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ABSTRACT

A reliable quantification of the potential effects of chemicals on freshwater ecosystems requires ecotoxicological response data for a large set of species which is typically not available in practice. In this study, we propose a method to estimate hazardous concentrations (HCs) of chemicals on freshwater ecosystems by combining two *in silico* approaches: quantitative structure activity relationships (QSARs) and interspecies correlation estimation (ICE) models. We illustrate the principle of our QSAR-ICE method by quantifying the HCs of 51 chemicals at which 50% and 5% of all species are exposed above the concentration causing acute effects. We assessed the bias of the HCs, defined as the ratio of the HC based on measured ecotoxicity data and the HC based on *in silico* data, as well as the statistical uncertainty, defined as the ratio of the 95th and 5th percentile of the HC. Our QSAR-ICE method resulted in a bias that was comparable to the use of measured data for three species, as commonly used in effect assessments: the average bias of the QSAR-ICE HC50 was 1.2 and of the HC5 2.3 compared to 1.2 when measured data for three species were used for both HCs. We also found that extreme statistical uncertainties ($> 10^5$) are commonly avoided in the HCs derived with the QSAR-ICE method compared to the use of three measurements with statistical uncertainties up to 10^{12}. We demonstrated the applicability of our QSAR-ICE approach by deriving HC50s for 1,223 out of the 3,077 organic chemicals of the USEtox database. We conclude that our QSAR-ICE method can be used to determine HCs without the need for additional in vivo testing to help prioritise which chemicals with no or few ecotoxicity data require more thorough assessment.

1. Introduction

The number of chemicals present on the market grows continuously. The Toxic Substances Control Act (TSCA) inventory of the United States has, for example, seen an increase in the listed substances from 62,000 in 1982 to around 85,000 in 2019 (U. S. Environmental Protection Agency, 2019). Many of these chemicals ultimately enter the environment, be it during manufacturing, use, or disposal. It is thus essential to know their potential effect on the receiving ecosystem.

For freshwater ecosystems, the potential effect of a chemical is expressed with a hazardous concentration (HC), i.e., the chemical concentration affecting a certain percentage of freshwater species. HCs may be derived from species sensitivity distributions (SSDs), a statistical description of the variation in sensitivity of multiple species to a chemical (Posthuma et al., 2002). SSDs rely primarily on species-specific

experiments which measure the relationship between chemical exposure concentrations and effects. Experimentally-derived ecotoxicity data for a chemical are often limited to a small number of species, typically three, covering different species groups, namely fish, daphnia, and algae (ECHA, 2008). This number of species is considered insufficient to adequately represent the ecosystem exposed to the chemical. In fact, Fantke et al. (2018) summarised in their review that the minimum number of ecotoxicity data necessary for a reliable prediction of a hazardous concentration lies between 5 and 10 depending on the legislation considered (Nugegoda and Kibria, 2013). In addition, several other authors have shown that the uncertainty of HCs derived from only three ecotoxicity values spans several orders of magnitude (Douziech et al., 2019; Golsteijn et al., 2012; Van Zelm et al., 2007). However, increasing the number of available measured ecotoxicity data is not straightforward because such experiments are time-consuming

and expensive as well as ethically controversial as they involve animal testing (Hartung, 2009). In addition, increased animal testing is against the "reduce, refine, or replace" principles of the USA and the EU, the goals of some companies, and the views of various stakeholder groups and consumers (European Commission, 2018; US EPA, 2018). One alternative to more testing is the use of *in silico* approaches, which estimate ecotoxicity values from chemical properties or species characteristics. Quantitative structure activity relationship models (QSARs), for example, relate physico-chemical properties to the species-specific toxicity of chemicals (e.g., Gramatica et al. (2016)). The majority of QSARs estimate acute ecotoxicity values for fish, daphnia, and algae, since most of the experimental data are available for these species and endpoints, as explained in e.g., Aurisano et al. (2019); May et al. (2016). Additional ecotoxicity data are therefore still required to derive reliable HCs. They can, for example, be generated with the Interspecies Correlation Estimation (ICE) models, which predict the ecotoxicity value of a chemical for an untested species based on the ecotoxicity value of that same chemical for a tested species (Raimondo et al., 2015).

Amongst others, Golsteijn et al. (2012), Awkerman et al. (2008, 2009), Dyer et al. (2008), Bejarano et al. (2017); Gredelj et al. (2018) combined ICE models with experimentally derived ecotoxicity values to estimate HCs. The derived HCs were comparable to those based on measured ecotoxicity values only, and their reliability increased. Barron et al. (2012) further derived HCs for 10 chemicals by combining QSAR and ICE estimates. The HCs did not match experimentally derived HCs well and Barron et al. (2012) stressed the need for additional research to reduce the uncertainty of HCs based on *in silico* approaches only. An extension of this work to more chemicals while propagating the uncertainties of the *in silico* methods used would therefore be a valuable addition. Similarly, He et al. (2017) recently mentioned the possibility to combine QSARs and ICE estimates to derive water quality criteria, but without addressing the representativeness and uncertainty of the results. In this paper, we take the analysis one step further by developing a methodology, which combines two *in silico* methods and assessing, for a large set of chemicals, the added value of this approach. The aim was to systematically assess the reliability and representativeness of hazardous concentrations for acute toxicity estimated with *in silico* methods applicable to a large set of organic chemicals.

Our method combines QSAR-derived ecotoxicity values for *Pimephales promelas*, *Daphnia magna*, and *Pseudokirchneriella subcapitata* with ICE models to estimate HCs for which 5% and 50% of the species included are exposed above their acute EC50, i.e HC5$_{EC50}$ and HC50$_{EC50}$. The EC50 describes the concentration causing effects in 50% of the population and is a common endpoint of *in silico* methods. Combining QSAR and ICE-based EC50s increases the number of species available for deriving the HCs and in turn ensures a better representation of the ecosystem (Nugegoda and Kibria, 2013). Spatial differences between species assemblages were not considered. We chose to estimate HC5$_{EC50}$ and HC50$_{EC50}$ as they are commonly used in comparative assessments of products as reviewed by, for example, Fantke et al. (2018) who showed how ecotoxicity is characterized across life cycle assessment studies, one of the frameworks available for such comparative assessments. Further, numerous studies exist that quantify the ecotoxicity of products for comparative assessments using acute HCs, sometimes extrapolated to chronic (Pennington et al., 2004; Rico et al., 2011; Rosenbaum et al., 2008; Saouter et al., 2017a, 2017b; Van de Meent and Huijbregts, 2005). Another motivation for using HC5$_{EC50}$ and HC50$_{EC50}$ is that both values can be used in the ecotoxicological screening of chemicals for which little or no experimental ecotoxicological data are available.

We compare our results with HC5$_{EC50}$ and HC50$_{EC50}$ of 51 chemicals with at least ten measured acute EC50s and within the applicability domains of the chosen QSARs, by quantifying two types of uncertainty: the statistical uncertainty and the bias. The statistical uncertainty of the HC5$_{EC50}$ and HC50$_{EC50}$ derived via our approach is a combination of the

QSAR and ICE uncertainty as well as the sampling uncertainty in the HCs. This sampling uncertainty directly relates to the number of datapoints available as well as the standard deviation between them. The uncertainty of the HCs derived from measured values is influenced by the sampling uncertainty only. The bias assesses the systematic difference between the estimated HCs using *in silico* methods and the HCs derived from all available measured acute ecotoxicity data.

2. Methods

We begin by describing the QSAR-ICE method, which combines *in silico* approaches to estimate hazardous concentrations for which 5% and 50% of the species are exposed above their acute EC50, i.e., HC5$_{EC50}$ and HC50$_{EC50}$ (Section 2.1). We then define the statistical uncertainty of the derived HC5$_{EC50}$ and HC50$_{EC50}$ (Section 2.2). Finally, we explain how the QSAR-ICE method was applied and how its performance was quantified (Section 2.3). Our analysis focuses on the performance of the QSAR-ICE method over all chemicals included and does not aim at describing the differences between the single chemicals.

