THESIS FOR THE DEGREE OF LICENTIATE OF ENGINEERING

ADVANCING THE INTEGRATION OF PERSISTENT AND MOBILE SUBSTANCES IN LIFE CYCLE IMPACT ASSESSMENT

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ABSTRACT

Among the many chemicals we use in daily life, 'forever chemicals' such as per- and polyfluoroalkyl substances (PFAS), can persist and accumulate in the environment and may cause long-term harm. These substances can remain with us for an extended period not only in the products we use but also as contaminants in the environment. They can also slowly transform to form toxic byproducts.

There is an increased awareness and concern about the use of these chemicals, leading to efforts at both the regulatory level and within academic, industrial, and societal spheres. Risk assessments for chemicals have typically focused on either emission during use or from manufacturing facilities rather than the whole life cycle. On the other hand, life cycle assessment (LCA) often considers greenhouse gas emissions but omits toxicity concerns. There is a need for tools that can quantify the impacts of these chemicals along product life cycles, integrating them into LCA. Without calculating toxicity characterization factors (CFs) using tools such as USEtox, LCA is unable to quantify ecotoxicity impacts of chemicals. In addition, there are limitations regarding the availability of ecotoxicity data required to calculate these CFs. Even when data is available, there are concerns about the applicability of existing tools to accurately calculate CFs.

The research presented in this licentiate thesis is based on two studies. The aims are to determine the gaps in the availability of ecotoxicity CFs for persistent and mobile substances, examine the influence of ecotoxicity data selection and harmonization alternatives on Effect factors (EFs), and explore the calculation of extrapolation factors to convert effect concentration indicators. The first study offers methodological contributions on the harmonization of ecotoxicity data of chemicals for use in calculating CFs. It also assesses whether QSAR-based data can effectively replace experimental data. Additionally, it offers practical use values for ecotoxicity CFs of persistent and mobile chemicals, which were previously unavailable in existing USEtox database, thus supporting their inclusion in LCA studies. The second study addresses the uncertainty in the extrapolation factors when converting different effect concentration indicators (endpoints). This aids in reducing the uncertainty of using generic extrapolation values for chemicals by providing species group-specific extrapolation values for EC10 and EC50 effect concentration indicators.

The study concludes that the coverage of persistent and mobile chemicals in the USEtox database (version 2.01) is only 28% for the chemicals in focus in this thesis (18 out of 64). Consequently, emissions of chemicals lacking CFs cannot be included in ecotoxicity impact assessments in LCA due to the absence of CFs. Additionally, the ecotoxicity data harmonization approach can significantly influence the calculation of EFs. A pragmatic harmonization approach is recommended to ensure the process is feasible without compromising the accuracy and reliability of the harmonized data. Furthermore, QSAR methods should be considered a last resort when experimental ecotoxicity values are unavailable. QSAR methods lack accuracy in estimating ecotoxicity values for EF calculations. Finally, extrapolation factors at the species group level differ considerably from those at the generic level, leading to the conclusion that species-level factors should be used to reduce uncertainty in the extrapolated effect concentration indicators.

Several challenges have also been identified which should be addressed for LCA to contribute to the quantification of impacts of forever chemicals. These include adaptation of the USEtox model to persistent and mobile chemicals, and the availability of ecotoxicity data for these chemicals.

LIST OF PUBLICATIONS

This licentiate thesis is based on the research work presented in the following papers. Manuscripts of the papers are appended at the end of the licentiate thesis.

PAPER 1
Aggarwal, R., Holmquist, H., Arvidsson, R., Reppas-Chrysovitsinos, E., & Peters, G. (2024). Influence of data selection on aquatic ecotoxicity characterization factors for selected persistent and mobile substances. *The International Journal of Life Cycle Assessment*, 29(2), 344-354

PAPER 2
Aggarwal, R., Gustavsson, M., Peters, G. & S., Molander (2024). Extrapolation factors for calculating ecotoxicity effects in LCA. *The International Journal of Life Cycle Assessment*

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LIST OF ABBREVIATIONS

AI	Artificial Intelligence
CAS	Chemical Abstracts Service
CF	Characterisation Factor
CLP	Classification, Labelling and Packaging
CNT	Carbon Nanotube
CTS	Chemical Transformation Simulator
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECF	Exposure Concentration Factor
ECHA	European Chemicals Agency
ECOSAR	Ecological Structure Activity Relationships
ECOTOX	Ecotoxicology Database
EF	Ecotoxicological Effect Factor
EFSA	European Food Safety Authority
ENM	Engineered Nanomaterial
EU	European Union
FF	Fate Factor
HF	Hazard Factor
ISO	International Organization for Standardization
IUCLID	International Uniform ChemicaL Information Database
LCA	Life Cycle Assessment
LCIA	Life Cycle Impact Assessment
LOEC	Lowest Observed Effect Concentration
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
OECD	Organisation for Economic Co-operation and Development
PAF	Potentially Affected Fraction of species
PBT	Persistent, Bioaccumulative and Toxic
PEF	Product Environmental Footprint
PFAS	Perfluoroalkyl and Polyfluoroalkyl Substance
PHF	Person-Hours Factor
PM	Persistent and Mobile
PMT	Persistent, Mobile, and Toxic
PNEC	Predicted No Effect Concentration
PPDB	Pesticides Properties DataBase
PSU	ProScale of Unit Process
PTFE	Polytetrafluoroethylene
QSAR	Quantitative Structure-Activity Relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RIVM	National Institute for Public Health and the Environment
SAICM	Strategic Alliance for International Chemicals Management
SB4N	SimpleBox4Nano
SETAC	Society of Environmental Toxicology and Chemistry
SVHC	Substances of Very High Concern

T.E.S.T.	US EPA Toxicity Estimation Software Tool
TP	Transformation Product
UNEP	United Nations Environment Programme
US-EPA	United States Environmental Protection Agency
VEGA	Variable Environment Genetic Algorithm
vPvB	Very Persistent, Very Bioaccumulative
vPvM	Very Persistent and Very Mobile
XF	Environmental Exposure Factors

1. INTRODUCTION

Chemicals play a dual role in modern society, both offering advantages and constituting hazards. On one hand, they have catalyzed progress across various industries such as energy, transportation, and healthcare, considerably improving the lifestyles of billions of people (Barrett, 2000; ICCA, 2011; Ogunseitan, 2023; UNEP, 2019). On the other hand, their widespread use has introduced risks to human health and the environment. These include toxic environmental contaminants and health issues such as hormone disruption and neurotoxicity, sometimes causing impacts exceeding those of major infectious diseases (Arp et al., 2021; Gerster et al., 2014; Landrigan et al., 2018; Levallois et al., 2018; Villarrubia-Gómez et al., 2018; Wu et al., 2020). The 1984 Bhopal methyl isocyanate incident, for example, had devastating consequences (Trushna & Tiwari, 2022). The Chemical Abstracts Service (CAS) has cataloged over 200 million chemical entities since the 1800s, with more than 350,000 chemicals or combinations thereof currently registered for commercial production and use (CAS, 2023; ECHA, 2023c; Wang et al., 2020). European Chemicals Agency (ECHA) alone has registrations for over 26,500 chemicals (ECHA, 2023c).

PMT (Persistent, Mobile, and Toxic) and vPvM (Very Persistent and Very Mobile) chemicals, which include the well-known per- and polyfluoroalkyl substances (PFAS) (colloquially known as 'forever chemicals'), are increasingly recognized as chemicals of concern due to their potential for environmental accumulation and considerable mobility in aquatic ecosystems (Neumann & Schliebner, 2019). PFAS are utilized in a wide array of applications such as fire-fighting foams, electroplating, ammunition, and climbing ropes (Aminot et al., 2023; Glüge et al., 2020). ECHA defines PFAS as substances containing at least one aliphatic CF2 or CF3 element, like PTFE (polytetrafluoroethylene) (ECHA, 2023a; Herzke et al., 2012; Wollin et al., 2023). Considering the widespread use of these chemicals, in 2020, the European Commission, in its Chemical Strategy for Sustainability, decided to categorize PMT/vPvM as Substances of Very High Concern (SVHC) under REACH by 2022, aiming for tighter control and potential restrictions (EU, 2020). By 2023, the Commission implemented a delegated regulation updating the Classification, Labelling and Packaging (CLP) regulation to include new hazard classifications for PMT and vPvM, mandating their new classifications by 2026 (ECHA, 2023b). PMT and vPvM classes are defined in detail by ECHA (2023b) under European Union (EU) hazard statements.

Chemical management and assessment tools including LCA, chemical alternatives assessment (CAA), comparative risk screening, and risk assessment are available for analyzing the toxicological effects of chemicals (Bare, 2006; P. Fantke et al., 2020; McCarty et al., 2018). These frameworks have differences in their aims, applications and underlying assumptions. Over the past decades, the LCA methodology, supported by standards like ISO 14040, has gained recognition for quantifying potential ecotoxicity impacts over a product lifecycle (Fantke & Ernstoff, 2018; Jacobs et al., 2016; Rosenbaum, 2015; Tickner et al., 2021). However, LCA methods for including ecotoxicity impacts of product system containing chemicals, including PMT/vPvM substances, depend on the availability of characterization factors (CFs) for each and every chemical substance (Henderson et al., 2011; Holmquist et al., 2020; Roos et al., 2017). These CFs are essential as they provide the necessary link between chemical emissions and potential ecotoxicity impacts as calculated by the LCIA (Pennington et al., 2004).

Calculation of ecotoxicity CFs depends on environmental fate, exposure, and ecotoxicological effects of chemicals (Jolliet et al., 2006; Rosenbaum et al., 2008). USEtox, an open-source life cycle impact assessment (LCIA) method, is widely utilized for determining ecotoxicity CFs. It is officially endorsed by the UNEP/SETAC Life Cycle Initiative, the European Commission, the World Business Council for Sustainable Development, and the United States Environmental Protection Agency (Fantke et al., 2017). The USEtox model (version 2.13) provides a consensus approach for determining freshwater ecotoxicity CFs for various chemicals (Fantke et al., 2017; Rosenbaum et al., 2008; USEtox, 2023). It establishes a cause-effect linkage tracing an environmental emission via fate and exposure to ecotoxicity impacts. Within USEtox, the CF [PAF.m³.d/kg emitted] is calculated in a matrix system including three factors: Fate factors (FF) in kg.kg-1.d-1, representing the residence time of a substance in different compartments; exposure factors (XF), which are unitless and represent the fraction of a substance dissolved in freshwater (i.e., bioavailable to aquatic species); and ecotoxicological EFs in PAF.m³.kg-1, illustrating the relationship between the potentially affected fraction (PAF) of aquatic species and the concentration of a substance. The CF is calculated according to the formula:

$$CF = FF \times XF \times EF \qquad \text{(Eq. 1)}$$

The official USEtox documentation by Fantke et al. (2017) provides detailed explanations of all equations, abbreviations, and input data used in the model to calculate chemical CFs. This licentiate thesis utilizes USEtox to determine aquatic CFs for 64 persistent and mobile (PM) chemicals, including 24 PFAS, 17 triazines, and 23 triazoles.

Despite the known risks that many chemicals pose to the natural environment, including freshwater, marine, and soil compartments, only a relatively small fraction of these chemicals have been integrated into LCA, leading to considerable data gaps and inaccuracies in the assessment of ecotoxicity impacts (Rosenbaum et al., 2017). This is underscored by the disparity between the 145,299 substances documented by the ECHA and the 3,104 substances (3,077 organic and 27 inorganic) available in the USEtox database (version 2.01) (Fantke et al., 2017; USEtox, 2023). Specifically, USEtox provides freshwater ecotoxicity Effect Factors (EFs) for only 2,499 substances, leaving many chemicals uncharacterized due to the scarcity of ecotoxicity data. In particular, among PFAS and other fluorinated compounds, the CompTox Chemicals Dashboard PFAS suspect list identifies 16,120 PFAS substances (CompTox, 2023; PubChem, 2023), yet the USEtox database (version 2.01) includes CFs for only 14 of these. This substantial gap in available CFs for PFAS substances severely hinders their integration into LCA. In past years, attempts were made to fill the data gaps, including experimental EFs and CFs, from studies conducted by Saouter et al. (2018), which calculated CFs for 6,711 chemicals. Additionally, Douziech et al. (2024) calculated EFs for 9,862 chemicals, with 8,876 using a default slope of 0.7 to derive SSDs to estimate HC20EC10, 701 based on Owsianiak et al. (2023) with intra-species extrapolated effect data, and 285 by combining intra-species extrapolated effect data and QSAR-based estimates to reach five species from three species groups.

2. AIM AND RESEARCH QUESTIONS

The overarching aim of this thesis is to advance the integration of chemical ecotoxicity impacts within LCAs, particularly focusing on persistent and mobile chemicals due to their potential to accumulate in the environment and cause long-term harm. The research is structured around three specific research questions.

Research Question 1: Are there important gaps in the availability of ecotoxicity CFs for PM substances?

The first research question addresses the gaps in the inclusion of PM substances' ecotoxicity impacts in LCA. PM substances are defined as those persistent in the environment and mobile in the aquatic environment based on combinations of their intrinsic properties. However, the criteria for classifying a substance as PM may vary depending on the qualifying values for these intrinsic properties as defined by regulatory organizations such as the German Environment Agency (UBA) or the ECHA (Hale et al., 2020). For a truly holistic life cycle assessment, the inclusion of ecotoxicity impacts from PM chemicals is essential, given their considerable effects on the environment. The USEtox database (version 2.01) has coverage of only 109 out of the 343 chemicals identified as PMT/vPvM by the UBA (Arp et al., 2023; Fantke et al., 2017; Rosenbaum et al., 2017). Paper 1 considers a group of 64 chemicals identified as chemicals of concern due to their persistence, mobility, and widespread use within Europe. For these selected PM chemicals, the coverage is only 18 out of 64 in USEtox. To overcome this lack of CFs for these chemicals, Paper 1 calculated CFs for all 64 chemicals, thus facilitating their inclusion in LCA studies.

Research Question 2: How does the selection of ecotoxicity data and its harmonization influence the calculation of EFs?

This research question explores the calculation of ecotoxicity CFs, which depends on matrix calculation of FF, XF, and EF. Studies have shown that CFs are particularly sensitive to the values of EFs (Holmquist et al., 2020; Roos et al., 2017), which are calculated using ecotoxicity data. However, ecotoxicity raw data need harmonization to standardize into the USEtox input format, as raw data often vary in terms of units, exposure durations, effect concentration indicators, and species tested. Paper 1 presents advancements in the harmonization of ecotoxicity data for chemicals to calculate EFs. A data harmonization strategy was developed for ecotoxicological effect data. Alternative data harmonization strategies were also developed

and tested to determine the influence of different steps in the EF calculation and different ecotoxicity data sources. Different in silico methods were also utilized and compared to experimental data to test the reliability of these methods in addressing data gaps.

Research Question 3: What are the challenges and opportunities with alternative ecotoxicity data translation and aggregation approaches in calculating extrapolation factors for CFs?

