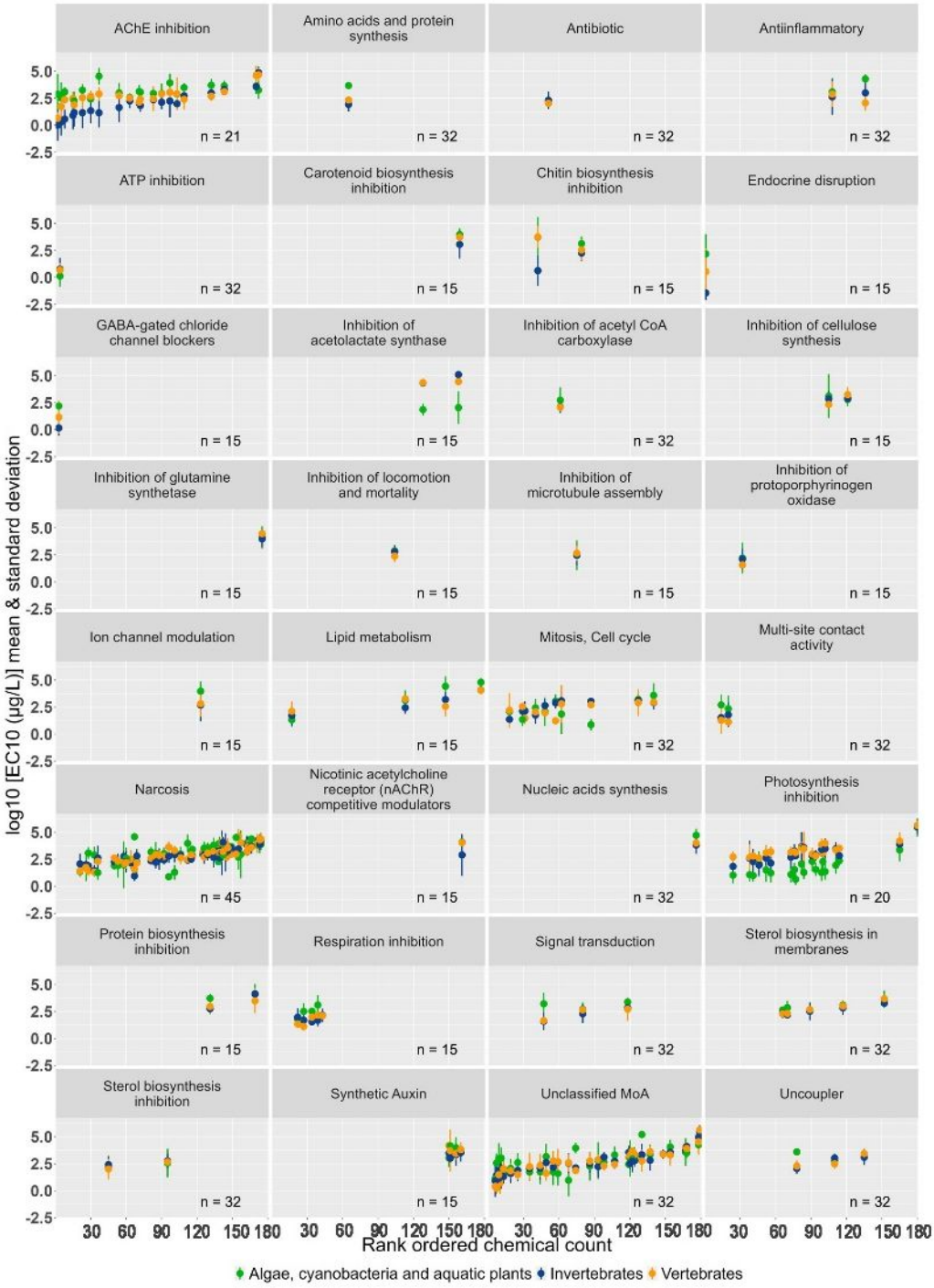

Figure S1 Distributions of chronic EC10-equivalent endpoint data for 180 chemicals, summarized as taxonomic group means and standard deviations, and considering different mechanistic modes of action (MoA). Chemicals in each plot are rank ordered based on the mean values calculated across all data per chemical. SSD-splitting grossly coincides (visually) with non-overlapping dots and standard deviations for different taxonomic groups (colors).

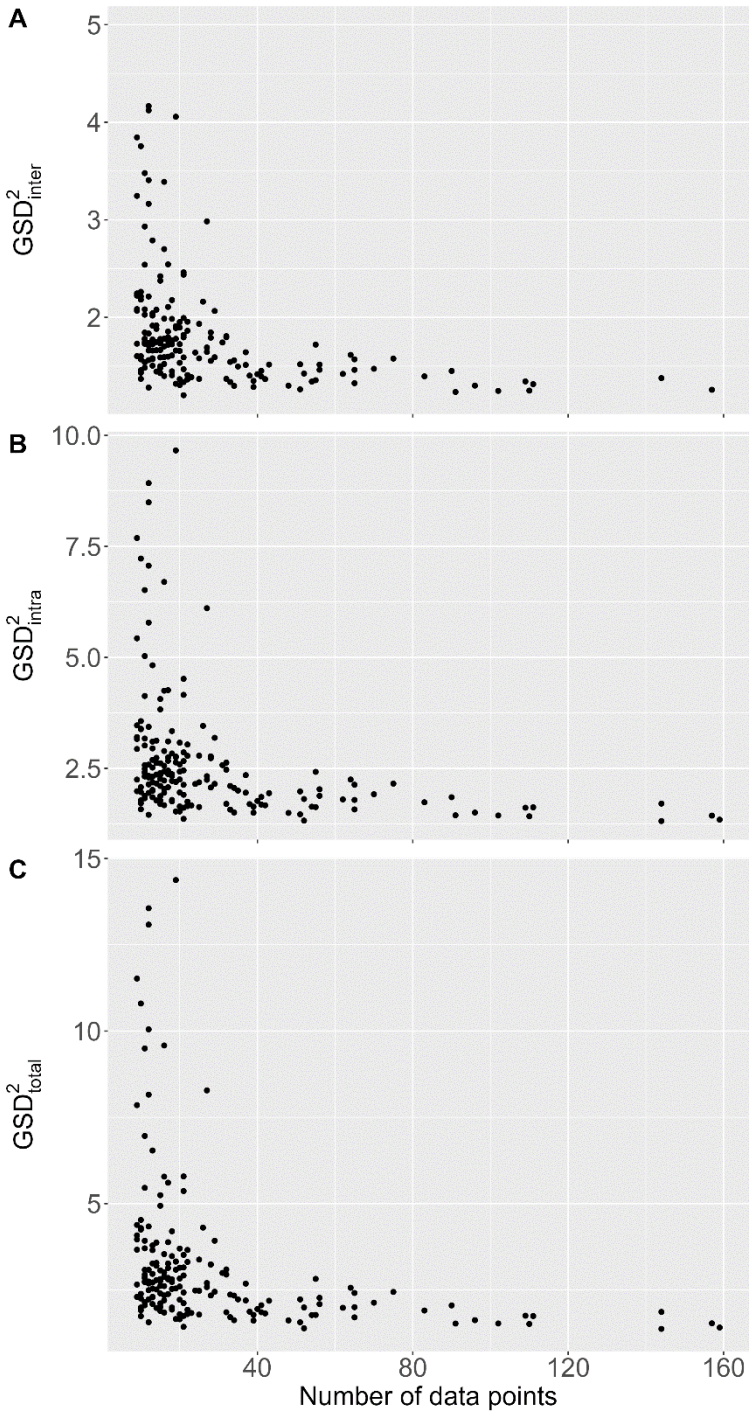

Figure S2 A. GSD^2_{inter} (inter-species variability), **B. GSD^2_{intra}** (intra-species variability) and **C. GSD^2_{total}** (total uncertainty) as a function of the number of data points available for each taxonomic group chemical combination. GSD^2_{total} combines both the GSD^2_{inter} and GSD^2_{intra}.