2.1. QSAR-ICE method

2.1.1. Hazardous concentrations
Following a lognormal species sensitivity distribution, HC related to p% of the species affected (HCp), in our case 5% and 50%, can be derived as shown in Eq. (1) (Aldenberg and Jaworska, 2000). A lognormal distribution was chosen as it is the most widely used type of SSD and the statistical uncertainty of this distribution is well studied (Aldenberg and Jaworska, 2000; Posthuma et al., 2002; Sarfraz Iqbal et al., 2013).

$$logHCp = \bar{x} - k_{p,N}\cdot s \tag{1}$$

where $\bar{x}$ is the average of the log-transformed EC50-values, $k_{p,N}$ is the extrapolation factor for p% of the species affected for sample size N, and s is the sample standard deviation of the log-transformed EC50 values.

We chose to derive HCs from acute EC50 values, rather than from chronic No Observed Effect Concentrations (NOECs), because of the debate around the usefulness of NOEC values (Isnard et al., 2001; Landis and Chapman, 2011), also valid for comparative assessments (Fantke et al., 2018). Moreover, EC50s are typical endpoints of the available *in silico* methods (Netzeva et al., 2007), and especially of the ICE equations our approach is based on. Finally, the limited number of other measured chronic endpoints was another motivation for the use of acute EC50 values, e.g., 7369 acute SSDs compared to 1,051 chronic SSDs derived from measured data collated in the database of Posthuma et al. (2019).

2.1.2. Quantitative Structure-Activity relationships (QSAR)
A literature review was conducted to identify QSARs predicting EC50 (effect concentration) values for a specific fish, daphnia, and algae species. Lethal concentration (LC50) were hereby also considered as a particular type of EC50. Chosen QSARs had to be publicly available and give a measure of the uncertainty of the estimates or provide enough information to quantify it (see Supplementary Information SI, S2 for more details on the uncertainty quantification). If multiple QSARs were available for the same species, QSARs were selected based on their applicability domain (AD), predictive performance, and size of their training data set. These criteria align with the current European guidelines according to which QSARs may be used instead of testing as long as the QSARs are validated scientifically and reliable documentation on the method is provided, the substance falls within the AD of the QSAR, and the results are adequate for classification and labelling (Netzeva et al., 2007). Our aim was to have a method applicable to the largest set of chemicals so that QSARs developed for specific modes of action (e.g., (Vighi et al., 2009)) were not considered. This likely influences the remaining uncertainty in the predicted effect concentrations, as explained in the discussion section.

The QSARs for *P. promelas* and *D. magna* available on the VEGA platform (v. 1.1.4) fulfilled these criteria (IRFMN, 2018). In addition, both QSARs were validated in an independent study and showed comparable performances to the other models assessed (Cappelli et al., 2015; Golbamaki et al., 2014). The software available for download on the VEGA platform estimates the ecotoxicity for *P. promelas* and *D. magna* from the SMILES notation using chemo-informatic predictors derived automatically by the software. In addition, the VEGA QSARs also provide a global applicability domain index (ADI) per estimated ecotoxicity value which is calculated by grouping several indicators taking a particular issue of the applicability domain into account (IRFMN, 2017a, 2017b). We considered a chemical as within the QSAR's applicability domain when the ADI was above 0.7 (Cappelli et al., 2015; Golbamaki et al., 2014; IRFMN, 2017a, 2017b).

The lack of a consistent dataset with experimental algal test results and the variability of these results make it difficult to derive globally-applicable QSARs for algal toxicity (Fu et al., 2015; Netzeva et al., 2007; Villain et al., 2014). The available models are either proprietary themselves or based on predictors requiring proprietary software (Bakire et al., 2018; Singh et al., 2014). We therefore chose to use two QSARs: one developed for pharmaceuticals and one for personal care products (Gramatica et al., 2016; Sangion and Gramatica, 2016). These QSARs estimate the ecotoxicity to *P. subcapitata* based on 2D chemoinformatic descriptors estimated with the PaDEL software (Yap, 2011). These QSARs were not applicable for a large set of chemicals but fulfilled all the other criteria. We used the QSARINS software v2.2.2 to choose the chemicals entirely within the AD of the QSAR (Gramatica et al., 2014, 2013). Whenever chemicals were within the AD of both QSARs, the QSAR derived for pharmaceuticals was preferred given the larger training set it was developed with.

2.1.3. Interspecies correlation estimation (ICE)

ICE models are available for aquatic species, including algae, and terrestrial birds and mammals. ICE models estimate the acute toxicity of a chemical to a species from the known toxicity of the chemical to another species (Raimondo et al., 2015). In our case, the QSAR-based EC50 values derived for *P. promelas, D. magna*, and *P. subcapitata* were used as input in the corresponding ICE models (Eq. (2))

$$log(EC50_{predictedspecies}) = a + b \cdot log(EC50_{surrogatespecies}) \qquad (2)$$

We used all existing ICE equations per surrogate toxicity data. With the logical exception of those for *D. magna* and *P. promelas*, we kept all the ICE estimates, as Bejarano et al. (2017) did not show any statistically significant difference between SSDs derived from all ICE equations or only ICE estimates meeting the criteria associated with greater predictive power. However, when multiple estimates of EC50 values were available for the same species, which was the case for 38 chemicals, we kept the optimal model according to the criteria described in Raimondo et al. (2015) (SI, S1). From the ICE models available from Raimondo et al. (2015), we were therefore left with 10 ICE models based on *P. subcapitata*, 27 based on *D. magna*, and 60 based on *P. promelas*.

2.2. Statistical uncertainty

The statistical uncertainty in the HC50$_{EC50}$ and HC5$_{EC50}$ is caused by uncertainty in the QSAR and ICE models as well as sampling uncertainty due to limited number of species available. We ran a Monte Carlo simulation with 10,000 iterations to propagate these three uncertainty sources into an uncertainty estimate for the HCs. This procedure comprises three consecutive steps:

1. EC50s were estimated from the available QSARs, including their associated uncertainty. This uncertainty was quantified following Mendenhall et al. (2009) (SI, S2), using the details of the QSAR training data sets and the reported mean squared errors (Gramatica et al., 2016; IRFMN, 2017a, 2017b; Sangion and Gramatica, 2016).

Per chemical, each Monte Carlo iteration resulted in three possible QSAR-based EC50 values (i.e. one for *D. magna*, one for *P. promelas* and one for *P. subcapitata*), drawn from their respective uncertainty distributions.

2. The set of possible QSAR-based EC50s was used as input in the ICE equations to estimate ICE-based EC50s for additional species. The uncertainty associated with these ICE-based EC50s was again determined following Mendenhall et al. (2009) (SI, S2) and assuming full correlation between the ICE equations from the same surrogate species. In fact, the available training data sets of the ICE models for *D. magna* and *P. promelas* showed that, in both cases, around 70% of the ecotoxicity data of a surrogate species for a given chemical was used to derive at least two ICE equations (Raimondo et al., 2016). The conservative assumption of full correlation between ICE estimates of the same surrogate species was therefore deemed appropriate. As such, each Monte Carlo iteration yielded a set of possible species-specific EC50s, drawn either from the uncertainty distribution of the three QSARs, or from the uncertainty distribution of the ICE equations used.

3. Chemical-specific HC50 and HC5 were then computed per iteration, based on their respective sets of possible EC50s (QSAR- and ICE-based). The sampling uncertainty was added to these HCs in a final step, so that one possible HC$_{50}$ and one possible HC$_5$ were computed per iteration. This sampling uncertainty is reflected by the extrapolation factor $k_{p,N}$ from a (non-)central t-distribution, as shown by Aldenberg and Jaworska (2000). This extrapolation factor accounts for the uncertainty due to the limited sample size.