This research question investigates the challenges in using ecotoxicity databases where chemicals have ecotoxicity values at different effect concentration indicators that cannot be directly used but require conversion into effect concentration indicators suitable for EF calculation. In USEtox, chronic EC50 (effective concentration inducing a 50% of the response) values are required. Available extrapolation factors usually consider the type of effect concentration indicators and exposure durations but generally fail to address variations across species groups, which can be considerable. Paper 2 delves into the current lack of species group-specific extrapolation factors to convert effect concentration indicators (EC10 eq and EC50 eq) to a chronic EC10 eq and chronic EC50 eq. The paper introduces both generic and species group-specific extrapolation factors. This advancement enables more precise ecotoxicity CF calculations in USEtox, enhancing its scope and accuracy. Along with proposing new generic and species group-specific extrapolation factors, Paper 2 also analyzes the influence of alternative ecotoxicity data aggregation approaches. It compares the methods of aggregation (geometric mean vs. arithmetic mean) and the classification of effect concentration indicators (EC10 eq and EC50 eq vs. NOEC, EC10, and EC50 eq). Additionally, the paper examines the differences in generic extrapolation factors across various chemical groups.

3. METHODS

3.1. Transformation products

Paper 1 made an attempt to include TPs in the calculation of CFs for chemicals, in particular those PM chemicals on the UBA list. Including every possible TP from the 64 chemicals considered in Paper 1 was infeasible. This is due to the limitations of TP prediction tools, which can only predict the probable TPs without providing their quantities, making it difficult to determine which TPs to include. To address this challenge, a simplified TP screening strategy was developed based on the chemical parameters of persistence in the freshwater environment and ecotoxicity values as shown in Figure 1. In this strategy, potential TPs up to three generations from the parent compound were estimated for each parent compound using two openly available TP prediction tools: the United States Environmental Protection Agency's Chemical Transformation Simulator (CTS) (Wolfe et al., 2016; Yuan et al., 2021) and enviPath (enviPath, 2022; Wicker et al., 2016). First, persistence data for all TPs was collected using the EPI Suite level III fugacity model with a fixed temperature of 25°C to estimate the half-life in water (Aronson et al., 2006; U.S.EPA, 2023b). TPs that were less persistent than their respective parent chemicals were excluded. Next, for the remaining persistent TPs, ecotoxicity data (LC50) for the fathead minnow over a 96-hour exposure duration was collected using the EPA T.E.S.T. QSAR (Quantitative Structure-Activity Relationship) method (U.S.EPA, 2020). TPs that were less toxic than their respective parent chemicals were excluded. The resulting relevant TPs were then added to the list of the 64 chemicals for the CF calculations. In total, three TPs were added to the list.

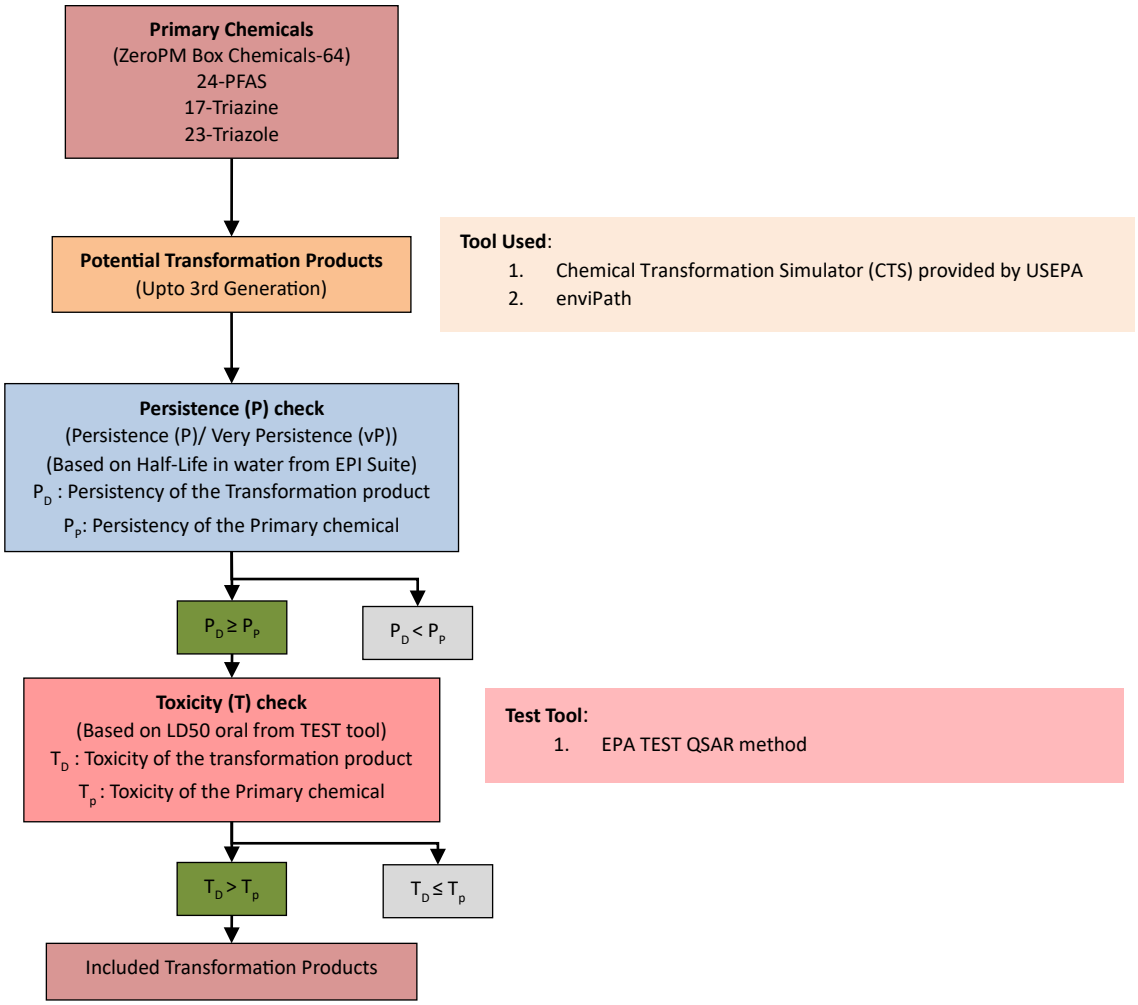

Figure 1: Simplified in silico PM transformation products screening-level assessment strategy.

3.2. Data sources

In Paper 1, the selection of 64 PM chemicals was based on a literature review by Hale, Kalantzi, et al. (2022), focusing on chemicals with a potential risk of contaminating drinking water sources due to their persistence and mobility, as well as their widespread use in Europe (Arp & Hale, 2022; Hale, Neumann, et al., 2022; Jin et al., 2020). These chemicals are classified into three groups: PFAS, triazines, and triazoles. For the calculation of EF, experimental ecotoxicity data was collected from purely experimental chemistry data sources including in the CompTox Version 2.1.1, which includes data from ToxValDB v9.1.1 (CompTox, 2022; Williams et al., 2017). A total of 5,002 ecotoxicity data points were collected, covering 15 PFAS, 12 triazines, and 21 triazoles. Additionally, QSAR-based ecotoxicity data was collected from various QSAR models, including ECOSAR™ Version 1.11 through EPI Suite v4.11 (Benfenati et al., 2013; U.S.EPA, 2023b), US EPA Toxicity Estimation Software Tool

(T.E.S.T.) (Mayo-Bean et al., 2012; U.S.EPA, 2020), VEGA (VEGA HUB, 2022), and the Danish (Q)SAR database (Danish (Q)SAR, 2022). Data collected from ECOSAR include effect concentration indicators such as LC50 96h for fish, LC50 48h for daphnia, and EC50 96h for green algae. Ecotoxicity data collected from US EPA T.E.S.T. v5.1.2 includes effect concentration indicators such as LC50 96h for fathead minnow, LC50 48h for Daphnia magna, and IC50 48h for Tetrahymena pyriformis.

Physicochemical data were retrieved from several sources: molecular weight (MW) from ChemSpider (Chemspider, 2022); pKa, chemical class (neutral, acid, base, amphoter), pKa.gain, and pKa.loss from ChemAxon (Chemaxon, 2022); and partition coefficients (KOW, KOC), Henry's Law constant at 25°C (KH25C), vapor pressure at 25°C (Pvap25), water solubility at 25°C (Sol25), and degradation rate constants in air, water, sediment, and soil (kdegA, kdegW, kdegSd, kdegSl) from EPI Suite v4.11 (U.S.EPA, 2023b).

In Paper 1, the EFs of the PM chemicals were calculated based on experimental data after harmonization. This dataset included, on average, data from 10 species and three species groups per chemical. Specifically, for the 14 PFAS compounds, data from an average of five species and two species groups were considered. For the 12 triazines, the dataset included data from 20 species and four species groups, and for the 21 triazoles, data from nine species and four species groups were used. The harmonized dataset covers a broad temporal span, from 1965 to 2020, with 58% of the data points from 2004 onwards, and 35% from 2010 onwards. The year 2004 is particularly notable as it can be considered as the cut-off year for most of the available input data in the USEtox database. The temporal distribution of data also varies across chemical groups. For PFAS, 90% of the data is from post-2004 and 77% from post-2010, highlighting that a substantial portion of PFAS data was generated in the past decade. In contrast, for triazines, 44% of the data is from post-2004 and 22% from post-2010. For triazoles, 69% of the data is from post-2004 and 39% from post-2010.

To address research question 2 regarding the influence of different ecotoxicity data sources on the EFs, two approaches were used. First, various QSAR methods were employed to calculate the EFs, which are then compared. Secondly, the results from all QSAR methods were combined and compared with experimental values to assess the reliability of QSAR models in calculating the EFs. The combined QSAR based ecotoxicity data from ECOSAR™ Version 1.11 [n=39], US EPA T.E.S.T. [n=44] and Danish (Q)SAR database [n=50] (Danish (Q)SAR Database, 2022) were collected and then harmonized. The harmonized data resulted in 524 data

points consisting of 161 data points for 21 PFAS, 158 data points for 17 triazines, and 205 data points for 21 triazoles.

To generate extrapolation factors in Paper 2, aquatic ecotoxicity data were collected from REACH dossiers and the CompTox database (Adkins, 2023; REACH, 2020; Williams et al., 2017). The REACH database accessed contained 225,517 ecotoxicity records for 12,411 chemicals identified by a European Commission Number. The CompTox data from the U.S. EPA ToxValDB (version 9.4) includes 517,067 ecotoxicity data points, for 8,640 chemicals identified by CAS number.

3.3. Harmonization of ecotoxicity data

The harmonization of ecotoxicity raw data is required for the calculation of the EFs. Ecotoxicity data harmonization refers to the process of standardizing ecotoxicity data from different sources to ensure consistency, comparability, and reliability across datapoints. This is usually done by converting the data from different formats to a standardized format based on the intended use of the data. In this study, the data is harmonized based on the format needed in USEtox. A data harmonization strategy for ecotoxicological effect data was developed in Paper 1, as illustrated by the decision tree in Figure 2.

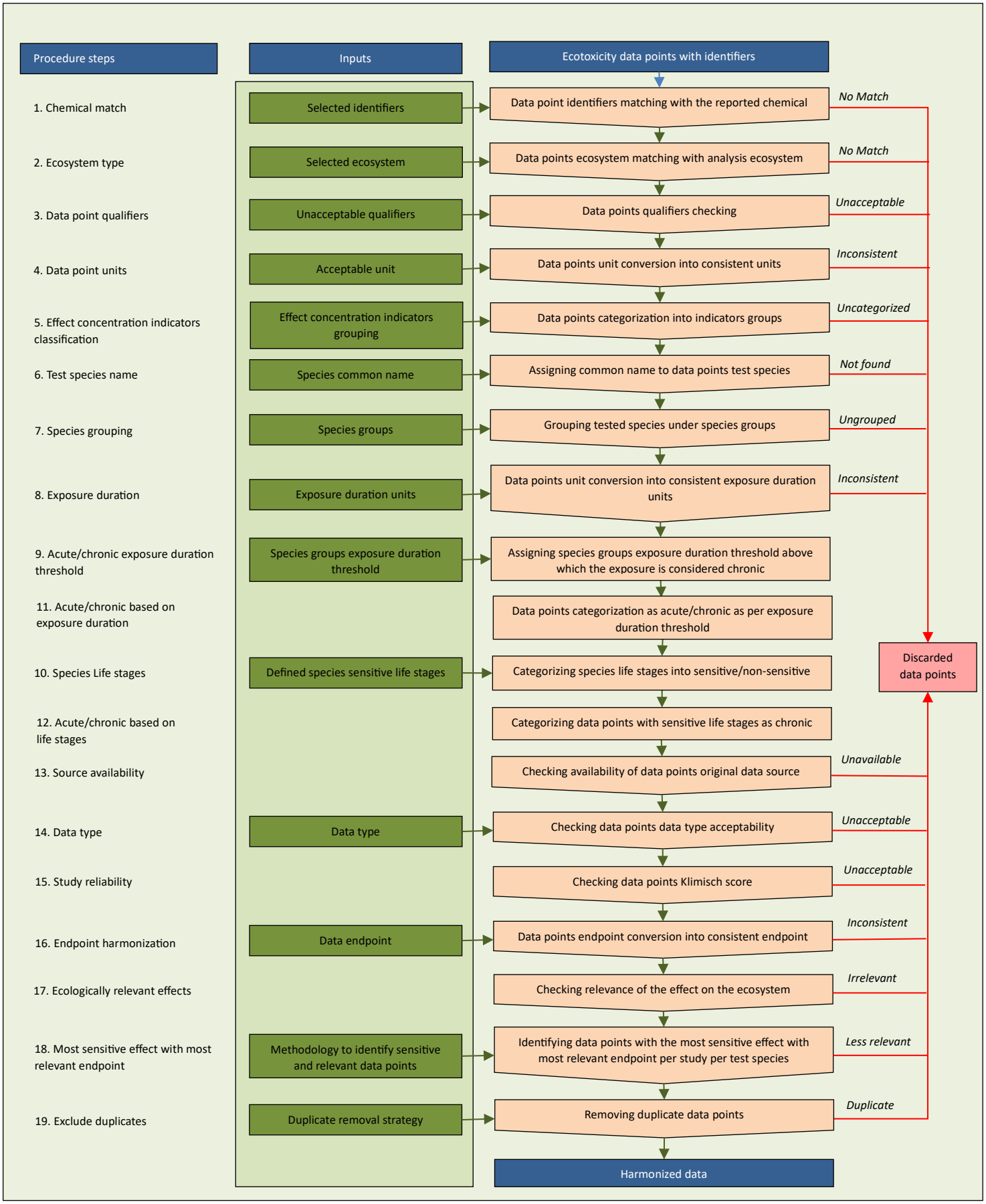

Figure 2: Decision tree for ecotoxicity data harmonization for effect factors calculation (Source: Paper 1)

The harmonization process begins with checking the chemical identifiers to ensure that the data corresponds to the chemical under consideration. This step is crucial because chemicals can have multiple identifiers, some unique and some not. Various databases use their own chemical identifiers, such as the European Commission number. To address this, commonly available chemical identifiers, specifically CAS registry numbers, were collected for all chemicals from the data sources and matched with the chemical under consideration. The next step is to ensure that the data pertains to the aquatic system since the CFs are to be calculated for the freshwater ecosystem as per the USEtox model. Data from other ecosystems were excluded. During data collection, some values may not be definitive, being either statistically estimated or outside the measurable range of instruments. This results in data with numeric qualifiers such as >, ≥, <, and ≤. To minimize uncertainty, only data points with the qualifier "=" were included. Subsequently, the effect values were harmonized to common units of mg/L for the calculation of the EFs. Data in units that could not be converted to mg/L, such as mg/day or mg/kg, were excluded due to the incompatibility with USEtox data requirements. Effect concentration indicators were harmonized into four groups: "NOEC eq," "EC10 eq," "EC50 eq," and "LOEC eq," based on available extrapolation factors to convert to chronic EC50 (Aurisano et al., 2019). Species names were standardized according to the US EPA ECOTOX knowledgebase (U.S.EPA, 2023a), and species were grouped into seven categories to determine chronic exposure duration thresholds for exposure type classification (Aurisano et al., 2019; Payet, 2004). Exposure duration was harmonized in days and then classified into different exposure type in multiple steps. First, a chronic exposure duration threshold was set as follows: >1 day for algae, cyanobacteria, and microorganisms; >4 days for invertebrates (crustaceans); and >7 days for fish, invertebrates (non-crustaceans), vertebrates, and aquatic plants other than algae, as given in Aurisano et al. (2019). Secondly, tests with early life stages (e.g., embryo, larva) were then combined with the chronic test class irrespective of test duration.