R code for split SSDs statistical analysis

```
###Library
library(readxl)
library(tidyverse)
library(broom)
library(car)
library(FSA)

#Import Excel Table S1
ecotox_data_CAS <- read_excel("... /Supplemental Excel File.xlsx", sheet = "Excel Table
S1", skip = 2)

## Split SSDs test
Step A: Check for Homogeneity of variance https://datatab.net/tutorial/levene-test
Step B: ANOVA test if Levene output is p>0.05
      B1. Do a post-hoc test -Tukey's HSD- post-hoc test for pairwise comparisons- to check
which group is different only if anova p<=0.05 (significant)
      B2. Compare two groups e.g A versus I using independent t test and A vs V+I --if Anova
p<=0.05
Step C: Kruskal-Wallis test if Levene output is p<0.05 (Non parametric equivalent of
ANOVA)
    C1. Do a post-hoc test (Dunn test- post-hoc test for pairwise comparisons- to check which
group is different only if Kruskal Wallis p<=0.05 (significant)
    C2. Compare two groups e.g A versus I using Mann Whitney U t test and A versus V+I --
if Kruskal Wallis p<=0.05
Step f: Linear regression with species groups as independent variables e.g A vs I and A vs
V+I --if Kruskal Wallis p<=0.05

### shorthand species group names
ecotox_data_CAS$renamed_speciesgroup <- case_when(
  ecotox_data_CAS$SpeciesGroup_Harmonized=="Algae, cyanobacteria and aquatic plants"
~ "A",
  ecotox_data_CAS$SpeciesGroup_Harmonized=="Invertebrates" ~ "I",
  .default = "V"
)

ecotox_data_CAS$SpeciesGroup_Harmonized.fac <-
factor(ecotox_data_CAS$renamed_speciesgroup)
#### merged groups
#For independent test-comparing means between 1 group vs two other groups

ecotox_data_CAS$merged_VI <-factor(ifelse(ecotox_data_CAS$renamed_speciesgroup
%in% c("V", "I"), "VI", "A"))
ecotox_data_CAS$merged_VA <-factor(ifelse(ecotox_data_CAS$renamed_speciesgroup
%in% c("V", "A"), "VA", "I"))
ecotox_data_CAS$merged_IA <-factor(ifelse(ecotox_data_CAS$renamed_speciesgroup
%in% c("I", "A"), "IA", "V"))

## Make a list to store the results
```

```
CASSPLIT <- aggregate(SpeciesName_Harmonized~CAS_Harmonized, data =
ecotox_data_CAS, FUN = length)

CASSPLIT$leveneTest <- NA
CASSPLIT$anovaTest <- NA
CASSPLIT$TukeyTest_I.A<-NA
CASSPLIT$TukeyTest_V.A<-NA
CASSPLIT$TukeyTest_V.I<-NA
CASSPLIT$Independent_V.IA <- NA
CASSPLIT$Independent_I.VA <- NA
CASSPLIT$Independent_A.VI <- NA
CASSPLIT$KruskalTest <-NA
CASSPLIT$dunnTest_I.A<-NA
CASSPLIT$dunnTest_V.A<-NA
CASSPLIT$dunnTest_V.I<-NA
CASSPLIT$ManW_V.IA <-NA
CASSPLIT$ManW_I.VA <-NA
CASSPLIT$ManW_A.VI <-NA
CASSPLIT$model_V.IA <-NA
CASSPLIT$model_I.VA <-NA
CASSPLIT$model_A.VI <-NA
CASSPLIT$model_V.IA_QC<-NA ##checking if the right p values are extracted
CASSPLIT$model_V.I <-NA
CASSPLIT$model_A.V <-NA
CASSPLIT$model_A.I <-NA



## for loop comparing means

for(irow in 1:nrow(CASSPLIT)) { #irow = 1
  iCAS <- CASSPLIT$CAS_Harmonized[[irow]]
  data <-ecotox_data_CAS %>%
                    filter(CAS_Harmonized == iCAS)

  varleveneTest <- leveneTest(log.EC10 ~ SpeciesGroup_Harmonized.fac, data=data)
  CASSPLIT$leveneTest[[irow]] <- varleveneTest$`Pr(>F)`[[1]]

  if(varleveneTest$`Pr(>F)`[[1]] > 0.05){

    varanovaTest <- aov(log.EC10 ~ SpeciesGroup_Harmonized.fac, data = data)
    sum_test<-unlist(summary(varanovaTest))
    CASSPLIT$anovaTest[[irow]] <- sum_test[["Pr(>F)1"]]

    if(sum_test[["Pr(>F)1"]] <= 0.05){
      varTurkeyTest<-TukeyHSD(varanovaTest, conf.level=.95)
      TukeyHSD_result <- varTurkeyTest[["SpeciesGroup_Harmonized.fac"]]
      CASSPLIT$TukeyTest_I.A[[irow]]<-TukeyHSD_result[["I-A","p adj"]]
      CASSPLIT$TukeyTest_V.A[[irow]]<-TukeyHSD_result[["V-A","p adj"]]
      CASSPLIT$TukeyTest_V.I[[irow]]<-TukeyHSD_result[["V-I","p adj"]]

```

```
184       }
185
186       if(sum_test[["Pr(>F)1"]] <= 0.05){
187         varIndependentTest_VI <- t.test(log.EC10 ~ merged_VI, data=data)
188         CASSPLIT$Independent_A.VI[[irow]]<-
189           varIndependentTest_VI[["p.value"]]
190
191       }
192
193       if(sum_test[["Pr(>F)1"]] <= 0.05){
194         varIndependentTest_VA <- t.test(log.EC10 ~ merged_VA, data=data)
195         CASSPLIT$Independent_I.VA[[irow]]<-
196           varIndependentTest_VA[["p.value"]]
197
198       }
199
200       if(sum_test[["Pr(>F)1"]] <= 0.05){
201         varIndependentTest_IA <- t.test(log.EC10 ~ merged_IA, data=data)
202         CASSPLIT$Independent_V.IA[[irow]]<-
203           varIndependentTest_IA[["p.value"]]
204
205       }
206
207
208     }
209
210     if(varleveneTest$`Pr(>F)`[[1]] < 0.05){
211       varKruskalTest <- kruskal.test(log.EC10 ~ SpeciesGroup_Harmonized.fac,
212                                      data = data)
213       CASSPLIT$KruskalTest[[irow]] <- varKruskalTest[["p.value"]]
214
215       if(varKruskalTest[["p.value"]] <= 0.05){
216         dunnTest_result<-
217           dunnTest(log.EC10 ~ SpeciesGroup_Harmonized.fac,
218                    data=data,method="bonferroni")
219         #dunn_result <- dunnTest_result[["res"]]
220         CASSPLIT$dunnTest_I.A[[irow]]<-
221           dunnTest_result[["res"]][["P.adj"]][1]
222
223         CASSPLIT$dunnTest_V.A[[irow]] <-
224           dunnTest_result[["res"]][["P.adj"]][2]
225
226          CASSPLIT$dunnTest_V.I[[irow]] <-
227           dunnTest_result[["res"]][["P.adj"]][3]
228
229
230
231       }
232
233       if(varKruskalTest[["p.value"]] <= 0.05){
234         varManW_VA <- tryCatch(
```

```
235             wilcox.test(log.EC10 ~ merged_VA, data=data),
236             error = stop,
237             warning = function(cond){
238               return(list(p.value = 0)) #or use jitter to suppress?