Following these steps, the variation in the final sets of 10,000 possible HC50$_{EC50}$ and 10,000 possible HC5$_{EC50}$ values reflects the uncertainty in the QSAR and ICE models as well as sampling uncertainty due to the limited number of species available. For the HCs derived from measured acute ecotoxicity values we only included the third step. As such, their resulting output distribution only reflects the sampling uncertainty and not uncertainty due to, e.g., experimental setup or measurement error. Further details on the statistical uncertainty in the QSAR and ICE estimates and the sampling uncertainty are given in the SI, S2.

2.3. Application of the QSAR-ICE method

2.3.1. Chemical selection

Organic chemicals with measured acute EC50 data available for at least 10 species covering 8 taxonomic groups were selected from the ecotoxicity database of Posthuma et al. (2019). We focused our analysis on organic chemicals and acute EC50 data because our aim was to use available *in silico* modelling techniques, which in their vast majority, estimate acute ecotoxicity values and are not able to estimate the ecotoxicity of metals (Netzeva et al., 2007). Furthermore, the data requirement of having ecotoxicity values from at least 10 different species covering 8 taxonomic groups was motivated by the current REACH guidelines to ensure a representative HC estimate (ECHA, 2008). Finally, the database collated by Posthuma et al. (2019) was chosen as starting point as it represents one of the most comprehensive open source ecotoxicity databases available to date with acute ecotoxicity values reported for specific species for 3,445 chemicals. From this data set, sufficient ecotoxicity data were available for 445 chemicals, which reflects the data limitations observed in other studies e.g., (Muller et al., 2017; Saouter et al., 2019a, 2019b). Further, acute QSAR-based EC50s could only be derived for 51 chemicals because of the limited applicability domains of the three QSARs used. More detailed explanation of the selection procedure and lists of the chemicals in- and outside the applicability domain of the QSARs are given in the SI, S3-5.

2.3.2. Scenarios

Prior to deriving the HCs, the suitability of using lognormal SSDs

Table 1

Combinations of EC50 values used to derive the hazardous concentrations. Number of EC50 shows the number of EC50s available per scenario to derive the hazardous concentrations. A stands for algae, F for fish, and D for daphnia.

	Scenario	Algae	Fish	Daphnia	Other	Number of EC50
(1)	AllMeasured	All available measured acute EC50 values				10–265
(2)	3Measured	measured	measured	measured	none	3
(3)	3QSAR	QSAR	QSAR	QSAR	none	3
(4)	3QSAR-ICE	QSAR	QSAR	QSAR	all ICE estimates	100
(5)	A-F-QSAR-ICE	QSAR	QSAR	none	all ICE estimates	73
(5)	A-D-QSAR-ICE	QSAR	none	QSAR	all ICE estimates	37
(5)	F-D-QSAR-ICE	none	QSAR	QSAR	all ICE estimates	87
(6)	A-QSAR-ICE	QSAR	none	none	all ICE estimates	11
(6)	F-QSAR-ICE	none	QSAR	none	all ICE estimates	61
(6)	D-QSAR-ICE	none	none	QSAR	all ICE estimates	28

was statistically tested and deemed reasonable according to a Kolmogorov-Smirnov test conducted on the available measured ecotoxicity values per chemical (SI, S5). Hazardous concentrations were derived (1) from all measured acute ecotoxicity values (AllMeasured, $N_{EC50} = 10$–265), (2) from three measured acute ecotoxicity values (3Measured, $N_{EC50} = 3$), (3) from three QSAR-based ecotoxicity values for *D. magna*, *P. promelas*, and *P. subcapitata* (3QSAR, $N_{EC50} = 3$), (4) from three QSAR-based ecotoxicity values and all available ICE equations (3QSAR-ICE, $N_{EC50} = 100$), (5) from two QSAR-based ecotoxicity values and all available ICE equations (A-F-QSAR-ICE; A-D-QSAR-ICE, F-D-QSAR-ICE, $N_{EC50} = 73$, 37, 87 respectively), and (6) from one QSAR-based ecotoxicity value and the available ICE equations (A-QSAR-ICE, F-QSAR-ICE, D-QSAR-ICE, $N_{EC50} = 11$, 61, 28 respectively) (Table 1). For the "3Measured" scenario, experimental acute EC50s for a fish, daphnia, and algae species were preferably used. If these were not available, data from species from other taxonomic classes were used instead (11 chemicals out of the 51, SI, S6).

Scenario "3Measured" reflects the number of measured ecotoxicity data typically available per chemical (Posthuma et al., 2019; Saouter et al., 2019b) and considered representative for current comparative risk assessments and life cycle impact assessment practice, while scenario "3QSAR" displays what can be achieved when HCs are estimated. The other scenarios were defined to investigate the added value of combining QSARs and ICE EC50s.

2.3.3. Indicators for bias and statistical uncertainty

Both bias and statistical uncertainty were quantified to assess the performance of the QSAR-ICE method. The bias compares the HC estimated from all available experimental ecotoxicity data ($HCp_{EC50,AllMeasured}$) to the HC derived per scenario ($HCp_{EC50,Scenario}$) without considering any source of statistical uncertainty (Eq. (3)).

$$Bias_{Scenario} = \frac{HCp_{EC50,AllMeasured}}{HCp_{EC50,Scenario}} \qquad (3)$$

A bias of 1 indicates perfect match between the $HCp_{EC50,AllMeasured}$ and the hazardous concentration derived for a scenario, while a bias below 1 means an overestimation of the $HCp_{EC50,AllMeasured}$ and a bias above 1 an underestimation.

The statistical uncertainty of the hazardous concentrations was defined as shown in Eq. (4).

$$Statistical\ uncertainty_{Scenario} = \frac{P95_{HCp_{EC50}}}{P5_{HCp_{EC50}}} \qquad (4)$$

where P95 represents the 95th percentile, P5 the 5th percentile of the $HC50_{EC50}$ or $HC5_{EC50}$ value derived for a given chemical and scenario.

2.3.4. Application to more chemicals

One aim of our approach was to develop a method applicable to a large number of chemicals. Based on the model evaluation for the 51 chemicals with 3 QSARs and sufficient experimental data available, we

investigated ways to extend the applicability of our approach to more chemicals. In a first step, we extended our model evaluation to more chemicals by adapting the 3QSAR-ICE method, according to the findings for the 51 chemicals, and re-running it on the chemicals included in the database provided by Posthuma et al. (2019). In a second step, we applied our method to derive $HC50_{EC50}$ for the chemicals included in the USEtox database to show the applicability potential of our approach. USEtox is a consensus model characterizing the human and ecotoxicological impacts of chemicals and is routinely applied in life cycle assessments (Fantke et al., 2018; Rosenbaum et al., 2008). We shall demonstrate how our approach can increase the confidence in the calculated HCs, also when limited or no measured ecotoxicity data are available. In fact, 27% of the 3,077 chemicals available in the organic's database of USEtox lack an ecotoxicological effect factor because of a complete lack of experimental data. Further, 44% of the ecotoxicological effect factors rely on ecotoxicity values for less than 4 species. From the 3,077 chemicals listed, 27 were stereoisomeric arrangements of another molecule, one was made of distinct sub-fragments. Further, 277 chemicals were either salts or contained elements other than carbon, hydrogen, oxygen, nitrogen, fluorine, chlorine, bromine, iodine, sulphur, phosphorus, silicon, arsenic, mercury, and tin. Our approach could therefore potentially be applied to a set of 2,772 chemicals.

3. Results

3.1. Bias of the hazardous concentration

In a first step, we assessed the representativeness of the HCs estimated with different QSAR and ICE combinations by comparing them to the HC derived from experimental data. Fig. 1 shows the calculated bias for the different scenarios, i.e: 3Measured, 3QSAR, and the different combinations of QSARs and ICE. The number of ecotoxicity values available per chemical therefore varies (N_{EC50}, Table 1). The range in the bias displayed in Fig. 1 reflects the variation in the single HCs bias computed, per chemical, for the 51 different chemicals included in our analysis.