Data source references were checked to ensure the data type was experimental only, followed by assessing study reliability based on the Klimisch score, accepting scores of either 1 (reliable without restriction) or 2 (reliable with restrictions) (Klimisch et al., 1997). If necessary, effect concentration indicators were extrapolated to chronic EC50 (Aurisano et al., 2019; Fantke et al., 2015). Only effects relevant to the aquatic ecosystem level were included, excluding effects such as biomarkers, as described in the data harmonization applied by Holmquist et al. (2020). Among multiple relevant effects, only the most sensitive effect per study, per chemical, and per test species was retained. Finally, all duplicate values were removed.

The ecotoxicity raw data retrieved from CompTox Version 2.1.1 in Paper 1 includes in total 5,002 experimental ecotoxicity data points. The harmonization steps including Step 2 (Ecosystem Type), Step 3 (Numeric Qualifiers), Step 4 (Data Point Unit), Step 5 (Effect concentration indicators Classification), Step 15 (Study Reliability), and Step 18 (Most Sensitive Effect) resulted in the exclusion of most of the data points in total that did not conform to the required format. As a result of these harmonization steps, the initial data set was notably reduced. The final harmonized dataset contained 1,189 data points: 174 for 14 PFAS, 668 for 12 triazines, and 347 for 21 triazoles.

Research question 2 addresses the impact of data selection and harmonization on the EFs. To illustrate this, Paper 1 developed alternative data harmonization strategies by modifying the baseline harmonization framework shown in Figure 2. Alternative Data Harmonization Strategy 1 included all data points regardless of the numeric qualifiers. By doing so, it assessed the impact of including data points with unacceptable numeric qualifiers (e.g., >, ≥, <, ≤). Alternative Data Harmonization Strategy 2 altered the exposure type classification by assuming all data points to be acute and then extrapolating them to a chronic equivalent. Alternative Data Harmonization Strategy 3 assumed all effects and effect concentration indicators to be equally sensitive and relevant, disregarding their specific distinctions. To assess the impact of these alternative strategies on the final CF results, a correlation analysis was conducted. This analysis involved log-transformed regression fits between the EFs calculated from the alternative strategies and those from the baseline harmonization strategy. The R^2 values were used to determine the extent of correlation.

Paper 2 developed a simplified framework for data selection and harmonization, building on the framework of Paper 1, for the calculation of extrapolation factors across effect concentration indicators, exposure type, and test species groups. Simplification was necessary because the aim of Paper 2 was to develop extrapolation factors rather than CFs. Consequently, steps such as effect concentration indicators harmonization, selection of the most sensitive effect, and other time-consuming processes like assessing species life stages and sensitivity and combining acute/chronic exposure based on life stages with risk assessment class, were excluded. The data harmonization process in Paper 2 included five main steps: chemical identification, data reliability control, data harmonization, consistency checking, and selection of ecologically relevant effects, as illustrated in Figure 3. The final harmonized dataset comprises 339,729 datapoints for 10,668 chemicals.

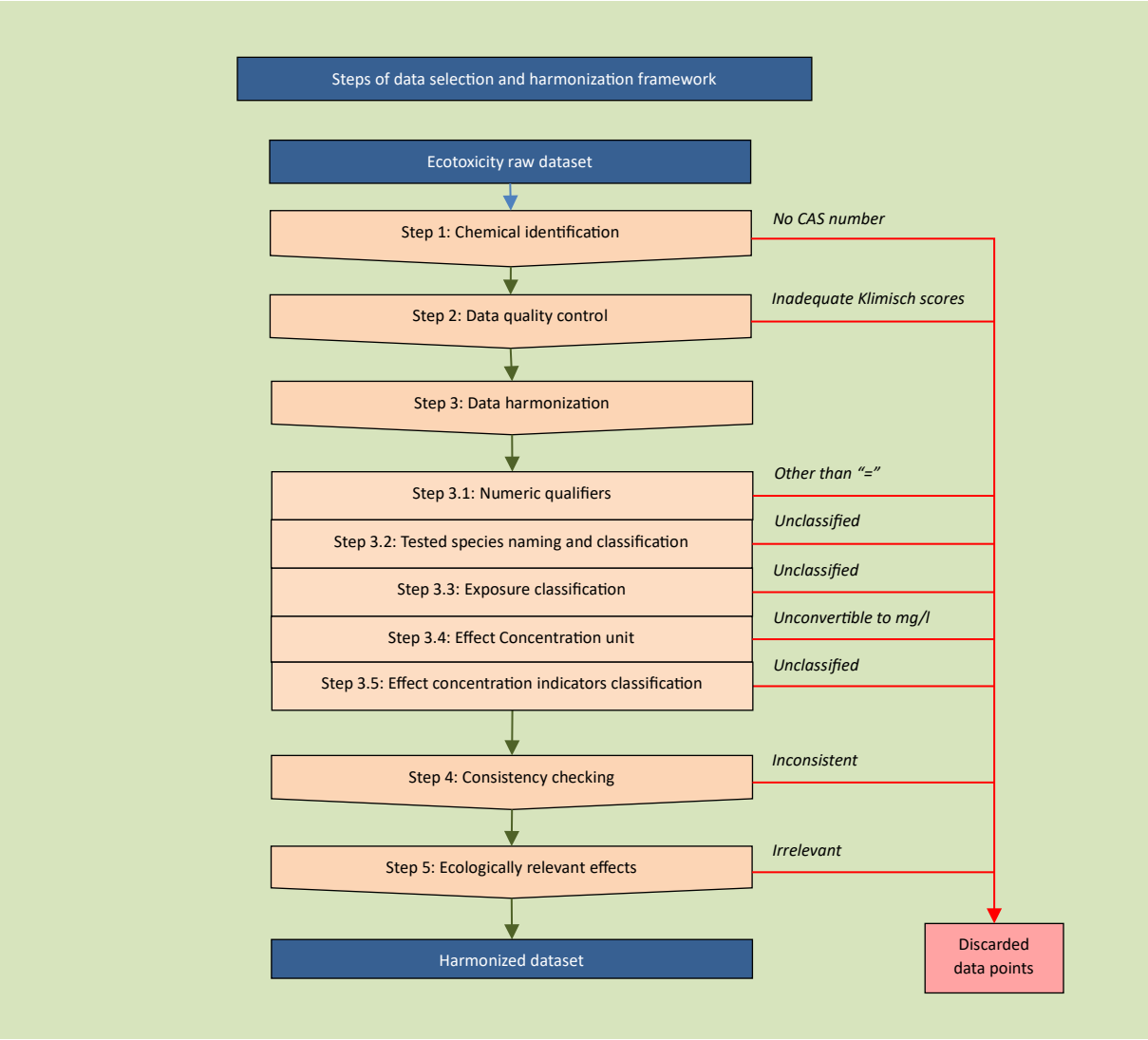

Figure 3: Decision tree for ecotoxicity data harmonization framework for extrapolation factors calculation (Source: Paper 2)

3.4. Extrapolation factor calculation

In the absence of data availability in the required format as required by USEtox, extrapolation factors are commonly employed to convert different effect concentration indicators (NOEC, EC50, and EC10) and exposure durations (acute and chronic). Current methods typically rely on predefined assessment factors (ECHA, 2012) or generic acute-to-chronic extrapolation ratios calculated from a limited chemical dataset (Fantke et al., 2017; Payet, 2004). However, these methods do not account adequately for variations across species groups, which can affect extrapolation accuracy. A number of studies have addressed the development of extrapolation factors in effect concentration indicators in aquatic environments. Aurisano et al. (2019) analyzed ECHA 2018 data involving 71,343 raw data points for 1,927 chemicals, leading to a harmonized dataset of 9,627 data points for 1,048 chemicals, calculating extrapolation factors

for three species groups—algae/cyanobacteria, crustaceans, and fish—covering effect concentration indicators such as EC50, NOEC, and EC10. Conversely, Payet (2004) data was collected from sources like ECETOC and the US-EPA, compiling 134,088 data points across species groups including plants and algae, vertebrates, and invertebrates, and effect concentration indicators EC50, LOEC, and NOEC. Saouter, Wolff, et al. (2019), used ECHA 2015 data, harmonized 305,068 raw data points to a set of 54,353, focusing on algae, crustaceans, and fish for effect concentration indicators like EC50 chronic, EC50 acute, and NOEC chronic.

In Paper 2, 339,729 harmonized datapoints for 10,668 chemicals were aggregated and then used to calculate the extrapolation factors. The data aggregation occurs at three levels: starting at the species level, followed by the species group level, and finally at the generic level with all organisms as one group using geometric means. The harmonized data aggregation at the species level yielded 79,001 aggregated effect concentration datapoints for 10,668 chemicals. At the species group level, there were 41,303 aggregated datapoints for these chemicals. Finally, at the generic level, there were 23,215 aggregated datapoints for the 10,668 chemicals. Then the aggregated, log10-transformed, harmonized dataset undergoes pairwise comparisons using linear regression analysis to calculate extrapolation factors. Linear regressions were applied in two different ways: one with a free slope and the other with a fixed slope of 1. The strength of the correlation was indicated by the coefficients of determination (R^2). Specifically, the free-slope regression was employed to derive the regression equation. Conversely, the regression with a fixed slope of unity was used to determine the default extrapolation factors. Initially, generic extrapolation factors for EC10 chronic were calculated from three effect concentration indicators: EC50 acute, EC10 acute, and EC50 chronic. Then, species group-specific extrapolation factors were calculated for different species for EC10 chronic from the same three effect concentration indicators: EC50 acute, EC10 acute, and EC50 chronic. Additionally, the study provided the best-fit regression equations for converting to EC10 chronic, enhancing the accuracy of extrapolation across various species and effect concentration indicators. In this study, the extrapolation factor is a multiplier. When applied to the effect concentration indicators to be extrapolated, it converts the value to the extrapolated chronic EC10.

4. RESULTS AND DISCUSSION

4.1. Influence of data harmonization strategies

The ecotoxicity data harmonization framework is the backbone of calculating the EFs, which dominate the CFs of a chemical. Ecotoxicity data varies due to the high diversity in factors affecting the data, ranging from different species, effect concentration indicators, and exposure types to units of measurement. In Paper 1, a detailed harmonization strategy is provided as the baseline, reducing the initial raw data from 5,002 experimental ecotoxicity data points to 1,189 data points, a reduction of 76.2%. This reduction is not due to the unreliability of the data points but because the data was collected for different purposes and lacked the required format for CF calculations. However, the harmonization framework might also exclude data points that could still be useful, leading to unnecessary data loss. In addition, three simplified data harmonization schemes that included more data were tested.

Alternative Data Harmonization Strategy 1 included data points regardless of numeric qualifiers, resulting in a total of 1,336 points for 48 substances. This strategy increased the data points from 23.8% to 26.7% in relation to the raw data points, a 2.9% increase. The calculated EFs were correlated with EFs calculated using the baseline harmonizing strategy, with an R^2 value of 0.94, indicating a strong correlation. This suggests that the removal of unacceptable numeric qualifiers had a low influence on most of the EFs, though this may not always be the case.

Alternative Data Harmonization Strategy 2 assumed all data points were acute and extrapolated them to a chronic equivalent exposure type, resulting in a total of 1,215 points for 47 substances. This strategy increased the data points from 23.8% to 24.3% in relation to the raw data points, a 0.5% increase. The correlation analysis showed an R^2 value of 0.99, indicating a very strong correlation. This suggests that the classification of acute and chronic effects did not notably affect most EFs, as the majority of the data points in the raw dataset are acute.

Alternative Data Harmonization Strategy 3 assumed all effects and effect concentration indicators to be equally sensitive and relevant, disregarding their specific distinctions, resulting in a total of 2,214 points for 47 substances. This strategy increased the data points from 23.8% to 55.7% in relation to the raw data points, a 31.9% increase. The correlation analysis showed an R^2 value of 0.94, indicating a strong correlation. This suggests that considering only sensitive effects and relevant effect concentration indicators does not have a notable influence

on most of the EFs. This result indicates that including all effect concentration indicators and effect types regardless of their sensitivity would not significantly alter the results. Therefore, if time and resources are limited, this step may be excluded in a simplified analysis and only included if detailed analysis or higher accuracy is needed.

4.2. Transformation product inclusion

The TPs identification strategy developed in Paper 1 was used. Using the TP prediction tools, a total of 322 TPs were determined, including 166 predicted with the CTS tool and 156 with enviPath. The identified TPs underwent a persistence test for 386 substances (64 primary chemicals and their 322 TPs), resulting in 78 TPs being equally or more persistent, and 242 being less persistent compared to their primary chemicals. Following the persistence test, the equally or more persistent TPs underwent an ecotoxicity test, resulting in 3 TPs being more toxic and 109 being less toxic compared to their primary chemicals.

In Paper 1, 3 TPs included were difenoconazole-ketone, 1-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-yl) ethenone, and 1-[(2Z)-3-(2-chlorophenyl)-2-(4-fluorophenyl)prop-2-en-1-yl]-1H-1,2,4-triazole. The CF values of these TPs were 9%, 7%, and 13% of their respective primary chemicals. It was reasonably assumed that the CF values of the TPs might be equal to or higher than the primary chemicals, especially since the TPs were selected based on having greater ecotoxicity than the primary chemicals, and EFs, which depend on ecotoxicity, play a dominant role in CFs. However, this was not the case. This result is due to the limitations in the ecotoxicity testing for TPs, which was based on a single species (fathead minnow), whereas the EF calculation aggregated all available data across different species. Using EFs in the ecotoxicity step to identify the most toxic TPs can improve the accuracy of the ecotoxicity step in the simplified TPs selection framework. Paper 1 emphasis the need for further research to refine this approach and develop more reliable tools and guidelines for the inclusion of TPs in CF calculations and LCAs.

4.3. QSAR data inclusion

Experimental data is costly and often scarce, particularly as the number of chemicals in the global marketplace continues to increase. To fill data gaps, many QSAR tools are available. However, the availability of these tools does not necessarily equate to their reliability. The final benefits come from tools that can reliably estimate ecotoxicity within a reasonably good applicability domain.