239             })
240           CASSPLIT$ManW_I.VA[[irow]]<-
241             varManW_VA[["p.value"]]
242         }
243
244         if(varKruskalTest[["p.value"]] <= 0.05){
245           varManW_IA <-
246             wilcox.test(log.EC10 ~ merged_IA, data=data)
247           CASSPLIT$ManW_V.IA[[irow]]<-
248             varManW_IA[["p.value"]]
249           }
250          if(varKruskalTest[["p.value"]] <= 0.05){
251           varManW_VI <-
252             wilcox.test(log.EC10 ~ merged_VI, data=data)
253           CASSPLIT$ManW_A.VI[[irow]]<-
254             varManW_VI[["p.value"]]
255
256         }
257       }
258
259   }
260
261   #NA's drop out -> test is not performed
262   cat("levene.test, equal variances p> 0.05")
263   table(CASSPLIT$leveneTest > 0.05)
264   cat("for levene.test p > 0.05  == FALSE")
265   cat("KruskalTest, differences in mean")
266   table(CASSPLIT$KruskalTest <= 0.05)
267   cat("dunnTest, differences in mean, 3way")
268   table(CASSPLIT$dunnTest_I.A <= 0.05 | CASSPLIT$dunnTest_V.A <= 0.05 |
269   CASSPLIT$dunnTest_V.I <= 0.05 )
270   cat("Mann Whitney, differences in mean, 3way")
271   table(CASSPLIT$ManW_A.VI <= 0.05 | CASSPLIT$ManW_I.VA  <= 0.05 |
272   CASSPLIT$ManW_A.VI <= 0.05 )
273   cat("full?")
274   table((CASSPLIT$ManW_A.VI <= 0.05) + (CASSPLIT$ManW_I.VA  <= 0.05) +
275   (CASSPLIT$ManW_A.VI <= 0.05) == 3 )
276
277   cat("for levene.test p > 0.05  == TRUE")
278   cat("One way ANOVA, differences in mean")
279   table(CASSPLIT$anovaTest <= 0.05)
280   cat("TukeyTest, differences in mean, 3way")
281   table(CASSPLIT$TukeyTest_I.A <= 0.05 | CASSPLIT$TukeyTest_V.A <= 0.05 |
282   CASSPLIT$TukeyTest_V.I <= 0.05 )
283   cat("Independent test, 3way")
284   table(CASSPLIT$Independent_V.IA <= 0.05 | CASSPLIT$Independent_I.VA <= 0.05 |
285   CASSPLIT$Independent_A.VI <= 0.05 )
```

```
cat("full?")
table((CASSPLIT$Independent_V.IA <= 0.05) + (CASSPLIT$Independent_I.VA <= 0.05) +
(CASSPLIT$Independent_A.VI <= 0.05) == 3)

###comparing models; 1 group vs 2 other groups

for(irow in 1:nrow(CASSPLIT)) {
  iCAS <- CASSPLIT$CAS_Harmonized[[irow]]
  data <- ecotox_data_CAS %>%
    filter(CAS_Harmonized == iCAS)
  model_IA <- lm(log.EC10 ~ merged_IA, data = data)

  tidy_model_IA <- tidy(model_IA)
  CASSPLIT$model_V.IA_QC[[irow]] <-
    unlist(tidy_model_IA[tidy_model_IA$term == "merged_IAV", "p.value"])
  #yes, it's the same

  CASSPLIT$model_V.IA[[irow]] <-
    summary(model_IA)$coefficients[, "Pr(>|t|)"][[2]]

}

for(irow in 1:nrow(CASSPLIT)) {
  iCAS <- CASSPLIT$CAS_Harmonized[[irow]]
  data <-ecotox_data_CAS %>%
                  filter(CAS_Harmonized == iCAS)
  model_VA <- lm(log.EC10 ~ merged_VA, data=data)
  CASSPLIT$model_I.VA[[irow]]<-
       summary(model_VA)$coefficients[,"Pr(>|t|)"][[2]]

}


for(irow in 1:nrow(CASSPLIT)) {
  iCAS <- CASSPLIT$CAS_Harmonized[[irow]]
  data <- ecotox_data_CAS %>%
                  filter(CAS_Harmonized == iCAS)

  model_VI <- lm(log.EC10 ~ merged_VI, data=data)

  CASSPLIT$model_A.VI[[irow]]<-
       summary(model_VI)$coefficients[,"Pr(>|t|)"][[2]]

}


###comparing 1 group vs 1 other group regression
##merged group model versus one used as an anchor
for(irow in 1:nrow(CASSPLIT)) {
  iCAS <- CASSPLIT$CAS_Harmonized[[irow]]
  data <-ecotox_data_CAS %>%
```

```
                    filter(CAS_Harmonized == iCAS &
                         merged_VI == "VI" )

  if(nrow(data>1)){
      merged_V.I <- lm(log.EC10 ~ SpeciesGroup_Harmonized.fac, data=data)
      CASSPLIT$model_V.I[[irow]]<-
        summary(merged_V.I)$coefficients[,"Pr(>|t|)"][[2]]
  }

}

for(irow in 1:nrow(CASSPLIT)) {
  iCAS <- CASSPLIT$CAS_Harmonized[[irow]]
  data <-ecotox_data_CAS %>%
                    filter(CAS_Harmonized == iCAS &
                         merged_VA == "VA" )
  merged_A.V <- lm(log.EC10 ~ SpeciesGroup_Harmonized.fac, data=data)
  CASSPLIT$model_A.V[[irow]] <-
       summary(merged_A.V)$coefficients[,"Pr(>|t|)"][[2]]

}


for(irow in 1:nrow(CASSPLIT)) {
   iCAS <- CASSPLIT$CAS_Harmonized[[irow]]
   data <-ecotox_data_CAS %>%
                    filter(CAS_Harmonized == iCAS&
                         merged_IA == "IA" )

   merged_A.I <- lm(log.EC10 ~ SpeciesGroup_Harmonized.fac, data=data)

   CASSPLIT$model_A.I[[irow]] <-
       summary(merged_A.I)$coefficients[,"Pr(>|t|)"][[2]]
}
```

R code for deriving split SSDs based on the process flow Experimental test data curation and harmonization

Table A1

```
###Library
library(ggplot2)
library(tidyverse)
library(readr)
library(scales)
library(tidyr)
library(readxl)
library(dplyr)
library(psych)
library(boot)
require(fitdistrplus)
```

```
library(cowplot)

## Input data
##All step for species taxonomy harmonization was done in excel; step 1a to 1e
ecotox_datain <- read_excel("... /Supplemental Excel File.xlsx", sheet = "Excel Table S1",
skip = 2)

###subset required data columns
ecotox_datain <-
ecotox_datain[,c("ID","test_id","CAS_Harmonized","NameHarmonized","Name","SpeciesNa
me_Harmonized", "SpeciesGroup_Harmonized","SpeciesName_Harmonized (distinct
species)","Genus_Harmonized",
"SpeciesGroupExposure_Harmonized","Conc1Harmonized.ug.L.",
"TypeToxData","Endpoint_Harmonized","EffCode",
"endpoint","HarmonizedExposureDuration.d.","ChronicDuration",
"Habitat_Aggregated_Harmonized")]

### select a chemical/s: Simazine used as an example

selected_CAS<-c("122-34-9")# Simazine

ecotox_data<- ecotox_datain %>%
  filter(Habitat_Aggregated_Harmonized == "Freshwater aquatic",CAS_Harmonized ==
selected_CAS)

ecotox_data %>%
  group_by(CAS_Harmonized) %>%
  slice_head()