The $HC50_{EC50}$ derived from three QSAR-based EC50s (scenario 3QSAR), as well as those derived using a combination of three QSAR-based and all ICE EC50s (scenario 3QSAR-ICE), have similar median bias as the $HC50_{EC50}$ derived from three measured EC50s (scenario 3Measured). The 3QSAR and 3QSAR-ICE scenarios result in $HC50_{EC50}$ with median bias of 0.7 (90% range [0.1–4.8]) and 1.2 (90% range [0.2–6.2]), respectively. These are close to the median bias of 1.2 (90% range of 0.3–5.4) associated with the $HC50_{EC50}$ derived from three measured values. Fig. 1 also shows small median bias and narrow 90% ranges for the scenarios combining at least two QSARs with ICE, e.g., for F-D-QSAR-ICE the median bias is 1.4 with 90% range [0.2–7.3]. In comparison, the 90% range of the $HC50_{EC50}$ bias combining only one QSAR-based EC50 with ICE is at least ten times larger than when two QSAR-based EC50s are used. In summary, combining at least two
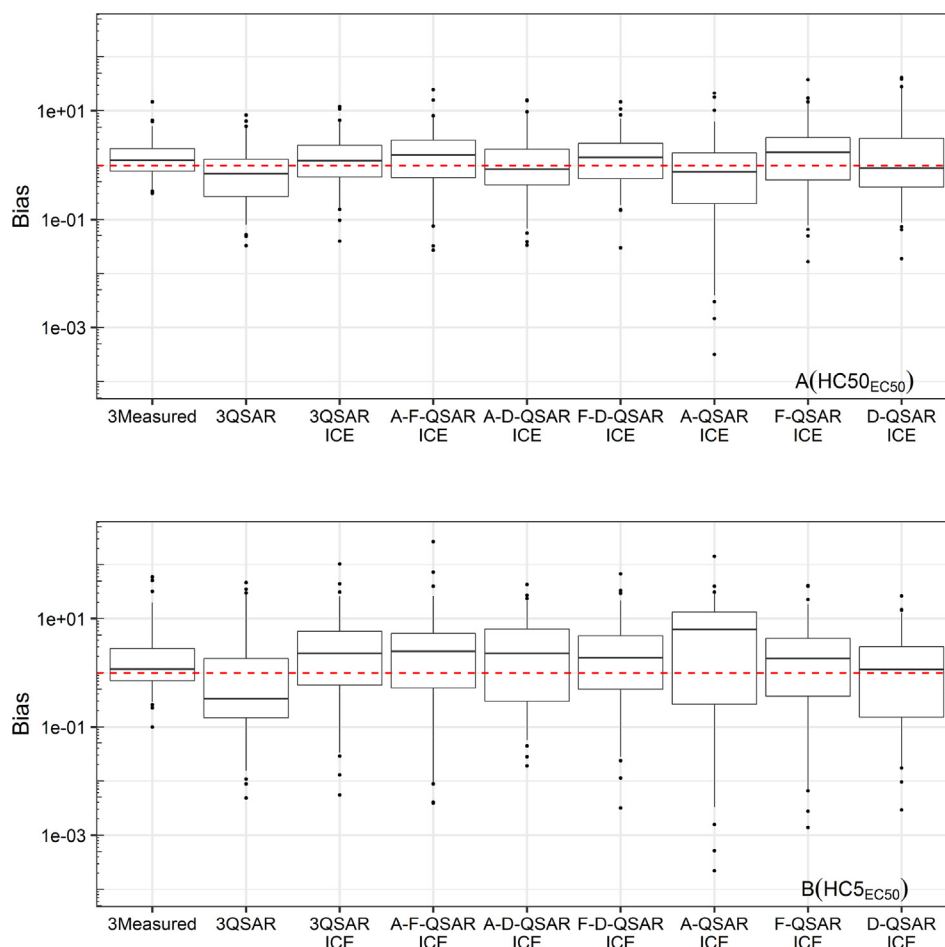
Fig. 1. Boxplots of the bias $HCp_{EC50,AllMeasured}/HCp_{EC50,Scenario}$ of the hazardous concentration related to 50% (A) and 5% (B) of the populations exposed above the concentration causing acute effects in 50% of the species' population (EC50) derived per chemical for the different scenarios. The boxplots show the 25th, 50th, and 75th percentiles and the whiskers represent the 5th and 95th percentiles. The x-axis lists the names of the scenarios: A stands for algae, F for fish, and D for daphnia.

QSAR- and ICE-based EC50s leads to $HC50_{EC50}$ values as representative as $HC50_{EC50}$ values based on three measured EC50s only. The median bias in the $HC5_{EC50}$ derived from three QSAR-based and all ICE EC50s (2.3) is larger than when only three QSAR-based (0.3) or experimental EC50s (1.2) are used. At the same time, the 90% range of the $HC5_{EC50}$ bias derived from three QSAR-based EC50s [0.02–32.1] is ten times larger than when three QSAR-based and all ICE EC50s are used [0.03–26.5]. The median bias and 90% range of the $HC5_{EC50}$ derived from the scenarios combining two QSARs with ICE are similar to the median bias and 90% range of the $HC5_{EC50}$ based on all three QSARs-ICE (Fig. 1). On the contrary, using only one QSAR-based EC50 and the corresponding ICE can lead to four times larger median bias and ten times larger 90% range compared to 3QSAR-ICE. A final observation from Fig. 1 is that the median bias and 90% range of the $HC5_{EC50}$ are larger than the ones of the $HC50_{EC50}$ for all QSAR-ICE scenarios.

In general, the bias was not dependent on the number of ecotoxicity values available. There was also no trend between the bias and the interspecies variability (i.e., the standard deviation of the ecotoxicity values) (SI, S8).

3.2. Statistical uncertainty of the hazardous concentrations

In a second step, we compared the statistical uncertainty of the HCs derived per chemical for the different scenarios (Fig. 2).

The median statistical uncertainty of the $HC50_{EC50}$ derived from three QSAR-based and the corresponding ICE EC50s (3QSAR-ICE) is one order of magnitude lower than when only three QSAR-based EC50s are

used (3QSAR) and the 90% range of the statistical uncertainty is approximately 1000 times smaller. The scenarios combining two QSAR-based and the corresponding ICE EC50s (A-F-QSAR ICE, A-D-QSAR ICE, F-D-QSAR ICE) have similar median statistical uncertainties and 90% ranges in the $HC50_{EC50}$ to the 3QSAR-ICE scenario (Fig. 2A). On the contrary, the $HC50_{EC50}$ derived from only one QSAR and the corresponding ICE EC50s (A-QSAR ICE, F-QSAR ICE, D-QSAR ICE) have one to two orders of magnitude larger median statistical uncertainties compared to the 3QSAR-ICE scenario. The median statistical uncertainty of the 3QSAR-ICE scenario is larger than when measured EC50s are available. However, in 25% of the cases, the 3QSAR-ICE scenario leads to less uncertain $HC50_{EC50}$ compared to using three measured ecotoxicity values.