To test this for the PM chemicals in Paper 1, two QSARs were evaluated by correlating their EFs with experimental data. First, one of the most applicable tools, ECOSAR QSAR from EPI Suite, estimated ecotoxicity data for 33 chemicals, resulting in an R^2 of 0.36 for the calculated EFs. This weak correlation prompted further testing for different chemical groups to classify them at a more specific level. ECOSAR could estimate ecotoxicity for only 2 PFAS, thus no correlation was calculated for this group of chemicals. For the triazines chemical group, the R^2 was 0.14 based on 12 chemicals, indicating a weak correlation. For triazoles, the R^2 was 0.46 for 19 chemicals, suggesting a moderate correlation.

The second QSAR model tested was T.E.S.T., resulting in an R^2 of 0.53 based on 33 chemicals, indicating a moderate correlation but better than ECOSAR. For different chemical groups, the R^2 values were 0.29 for 12 PFAS, 0.62 for 8 triazines, and 0.66 for 13 triazoles.

Combining different QSARs might fill data gaps by covering more chemicals. In Paper 1, a comparison was made between experimental and combined QSAR-based EFs by integrating results from five QSARs with data availability for 45 chemicals. The resulting R^2 was 0.37, indicating a weak correlation. Additionally, the QSAR-based EFs were within two orders of magnitude of the experimental EFs. This result suggests that combining different QSARs may not be an effective approach to fill data gaps, as the uncertainty in the QSARs may be amplified by combining them.

4.4. Effect factor results

One reliable source that is available for the CFs in the form of a database is the USEtox database. In Paper 1, we compared the EFs calculated in Paper 1 to the EFs in the USEtox database. We found that 18 of the chemicals in Paper 1 overlapped with the USEtox database, so the study compared them with the USEtox organic substances database (version 2.01). For the comparison, linear regression analysis was used with R^2 as the indicator of the degree of correlation. The result was an R^2 of 0.63, showing a moderate correlation.

However, given that the CFs matrix equation involves three factors in the calculation of CFs, the study aimed to determine the influence of the other factors, or in general terms, the impact of other physical and chemical properties except for ecotoxicity. The study input the physical and chemical properties for the overlapping 18 chemicals from the USEtox database and used the EFs calculated in the study. The correlation between the initial calculated CFs with USEtox inputs and EFs, compared to the CFs calculated in Paper 1, was $R^2 = 0.99$. This indicates that

the physical and chemical properties used are similar to those in USEtox and that the differences are primarily due to the EF values, which depend on the ecotoxicity values. This observation implies that changes in CFs are predominantly related to differences in ecotoxicity values rather than other input data, aligning with previous findings (Holmquist, 2020; Roos et al., 2017).

The original ecotoxicity factors in USEtox were calculated using ecotoxicity data from two sources: the e-toxBase database from the National Institute for Public Health and the Environment (RIVM) by van Zelm et al. (2009); Zelm et al. (2007); and the ECOTOX and IUCLID databases as referenced by Payet (2004). The USEtox database was thus created using older data and may need updating. In Paper 1, more recent data was used, which may lead to changes. However, these changes will not necessarily increase or decrease the factors uniformly, as new ecotoxicity data can result in either an increase or decrease in factors.

4.5. Characterization factor results

In Paper 1, CFs were calculated for 67 chemicals, including 64 primary chemicals and 3 TPs. The chemicals consisted of 24 PFAS, 17 triazines, and 23 triazoles. The 67 CFs calculated in Paper 1 ranged over almost six orders of magnitude. The main results led to the conclusion that there is no specific relationship between a chemical belonging to a particular chemical class and its CFs. All three chemical classes exhibited diversity across the minimum to maximum range, indicating that chemicals in a particular class do not necessarily have relatively high or low CFs compared to other classes.

Secondly, the findings highlight that at that time available CF databases lack coverage of PM chemicals. Out of the 64 primary chemicals, there was only a 28% overlap (18/64) with the USEtox organic substances database (version 2.01). This includes none of the 24 PFAS, 10 out of 17 triazines, and 8 out of 23 triazoles. The changes in CF values are due to differences in EF values, with temporal differences in the ecotoxicity data. This indicates that the USEtox database has limited coverage of certain chemical groups, especially PFAS.

Another result is that ecotoxicity data sources' lack of experimental data, resulting in only 70% of the CFs (47/67) being calculated using experimental ecotoxicity data for 14 PFAS, 12 triazines, and 21 triazoles. This leaves a gap of 30%. To fill this gap, ecotoxicity data from QSARs were used, providing CFs for an additional 17 chemicals, including 8 PFAS, 5 triazines, 1 triazole, and 3 TPs. However, the QSARs were unable to predict values for two

PFAS and one triazole. Two methods were adopted to estimate their CFs. First, for the PFAS, a simplified regression analysis was developed relating the number of perfluorinated carbons with the calculated CFs to estimate the CFs for the PFAS with unknown CFs. However, the correlation was low due to the diverse nature of PFAS, and the number of perfluorinated carbons was not directly related to ecotoxicity. The correlation might be improved if PFAS were subdivided, but due to the lack of PFAS with experimental CFs, this was not explored. The second method involved averaging the CFs within a chemical group and assigning this average to the chemicals in that group with unknown CFs. In the study, the average of 22 PFAS within the PFAS group was used to fill the data gaps for the two PFAS, and the average CF of the 22 triazoles was used for one triazole.

To understand the relative freshwater ecotoxicity potential of the PM chemicals in Paper 1 compared to all the other chemicals in the USEtox 2.01 organic substances database, the harmonized datasets for PFAS, triazines, and triazoles are ranked against the USEtox 2.01 dataset. The study also examines the influence of being grouped in a particular group of PM chemicals, such as PFAS, triazines, and triazoles, and related ecotoxicity potential range. The plotting of calculated CFs against USEtox CFs (version 2.01) [n=2499] shows the range of CF values for different PM chemical groups as presented in Paper 1. The plots highlight variability and diversity in CF values across different groups, indicating that no particular group consistently has higher or lower CFs. However, all the CFs for the different groups fall within the range of USEtox CF values and vary widely from lower to higher, rather than being concentrated in a narrow range.

4.6. Extrapolation factor results

Paper 2 advances previous research by utilizing a more extensive database of experimental ecotoxicity data, comprising 339,729 data points across 10,668 chemicals. It also implements a modified curation process and delivers extrapolation factors that convert various effect concentration indicators to a chronic EC10, which is recommended for calculating USEtox EFs. Paper 2 calculated three generic and 24 species group-specific extrapolation factors, for two effect concentration indicators and two exposure type. These facilitate the extrapolation of effect concentration indicators (EC10 eq and EC50 eq) to a chronic EC10, thereby enabling more accurate ecotoxicity CF calculations in USEtox.

The first results of the extrapolation factors include the calculation of both generic and species group-specific extrapolation factors. There are two main types of extrapolation factors calculated: Firstly, extrapolation to EC10 chronic from different effect concentration indicators, in line with the recommended USEtox methodology for calculating CFs. Secondly, extrapolation to EC50 chronic from different effect concentration indicators, following the existing USEtox methodology for calculating CFs. The values of the default extrapolation factor, along with the extrapolation equations, are provided in Paper 2 and its supplementary information. It is recommended to use extrapolation factors equations instead of the default factors for accuracy and reliability.

There are two important aspects related to the calculated extrapolation factors. Firstly, due to a lack of data for certain species groups, such as aquatic plants, fungi, moss, hornworts, and reptiles, extrapolation factors were not calculated for these groups. It is recommended that users either use generic extrapolation factors or judgment to apply extrapolation factors from closely related species groups. Secondly, the overall reliability was not high, with R^2 values less than 0.80. However, there were also differences based on the exposure type, with weaker correlations observed for acute effect extrapolation compared to chronic effect extrapolation. This suggests that extrapolation within the same exposure type is more reliable than across different exposure types.

The second part of the results involves comparing generic and species group-specific extrapolation factors. Studies often use generic extrapolation factors instead of species group-specific ones, which may lead to uncertainty. In this thesis, the level of uncertainty is assessed by comparing generic extrapolation factors to different species group-specific extrapolation factors for different effect concentration indicators. Figure 4 and Table 1 shows the differences in default extrapolation factors for different species groups compared to generic extrapolation factors for converting EC10 acute, EC50 acute and EC50 chronic effect concentration indicators to EC10 chronic. The comparison indicates that species group-specific extrapolation factors may provide different results, so it is recommended to use them if available instead of diluting the extrapolation using generic factors. However, the difference also varies between species group, effect concentration indicators, exposure duration, and the number of data points considered in the calculation of the extrapolation factor. For example, converting EC10 acute data for algae using the generic factor would underestimate EC10 chronic by a factor of 2.6.

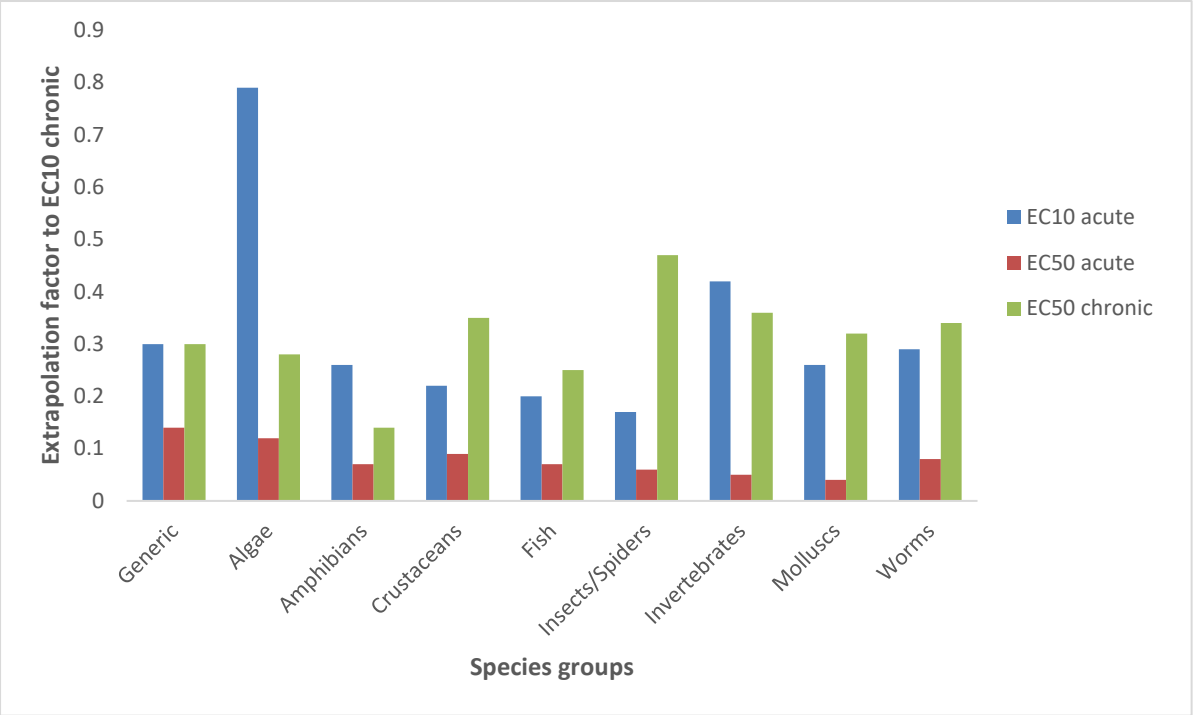

Figure 4: Generic and species group specific extrapolation factors to convert EC10 acute, EC50 acute and EC50 chronic effect concentration indicators to EC10 chronic

The third part of the results is the comparison of the generic and species group-specific extrapolation factors calculated in Paper 2 with other available literature. Only Aurisano et al. (2019) and Saouter, Wolff, et al. (2019) provide species group-specific extrapolation factors. While the factors were not vastly different, they were distinct enough to be notable. The species groups specific extrapolation factors differ maximum with a factor of 4 for algae, 2.6 for crustaceans, 2.6 for fish, and 6 for Invertebrates and the default generic extrapolation factors available for EC50eq acute to EC10eq chronic are within a factor of 4.2 (0.25/0.06). These differences can be attributed to several factors: Firstly, how the ecotoxicity data is aggregated. Different studies use different aggregation methods to combine data points. Aurisano et al. (2019) used arithmetic means, whereas Saouter, Wolff, et al. (2019) used geometric means. Paper 2 also used geometric means, as recommended by REACH and also used in the USEtox methodology. Secondly, which extrapolation factor calculation methodology was used. Aurisano et al. (2019) and Payet (2004) relied on regression analyses, while Saouter, Wolff, et al. (2019) used the geometric mean of the ratios of the compared effect concentration indicators. In Paper 2, we employed regression analyses with a free slope for best-fit regression equations and a slope with unity for default factors. Thirdly, how effect concentration indicators are classified. The classification of effect concentration indicators in Paper 2 was different from previous studies. We combined EC10 and NOEC to EC10eq based on literature

suggesting that the biological effects observed at concentrations reported as NOECs typically range from 10 to 30% (Crane & Newman, 2000; Moore & Caux, 1997; US EPA, 1991). This approach aligns with risk assessment-based regulations such as the European Union's REACH regulation, the plant protection product regulation, and the Water Framework Directive, which use NOEC and EC10 interchangeably (ECHA, 2008; EFSA, 2013; European Commission, 2011).

Table 1: Overview of statistical parameters in calculation of generic and species group specific extrapolation factors to convert EC10 acute, EC50 acute and EC50 chronic effect concentration indicators to EC10 chronic