# Function for saving and displaying a plot.

import::from(ggplot2, ggsave)


save_and_display <- function(fig, filename, width = 6, height = 4){
  filepath <- paste("Figures/", filename, ".", "png", sep = "")
  ggsave(filepath, fig, width = width, height = height)
  return(fig)
}

####create a path for figures
plot_directory <- "Figures"

unlink(paste(plot_directory, "/*", sep = ""))
dir.create(plot_directory, recursive = TRUE)

##Step1f:Exposure duration
#Exposure duration type allocation- acute or chronic
#We used extrapolation factors from Aurisano et al. 2019
```

```
ecotox_data$HarmDur2 <-
as.numeric(as.character(ecotox_data$HarmonizedExposureDuration.d.))

ecotox_data$duration_type.curated <- ifelse (
  ecotox_data$HarmDur2 <= 1 &
    (ecotox_data$SpeciesGroupExposure_Harmonized == ("Algae and cyanobacteria") |
       ecotox_data$SpeciesGroupExposure_Harmonized == "Other microorganism") ,"Acute",
  ifelse(
    ecotox_data$HarmDur2> 1 &
      (ecotox_data$SpeciesGroupExposure_Harmonized == "Algae and cyanobacteria" |
         ecotox_data$SpeciesGroupExposure_Harmonized =="Other microorganism"),
"Chronic",
    ifelse(
      ecotox_data$HarmDur2<= 4  &
        ecotox_data$SpeciesGroupExposure_Harmonized == "Invertebrates", "Acute",
      ifelse(
        ecotox_data$HarmDur2 > 4 &
          ecotox_data$SpeciesGroupExposure_Harmonized == "Invertebrates",  "Chronic",
        ifelse(
          ecotox_data$HarmDur2 <= 7 &
            (ecotox_data$SpeciesGroupExposure_Harmonized == "Other plants" |
               ecotox_data$SpeciesGroupExposure_Harmonized =="Vertebrates"), "Acute",
          ifelse(
            ecotox_data$HarmDur2 > 7 &
              (ecotox_data$SpeciesGroupExposure_Harmonized == "Other plants" |
                 ecotox_data$SpeciesGroupExposure_Harmonized =="Vertebrates"), "Chronic",
            NA
          ))))))


##### Exposure durations with NAs


ecotox_data$duration_type.curated.2 <- ifelse(ecotox_data$ChronicDuration == "TRUE",
"Chronic", "Acute")

ecotox_data$Harmonised_effect.type<- (ifelse(is.na(ecotox_data$duration_type.curated),
paste(ecotox_data$duration_type.curated.2, ecotox_data$Endpoint_Harmonized, sep=" "),
                                      paste(ecotox_data$duration_type.curated,
ecotox_data$Endpoint_Harmonized
                                            , sep=" ")))

####Remove ambigous endpoints

ecotox_data<-ecotox_data [ecotox_data$Harmonised_effect.type %in% c("Acute EC10",
"Chronic EC10","Acute NOEC","Chronic NOEC" ,"Acute EC50", "Chronic EC50"), ]

##Step2a:Extrapolation to chronic EC10 equivalents: We applied full regression equation
(slope not assumed to unity)
ecotox_data_extrapolated <- ecotox_data
```

```
ecotox_data_extrapolated$EC10 = ifelse(ecotox_data_extrapolated$Harmonised_effect.type
%in% "Acute NOEC",ecotox_data_extrapolated $Conc1Harmonized.ug.L.^0.816/ 0.953,
                                ifelse(ecotox_data_extrapolated$Harmonised_effect.type %in%
"Chronic NOEC",ecotox_data_extrapolated $Conc1Harmonized.ug.L.^0.965/1.394,
                                      ifelse(ecotox_data_extrapolated$Harmonised_effect.type %in%
"Acute EC50",ecotox_data_extrapolated $Conc1Harmonized.ug.L.^0.869/3.219,
                                            ifelse(ecotox_data_extrapolated$Harmonised_effect.type
%in% "Chronic EC50",ecotox_data_extrapolated $Conc1Harmonized.ug.L.^0.872/0.185,

ifelse(ecotox_data_extrapolated$Harmonised_effect.type %in% "Acute
EC10",ecotox_data_extrapolated $Conc1Harmonized.ug.L.^0.813/0.108,

ifelse(ecotox_data_extrapolated$Harmonised_effect.type %in% "Chronic
EC10",ecotox_data_extrapolated $Conc1Harmonized.ug.L./1,
                                                  NA))))))

## Aggregation of data points per species
#Step 2: First average or geometric mean for the same effect level (NOEC vs. EC10 vs.
EC50) and then conversion to EC10eq?