Whether the $HC5_{EC50}$ is derived from one, two, or three QSAR-based EC50s, combined with the corresponding ICE-based EC50s, makes little difference for the resulting median statistical uncertainties and 90% ranges. Indeed, the median uncertainty using three QSAR-based EC50s (3QSAR-ICE scenario) is $3.4·10^3$ with a 90% range of $1.3·10^3$-$1.0·10^5$, while the median uncertainty using only the QSAR-based EC50 for fish (F-QSAR-ICE scenario) is $6.6·10^3$ with a 90% range of $4.2·10^3$-$2.9·10^5$. These values are smaller than the statistical uncertainty of the $HC5_{EC50}$ when derived from three EC50s (either measured or QSAR-based), and corresponding ICE EC50s are not considered. Based on three measured EC50s only, the median statistical uncertainty is $9.0·10^3$ with a 90% range of 10.2–$1.6·10^{12}$; based on three QSAR-based EC50s only, this was $5.6·10^7$ with a 90% range of $6.1·10^6$-$8.0·10^{14}$. Finally, Fig. 2 shows a larger statistical uncertainty of the $HC5_{EC50}$ compared to the
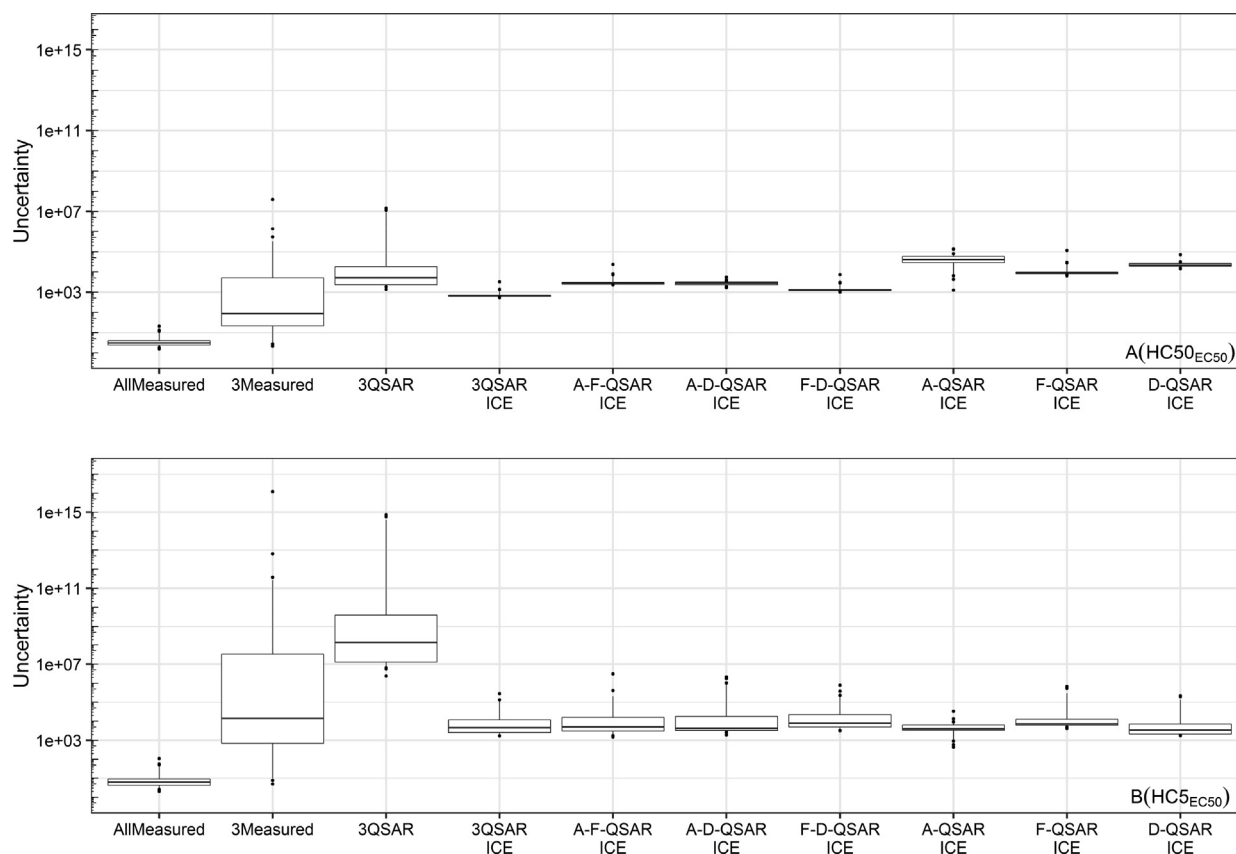
Fig. 2. Boxplots of the statistical uncertainty P_{95HCp}/P_{5HCp} of the hazardous concentration related to 50% (A) and 5% (B) of the populations exposed above the concentration causing acute effects in 50% of the species' population (EC50) derived per chemical for the different scenarios. The boxplots show the 25th, 50th, and 75th percentiles and the whiskers represent the 5th and 95th percentiles. The x-axis lists the names of the scenarios: A stands for algae, F for fish, and D for daphnia.

$HC50_{EC50}$.

In a last step, the influence of accounting for the QSAR uncertainty when describing the statistical uncertainty of the estimated HCs was quantified (Fig. 3) and the implications of the correlation in ICE estimates assessed (SI, S7).

The influence of the QSAR uncertainty on the statistical uncertainty of the $HC50_{EC50}$ is small compared to the uncertainty in the ICE estimates. In fact, for all scenarios combining QSAR and ICE estimates, the statistical uncertainty increases on average by 8.5 when the QSAR uncertainty is included (Fig. 3). Accounting for the QSAR uncertainty influences the statistical uncertainty of the $HC5_{EC50}$ more than the $HC50_{EC50}$ with an average increase in statistical uncertainty of 10.7. This relates to the typically larger uncertainty in the 5th percentile of a distribution compared to the median.

Applying correlated ICE estimates for the same surrogate species led to a one order of magnitude larger statistical uncertainty of the HCs for all scenarios, on average, except for two of the $HC5_{EC50}$. Combination of either *P. subcapitata* or *D. magna* QSAR EC50s with ICE to estimate the $HC5_{EC50}$, assuming correlated ICE for the same surrogate species led to similar median statistical uncertainties (SI, S7).

Finally, we found that the statistical uncertainty of the $HC50_{EC50}$ was significantly correlated to the inter-species variation ($p < 0.05$) with an increased statistical uncertainty with increased interspecies variability (SI, S9). Further, an increase in the number of ecotoxicity values tends to reduce the uncertainty of the $HC50_{EC50}$ values. Similar relationships were found for the uncertainty in the $HC5_{EC50}$ (SI, S9).

3.3. Application to more chemicals

Our results showed that combining QSAR- and ICE-based EC50s led to HCs with similar bias and uncertainties irrespective of whether two or three QSARs were used as starting points. We used this outcome to investigate for how many additional chemicals HCs can be estimated if at least two QSARs and the corresponding ICE estimates are used. We called this scenario QSAR-ICE.

#### 3.3.1. Database of Posthuma et al. (2019)

From the set of 445 chemicals extracted from (Posthuma et al., 2019)'s database, we derived HCs for 202 of them by basing the HCs on a combination of at least two QSARs and the corresponding ICE. Figs. 4 and 5 show a comparison of the bias and statistical uncertainty computed for the HCs derived for the 202 chemicals from the QSAR-ICE scenario as well as for the 3 Measured and All Measured scenarios. For sake of completeness, we also include a comparison to the 3QSAR-ICE scenario, which was however applied only to 51 chemicals.

3.3.2. USEtox's organics database

After consideration of the applicability domains of all three QSARs and estimating the $HC50_{EC50}$ from a combination of at least two QSARs and corresponding ICE-based EC50s, it was possible to estimate the $HC50_{EC50}$ for 1,223 chemicals out of the 3,077 organic chemicals of the USEtox database. The resulting log $HC50_{EC50}$ are shown in Fig. 6 per chemical together with the 25th and 75th percentiles. Numerical values can be found in the SI, S10.