Extrapolation to EC10 chronic from different effect concentration indicators	Species group	Default extrapolation factor	Datapoints	Intercept	Intercept Lower 95%	Intercept Upper 95%	Slope	Slope Lower 95%	Slope Upper 95%	Correlation (r)	Rsquare (R2)	Intercept* (Slope = 1)	Intercept* Lower 95%	Intercept* Upper 95%
EC10 acute	Generic	0.30	3192	-0.37	-0.40	-0.33	0.76	0.73	0.78	0.74	0.55	-0.52	-0.55	-0.48
EC50 acute	Generic	0.14	3679	-0.64	-0.68	-0.61	0.76	0.74	0.79	0.71	0.51	-0.86	-0.89	-0.83
EC50 chronic	Generic	0.30	3543	-0.42	-0.44	-0.39	0.82	0.80	0.83	0.84	0.70	-0.53	-0.55	-0.50
EC10 acute	Algae	0.79	342	-0.07	-0.17	0.04	0.66	0.60	0.72	0.77	0.59	-0.10	-0.22	0.02
EC50 acute	Algae	0.12	425	-0.53	-0.64	-0.42	0.53	0.47	0.59	0.63	0.40	-0.93	-1.05	-0.81
EC50 chronic	Algae	0.28	2937	-0.47	-0.50	-0.44	0.90	0.88	0.92	0.88	0.77	-0.55	-0.57	-0.52
EC10 acute	Amphibians	0.26	154	-0.56	-0.74	-0.37	0.67	0.55	0.79	0.66	0.44	-0.58	-0.78	-0.38
EC50 acute	Amphibians	0.07	143	-0.74	-0.97	-0.52	0.45	0.32	0.58	0.49	0.24	-1.16	-1.40	-0.92
EC50 chronic	Amphibians	0.14	59	-0.77	-1.02	-0.52	0.83	0.65	1.00	0.78	0.61	-0.87	-1.10	-0.63
EC10 acute	Crustaceans	0.22	1133	-0.61	-0.66	-0.55	0.76	0.73	0.80	0.80	0.64	-0.66	-0.72	-0.60
EC50 acute	Crustaceans	0.09	1514	-0.93	-0.98	-0.88	0.81	0.78	0.84	0.79	0.63	-1.04	-1.09	-0.99
EC50 chronic	Crustaceans	0.35	859	-0.44	-0.49	-0.40	0.87	0.84	0.90	0.89	0.79	-0.45	-0.50	-0.41
EC10 acute	Fish	0.20	1119	-0.64	-0.71	-0.58	0.77	0.73	0.81	0.73	0.54	-0.69	-0.76	-0.62
EC50 acute	Fish	0.07	1205	-1.04	-1.10	-0.98	0.84	0.80	0.88	0.77	0.59	-1.16	-1.22	-1.11
EC50 chronic	Fish	0.25	443	-0.55	-0.63	-0.47	0.75	0.69	0.80	0.79	0.63	-0.61	-0.69	-0.52
EC10 acute	Insects/Spiders	0.17	167	-0.84	-1.00	-0.68	0.60	0.52	0.69	0.74	0.55	-0.77	-0.97	-0.57
EC50 acute	Insects/Spiders	0.06	224	-1.10	-1.26	-0.94	0.57	0.48	0.66	0.66	0.43	-1.19	-1.38	-1.00
EC50 chronic	Insects/Spiders	0.47	107	-0.60	-0.79	-0.40	0.71	0.62	0.80	0.83	0.70	-0.33	-0.53	-0.12
EC10 acute	Invertebrates	0.42	152	-0.46	-0.64	-0.27	0.80	0.67	0.92	0.70	0.49	-0.38	-0.56	-0.20
EC50 acute	Invertebrates	0.05	119	-1.13	-1.37	-0.90	0.59	0.42	0.77	0.53	0.28	-1.27	-1.52	-1.02
EC50 chronic	Invertebrates	0.36	55	-0.54	-0.75	-0.33	0.63	0.47	0.79	0.73	0.54	-0.44	-0.69	-0.20
EC10 acute	Molluscs	0.26	253	-0.75	-0.90	-0.60	0.60	0.51	0.69	0.63	0.40	-0.58	-0.75	-0.42
EC50 acute	Molluscs	0.04	221	-1.27	-1.42	-1.11	0.68	0.57	0.79	0.64	0.40	-1.41	-1.56	-1.25
EC50 chronic	Molluscs	0.32	107	-0.60	-0.74	-0.45	0.80	0.69	0.92	0.80	0.65	-0.50	-0.64	-0.35
EC10 acute	Worms	0.29	120	-0.39	-0.59	-0.18	0.61	0.48	0.75	0.64	0.40	-0.54	-0.76	-0.32
EC50 acute	Worms	0.08	98	-0.69	-1.03	-0.35	0.52	0.30	0.74	0.43	0.18	-1.09	-1.40	-0.77
EC50 chronic	Worms	0.34	55	-0.45	-0.62	-0.29	0.93	0.78	1.08	0.86	0.75	-0.47	-0.63	-0.31

Note:* statistical parameters with slope as unity

The last part of the results focuses on understanding the influence of different choices in the calculation of the extrapolation factors including extrapolation factors calculated for different chemical groups. Similar to species group-specific extrapolation factors, chemical group-specific factors may be more accurate than generic factors. However, this approach has limitations: Firstly, a lack of data availability can make the extrapolation factor calculation less

robust. Secondly, some chemical groups, like PFAS, are very diverse and very large - which is a reason for the huge variations and may need further sub-grouping for more comprehensive results. An attempt was made to calculate generic extrapolation factors to EC50eq chronic for 15 chemical groups with median values, 0.99 from EC10 acute, 3.48 from EC10 chronic, and 0.42 from EC50 acute. These values correspond to generic values of 0.99, 3.51, and 0.45 calculated for all organic chemicals in the freshwater ecosystem in Paper 2. For extrapolation to EC10eq chronic as shown in Figure 5 and Table 2, the median values of the extrapolation factors were 0.30 from EC10 acute, 0.11 from EC50 acute, and 0.29 from EC50 chronic, corresponding to generic values of 0.29, 0.13, and 0.29 calculated for all organic chemicals in the freshwater ecosystem, respectively. These results indicate that while generic extrapolation factors can provide a baseline, chemical group-specific extrapolation factors may offer more accuracy and should be used when available to reduce uncertainty. However, the differences will not vary significantly for many chemical groups at generic level, as shown in Figure 5, except for groups that are diverse, such as PFAS.

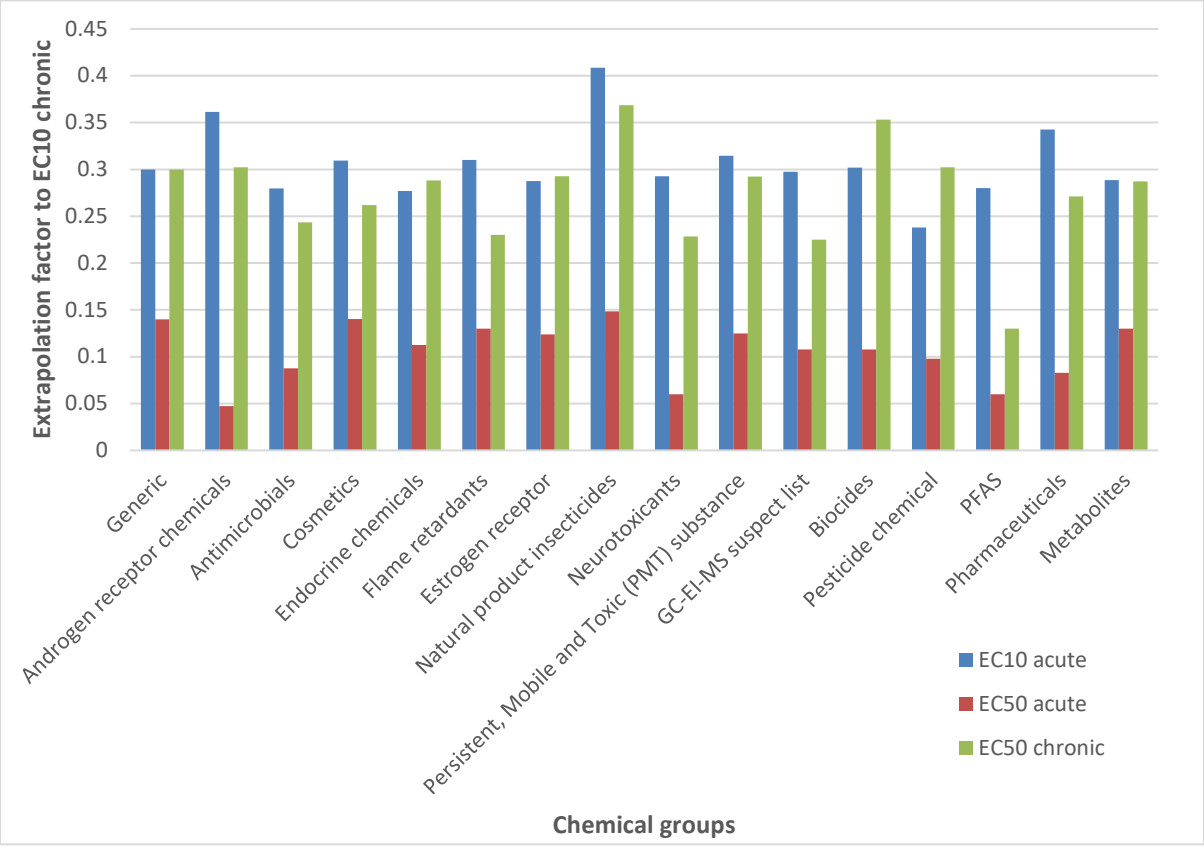

Figure 5: Default generic extrapolation factors for different chemical groups to convert EC10 acute, EC50 acute and EC50 chronic effect concentration indicators to EC10 chronic

Table 2: Overview of statistical parameters in calculation of default generic extrapolation factors for different chemical groups to convert EC10 acute, EC50 acute and EC50 chronic effect concentration indicators to EC10 chronic

Extrapolation to EC10 chronic from different effect concentration indicators	Chemical groups	Default extrapolation factor	Datapoints	Intercept	Intercept Lower 95%	Intercept Upper 95%	Slope	Slope Lower 95%	Slope Upper 95%	Correlation (r)	Rsquare (R^2)	Intercept* (Slope =1)	Intercept* Lower 95%	Intercept* Upper 95%
EC10 acute	Generic	0.30	3192	-0.37	-0.40	-0.33	0.76	0.73	0.78	0.74	0.55	-0.52	-0.55	-0.48
EC50 acute		0.14	3679	-0.64	-0.68	-0.61	0.76	0.74	0.79	0.71	0.51	-0.86	-0.89	-0.83
EC50 chronic		0.30	3543	-0.42	-0.44	-0.39	0.82	0.80	0.83	0.84	0.70	-0.53	-0.55	-0.50
EC10 acute	Androgen Receptor Chemicals	0.36	71	-0.66	-0.88	-0.43	0.68	0.51	0.85	0.69	0.48	-0.44	-0.65	-0.23
EC50 acute		0.05	63	-1.28	-1.50	-1.05	0.85	0.61	1.09	0.67	0.45	-1.33	-1.54	-1.11
EC50 chronic		0.30	50	-0.61	-0.81	-0.42	0.80	0.64	0.97	0.82	0.67	-0.52	-0.70	-0.33
EC10 acute	Antimicrobials	0.28	42	-0.51	-0.79	-0.23	0.92	0.73	1.12	0.83	0.69	-0.55	-0.81	-0.30
EC50 acute		0.09	43	-0.94	-1.23	-0.66	0.89	0.73	1.06	0.86	0.75	-1.06	-1.29	-0.83
EC50 chronic		0.24	34	-0.55	-0.72	-0.37	0.90	0.78	1.01	0.94	0.89	-0.61	-0.78	-0.45
EC10 acute	Cosmetics	0.31	454	-0.24	-0.35	-0.13	0.76	0.70	0.82	0.74	0.54	-0.51	-0.60	-0.42
EC50 acute		0.14	509	-0.55	-0.66	-0.43	0.77	0.71	0.84	0.73	0.54	-0.85	-0.93	-0.77
EC50 chronic		0.26	438	-0.39	-0.47	-0.31	0.82	0.77	0.87	0.85	0.73	-0.58	-0.65	-0.52
EC10 acute	Endocrine chemicals	0.28	1416	-0.41	-0.46	-0.35	0.73	0.70	0.77	0.74	0.54	-0.56	-0.61	-0.50
EC50 acute		0.11	1524	-0.73	-0.79	-0.67	0.77	0.73	0.81	0.73	0.53	-0.95	-1.00	-0.90
EC50 chronic		0.29	1376	-0.43	-0.47	-0.39	0.80	0.77	0.83	0.84	0.70	-0.54	-0.58	-0.50
EC10 acute	Flame Retardants	0.31	51	-0.45	-0.69	-0.20	0.87	0.70	1.05	0.82	0.67	-0.51	-0.74	-0.28
EC50 acute		0.13	43	-0.51	-0.85	-0.18	0.62	0.42	0.82	0.70	0.49	-0.87	-1.19	-0.55
EC50 chronic		0.23	36	-0.51	-0.78	-0.24	0.83	0.64	1.02	0.84	0.71	-0.63	-0.87	-0.39
EC10 acute	Estrogen Receptor	0.29	1683	-0.38	-0.43	-0.32	0.73	0.70	0.77	0.73	0.53	-0.54	-0.59	-0.49
EC50 acute		0.12	1801	-0.66	-0.72	-0.61	0.75	0.71	0.78	0.71	0.50	-0.91	-0.95	-0.86
EC50 chronic		0.29	1717	-0.42	-0.46	-0.39	0.83	0.81	0.85	0.85	0.73	-0.53	-0.57	-0.50
EC10 acute	Natural Product Insecticides	0.41	41	-0.59	-0.86	-0.32	0.73	0.54	0.93	0.77	0.60	-0.39	-0.64	-0.14
EC50 acute		0.15	44	-0.87	-1.10	-0.64	0.90	0.69	1.10	0.80	0.65	-0.83	-1.05	-0.61
EC50 chronic		0.37	39	-0.75	-1.09	-0.41	0.66	0.43	0.88	0.69	0.48	-0.43	-0.73	-0.14
EC10 acute	Neurotoxicants	0.29	134	-0.43	-0.59	-0.27	0.79	0.69	0.90	0.81	0.65	-0.53	-0.69	-0.37
EC50 acute		0.06	126	-1.06	-1.26	-0.85	0.86	0.74	0.97	0.79	0.63	-1.22	-1.38	-1.06
EC50 chronic		0.23	108	-0.53	-0.70	-0.35	0.81	0.71	0.92	0.83	0.69	-0.64	-0.81	-0.48
EC10 acute	Persistent, Mobile and Toxic (PMT) Substance	0.31	304	-0.30	-0.43	-0.17	0.81	0.73	0.90	0.74	0.55	-0.50	-0.60	-0.40
EC50 acute		0.12	334	-0.70	-0.85	-0.55	0.85	0.77	0.94	0.74	0.55	-0.90	-1.00	-0.81
EC50 chronic		0.29	309	-0.40	-0.49	-0.31	0.86	0.81	0.92	0.86	0.75	-0.53	-0.61	-0.46
EC10 acute	GC-EI-MS suspect list	0.30	518	-0.37	-0.46	-0.29	0.76	0.71	0.82	0.76	0.58	-0.53	-0.61	-0.44
EC50 acute		0.11	575	-0.73	-0.83	-0.63	0.80	0.74	0.85	0.75	0.56	-0.97	-1.05	-0.89
EC50 chronic		0.22	475	-0.50	-0.57	-0.42	0.80	0.75	0.85	0.83	0.68	-0.65	-0.72	-0.58
EC10 acute	Biocides	0.30	88	-0.62	-0.82	-0.42	0.79	0.66	0.92	0.79	0.62	-0.52	-0.72	-0.32
EC50 acute		0.11	89	-0.97	-1.16	-0.78	0.81	0.67	0.95	0.77	0.60	-0.97	-1.16	-0.77
EC50 chronic		0.35	77	-0.54	-0.71	-0.37	0.84	0.73	0.95	0.87	0.75	-0.45	-0.62	-0.28
EC10 acute	Pesticide Chemical	0.24	755	-0.55	-0.62	-0.48	0.70	0.65	0.74	0.71	0.50	-0.62	-0.70	-0.54
EC50 acute		0.10	784	-0.85	-0.93	-0.77	0.74	0.70	0.79	0.73	0.53	-1.01	-1.08	-0.94
EC50 chronic		0.30	686	-0.49	-0.55	-0.42	0.77	0.73	0.81	0.80	0.64	-0.52	-0.59	-0.45
EC10 acute	PFAS	0.28	66	-0.27	-0.55	0.01	0.41	0.25	0.58	0.53	0.28	-0.56	-0.91	-0.20
EC50 acute		0.06	47	-0.83	-1.26	-0.40	0.70	0.46	0.95	0.66	0.43	-1.21	-1.52	-0.89
EC50 chronic		0.13	41	-0.57	-0.87	-0.27	0.72	0.55	0.89	0.81	0.66	-0.89	-1.15	-0.63
EC10 acute	Pharmaceuticals	0.34	321	-0.36	-0.48	-0.24	0.81	0.74	0.88	0.78	0.61	-0.47	-0.58	-0.35
EC50 acute		0.08	319	-0.86	-1.02	-0.71	0.83	0.75	0.91	0.74	0.54	-1.08	-1.20	-0.96
EC50 chronic		0.27	264	-0.41	-0.52	-0.31	0.81	0.75	0.87	0.85	0.73	-0.57	-0.67	-0.47
EC10 acute	Metabolites	0.29	1534	-0.41	-0.46	-0.36	0.74	0.71	0.78	0.73	0.53	-0.54	-0.59	-0.49
EC50 acute		0.13	1643	-0.68	-0.74	-0.63	0.75	0.72	0.79	0.71	0.50	-0.89	-0.94	-0.84
EC50 chronic		0.29	1619	-0.46	-0.49	-0.42	0.84	0.82	0.87	0.86	0.74	-0.54	-0.58	-0.51

Note:* statistical parameters with slope as unity

In this study the geometric mean was chosen for the aggregation of data points. To understand the influence of using geometric mean over arithmetic mean, Paper 2 compared both aggregation methods. Additionally, the impacts of classifying chemicals into different types (organic and inorganic versus only organic chemicals) and classifying compartments (freshwater, marine, or both freshwater and marine as aquatic) were examined. Effect concentration indicators classification into only EC10 and EC50 versus NOEC, EC10, and EC50 was also considered. Table 3 provides all the scenarios considered in Paper 2 to determine the uncertainty in different choices in the calculation of the extrapolation factors.