ecotox_data_extrapolated <- ecotox_data_extrapolated %>%
  group_by(CAS_Harmonized, SpeciesName_Harmonized) %>%
  mutate(n = n(),
         EC10_C = mean(EC10,na.rm = TRUE ),
         max_c = max(EC10),
         min_c = min(EC10)) %>%
  ungroup()
####Keeping distict species values
#Using species-chemical chemical combination

ecotox_data_extrapolated <- ecotox_data_extrapolated %>%
  group_by(CAS_Harmonized) %>%
  distinct_at('SpeciesName_Harmonized',.keep_all = TRUE)


#### Convert EC10 to logEC10;log and log 10 of values is different in r but same in excel

ecotox_data_extrapolated_logged <- ecotox_data_extrapolated %>%
  mutate(log.EC10=log10(EC10_C))

##Step2b:Data poor and data rich chemicals
#Set data poor and data rich chemicals, by first summing the total number of species  per
chemical and species group

ecotox_data_extrapolated_logged  <-ecotox_data_extrapolated_logged %>%
  group_by(CAS_Harmonized) %>%
  mutate(n_total=n(),
         tax.count = (n_distinct(SpeciesGroup_Harmonized)))

ecotox_data_extrapolated_logged  <-ecotox_data_extrapolated_logged %>%
  group_by(CAS_Harmonized,SpeciesGroup_Harmonized) %>%
```

```
  mutate(species.count = n())

###step2c:Identification of data poor chemicals
#Criteria definition for minimum number of data points

ecotox_data_extrapolated_logged  <-ecotox_data_extrapolated_logged  %>%
  mutate(
    newcol = case_when(
      tax.count < 3  ~ 'poor',
      species.count < 3  ~ 'poor',
      tax.count >= 3 & species.count < 3 ~ 'poor',
      tax.count >= 3 & species.count >= 3 ~ 'rich'

    )
  )

d <- ecotox_data_extrapolated_logged %>% group_by(CAS_Harmonized) %>%
  summarise(Condition = sum(newcol == "poor")) %>% mutate(Adequate.data =
ifelse(Condition > 0 , "poor","rich"))
d

ecotox_data_extrapolated_logged<- left_join(ecotox_data_extrapolated_logged, d, by=
c("CAS_Harmonized"= "CAS_Harmonized"))

### Overview of the number of data poor and data rich chemicals:select data rich chemicals

poor_richchem <-ecotox_data_extrapolated_logged %>%
  group_by(CAS_Harmonized) %>%
  count(Adequate.data)
poor_richchem

ecotox_data_rich <- filter(ecotox_data_extrapolated_logged,
                           Adequate.data=="rich")

## Step3:Effect calculation
##one can use order, arrange or sort to get frac or cumulative concentrations==PAF

ecotox_data_rich_CAS <- ecotox_data_rich %>%
  group_by(CAS_Harmonized) %>%
  arrange((log.EC10), .by_group = TRUE) %>%
  mutate(frac_C=ppoints(log.EC10,0.5)) %>%
  ungroup()


ecotox_data_rich_taxa<-  ecotox_data_rich %>%
  group_by(CAS_Harmonized, SpeciesGroup_Harmonized)%>%
  arrange((log.EC10), .by_group = TRUE) %>%
  mutate(frac_t=ppoints(log.EC10,0.5)) %>%
  ungroup()
```

```
## Calculating deterministic HC20
### Calculating deterministic HC20 per chemical

ecotox_data_CAS <-  ecotox_data_rich_CAS %>%
  group_by(CAS_Harmonized) %>%
  mutate(n_C = n(),
         mu_C = mean(log.EC10),
         SDV_C = sd(log.EC10),
         lwr=mu_C-SDV_C,
         upr=mu_C+SDV_C) %>%
  ungroup()
ecotox_data_CAS <- ecotox_data_CAS %>%
  mutate(HC20_C = qnorm(0.2, mean=mu_C, sd=SDV_C),
         HC5_C=qnorm(0.05, mean=mu_C, sd=SDV_C))

### Deterministic HC20 per chemical-Speciesgroups
#We want the mean and SD per chemical AND species group and HC20 per species group

ecotox_data_TAXA <-ecotox_data_rich_taxa %>%
  group_by(CAS_Harmonized, SpeciesGroup_Harmonized) %>%
  mutate(n_t = n(),
         mu_t = mean(log.EC10),
         SDV_t = sd(log.EC10),
         lwr_t=mu_t-SDV_t,
         upr_t=mu_t+SDV_t) %>%
  ungroup()

ecotox_data_TAXA <- ecotox_data_TAXA%>%
  mutate(HC20_t = qnorm(0.2, mean=mu_t, sd=SDV_t),
         HC5_t = qnorm(0.05, mean=mu_t, sd=SDV_t))

##Step3
###Plotting species distribution per chemicals

cols <- c( "#00B612" , "#0F4392","#ff9900")
cols.2 <- c( "#0F4392","#575757")
cols.3<- c( "#00B612", "#575757")
cols.4<- c( "#575757","#ff9900")

newxs <- seq(-2.5, 5.0, length.out = 1000)
ecotox_data_CAS$labels <- paste0("n", " = ", ecotox_data_CAS$n_total)

new_label_No_split <- as_labeller(c(`Simazine` = "No split"))

fig0<- ggplot(data = ecotox_data_CAS)+
  geom_point(aes(x=log.EC10, y=frac_C, color = SpeciesGroup_Harmonized,
shape=SpeciesGroup_Harmonized),size=5)+
  scale_color_manual(values = cols)+
  labs(x=expression(paste('log10 [EC10 (µg/L)]')),
       y='Potentially affected species fraction') +
  annotation_logticks(sides='b')+
```

```
  facet_wrap(.~Name, ncol=1, labeller = new_label_No_split) +
  theme(strip.text.x = element_text(size =20))+


  geom_text(data=ecotox_data_CAS, aes(1, 0.95,label=labels), size=4)

###Deterministic curve per chemical

d_fit_chemical <- c(unique(ecotox_data_CAS$CAS_Harmonized))
d_fit_chemical <- as.data.frame(d_fit_chemical)
colnames(d_fit_chemical) [1] <- "CAS_Harmonized"

distribution_prameters_C <- ecotox_data_CAS %>%
  group_by(CAS_Harmonized) %>%
  slice(1)


d_fit_chemical <- d_fit_chemical %>%
  left_join(distribution_prameters_C[,c("CAS_Harmonized", "mu_C", "SDV_C","Name")],
            by=c("CAS_Harmonized")) %>%
  right_join(
    tidyr::expand(d_fit_chemical, CAS_Harmonized, EC = newxs),
    by = c("CAS_Harmonized"))


d_fit_chemical <- d_fit_chemical %>%
  mutate(PAF_C=pnorm(EC, mean=mu_C, sd=SDV_C))
# mutate(PAF_C=pnorm(EC, mean=log.EC10$estimate[1], #sd=log.EC10$estimate[2]))

SSD_chemical <- ggplot()+

  scale_color_manual(values = cols)+
  geom_line(data=d_fit_chemical,
            aes(x=EC,
                y=PAF_C), color="DARK GRAY") +
  geom_smooth() +
  labs(x = expression(paste('log10 [EC10 (µg/L)]')),
       y = 'Potentially affected species fraction')+
  geom_segment(aes(x =ecotox_data_CAS$HC20_C, y = 0.0, xend =
ecotox_data_CAS$HC20_C,  yend = 0.2),color ="red", size=0.9)+

  geom_segment(aes(x =ecotox_data_CAS$HC5_C, y = 0.0, xend =
ecotox_data_CAS$HC5_C,  yend = 0.05),color ="black",  size=0.9)+#1.2

  facet_wrap(.~Name, ncol=1, labeller=new_label_No_split) +
  theme(strip.text.x = element_text(size = 20))