4. Discussion

Deriving hazardous concentrations from a set of estimated ecotoxicity values potentially introduces bias and uncertainty in these concentrations. The aim of this work was to systematically assess the representativeness (e.g., small bias) and reliability (e.g., small statistical uncertainty) of HCs estimated from a combination of QSAR and ICE
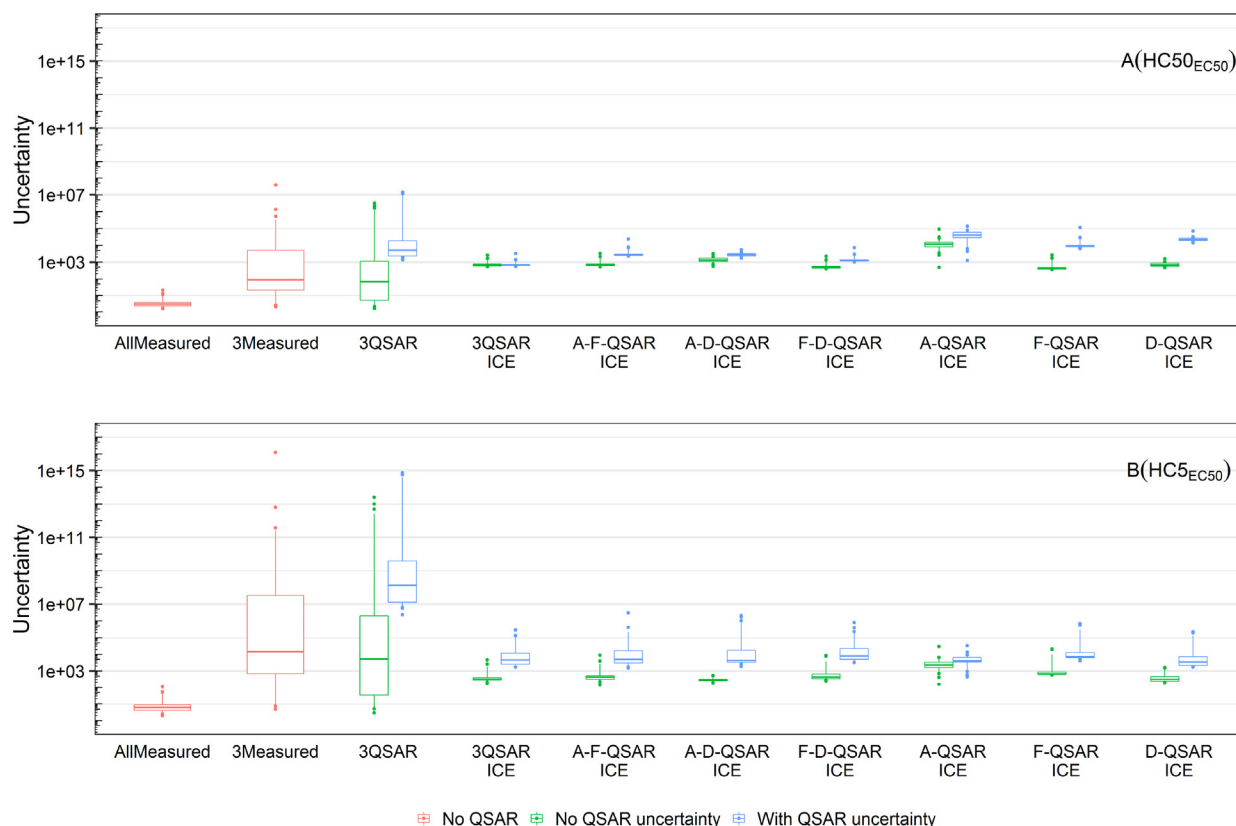
Fig. 3. Comparison of the boxplots of the statistical uncertainty computed per chemical while taking into account (1) the QSAR uncertainty and the ICE uncertainty ("With QSAR uncertainty", blue) and (2) only the ICE uncertainty ("No QSAR uncertainty", green) for the hazardous concentration related to 50% (A) and 5% (B) of the populations exposed above the concentration causing acute effects in 50% of the species' population (EC50) for the different scenarios analysed. The x-axis lists the names of the scenarios: A stands for algae, F for fish, and D for daphnia.

models. The following paragraphs discuss the representativeness, reliability, and potential applications and limitations of our method.

4.1. Representativeness of the hazardous concentrations

Hazardous concentrations derived by combining at least two QSARs and their corresponding ICE equations were comparable to those derived from measured ecotoxicity data for at least 10 species covering 8
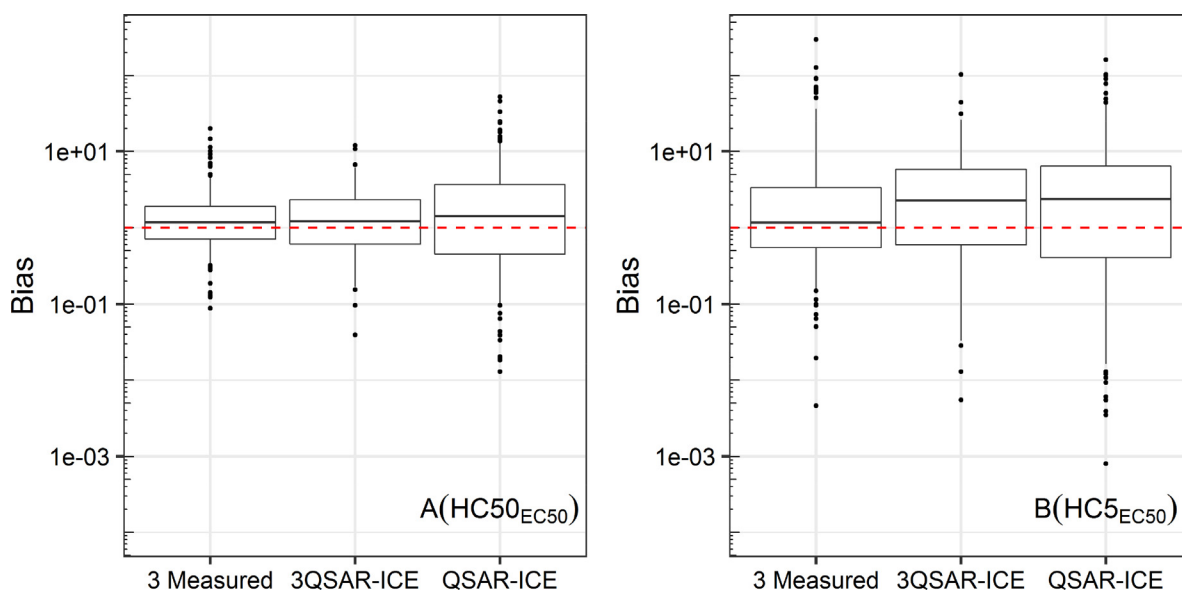
Fig. 4. Boxplots of the bias $HCp_{EC50,AllMeasured}/HCp_{EC50,Scenario}$ of the hazardous concentration related to 50% (A) and 5% (B) of the populations exposed above the concentration causing acute effects in 50% of the species' population (EC50) derived per chemical for the different scenarios. The boxplots show the 25th, 50th, and 75th percentiles and the whiskers represent the 5th and 95th percentiles. The x-axis lists the names of the scenarios.