Table 3: Scenarios considered in the study for calculating extrapolation factors

Scenario classification	Chemical type	Compartment classification	Aggregation method
Scenario 1	Organic and Inorganic	Aquatic (Freshwater & Marine)	Geometric mean
Scenario 2	Only Organic	Aquatic (Freshwater & Marine)	Geometric mean
Scenario 3	Organic and Inorganic	Aquatic (Freshwater & Marine)	Arithmetic mean
Scenario 4	Only Organic	Aquatic (Freshwater & Marine)	Arithmetic mean
Scenario 5	Organic and Inorganic	Freshwater	Geometric mean
Scenario 6	Only Organic	Freshwater	Geometric mean
Scenario 7	Organic and Inorganic	Freshwater	Arithmetic mean
Scenario 8	Only Organic	Freshwater	Arithmetic mean
Scenario 9	Organic and Inorganic	Marine water	Geometric mean
Scenario 10	Only Organic	Marine water	Geometric mean
Scenario 11	Organic and Inorganic	Marine water	Arithmetic mean
Scenario 12	Only Organic	Marine water	Arithmetic mean

In Figure 6 and Table 4, the extrapolation factors are given for different scenarios as outlined in Table 3 for the effect concentration indicators classification of EC10 and EC50. Figure 7 and Table 5 also shows extrapolation factors calculated for different scenarios, for effect concentration indicators classification into NOEC, EC10, and EC50. The reliability of the extrapolation factors in marine water is lower due to the smaller number of data points available. Scenarios reflect the various combinations of chemical type classifications, compartment classifications, effect concentration indicator classifications, and aggregation methods considered in the study to calculate extrapolation factors. This approach helps to assess the influence of different factors on the reliability and accuracy of extrapolation factors calculated. In the analysis, as shown in Figure 6, the extrapolation from EC50 chronic to EC10 chronic with geometric mean-based aggregation differs significantly compared to arithmetic mean-based aggregation. This discrepancy is mainly due to the EC10 acute equivalent, which combines both NOEC acute and EC10 acute. In Figure 7, it is shown that extrapolation from EC10 acute to EC10 chronic is higher, but NOEC acute to EC10 chronic is lower in arithmetic mean-based aggregation. By combining both effect concentration indicators, the differences

balance out, making the results similar to geometric mean-based aggregation. This may explain why there is a difference based on how effect concentration indicators are combined in the EC10 acute which is not in EC50 chronic.

Table 4: Overview of statistical parameters in calculation of default generic extrapolation factors for different scenarios with the effect concentration indicators classification limited to EC10 and EC50 to convert EC10 acute, EC50 acute and EC50 chronic effect concentration indicators to EC10 chronic

Extrapolation to EC10 chronic from different effect concentration indicators	Different scenarios	Default extrapolation factor	Datapoints	Intercept	Intercept Lower 95%	Intercept Upper 95%	Slope	Slope Lower 95%	Slope Upper 95%	Correlation (r)	Rsquare (R^2)	Intercept* (Slope =1)	Intercept* Lower 95%	Intercept* Upper 95%
EC10 acute	Scenario 1	0.30	3192	-0.37	-0.40	-0.33	0.76	0.73	0.78	0.74	0.55	-0.52	-0.55	-0.48
EC50 acute		0.14	3679	-0.64	-0.68	-0.61	0.76	0.74	0.79	0.71	0.51	-0.86	-0.89	-0.83
EC50 chronic		0.30	3543	-0.42	-0.44	-0.39	0.82	0.80	0.83	0.84	0.70	-0.53	-0.55	-0.50
EC10 acute	Scenario 2	0.31	2608	-0.37	-0.41	-0.33	0.75	0.72	0.77	0.74	0.55	-0.51	-0.55	-0.47
EC50 acute		0.13	2965	-0.65	-0.69	-0.61	0.75	0.72	0.77	0.71	0.50	-0.87	-0.91	-0.84
EC50 chronic		0.29	2829	-0.43	-0.46	-0.40	0.81	0.79	0.83	0.83	0.69	-0.54	-0.57	-0.51
EC10 acute	Scenario 3	0.31	3192	-0.18	-0.23	-0.13	0.68	0.65	0.70	0.66	0.44	-0.52	-0.55	-0.48
EC50 acute		0.14	3679	-0.41	-0.45	-0.36	0.66	0.63	0.68	0.63	0.39	-0.84	-0.88	-0.81
EC50 chronic		0.40	3543	-0.19	-0.22	-0.16	0.76	0.74	0.78	0.78	0.61	-0.39	-0.42	-0.37
EC10 acute	Scenario 4	0.31	2608	-0.18	-0.23	-0.13	0.66	0.63	0.68	0.65	0.43	-0.51	-0.56	-0.47
EC50 acute		0.14	2965	-0.39	-0.44	-0.34	0.63	0.60	0.66	0.61	0.38	-0.86	-0.90	-0.81
EC50 chronic		0.40	2829	-0.20	-0.23	-0.16	0.75	0.73	0.78	0.77	0.59	-0.40	-0.43	-0.37
EC10 acute	Scenario 5	0.29	2698	-0.38	-0.42	-0.34	0.74	0.71	0.76	0.72	0.52	-0.54	-0.58	-0.50
EC50 acute		0.13	2977	-0.65	-0.69	-0.60	0.76	0.73	0.79	0.69	0.48	-0.87	-0.91	-0.83
EC50 chronic		0.30	2960	-0.43	-0.46	-0.41	0.85	0.83	0.87	0.86	0.74	-0.53	-0.55	-0.50
EC10 acute	Scenario 6	0.29	2196	-0.38	-0.42	-0.33	0.73	0.70	0.76	0.73	0.53	-0.53	-0.58	-0.49
EC50 acute		0.13	2368	-0.65	-0.70	-0.60	0.74	0.71	0.77	0.69	0.48	-0.89	-0.93	-0.84
EC50 chronic		0.29	2340	-0.45	-0.48	-0.41	0.84	0.82	0.86	0.86	0.73	-0.54	-0.57	-0.52
EC10 acute	Scenario 7	0.31	2698	-0.20	-0.25	-0.15	0.68	0.65	0.71	0.65	0.43	-0.51	-0.56	-0.47
EC50 acute		0.14	2977	-0.43	-0.49	-0.38	0.68	0.65	0.71	0.62	0.38	-0.84	-0.88	-0.80
EC50 chronic		0.39	2960	-0.24	-0.27	-0.21	0.80	0.78	0.82	0.79	0.63	-0.41	-0.44	-0.38
EC10 acute	Scenario 8	0.31	2196	-0.20	-0.25	-0.14	0.67	0.63	0.70	0.66	0.43	-0.51	-0.56	-0.46
EC50 acute		0.14	2368	-0.42	-0.48	-0.36	0.66	0.62	0.69	0.61	0.38	-0.85	-0.89	-0.80
EC50 chronic		0.37	2340	-0.27	-0.30	-0.23	0.81	0.78	0.83	0.80	0.64	-0.43	-0.46	-0.40
EC10 acute	Scenario 9	0.23	599	-0.66	-0.76	-0.56	0.75	0.69	0.82	0.69	0.48	-0.63	-0.74	-0.53
EC50 acute		0.09	584	-0.94	-1.05	-0.83	0.75	0.68	0.82	0.66	0.44	-1.04	-1.14	-0.93
EC50 chronic		0.23	461	-0.64	-0.73	-0.56	0.82	0.77	0.88	0.81	0.66	-0.65	-0.73	-0.56
EC10 acute	Scenario 10	0.21	466	-0.74	-0.85	-0.62	0.73	0.66	0.81	0.67	0.44	-0.67	-0.80	-0.55
EC50 acute		0.07	445	-1.05	-1.17	-0.92	0.75	0.67	0.82	0.66	0.44	-1.13	-1.26	-1.00
EC50 chronic		0.20	343	-0.72	-0.82	-0.62	0.80	0.74	0.86	0.80	0.65	-0.69	-0.80	-0.58
EC10 acute	Scenario 11	0.19	599	-0.56	-0.68	-0.45	0.63	0.56	0.70	0.57	0.33	-0.73	-0.85	-0.61
EC50 acute		0.08	584	-0.75	-0.88	-0.61	0.61	0.53	0.68	0.53	0.28	-1.09	-1.22	-0.97
EC50 chronic		0.36	461	-0.37	-0.47	-0.27	0.75	0.69	0.81	0.74	0.55	-0.44	-0.54	-0.34
EC10 acute	Scenario 12	0.15	466	-0.68	-0.81	-0.54	0.59	0.50	0.67	0.53	0.28	-0.81	-0.96	-0.67
EC50 acute		0.06	445	-0.88	-1.03	-0.72	0.59	0.49	0.68	0.51	0.26	-1.21	-1.36	-1.06
EC50 chronic		0.34	343	-0.45	-0.57	-0.34	0.73	0.66	0.80	0.73	0.54	-0.47	-0.60	-0.35

Note:* statistical parameters with slope as unity

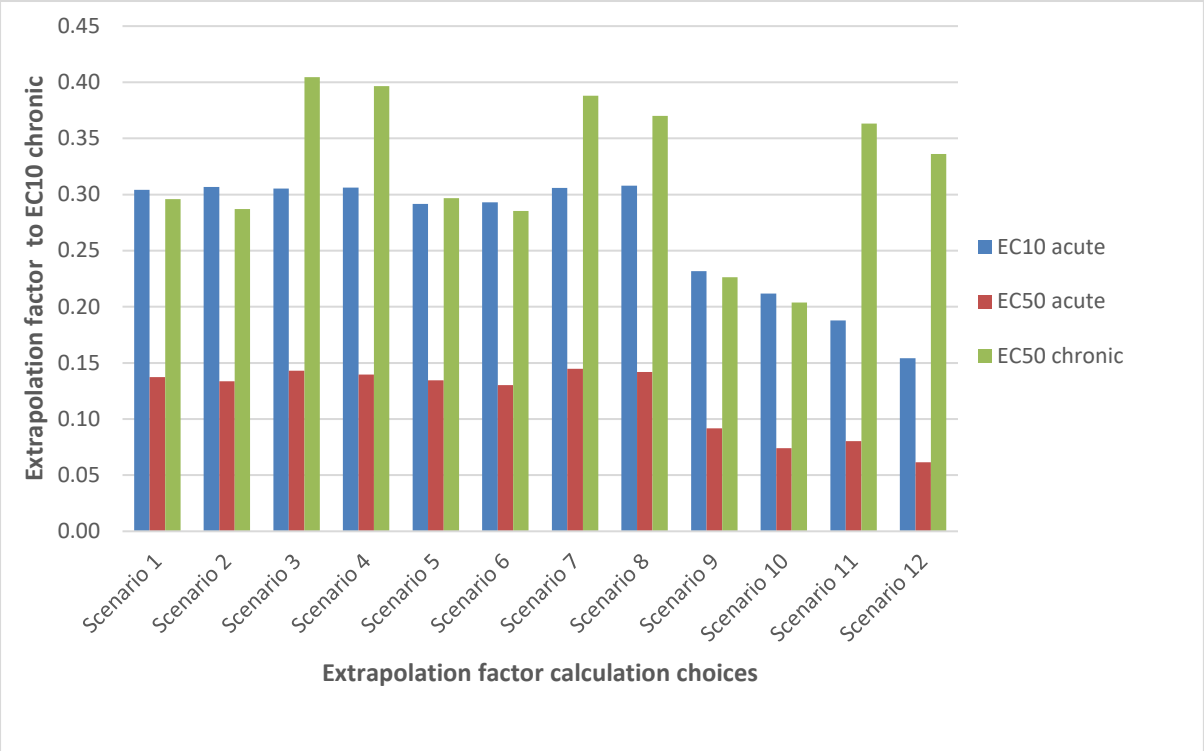

Figure 6: Default generic extrapolation factors for different scenarios with the effect concentration indicators classification limited to EC10 and EC50 to convert EC10 acute, EC50 acute and EC50 chronic effect concentration indicators to EC10 chronic

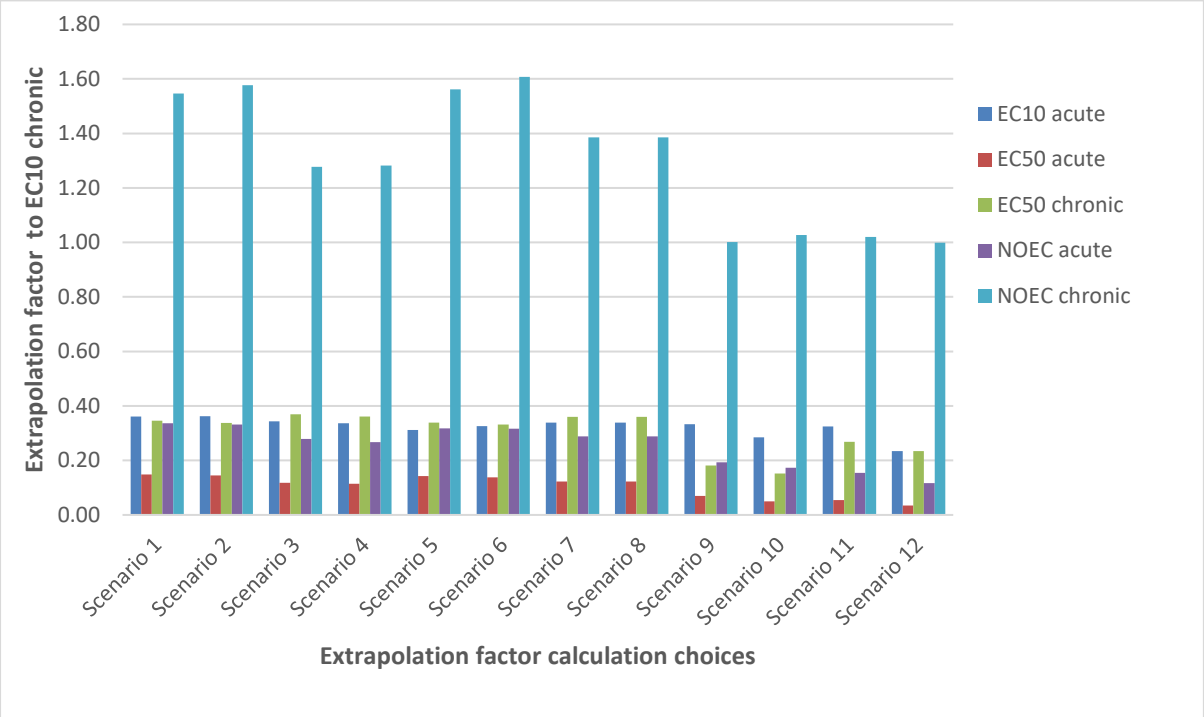

Figure 7: Default generic extrapolation factors for different scenarios with the effect concentration indicators classification limited to NOEC, EC10, and EC50, to convert EC10 acute, EC50 acute, EC50 chronic, NOEC acute, and NOEC chronic effect concentration indicators to EC10 chronic