### Species group determistic curves:Creating plots per chemicals and species group
combination to create deterministic fit

```

```
new_label_full_split <- as_labeller(c(`Simazine` = "Full split"))

d_fit_taxa <- unique(ecotox_data_TAXA[c("CAS_Harmonized")])
d_fit_taxa <- as.data.frame(d_fit_taxa)
colnames(d_fit_taxa) [1] <- c("CAS_Harmonized")

distribution_prameters_t <- ecotox_data_TAXA %>%
  group_by(CAS_Harmonized, SpeciesGroup_Harmonized) %>%
  slice(1)


d_fit_taxa <- d_fit_taxa %>%
  left_join(distribution_prameters_t[,c( "CAS_Harmonized","SpeciesGroup_Harmonized",
"mu_t","SDV_t", "Name")],
            by=c("CAS_Harmonized")) %>%
  right_join(
    tidyr::expand(d_fit_taxa, CAS_Harmonized,EC = newxs),
    by = c("CAS_Harmonized"))


d_fit_taxa <- d_fit_taxa %>%
  mutate(PAF_t=pnorm(EC, mean=mu_t, sd=SDV_t))

SSD_taxa <- ggplot()+
  #geom_point(data = ecotox_data_TAXA, aes(x = log.EC10, y = frac_t, colour
=SpeciesGroup_Harmonized)) +
  scale_color_manual(values = cols)+
  geom_line(data=d_fit_taxa,
            aes(x=EC,
                y=PAF_t,
                color= SpeciesGroup_Harmonized,
                Size=1)) +
  labs(x = expression(paste('log10 [EC10 (µg/L)]')),
       y = 'Potentially affected species fraction')+

  facet_wrap(.~Name, ncol=1, labeller = new_label_full_split ) +
  theme(strip.text.x = element_text(size = 20))

SSD_taxa

###save output with points

SSD_taxa_points <- ggplot()+
  geom_point(data = ecotox_data_TAXA, aes(x = log.EC10, y = frac_t, colour
=SpeciesGroup_Harmonized),size=5) +
  scale_color_manual(values = cols)+
  geom_line(data=d_fit_taxa,
            aes(x=EC,
                y=PAF_t,
                color= SpeciesGroup_Harmonized,
                Size=2)) +
```

```
743
744   labs(x = expression(paste('log10 [EC10 (µg/L)]')),
745        y = 'Potentially affected species fraction')+
746
747   facet_wrap(.~Name, ncol=1,labeller = new_label_full_split ) +
748   theme(strip.text.x = element_text(size = 20))
749
750
751
752 save_and_display(
753   SSD_taxa_points,
754   "Herbicide_1-8.single SSD_no CI_no split",
755   width = 7,
756   height = 18)
757
758 SSD_taxa_points
759
760 ### Adding CI to chemicals deterministic curve
761 #for herbicides newxs <- seq(0.001, 100, length.out = 1000)
762 #for insecticides newxs <- seq(-0.1, 10, length.out = 1000)
763 #insectides requires much lower starting scales 0.001
764
765 d.in <-ecotox_data_CAS
766 myboot2 <- function(d.in, newxs){
767   xr <- sample(d.in$log.EC10, length(d.in$log.EC10), replace = TRUE)
768
769   # fit distribution to new data
770   fitr <- fitdistr(xr, 'normal')
771
772   ##predict PAF for new data
773   pyr <- pnorm(newxs, mean = fitr$estimate[1], sd = fitr$estimate[2])
774   return(pyr)
775 }
776
777 pdat.final <- NULL
778 bootdat.final <- NULL
779 set.of.CAS_Harmonized <- unique(d.in$CAS_Harmonized)
780 id <- set.of.CAS_Harmonized[1]
781
782 for(id in set.of.CAS_Harmonized){
783   data.sub <- d.in[which(d.in$CAS_Harmonized %in% id),]
784
785   boots <- replicate(1000, myboot2(d.in=data.sub, newxs))
786
787   # extract bootstrap values
788   bootdat <- data.frame(boots)
789   bootdat$newxs <- newxs
790   bootdat <- reshape2::melt(bootdat, id = 'newxs')
791   # extract CI
792   cis <- apply(boots, 1, quantile, c(0.025, 0.975))
793   rownames(cis) <- c('lwr', 'upr')
```

```
  # add fitted values
  pdat <- data.frame(newxs, py = pnorm(newxs, mean = data.sub$mu_C, sd =
data.sub$SDV_C))
  # add CI
  pdat <- cbind(pdat, t(cis))
  pdat$CAS_Harmonized <- id

  bootdat$CAS_Harmonized <- id

  pdat.final <- rbind(pdat.final, pdat)
  bootdat.final <- rbind(bootdat.final, bootdat)

}

#### Chemical CI

SSD_CAS_CI <- SSD_chemical

for(id in set.of.CAS_Harmonized){
  print(id)

  SSD_CAS_CI <- SSD_CAS_CI +

    geom_ribbon(data = pdat.final[which(pdat.final$CAS_Harmonized %in% id),], aes(x
=newxs, ymin =lwr,ymax = upr),
              fill="black",  alpha=0.2)

}

SSD_CAS_CI

##Add original data
SSD_chemical2 <- SSD_CAS_CI +
  geom_segment(aes(x =ecotox_data_CAS$HC20_C, y = 0.0, xend =
ecotox_data_CAS$HC20_C,  yend = 0.2),color ="red", size=0.9) +

  geom_segment(aes(x =ecotox_data_CAS$HC5_C, y = 0.0, xend =
ecotox_data_CAS$HC5_C,  yend = 0.05),color ="black",  size=0.9) +
  geom_point(data = ecotox_data_CAS, aes(x = log.EC10, y = frac_C, colour
=SpeciesGroup_Harmonized, shape=SpeciesGroup_Harmonized),size=5) +
  annotation_logticks(sides='b')+
  theme(legend.key.size = unit(0.5, 'cm'),
        legend.key.height = unit(0.5, 'cm'),
        legend.key.width = unit(0.5, 'cm'),
        legend.title = element_text(size=20),
        legend.text = element_text(size=20))+
  geom_errorbarh(data=ecotox_data_CAS, mapping=aes(y=frac_C, x=log.EC10,
xmin=log10(min_c), xmax=log10(max_c), colour = SpeciesGroup_Harmonized),
height=0.02, size=0.4)+
  geom_text(data=ecotox_data_CAS, aes(-1, 0.9,label=labels), size=5.5)+

```

```
  scale_x_continuous(limits = c(-2,6))


SSD_chemical2

#### CI around taxonomic groups fitted curves

d.in <-ecotox_data_TAXA
myboot2 <- function(d.in, newxs){
  xr <- sample(d.in$log.EC10, length(d.in$log.EC10), replace = TRUE)

  # fit distribution to new data
  fitr <- fitdistr(xr, 'normal')

  ##predict PAF for new data
  pyr <- pnorm(newxs, mean = fitr$estimate[1], sd = fitr$estimate[2])
  return(pyr)
}

pdat.final <- NULL
bootdat.final <- NULL
set.of.CAS_Harmonized <- unique(d.in$CAS_Harmonized)
id <- set.of.CAS_Harmonized[1]

set.of.groups <- (unique(d.in$SpeciesGroup_Harmonized))
input.group <-set.of.groups
input.group
input.group<-set.of.groups[2]

for(id in set.of.CAS_Harmonized){
  data.sub <- d.in[which(d.in$CAS_Harmonized %in% id),]


  for(input.group in set.of.groups){
    data.sub.in <-  data.sub[which(data.sub$SpeciesGroup_Harmonized %in% input.group),]


    boots <- replicate(1000, myboot2(d.in=data.sub.in, newxs))

    # extract bootstrap values
    bootdat <- data.frame(boots)
    bootdat$newxs <- newxs