Fig. 5. Boxplots of the statistical uncertainty $P95_{HCp}/P5_{HCp}$ of the hazardous concentration related to 50% (A) and 5% (B) of the populations exposed above the concentration causing acute effects in 50% of the species' population (EC50) derived per chemical for the different scenarios. The boxplots show the 25th, 50th, and 75th percentiles and the whiskers represent the 5th and 95th percentiles. The x-axis lists the names of the scenarios.
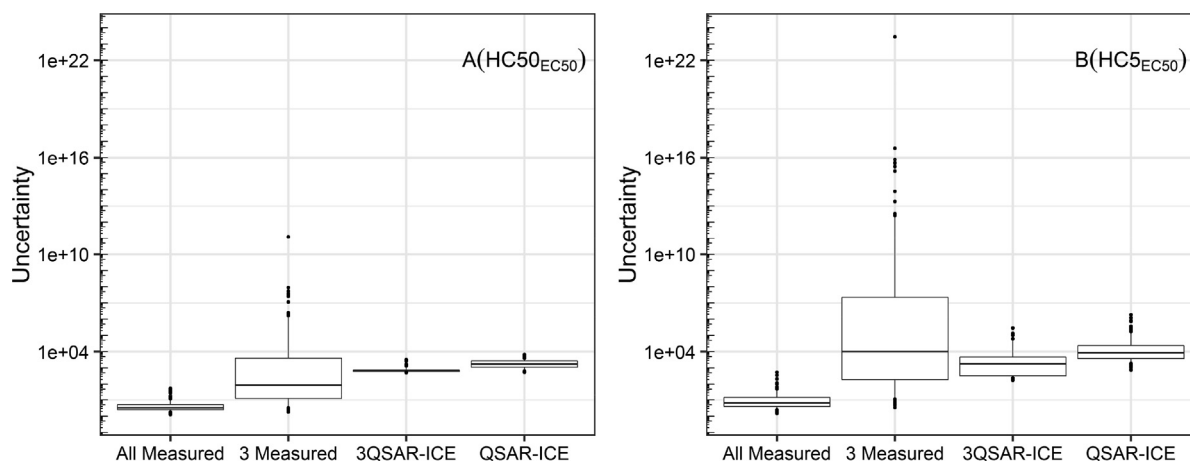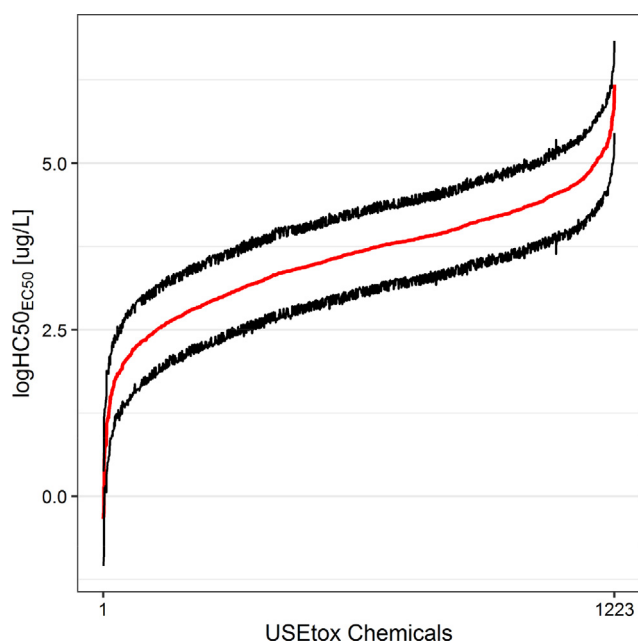

Fig. 6. log $HC50_{EC50}$ derived using our QSAR-ICE approach for the chemicals included in the organic's database of USEtox. The red line represents the average of the estimated EC50 values and the black lines the 75th and 25th percentiles of the Monte Carlo simulation.

taxonomic groups. Further, the HCs derived from at least two QSAR-based and corresponding ICE EC50s had a smaller or comparable bias compared to using three QSAR-based EC50s. Overall, the representativeness was smaller for the $HC5_{EC50}$ compared to the $HC50_{EC50}$, thus potentially limiting the applicability of our QSAR-ICE approach to estimate $HC5_{EC50}$. A potential explanation lies in the different species included in the calculation of the HC from QSAR-ICE and from measured ecotoxicity data. For example, 11 out of the 51 chemicals did not have measured ecotoxicity data for an algae species. Still, no clear difference in the estimated bias for the $HC5_{EC50}$ for these chemicals was observed (SI, S8). Barron et al. (2012) also highlighted that the bias between HCs based on *in silico* or experimental EC50s was smaller when similar species were included in both sets. A more thorough analysis of the species combination per chemical would, however, be necessary to better understand the drivers of these differences, but this was outside the scope of this paper. Another explanation could relate to the fact that the bias in the $HC5_{EC50}$ vary over a larger range than the bias of the

$HC50_{EC50}$, so that the median bias of the $HC5_{EC50}$ is likely to deviate more from the benchmark than the median bias of the $HC50_{EC50}$. Our analysis also showed that using only the *P. subcapitata* QSAR EC50 combined with the available ICE equations resulted in lower $HC5_{EC50}$, thus more protective hazardous concentrations. One explanation could be that the ICE equations related to *P. subcapitata* estimate EC50 only for other algae species and no other taxonomic group (Raimondo et al., 2015). Weyers et al. (2000) for example showed that, for around 2500 chemicals, the algae growth inhibition test was the most sensitive in 43.5% of the cases. We should also stress that we assumed that HCs based on at least 10 ecotoxicity data are a good representation of reality. We based this assumption on current legislation and simulation exercises showing a stabilisation of the derived HCs around this number (ECHA, 2008; Wheeler et al., 2002). We believe that given the current state of knowledge and data availability, this is the best approximation of the real ecotoxicological impact one can derive.

Bejarano et al. (2017) reported that in 58% of the cases, the ICE-supplemented SSDs based on experimental EC50s produced HC5s within a three-fold difference to the estimates from SSDs based on measured data only. With our approach, 41% of the QSAR-ICE estimated $HC5_{EC50}$ were within a three-fold difference to the measured $HC5_{EC50}$, thus had a bias between 0.3 and 3. Given the increased reliability observed in the study of Bejarano et al. (2017) and the conclusions from Barron et al. (2012), combining experimental values with ICE estimates might lead to more representative hazardous concentrations. The findings of Dyer et al. (2006), who reported that the ICE-based predicted HC5s of five different chemicals were generally within a factor 10 of the HC5s derived from experimental ecotoxicity values, are closer to ours. Further, in accordance to Dyer et al. (2006), we showed that the bias in the hazardous concentrations did not depend on the standard deviation of the ecotoxicity values used nor on the number of ecotoxicity values (SI, S8). Overall, our work and the findings of previous research highlight that complementing SSDs with ICE-based EC50s leads to representative HCs estimates in the majority of the cases. Future research exploring e.g. the use of traits in the ecotoxicity predictions for different species might improve the accuracy of ICE equations and therefore the representativeness of our approach (van den Berg et al., 2019).

4.2. Reliability of the hazardous concentrations

Hazardous concentrations estimated by combining QSAR and ICE-derived EC50s are influenced by uncertainty in the QSAR and the ICE estimates, and by sampling uncertainty resulting from the limited number of species (i.e., available EC50s). Because we assumed ICE

estimates for the same surrogate species to be correlated, the uncertainty in the HC50$_{EC50}$ estimated from only one QSAR and its corresponding ICE equations is relatively large, e.g., larger than when only three ecotoxicity values are used. In this case, the increase of uncertainty in the HC50$_{EC50}$ resulting from the ICE models is too large to be compensated by the reduced sampling uncertainty. This is different when at least two QSAR EC50s are combined with ICE models. The median statistical uncertainty of the HC50$_{EC50}$ is then comparable to the values of the three QSAR scenario (A-F-QSAR-ICE, A-D-QSAR-ICE, F-D-QSAR-ICE vs 3QSAR; Fig. 2).

Overall, the uncertainty in the ICE estimates contributed most to the uncertainty of the HCs derived with our approach. Because of the importance of the ICE uncertainty and our assumption of correlated ICE estimates for the same surrogate species, the statistical uncertainty of both hazardous concentrations was similar. In fact, assuming correlated ICE estimates led to small sampling uncertainties (< 1.2) especially when only one QSAR EC50 was combined with ICE. The small sampling uncertainty combined with the large number of available EC50s (minimum of 11) led to similar statistical uncertainties of the HC5$_{EC50}$ compared to the HC50$_{EC50}$. The uncertainty in the ICE estimates is hereby likely to be driven by the assumption of a fixed relationship between two species exposed to a chemical, irrespective of the chemical considered.