Table 5: Overview of statistical parameters in calculation of default generic extrapolation factors for different scenarios with the effect concentration indicators classification limited to NOEC, EC10, and EC50, to convert EC10 acute, EC50 acute, EC50 chronic, NOEC acute, and NOEC chronic effect concentration indicators to EC10 chronic

Extrapolation to EC10 chronic from different effect concentration indicators	Different scenarios	Default extrapolation factor	Datapoints	Intercept	Slope	Correlation (r)	Rsquare (R^2)	Intercept* (Slope = 1)
EC10 acute	Scenario 1	0.36	1452	-0.38	0.70	0.71	0.51	-0.44
EC50 acute		0.15	2666	-0.65	0.79	0.70	0.49	-0.83
EC50 chronic		0.35	2650	-0.38	0.85	0.82	0.67	-0.46
NOEC acute		0.34	2254	-0.37	0.77	0.72	0.52	-0.47
NOEC chronic		1.55	2688	0.17	0.93	0.88	0.77	0.19
EC10 acute	Scenario 2	0.36	1156	-0.40	0.68	0.70	0.49	-0.44
EC50 acute		0.14	2171	-0.65	0.78	0.69	0.48	-0.84
EC50 chronic		0.34	2147	-0.38	0.84	0.81	0.65	-0.47
NOEC acute		0.33	1841	-0.38	0.77	0.72	0.52	-0.48
NOEC chronic		1.58	2128	0.18	0.93	0.87	0.75	0.20
EC10 acute	Scenario 3	0.34	1452	-0.19	0.63	0.63	0.40	-0.46
EC50 acute		0.12	2666	-0.51	0.68	0.61	0.37	-0.93
EC50 chronic		0.37	2650	-0.27	0.81	0.77	0.59	-0.43
NOEC acute		0.28	2254	-0.26	0.68	0.64	0.41	-0.55
NOEC chronic		1.28	2688	0.14	0.86	0.82	0.67	0.11
EC10 acute	Scenario 4	0.34	1156	-0.21	0.60	0.61	0.37	-0.47
EC50 acute		0.11	2171	-0.50	0.65	0.59	0.35	-0.94
EC50 chronic		0.36	2147	-0.27	0.80	0.75	0.57	-0.44
NOEC acute		0.27	1841	-0.28	0.66	0.62	0.38	-0.57
NOEC chronic		1.28	2128	0.13	0.85	0.80	0.64	0.11
EC10 acute	Scenario 5	0.31	1221	-0.43	0.68	0.70	0.49	-0.51
EC50 acute		0.14	2286	-0.67	0.80	0.69	0.48	-0.85
EC50 chronic		0.34	2296	-0.39	0.86	0.84	0.70	-0.47
NOEC acute		0.32	1940	-0.38	0.76	0.71	0.50	-0.50
NOEC chronic		1.56	2337	0.18	0.93	0.88	0.77	0.19
EC10 acute	Scenario 6	0.33	955	-0.43	0.66	0.69	0.47	-0.49
EC50 acute		0.14	1840	-0.66	0.78	0.68	0.47	-0.86
EC50 chronic		0.33	1844	-0.39	0.86	0.83	0.69	-0.48
NOEC acute		0.32	1571	-0.39	0.76	0.71	0.51	-0.50
NOEC chronic		1.61	1839	0.19	0.93	0.87	0.75	0.21
EC10 acute	Scenario 7	0.34	1221	-0.22	0.64	0.63	0.39	-0.47
EC50 acute		0.12	2286	-0.56	0.72	0.62	0.39	-0.91
EC50 chronic		0.36	2296	-0.30	0.82	0.78	0.62	-0.44
NOEC acute		0.29	1940	-0.27	0.69	0.64	0.41	-0.54
NOEC chronic		1.39	2337	0.16	0.86	0.83	0.69	0.14
EC10 acute	Scenario 8	0.34	1221	-0.22	0.64	0.63	0.39	-0.47
EC50 acute		0.12	2286	-0.56	0.72	0.62	0.39	-0.91
EC50 chronic		0.36	2296	-0.30	0.82	0.78	0.62	-0.44
NOEC acute		0.29	1940	-0.27	0.69	0.64	0.41	-0.54
NOEC chronic		1.39	2337	0.16	0.86	0.83	0.69	0.14
EC10 acute	Scenario 9	0.33	331	-0.59	0.76	0.69	0.48	-0.48
EC50 acute		0.07	416	-1.11	0.81	0.67	0.45	-1.15
EC50 chronic		0.18	338	-0.75	0.82	0.75	0.56	-0.74
NOEC acute		0.19	403	-0.79	0.79	0.71	0.50	-0.72
NOEC chronic		1.00	501	-0.11	0.87	0.88	0.77	0.00
EC10 acute	Scenario 10	0.28	233	-0.73	0.74	0.68	0.47	-0.55
EC50 acute		0.05	300	-1.28	0.80	0.68	0.46	-1.30
EC50 chronic		0.15	236	-0.87	0.78	0.73	0.53	-0.82
NOEC acute		0.17	292	-0.90	0.77	0.68	0.46	-0.76
NOEC chronic		1.03	355	-0.13	0.88	0.88	0.77	0.01
EC10 acute	Scenario 11	0.32	331	-0.46	0.58	0.53	0.28	-0.49
EC50 acute		0.05	416	-0.97	0.63	0.52	0.27	-1.26
EC50 chronic		0.27	338	-0.48	0.70	0.64	0.41	-0.57
NOEC acute		0.15	403	-0.75	0.71	0.62	0.38	-0.81
NOEC chronic		1.02	501	-0.08	0.81	0.82	0.67	0.01
EC10 acute	Scenario 12	0.23	233	-0.68	0.52	0.48	0.23	-0.63
EC50 acute		0.03	300	-1.20	0.62	0.51	0.26	-1.46
EC50 chronic		0.23	236	-0.61	0.68	0.62	0.39	-0.63
NOEC acute		0.12	292	-0.93	0.68	0.58	0.34	-0.93
NOEC chronic		1.00	355	-0.14	0.81	0.81	0.66	0.00

Note:* statistical parameters with slope as unity

4.7. Challenges in calculating characterization factors

This section focuses on the limitations related to the CFs and ecotoxicity data used in the calculation of EFs and extrapolation factors. However, in a broader sense, if one considers LCA as a whole, there are additional limitations beyond CF availability. One limitation is related to how to account for and include the inventory of chemical emissions in an LCA study, including both direct and indirect emissions in different compartments. Accurate measurement and reporting of emissions are crucial for a comprehensive LCA, yet often challenging due to data gaps and variability in emission sources and pathways. Another limitation arises once the analysis provides the ecotoxicity impacts. Interpreting these results involves understanding the varying levels of impact from different types of chemical emissions. Some emissions have more significant impacts than others based on the amount released, the compartment into which they are emitted, and the CFs values of those emissions. This complexity requires careful consideration to ensure accurate representation of ecotoxicity impacts. All these limitations related to the inclusion of ecotoxicity impacts in LCA are broader. However, this study is limited in scope, and the limitations are focused on the specific aspects covered within the study's context of calculation of CFs.

4.7.1. Applicability of the characterization factor methods

There is uncertainty regarding the suitability of USEtox for chemical substances such as inorganic anions and oxoanions, reactive gases, nanoparticles, ionic liquids, and PFAS (Owsianiak et al., 2023). USEtox is generally recognized as a global consensus model suitable for a wide range of chemicals but not one tailored for specific chemical groups (Rosenbaum et al., 2008). Paper 1 focuses on PM chemicals, noting their unique challenges due to their persistency and mobility. As noted by Holmquist et al. (2020), USEtox does not specifically accommodate the unique characteristics of PFAS chemicals, which are a notable group of PM chemicals. To address these limitations, Holmquist et al. (2020) developed a PFAS-adapted version of the USEtox model (version 2.1), introducing several enhancements to better assess the ecotoxicity impacts of PFAS.

A solution for calculating the CFs for PM chemicals would be to utilize the PFAS-adapted model for calculating CFs, which is expected to provide more relevant results for PFAS chemicals. However, the PFAS-adapted model also introduces notable challenges due to its requirements for more input data: 31 values per chemical as compared to 18 in the default USEtox model. This motivates an evaluation of trade-offs between the additional data

requirements of the PFAS-adapted model and how much these improve the calculated CFs. In Paper 1, a comparison was made between the PFAS-adapted model and USEtox (version 2.13) to calculate CFs for three specific PFASs: perfluorooctanoic acid (PFOA), perfluorohexanoic acid (PFHxA), and perfluorobutanesulfonic acid (PFBS) (Aggarwal et al., 2024). The CFs were calculated for the freshwater compartment, disregarding species richness and groundwater recirculation aspects in the PFAS-adapted model. This comparison resulted in an average difference of only 2.5%, with a range from 0 to 7%, leading to the indication that the PFAS-adapted model and USEtox CFs are not notably different. Given these similarities and the additional data requirements of the PFAS-adapted model, it was decided to use USEtox version 2.13 without PFAS-specific adaptations in Paper 1.

Additionally, recent advancements by Owsianiak et al. (2023) introduced further modifications for ecotoxicity CF calculation in USEtox. These recommendations resulted from collaborative work by the Ecotoxicity Task Force and the SETAC Pellston Workshop (Owsianiak et al., 2019). These recommendations shift from a traditional HC50EC50eq based approach, which uses chronic EC50 values, to an HC20EC10eq based approach utilizing chronic EC10 equivalents. HC20 represents the environmental concentration affecting 20% of species, quantified using the equation: EF = 0.2/HC20EC10eq [PAF m^3 kg-1]. Although these recommendations have not yet been incorporated into the official USEtox model, they have been implemented in the product environmental footprint (PEF) methodology for calculating CFs related to freshwater ecotoxicity in the EU environmental footprint version 3.0 (Sala et al., 2022; Saouter et al., 2018). Thus, Paper 1 adheres to the established methodologies of USEtox (version 2.13) while acknowledging these emerging recommendations.

Overall, while theoretical CFs offer a methodological framework for evaluating chemical impacts, their practical verification in environmental settings remains challenging. The reliability and accuracy of these theoretical values are difficult to verify, underscoring the need for a universally acceptable methodology that allows for comparative and relative assessments of CFs. Resolving these uncertainties is particularly crucial for PM chemicals, as the current methodologies may not adequately reflect their environmental behaviors and effects.

4.7.2. The temporal validity of characterization factors

In Paper 1, the comparison of newly calculated CFs with those available in the USEtox database serves as a temporal sensitivity test, illustrating the effects of incorporating additional ecotoxicity data. In Paper 1, 51% of the datapoints for 18 analyzed chemicals were collected

since 2005, and 28% since 2011. The introduction of this new data notably influences the CFs, largely due to the crucial role of EFs in the CFs calculation.

The original USEtox CFs were derived from ecotoxicity datasets that are now over a decade old (Payet, 2004; van Zelm et al., 2009; Zelm et al., 2007). Compared to these, CFs calculated with updated data exhibit substantial changes. It is crucial to recognize the variability in EF values, which depend on both the quantity and the diversity of the ecotoxicity data used (Roos et al., 2017). This diversity spans different species and species groups covered, as well as the methodologies employed and the environmental conditions of the tests (Saouter et al., 2017). Unlike other physical-chemical properties that are determined using standardized methods yielding consistent values with less variability and reflecting intrinsic properties, ecotoxicity assessments vary considerably (von Borries et al., 2023). This variability leads to uncertainty in EF values and consequently, CF values, as EFs are a dominant influence on CF calculations (Roos et al., 2017). Moreover, including data for a broader range of species has made the EFs more representative of average ecotoxicity across species, thereby enhancing both the accuracy and relevance of the CFs. This underscores the need for ongoing updates and refinements in CF calculations to better reflect the latest ecotoxicological data and account for biological diversity.

Given the online availability of various ecotoxicity databases and the increasing focus on transparency and accessibility of data (Peter Fantke et al., 2020; Grulke et al., 2019; Olker et al., 2022; Williams et al., 2017), it is important to view the calculated CFs as provisional values that can be improved with more comprehensive ecotoxicity data. This perspective acknowledges that CFs derived from limited data sets may not fully capture the complexity of environmental impacts and highlights the need for continuous updates and refinement as new data becomes available. As a result, CFs should be regularly updated based on the latest available ecotoxicity databases and in response to new recommendations in calculation methodologies. This includes different harmonization processes, conversion and extrapolation factors such as species distribution, and moving from acute to chronic conversions from the generic level to the species group level (Oginah et al., 2023). This necessitates the creation of a dynamic database for CFs that includes regular updates, rather than relying on static values. Such an approach would ensure that the CFs remain current and scientifically robust, reflecting the latest advancements and data in ecotoxicity.

However, there is a practical perspective to consider about the stability of CF values. CFs should remain stable to ensure their applicability and comparability with previous literature, avoiding the need to constantly update CFs (Arvidsson et al., 2020). Temporal reliability of CFs is crucial over the long term, as temporally unstable CFs will result in changing assessment conclusions. In USEtox, CFs depend on the availability of ecotoxicity data. Due to the lack of data for many chemicals, some CFs are recommended while others are interim, which may change as more data becomes available. In an LCA study, multiple chemicals are often involved, leading to chemical emissions and the use of CFs, with some recommended and some interim that can be improved over time. It is not practical from an accuracy standpoint to use less accurate CFs if more accurate CFs are available. Therefore, a pragmatic approach is needed to ensure transparency in the Life Cycle Inventory (LCI) data used, allowing users to update results with new CFs as needed. Additionally, there should be a predefined minimum data requirement for the calculation of CFs to maintain stability. This means that CFs for chemicals with already sufficient data should not change, while those with insufficient data can be updated when new information becomes available. Balancing transparency in LCI to update results and avoiding unnecessary changes to CFs for well-documented chemicals can be a way forward. Indicating which CFs are not recommended due to insufficient data can help maintain scientific integrity and practicality without compromising the accuracy of the results.

4.7.3. Lack of ecotoxicity data

One limitation in the calculation of CFs is non-availability of ecotoxicity values for various species groups at desired effect concentration indicators (Douziech et al., 2024; Oginah et al., 2023; Posthuma et al., 2019; Saouter, Biganzoli, et al., 2019). This gap is the main cause of the lack of CFs in LCA studies for different chemicals. There are very few ecotoxicity data produced specifically to calculate CFs. To address this, data originally collected for other purposes, such as risk assessment, can be used for CF calculations (Müller et al., 2017). However, this introduces the challenge of data harmonization. It is not just about the data availability but also about standardizing it in the USEtox format to make it usable in CF calculations. For example, Paper 1 began with ecotoxicity data from CompTox Version 2.1.1, initially retrieving 5002 data points, which, after harmonization, were reduced to 1189 data points, categorized by chemical groups: PFAS, triazines, and triazoles. Similarly, in Paper 2, data from REACH and CompTox underwent rigorous harmonization, resulting in a 51% reduction in data points.