    bootdat <- reshape2::melt(bootdat, id = 'newxs')
    # extract CI
    cis <- apply(boots, 1, quantile, c(0.025, 0.975))
    rownames(cis) <- c('lwr', 'upr')
    # add fitted values
    pdat <- data.frame(newxs, py = pnorm(newxs, mean = data.sub.in$mu_t, sd =
data.sub.in$SDV_t))
    # add CI
    pdat <- cbind(pdat, t(cis))
```

```
    pdat$CAS_Harmonized <- id
    pdat$SpeciesGroup_Harmonized <- input.group


    bootdat$CAS_Harmonized <- id
    bootdat$SpeciesGroup_Harmonized <- input.group


    pdat.final <- rbind(pdat.final, pdat)
    bootdat.final <- rbind(bootdat.final, bootdat)

  }
}


####Species group CI

SSD_taxa_CI <- SSD_taxa
for(id in set.of.CAS_Harmonized){
  print(id)
  for(input.group in set.of.groups){
    print(input.group)

    SSD_taxa_CI <- SSD_taxa_CI +

      geom_ribbon(data = pdat.final[which(pdat.final$CAS_Harmonized %in% id &
pdat.final$SpeciesGroup_Harmonized %in% input.group),], aes(x =newxs, ymin =lwr,ymax
= upr),
                  fill="black",  alpha=0.2)

  }
}
SSD_taxa_CI

###Observation per species groups
ecotox_data_TAXA$reduced_species.group.name <-
ifelse(ecotox_data_TAXA$SpeciesGroup_Harmonized=="Invertebrates", "I",

ifelse(ecotox_data_TAXA$SpeciesGroup_Harmonized=="Vertebrates", "V",

                                          "A"))

ecotox_data_TAXA$labels.taxa <- paste0(ecotox_data_TAXA$reduced_species.group.name,
" = ", ecotox_data_TAXA$n_t)

##position of the text
ecotox_data_TAXA$y_position <-
as.numeric(factor(ecotox_data_TAXA$reduced_species.group.name)) *0.05 + 0.80#0.65

###Add original data
SSD_taxa2 <- SSD_taxa_CI +
```

```
  geom_segment(aes(x =ecotox_data_TAXA$HC20_t, y = 0.0, xend =
ecotox_data_TAXA$HC20_t,  yend = 0.2),color ="red", size=0.9) +

  geom_segment(aes(x =ecotox_data_TAXA$HC5_t, y = 0.0, xend =
ecotox_data_TAXA$HC5_t,  yend = 0.05),color ="black",  size=0.9) +
  geom_point(data = ecotox_data_TAXA, aes(x = log.EC10, y = frac_t, colour
=SpeciesGroup_Harmonized, shape=SpeciesGroup_Harmonized),size=5) +
  scale_color_manual(values = cols) +
  annotation_logticks(sides='b')+
  theme(legend.key.size = unit(0.5, 'cm'),
        legend.key.height = unit(0.5, 'cm'),
        legend.key.width = unit(0.5, 'cm'),
        legend.title = element_text(size=15),
        legend.text = element_text(size=15))+
  geom_errorbarh(data=ecotox_data_TAXA, mapping=aes(y=frac_t, x=log.EC10,
xmin=log10(min_c), xmax=log10(max_c), colour = SpeciesGroup_Harmonized),
height=0.02, size=0.4)+
  geom_text(aes(-0.7, ecotox_data_TAXA$y_position,label=ecotox_data_TAXA$labels.taxa),
size=5.5,family="Sans", fontface="plain")+
  scale_x_continuous(limits = c(-2,6))


### Grid arrange first split plots
#Here all species groups are plotted separately

p <- plot_grid (SSD_chemical2  + theme(legend.position="none", axis.text =
element_text(size = 15),axis.title = element_text(size = 17)),
                SSD_taxa2 + theme(legend.position="right", axis.text = element_text(size =
15),axis.title = element_text(size = 17)),

                labels = c(","),
                label_x = 0.4,
                ncol = 2,
                rel_widths = c(1.4, 2.0),
                rel_heights = 10,
                align = "h",
                label_size = 25)

## Second splitting
#calculating PAF after combining vertebrates and other plants species groups
#This is where we combined data points for various groups based on the plot output
#Always check the species that need s to be split and change color sceme accordingly

ecotox_data_rich$Merged_group <-
ifelse(ecotox_data_rich$SpeciesGroup_Harmonized=="Algae, cyanobacteria and aquatic
plants", "Algae, cyanobacteria and aquatic plants", "Others")

ecotox_data_rich %>%
  group_by(CAS_Harmonized, Merged_group)%>%
  arrange((log.EC10), .by_group = TRUE) %>%
  mutate(frac_t=ppoints(log.EC10,0.5)) %>%
```

```
  ungroup()->ecotox_data_rich_taxa.2

###New effect factors
ecotox_data_rich_taxa.2 <-ecotox_data_rich_taxa.2 %>%
  group_by(CAS_Harmonized, Merged_group) %>%
  mutate(n_t = n(),
         mu_t = mean(log.EC10),
         SDV_t = sd(log.EC10)) %>%

  ungroup()

###Observation per merged species groups
ecotox_data_rich_taxa.2$reduced_species.group.name.2 <-
ifelse(ecotox_data_rich_taxa.2$Merged_group=="Algae, cyanobacteria and aquatic plants",
"A",

                                          "Other")


ecotox_data_rich_taxa.2$labels.taxa.2 <-
paste0(ecotox_data_rich_taxa.2$reduced_species.group.name.2, " = ",
ecotox_data_rich_taxa.2$n_t)

ecotox_data_rich_taxa.2


##position of the text
ecotox_data_rich_taxa.2$y_position <-
as.numeric(factor(ecotox_data_rich_taxa.2$reduced_species.group.name.2))*0.05 + 0.85

ecotox_data_rich_taxa.2 <- ecotox_data_rich_taxa.2%>%
  mutate(HC20_t = qnorm(0.2, mean=mu_t, sd=SDV_t),
         HC5_t = qnorm(0.05, mean=mu_t, sd=SDV_t))


### Merged group deterministic curve
#Change the color depending on the grouping selected

d_fit_taxa.2 <- unique(ecotox_data_rich_taxa.2[c("CAS_Harmonized")])
d_fit_taxa.2 <- as.data.frame(d_fit_taxa.2)
colnames(d_fit_taxa.2) [1] <- c("CAS_Harmonized")

distribution_prameters_t <- ecotox_data_rich_taxa.2 %>%
  group_by(CAS_Harmonized, Merged_group) %>%
  slice(1)
###facet_grid new labels (manually done)
new_label <- as_labeller(c(`Simazine` = "Responsible split"))

d_fit_taxa.2 <- d_fit_taxa.2 %>%
  left_join(distribution_prameters_t[,c( "CAS_Harmonized","Merged_group",
"mu_t","SDV_t", "Name")],
```

```
            by=c("CAS_Harmonized")) %>%
  right_join(
    tidyr::expand(d_fit_taxa.2, CAS_Harmonized,EC = newxs),
    by = c("CAS_Harmonized"))


d_fit_taxa.2 <- d_fit_taxa.2 %>%
  mutate(PAF_t=pnorm(EC, mean=mu_t, sd=SDV_t))

SSD_taxa.2 <- ggplot()+
  #geom_point(data = ecotox_data_rich_taxa.2, aes(x = log.EC10, y = frac_t, colour
=Merged_group)) +
  scale_color_manual(values = cols.3)+
  geom_line(data=d_fit_taxa.2,
            aes(x=EC,
                y=PAF_t,
                color= Merged_group,