The observed spread in the statistical uncertainty is similar to the findings of Golsteijn et al. (2012) for hazardous doses (HD50) derived from three ecotoxicity values only. HD50 is the dose of a chemical toxic to at least 50% of the individuals in 50% of all warm-blooded species considered, as opposed to the concentration (HC50) relevant for freshwater species. They reported statistical uncertainties of HD50 ranging from 1 to 10^{10} when three ecotoxicity values are available, while 90% of the statistical uncertainties of the HC50$_{EC50}$ from ICE were between 10^3 and 10^5. On the other hand, Golsteijn et al. (2012) found a statistical uncertainty in the HD50 that was smaller than 10 when more than 10 ecotoxicity values were measured, while in our case this is between two and three orders of magnitude. This difference is the result of the uncertainty in the QSAR estimates, not included in Golsteijn et al. (2012), who combined ICE estimates with experimentally derived ecotoxicity values (Fig. 3). The larger spread and median uncertainties observed for the HC5$_{EC50}$ compared to the HC50$_{EC50}$ is related to the extrapolation factor $k_{p,N}$ in Eq. (1) which is more uncertain for the HC5$_{EC50}$ compared to the HC50$_{EC50}$ for the same N (Fantke et al., 2018; Saouter et al., 2017a).

Our results also suggested an increase in the uncertainty of the hazardous concentrations with increasing interspecies variability, especially when more than two QSARs are combined with ICE. This reflects the increased uncertainty of the interspecies variability resulting from the use of uncertain EC50 values.

Finally, statistical uncertainty can be kept low by a conscious species selection for the QSAR development or application, to optimally leverage the number of ICE equations available. This aligns to previous research, e.g., (Golsteijn et al., 2012; Van Zelm et al., 2007), who also stressed the importance of choosing species for which many ICE estimates are available to define or use QSARs. In fact, while *P. promelas* estimates effect concentrations for up to 76 different species, *Danio rerio* would only provide 2 additional estimates (Raimondo et al., 2015).

4.3. Potential applications and limitations

Our approach can be used to derive screening values for substances for which ecotoxicity data are lacking, whether these chemicals are existing or newly introduced on the market (Posthuma et al., 2019; Saouter et al., 2017a, 2017b). The bias of the HC50$_{EC50}$ derived with our approach were namely comparable to the HC50$_{EC50}$ based on three measured EC50s and in 25% of the cases, the statistical uncertainty of the HC50$_{EC50}$ based on our combined QSAR-ICE approach was lower than when using three measured ecotoxicity values. Typically,

experimental ecotoxicity values are, however, not available for three distinct species (Saouter et al., 2019b). In those cases, we showed that combining at least two QSARs and ICE estimates led to HC50$_{EC50}$ and HC5$_{EC50}$ estimates with smaller median uncertainties and comparable biases than when only three QSARs are used. In addition, the combination of QSARs and ICE reduced the number of very uncertain hazardous concentration, also observed when three measured ecotoxicity values were used. Our results, therefore, highlight two advantages of combining at least two QSARs and ICE estimates to estimate HC5$_{EC50}$ compared to only three QSARs: (1) the reduced number of highly uncertain HC5$_{EC50}$, and (2) the increased applicability of this approach as limited by the applicability domains of two QSARs only. We illustrated this second advantage by determining by how much the set of chemicals increases, if at least two QSARs are combined with ICE instead of three. Applying this alternative method to the initial set of 445 chemicals allowed us to estimate the HCs for 202 chemicals. The resulting median bias of the HC50$_{EC50}$ was 1.4 and 2.4 for the HC5$_{EC50}$, which is very close to the values derived for the 3QSARICE scenario, namely 1.2 and 2.3 for the HC50$_{EC50}$ and HC5$_{EC50}$ respectively. A similar behaviour was observed between the statistical uncertainty of the HCs derived from two to three QSAR-EC50 and the corresponding ICE and from the 3QSARICE scenario. In addition, we demonstrated how our approach can be used to estimate HCs of organic chemicals with limited or no measured ecotoxicity data, such as the database provided in the USEtox model. We were in fact able to estimate HCs for 1,028 chemicals and provide a measure of the uncertainty of these estimates. Among the 1,028 chemicals, 195 did not have any reported HC50 value so far. These additional HC50s are a valuable addition, also because a quantification of the statistical uncertainty is included, but it is clear that the applicability of our approach is limited by the applicability domains of the QSARs included. Refining our approach with QSARs better suited for specific chemicals or with larger applicability domains would allow to derive HCs for more chemicals.

Including our approach in available tools such as the screening level Risk Assessment, IDentification, And Ranking (RAIDAR) model (Arnot et al., 2006), could lead to more robust outcomes as it would quantify the uncertainty in the assessed risk, while keeping it to a minimum. In addition, combining QSAR-based and ICE EC50s could increase the number of chemicals for which ecotoxicological impacts can be quantified within the life cycle impact assessment framework (Huijbregts et al., 2010). Ideally, comparative assessments would describe long-term effects of chemicals and therefore be based on chronic ecotoxicity data. However, given the current limited availability of chronic NOEC and EC10 ecotoxicity data (Posthuma et al., 2019), it is not possible to follow our approach based on chronic ecotoxicity values for a larger set of chemicals. Using acute-to-chronic extrapolation factors, as done for example in the USEtox model (Huijbregts et al., 2010), could be a way to derive long-term effect estimates with our approach, given that a thorough assessment of the uncertainty of these factors is conducted (Aurisano et al., 2019; Hoff et al., 2010; May et al., 2016).

Another limitation of our approach is its inability to cover specific modes of action (MoA). On the one hand, this is because we used acute data that often do not capture a chemical's specific MoA. On the other hand, ICE models assume a linear relationship between the response of different species to the same chemicals, thus neglecting potential specific actions of chemicals on distinct species. Higher accuracy could be achieved by developing MoA specific models for less related taxonomic groups (Raimondo et al., 2010). We showed that particular care should be given to developing reliable ICE estimates, in light of the larger influence of the ICE uncertainty compared to the QSAR uncertainty on the HC statistical uncertainty. Considering the findings of Bejarano et al. (2017), using a reduced set of ICE equations, based on the goodness-of-fit criteria exposed in Raimondo et al. (2015), will most likely not reduce the uncertainty in the HCs. Instead, the integration of a mechanistic understanding of the toxicological processes within the ICE equations, as done with so-called Interspecies Quantitative Structure-

Toxicity Relationships (Khan and Roy, 2017), could increase the reliability of ICE equations and in turn the applicability of our method.

5. Conclusions

The research presented here estimated hazardous concentrations from a combination of ecotoxicity values derived from QSAR and ICE equations. We showed comparable or even smaller bias and statistical uncertainty of the hazardous concentrations derived from at least two QSAR-estimated EC50s and their corresponding ICE estimates compared to when only QSAR estimates were used. The method presented here also has the advantage of reducing the number of very uncertain hazardous concentrations observed when only three measured or estimated ecotoxicity values are used. Finally, our results already showed an added value when just two QSAR estimates were combined with ICE, allowing us to apply the approach to a larger set of chemicals compared to the initial one (N = 202 vs. 51 chemicals). We were also able to estimate acute hazardous concentrations for 1,223 chemicals out of the 3,077 chemicals available in the organic chemicals' database of USEtox. Still, the feasibility of our approach is directly related to the applicability domains of the QSARs included. Our QSAR-ICE approach can be used to derive toxicity values for substances newly introduced on the market or for which measured or estimated ecotoxicity data are lacking and thus help prioritize chemicals, as long as the applicability domain of the QSARs is verified. The domain of applicability of our approach namely directly relates to the applicability domains of the QSARs used and needs to be assessed per chemical depending on the specific QSAR definitions.

CRediT authorship contribution statement

Mélanie Douziech: Software, Validation, Formal analysis, Methodology. **Ad M.J. Ragas:** Methodology. **Rosalie van Zelm:** Methodology. **Rik Oldenkamp:** Methodology. **A. Jan Hendriks:** Methodology. **Henry King:** Supervision, Funding acquisition. **Rafika Oktivaningrum:** Validation. **Mark A.J. Huijbregts:** Methodology, Supervision, Funding acquisition.

Declaration of Competing Interest

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

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105334.

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