Complexities also arise from challenges and gaps in available information. For instance, outdated species names create uncertainty in naming and classification, while exposure classifications also show inconsistencies. For example, algae tests typically do not differentiate between acute and chronic effects due to the rapid reproduction rate of algae, suggesting a leaning towards chronic evaluation (Hahn et al., 2014). Moreover, the classification of effect concentration indicators remains ambiguous, with uncertainties especially at the lower range of species sensitivity distributions, complicating the statistical distinction between NOEC, LOEC, and EC1-10 values (Iwasaki et al., 2015).

Once data is harmonized, there are additional challenges with conversion factors needed to transform the harmonized data into the required format of exposure type and effect concentration indicator type. Another limitation is determining which data is relevant in terms of the type of effects considered. This is critical even if all effects are chronic, as the sensitivity of these effects can vary and lead to inconsistencies that can result in data exclusion. These limitations related to data and its harmonization need to be addressed to find a feasible way to mitigate these issues in the CF calculations.

5. CONCLUSIONS

This licentiate thesis aimed to summarize three research questions answered in Paper 1 and Paper 2: 1) Are there important gaps in the availability of ecotoxicity CFs for PM substances? 2) How does the selection of ecotoxicity data and its harmonization influence the calculation of EFs? and 3) What are the challenges and opportunities with alternative ecotoxicity data translation and aggregation approaches in calculating extrapolation factors for CFs?

To address Research Question 1, Paper 1 presented the results of the EFs and CFs calculations. It evaluates the coverage of PM chemicals in the USEtox database, which has only 18 chemicals available out of 64. The findings indicate that the coverage of PM chemicals is low in the USEtox database, highlighting the need to increase the coverage of PM chemicals. As a result, in Paper 1, ecotoxicity CFs for 67 chemicals were provided, including 49 that were previously not characterized. In total, it provided CFs for 24 PFAS, 17 triazines, 23 triazoles, and 3 TPs. Benchmarking these CFs against all pre-calculated CFs from the USEtox database shows that the new CFs fall within the existing range of USEtox CFs. Paper 1 also compares the available 18 chemicals with the calculated EFs and CFs to understand the influence of including additional ecotoxicity data since the availability of the USEtox database. The comparison indicates the visible influence of including up-to-date ecotoxicity data. The addition of new data influences the CFs and underscores the relevance of up-to-date toxicological research and the regular updating of ecotoxicity data in the USEtox database to ensure more reliability in ecotoxicity CFs.

Research question 2 addresses the influence of ecotoxicity data harmonization alternatives on EFs. A data harmonization strategy for ecotoxicological effect data was developed in Paper 1. The analysis revealed that non-harmonized ecotoxicity data, once harmonized into the format of USEtox, resulted in over a 70% reduction of the raw data. This mirrors results from other literature studies, where harmonized data often leads to a reduction in the data available and, in some cases, also the chemicals, as the data is not in a format that is easily harmonized.

The second part of the research question 2 explores the impact of three alternative data harmonization strategies on the EFs. By removing certain resource- and time-intensive steps in the EF calculations, the number of ecotoxicity data points increased without reducing the reliability of the data compared to baseline harmonization. The conclusions from the comparison of alternative data harmonization strategies with baseline strategy indicate that

adopting a highly detailed harmonization strategy does not notably alter the relative ranking of CFs. However, it can lead to the removal of data points, thus reducing the data available for analysis. This suggests that a pragmatic approach is necessary to balance the trade-offs between removing inconsistent data and retaining enough data for meaningful analysis. The goal is to harmonize data to a level where inconsistencies do not considerably influence the final CF values, without excessively eliminating useful data.

The last part of research question 2, regarding the selection of different ecotoxicity data sources on the EFs, was addressed by employing various QSARs to calculate the EFs and comparing them with experimental EFs. The findings indicate that in this case QSARs have low reliability in calculating the EFs. Comparisons between EFs based on experimental ecotoxicity data and those derived from QSAR models revealed a weak correlation. This indicates that QSAR models may not yet be mature or reliable enough to provide ecotoxicity data suitable for accurate CF calculations. This points to a need for further development of in-silico tools for CF calculations.

Research Question 3 explores the challenges and opportunities with alternative ecotoxicity data translation and aggregation approaches on the calculation of extrapolation factors for CFs. The findings indicate that species group-specific extrapolation factors exhibit variations both within different species groups and as compared to the generic level. The extent varies depending on factors such as the species group and type of exposure considered as well as data availability. For instance, using a generic factor to convert acute EC10 data for algae could result in an underestimation of EC10 by a factor of 2.6. Paper 2 also compares the influence of different choices in extrapolation factors on the results, beginning with the calculation of extrapolation factors for chemical groups. The comparison of the generic extrapolation factors calculated for all chemicals versus those calculated for different chemical groups does not show significant differences. However, the comparison between generic extrapolation factors for all chemicals and species group-specific extrapolation factors reveals notable differences. This leads to the recommendation that species group-specific extrapolation factors should be used when available to reduce uncertainty. The study also compares the use of geometric mean over arithmetic mean in the aggregation of data points. Additionally, the impacts of classifying chemicals into different types (organic and inorganic versus only organic chemicals) and classifying compartments (freshwater, marine, or both freshwater and marine as aquatic) were examined. Effect concentration indicators classification into only EC10 and EC50 versus

NOEC, EC10, and EC50 was also considered. The study evaluated 12 different scenarios. The conclusion is that detailed classifications, such as species group-specific, chemical group-specific, aquatic compartment-specific, and chemical type-specific detailing, can provide better reliability and accuracy. However, this must be balanced with the challenge of data availability, as a lack of data can make detailed classifications less robust and lead to uncertainty.

6. SUGGESTIONS FOR FUTURE RESEARCH

6.1. AI for generating ecotoxicity data

A large number of chemicals remain uncharacterized, primarily due to the scarcity of ecotoxicity data, such as chronic EC50 and EC10 values needed for CF calculations. In the absence of experimental data, QSAR-based data are increasingly used as fast and cost-efficient alternatives to traditional methods (Cherkasov et al., 2014; Hou et al., 2020; Muratov et al., 2020). However, they often exhibit limitations in accurately predicting ecotoxicity data, highlighting the need for improved methodologies (Benfenati et al., 2013; Martin, 2020; Mayo-Bean et al., 2012). The availability of experimental data for tens of thousands of chemicals across various species has enabled the use of advanced deep learning methods, which hold the potential to enhance computational predictions of chemical ecotoxicity for these species groups (Gustavsson et al., 2024).

One such advanced artificial intelligence (AI) tool is TRIDENT, developed by Gustavsson et al. (2024) for aquatic ecotoxicity data. It has demonstrated superior performance compared to commonly used QSAR methods such as ECOSAR v2.2, VEGA v1.1.5, and T.E.S.T. v5.1.1.0, with a broader applicability domain and notably lower error rates. TRIDENT is freely accessible and processes inputs in SMILES format, outputting predicted effect concentrations. TRIDENT employs transformers to extract ecotoxicity-specific features from chemical structures and utilizes deep neural networks to predict EC50 and EC10 effect concentrations, focusing on organisms across three trophic levels—algae, aquatic invertebrates, and fish. It also accommodates various effects such as development, growth, intoxication, mortality, morphology, population, and reproduction, and can handle these effects across acute and chronic exposure durations.

In calculating the EFs, new AI tools like TRIDENT may help overcome the limitations of traditional QSAR models. Additionally, the use of AI tools facilitates the easy integration of newly generated data into the training dataset, allowing for regular updates to dynamically predict data for EFs, ensuring reliable and up-to-date EF calculations.

6.2. Inclusion of transformation products in CFs

Traditional chemical regulations have focused on individual parent chemical evaluations rather than assessing their holistic impact including TPs. While a large body of information is available on the environmental effects of parent chemicals, we know much less about the

effects of TPs since it is very complicated to perform the experiments to identify TPs, which are furthermore dependent on several different environmental conditions. The detrimental impact of TPs to environmental and human health is not a recent issue (Sinclair & Boxall, 2009). Some of the most noticed historical cases of environmental and human health effects of pesticides have been shown to be due to TPs rather than the parent compounds (Walker et al., 2005). The need to include stable and/or toxic TPs in risk assessment is mentioned in several regulatory assessment schemes, including the Pesticide Directive and REACH (Escher & Fenner, 2011; EU, 2009; EU, 2006). The ecotoxicity impacts of the parent chemical are compounded if the chemicals also transform in the environment, and some of the TPs are known to be more abundant in the aquatic environment than their parent compounds (Boxall, 2009; Boxall et al., 2004). The additional concern is that the majority of TPs of the parent chemicals have not even been identified yet, leading to exposure of the aquatic environment to the variable ecotoxicity of the parent chemical with unidentified TPs. TPs may contribute considerably to the risk posed by the parent compound (i) if they are formed with a high yield; (ii) if they are more persistent or more mobile than the parent compounds; or (iii) if they have a high toxicity (Escher & Fenner, 2011). TPs cannot be regarded in isolation from their parent compounds as they often exhibit the same mode of toxic action and act concentration-additive in mixtures, meaning that the effects from TPs and parent compounds must conservatively be considered additive, but synergistic effects could even enhance overall ecotoxicity (Escher & Fenner, 2011; Neuwoehner et al., 2009; Neuwoehner et al., 2010).

There is currently no well-defined approach for including TPs in LCA studies. One method, as applied in Paper 1, involves identifying relevant TPs and incorporating them into the CF calculations, including their emissions when known. An alternative approach, proposed by Van Zelm et al. (2010), suggests increasing the parent compound CF in proportion to the CFs of its TPs. This method acknowledges that the total environmental impact of a chemical includes both its own impact and that of its TPs. Another approach involves adding TPs that translate emissions of primarily emitted substances, such as PFASs, into their highly persistent terminal degradation products, as discussed by Holmquist et al. (2020). This method aims to account for the entire lifecycle impact of the chemical, including all known degradation pathways. Each of these approaches is a way to reflect the environmental impact of chemicals more accurately, including their TPs, in LCA studies. However, there is a need for further research to develop an acceptable method for including both parent chemicals and their TPs in LCA assessments.

This is crucial to avoid underestimating the impacts due to not including TPs of the parent chemicals.

6.3. LCA of engineered nanomaterials and CFs calculations

There is a potential new field of research developing nanomaterials that can match or surpass the functionalities and performance levels of existing technologies, materials, and chemicals. These engineered nanomaterial (ENM)-enabled products are emerging as high-performance alternatives to conventional materials and chemicals (Falinski et al., 2018). For example, graphene oxide (GO) shows promise in applications such as semiconductors, batteries, and electronics due to its exceptional material properties (Chen et al., 2012). Similarly, carbon nanotube (CNT) products are recognized for their potential societal benefits (Upadhyayula et al., 2012). However, alongside these potential advantages, these emerging nanomaterials could also pose undesired negative impacts on public health and the environment once released (Zhang et al., 2023). With commercial production of these ENMs already underway and expected to increase rapidly, there is a growing concern about their environmental concentrations and the associated risks (Deng et al., 2017).

There is a need to conduct impact assessment of products containing ENMs across their entire life cycle to adequately quantify their negative impacts and balance these against the benefits they provide. LCA has been recognized as a systematic approach to evaluate and identify potential environmental and human health impacts of products containing nanomaterials (Klöpffer et al., 2007; Zhang et al., 2023). A key limitation in this process is the absence of CFs that effectively model the environmental impacts of nanomaterials, highlighting the need for calculating such factors to support life cycle impact assessments of nano-enabled products (Salieri et al., 2018). A number of studies have been undertaken to calculate CFs for various nanomaterials as summarized by Zhang et al. (2023). Notable examples include CFs for silver nanoparticles (Hicks et al., 2015; Meyer et al., 2011; Miseljic, 2014; Pourzahedi & Eckelman, 2015; Temizel-Sekeryan & Hicks, 2020, 2021; Walser et al., 2011), carbon nanotubes (Eckelman et al., 2012), graphene oxide (Deng et al., 2017), copper nanoparticles (Pu et al., 2016), and titanium dioxide nanoparticles (Buist et al., 2017; Ettrup et al., 2017; Miseljic & Olsen, 2014; Pini et al., 2016; Salieri et al., 2015). These studies provide a foundation for developing comprehensive CFs that encompass a broader range of nanomaterials, essential for fully assessing their life cycle impacts.

As ENMs behave differently from traditional materials, they may require separate, specialized approaches. USEtox is a fate-effect model for calculating CFs, but it cannot be directly applied to ENMs because the parameters of USEtox are not suitable for multimedia fate modeling of nanomaterials (Praetorius et al., 2014). Currently, the literature identifies several methods for calculating the fate of nanoparticles. One increasingly used tool is SimpleBox4Nano (SB4N), a fate model specifically designed to handle nanomaterials (Blázquez et al., 2022; Meesters et al., 2014; Meesters et al., 2016). Methodological studies have been conducted to integrate SimpleBox4.0-Nano and USEtox to create a compatible nano-specific version of USEtox, referred to as 'USEtox4Nano' (Blázquez et al., 2022; Salieri et al., 2019). This adaptation seeks to bridge the gap between traditional chemical fate models and the unique behavior of ENMs in the environment. This represents a future area of research for integrating nanomaterial CFs into LCA frameworks.

6.4. ProScale for assessing toxicity

Efforts are ongoing to develop alternative methods to USEtox for estimating the life cycle impacts of chemicals. An increasingly popular alternative is ProScale, developed by the ProScale Consortium. Formed in 2015, the consortium aims to create a life cycle method that uses a hazard and exposure-based scoring system to compare chemical risks associated with products from a life cycle perspective (Lexén et al., 2021). The proposed scoring system incorporates four combined characteristics: the toxicity of a product's ingredients, the exposure potential of these ingredients, the application of life cycle thinking from cradle to grave, and the aggregation of data from individual ingredients to the product level. It is designed to encompass both human and ecotoxicity as well as to assess the toxicological exposure potential of hazardous substances in products across their entire lifecycle. ProScale scores can be calculated at various levels of aggregation and can be declared separately for different exposure routes (inhalation, oral, and dermal).

This method focuses on direct exposure to hazardous substances at each stage of the product's life. The methodology utilizes four key parameters to determine the ProScale score: the hazard factor (HF), exposure concentration factor (ECF), person-hours factor (PHF), and mass flow (Lexén et al., 2021). Each of these factors is calculated for every substance involved in each unit process within the lifecycle and then combined to establish the ProScale score. ProScale scores can be computed at two different levels of aggregation: ProScale of unit process (PSU), which analyzes individual processes, and ProScale of product, which assesses the overall

product. This dual approach allows for evaluations of specific processes as well as assessments of the entire product.

This easy-to-use method for aggregating ecotoxicity impacts is increasingly utilized in assessments. Not all decision-makers require detailed analysis of ecotoxicity impacts from chemicals like that provided by USEtox; sometimes a simplified analysis is sufficient to make decisions or to identify areas needing more detailed analysis. Research to clarify the applicability domain of simplified methods and enhance their usability is warranted.

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