                Size=2)) +
  # scale_x_continuous(limits = c(0.010, 10),
  #   trans = log10_trans()) +

  labs(x = expression(paste('log10 [EC10 (µg/L)]')),
       y = 'Potentially affected species fraction')+
  #facet_grid(cols=vars(Name))+
  facet_wrap(.~Name, ncol=1, labeller=new_label) +
  theme(strip.text.x = element_text(size = 20))


SSD_taxa.2

#### CI around taxonomic groups fitted curves
###combined groups after splitting

d.in <-ecotox_data_rich_taxa.2
myboot2 <- function(d.in, newxs){
  xr <- sample(d.in$log.EC10, length(d.in$log.EC10), replace = TRUE)

  # fit distribution to new data
  fitr <- fitdistr(xr, 'normal')

  ##predict PAF for new data
  pyr <- pnorm(newxs, mean = fitr$estimate[1], sd = fitr$estimate[2])
  return(pyr)
}

pdat.final <- NULL
bootdat.final <- NULL
set.of.CAS_Harmonized <- unique(d.in$CAS_Harmonized)
id <- set.of.CAS_Harmonized[1]

set.of.groups <- (unique(d.in$Merged_group))
```

```
input.group <-set.of.groups
input.group
input.group<-set.of.groups[2]

for(id in set.of.CAS_Harmonized){
  data.sub <- d.in[which(d.in$CAS_Harmonized %in% id),]
  #set.of.groups <- as.character(unique(data.sub$SpeciesGroup_Harmonized))

  for(input.group in set.of.groups){
    data.sub.in <-  data.sub[which(data.sub$Merged_group %in% input.group),]

    # new data to predict
    #newxs <- seq(0.01, max(data.sub.in$log.EC10), length.out = 1000)
    boots <- replicate(1000, myboot2(d.in=data.sub.in, newxs))

    # extract bootstrap values
    bootdat <- data.frame(boots)
    bootdat$newxs <- newxs
    bootdat <- reshape2::melt(bootdat, id = 'newxs')
    # extract CI
    cis <- apply(boots, 1, quantile, c(0.025, 0.975))
    rownames(cis) <- c('lwr', 'upr')
    # add fitted values
    pdat <- data.frame(newxs, py = pnorm(newxs, mean = data.sub.in$mu_t, sd =
data.sub.in$SDV_t))
    # add CI
    pdat <- cbind(pdat, t(cis))
    pdat$CAS_Harmonized <- id
    pdat$Merged_group <- input.group

    bootdat$CAS_Harmonized <- id
    bootdat$Merged_group <- input.group


    pdat.final <- rbind(pdat.final, pdat)
    bootdat.final <- rbind(bootdat.final, bootdat)

  }
}
#### Add CI to the merged species groups
SSD_taxa_CI.2 <- SSD_taxa.2
for(id in set.of.CAS_Harmonized){
  print(id)
  for(input.group in set.of.groups){
    print(input.group)

    SSD_taxa_CI.2 <- SSD_taxa_CI.2 +


      geom_ribbon(data = pdat.final[which(pdat.final$CAS_Harmonized %in% id &
pdat.final$Merged_group %in% input.group),], aes(x =newxs, ymin =lwr,ymax = upr),
```

```
                fill="black",  alpha=0.2)


  }
}

SSD_taxa.2. <- SSD_taxa_CI.2 +
  geom_segment(aes(x =ecotox_data_rich_taxa.2$HC20_t, y = 0.0, xend =
ecotox_data_rich_taxa.2$HC20_t,  yend = 0.2),color ="red",  size=0.9) +
  geom_segment(aes(x =ecotox_data_rich_taxa.2$HC5_t, y = 0.0, xend =
ecotox_data_rich_taxa.2$HC5_t,  yend = 0.05),color ="black", size=0.9) +
  geom_point(data = ecotox_data_rich_taxa.2, aes(x = log.EC10, y = frac_t, colour
=Merged_group, shape=SpeciesGroup_Harmonized),size=5) +
  scale_color_manual(values = cols.3) +
  annotation_logticks(sides='b')+
  theme(legend.key.size = unit(0.5, 'cm'),
        legend.key.height = unit(0.5, 'cm'),
        legend.key.width = unit(0.5, 'cm'),
        legend.title = element_text(size=15),
        legend.text = element_text(size=15))+

  geom_text(aes(-0.7,
ecotox_data_rich_taxa.2$y_position,label=ecotox_data_rich_taxa.2$labels.taxa.2), size=5.5,
            family="Sans", fontface="plain", lineheight=.8)+
  geom_errorbarh(data=ecotox_data_rich_taxa.2, mapping=aes(y=frac_t, x=log.EC10,
xmin=log10(min_c), xmax=log10(max_c), colour = Merged_group), height=0.02, size=0.4)+

  scale_x_continuous(limits = c(-2,6))

### Grid arrange all the three plots
SSD_taxa2 <- SSD_taxa2 + theme(
  axis.text.y = element_blank(),
  axis.ticks.y = element_blank(),
  axis.title.y = element_blank())

SSD_taxa.2. <- SSD_taxa.2. + theme(
  axis.text.y = element_blank(),
  axis.ticks.y = element_blank(),
  axis.title.y = element_blank())

merged.figure <- plot_grid (SSD_chemical2 + theme(legend.position="none", axis.text =
element_text(size = 20),axis.title = element_text(size = 20)),
                        SSD_taxa2 + theme(legend.position="none", axis.text = element_text(size =
20),axis.title = element_text(size = 20)),
                        SSD_taxa.2.  + theme(legend.position="none", axis.text = element_text(size
= 20),axis.title = element_text(size = 20)),
                        labels = c(","),
                        label_x = 0.4,
                        ncol = 3,
                        rel_widths = c(0.6, 0.6, 0.6), ##1.2
                        rel_heights = 10,
```

```
                    align = "h",
                    label_size = 29)

title <- ggdraw() + draw_label("a.Simazine, CAS:122-34-9 (Photosynthesis inhibition
MoA):split algae, cyanobacteria and aquatic plants from the rest", fontface='bold', size=20)

merged.figure <- plot_grid(title, merged.figure, ncol=1, rel_heights=c(0.1, 1)) # rel_heights
values control title margins

save_and_display(
  merged.figure,
  "Split_ssds_Simazine",
  width = 21,
  height = 7)
```

References

(1) Venables, W. N.; Ripley, B. D. *Modern Applied Statistics with S*, Fourth.; Springer: New York, 2002.

(2) Emara, Y.; Jolliet, O.; Finkbeiner, M.; Heß, S.; Kosnik, M.; Siegert, M.-W.; Fantke, P. Comparative Selective Pressure Potential of Antibiotics in the Environment. *Environ. Pollut.* **2023**, *318* (October 2022), 120873. https://doi.org/10.1016/j.envpol.2022.120873.

(3) Rosenbaum, R. K.; Georgiadis, S.; Fantke, P. Uncertainty Management and Sensitivity Analysis. In *Life Cycle Assessment: Theory and Practice*; Hauschild, M. Z., Rosenbaum, R. K., Olsen, S. I., Eds.; Springer International Publishing: Cham, 2018; pp 271–321. https://doi.org/10.1007/978-3-319-56475-3_11.

Excel Document with 180 underlying data and splitting SSD statistical results are provided as a supplementary Excel file.