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Hierarchical Bayesian Approach To Reduce Uncertainty in the Aquatic Effect Assessment of Realistic Chemical Mixtures

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Supporting Information

ABSTRACT: Species in the aquatic environment differ in their toxicological sensitivity to the various chemicals they encounter. In aquatic risk assessment, this interspecies variation is often quantified via species sensitivity distributions. Because the information available for the characterization of these distributions is typically limited, optimal use of information is essential to reduce uncertainty involved in the assessment. In the present study, we show that the credibility intervals on the estimated potentially affected fraction of species after exposure to a mixture of chemicals at environmentally relevant surface water concentrations can be extremely wide if a classical approach is followed, in which each chemical in the mixture is considered in isolation. As an alternative, we propose a hierarchical Bayesian approach, in which knowledge on the toxicity of chemicals other than those assessed is incorporated. A case study with a mixture of 13 pharmaceuticals demonstrates that this hierarchical approach results in more realistic estimations of the potentially affected fraction, as a result of reduced uncertainty in species sensitivity distributions for data-poor chemicals.

INTRODUCTION

Species vary in their sensitivity to chemical substances. Under the assumption that this spread in sensitivities can be described by a statistical distribution, it is often quantified using chemical-specific species sensitivity distributions (SSDs). SSDs are typically constructed based on a sample of toxicity data reflecting the relative sensitivities of individual species. A common choice for this is the median effect concentration (EC50), which is the concentration having a specified effect for 50% of the individuals of a single species. If the concentration of a chemical in the environment is known, SSDs can be used to predict the fraction of species for which the EC50 is being exceeded. This is the so-called potentially affected fraction of species, or PAF.13

The PAF can be calculated not only for single chemicals but also for a mixture, and it is then referred to as the multisubstance PAF (msPAF).2 To aggregate the individual contributions of single chemicals into an msPAF, the principles of response addition3 and concentration addition4 can be followed, or a hybrid form of the two in which concentration addition principles are followed for chemicals with the same toxic mode of action (TMoA) and response addition principles for chemicals that have a different TMoA.2,7–9

The confidence that can be attributed to an msPAF depends, among other things, on how accurately the parameters of the underlying SSDs can be estimated from the available data, i.e., how well the sample of test species represents the community of interest.10 The results of single substance SSD analyses appear to stabilize at 10–15 data points,11 but chemicals for which less toxicity data are available may show highly uncertain PAF values. This is evident when an environmentally realistic mixture consisting of a large amount of chemicals is being assessed. In earlier studies,12,13 application of the classical approach has led to the conclusion that adverse effects on all aquatic life cannot be excluded (i.e., msPAF = 1). However, empirical data show that a large number of species are currently doing relatively well in European surface waters such as the
river Rhine. This triggers the question whether estimations using only toxicity data on the mixture chemicals, i.e., considering them in isolation, adequately reflects our state of knowledge on the toxicity of complex mixtures. One option to improve upon the current approach is by including our extensive knowledge on the toxicity of other chemicals via a hierarchical Bayesian approach. Bayesian inference of SSD parameters in single substance analysis has been applied before, e.g., using noninformative prior distributions,\textsuperscript{14,15} prior distributions based on expert elicitation,\textsuperscript{16} or informative priors based on data from other chemicals.\textsuperscript{7–19} Similarly, Roelofs et al.\textsuperscript{20} derived PNEC values via Bayesian inference of acute-to- chronic assessment factors from a limited set of acute toxicity data and informative prior distributions derived from an ecotoxicological database.

The aim of the present paper is to explore whether and how credibility intervals on msPAF values for realistic mixtures can be reduced using a hierarchical Bayesian model. This is done in a case study in which aquatic msPAF values are calculated for a realistic mixture of antibiotics (ABs) and anticancer drugs (ACs), based on surface water concentrations predicted for the Ruhr area in Western Germany. This location was chosen because it is relatively densely populated and because all pharmaceuticals in the mixture are actually prescribed in Germany. The hierarchical model is populated with a chemical data inventory containing toxicity data on more than 2000 chemicals. Different data (sub)sets of this inventory are used to populate the hierarchical model, in order to assess the influence of the representativeness of these data for the chemicals in the mixture. The uncertain msPAF values are calculated on the basis of the principles of response addition, concentration addition, and a hybrid form of the two.

\section*{METHODS}

\textbf{Species Sensitivity Distributions and msPAF Calculations.} The use of species sensitivity distributions (SSDs) is based on the assumption that, for each chemical, the interspecies variation in sensitivity can be described by a statistical distribution. The available toxicity data are considered a sample from this distribution and are used to estimate the parameters of the SSD.\textsuperscript{1} The resulting SSD can be used to assess the potentially affected fraction (PAF) of all species at a certain environmental concentration. Statistical distributions that are commonly used to describe the spread in sensitivity between species are the log-normal,\textsuperscript{14,21,22} the log-logistic,\textsuperscript{23–25} and the Burr Type III distribution.\textsuperscript{26} Here, we assume log-normal SSDs based on the central limit theorem (i.e., the product of a large number of independent variables will be log-normally distributed). Additionally, well-known sampling distributions are available for the characterization of the uncertainty in the parameters of the log-normal distribution.\textsuperscript{27}

We use EC50 values as a measure of the relative sensitivity of individual species. While EC50s have been criticized for their lack of ecological relevance,\textsuperscript{26,29} they are relatively widely available and statistically preferable over other toxicity values such as NOECs.\textsuperscript{30,31} The log-normal SSDs are described by the location parameter $\mu_{\logEC50}$ (i.e., the mean over the log-transformed EC50 values) and the scale parameter $\sigma_{\logEC50}$ (i.e., the standard deviation over the log-transformed EC50 values). In our case study, single chemical hazard units (HUs)\textsuperscript{eq 4} and PAFs are calculated at realistic environmental concentrations and integrated into msPAFs based on principles of response addition (msPAF$_{ra}$, \textit{eq 1}\textsuperscript{2}) and concentration addition (msPAF$_{ca}$, \textit{eq 2}), respectively. Response addition is based on the supposition of dissimilar action; i.e., all chemicals in the mixture act independently and exert their own toxic effect. Concentration addition, on the contrary, is based on the supposition of similar action; i.e., all chemicals in the mixture act in the same way and only differ in their potency.\textsuperscript{25} Additionally, a hybrid form of the two is used in which concentration addition principles are applied to chemicals sharing the same TMoA and response addition principles are applied to aggregate these groups of chemicals (msPAF$_{hyb}$ \textit{eq 3}).\textsuperscript{8}

\begin{align*}
\text{msPAF}_{ra} &= 1 - \prod_i (1 - \text{PAF}_i) \quad (1) \\
\text{msPAF}_{ca} &= \frac{1}{\sigma \sqrt{2\pi} \text{TU} \times \ln 10} \int_0^{\text{TU}} e^{-\left(\frac{1}{2}\left(\frac{\log(TU)}{\sigma}\right)^2\right)} d\text{TU} \quad (2) \\
\text{msPAF}_{hyb} &= 1 - \prod_i (1 - \text{msPAF}_{ca,i}) \quad (3)
\end{align*}

Table 1. Names, CAS Registration Numbers (CAS RN), Number of Species for which EC50s Are Available ($n_{\text{species}}$), SSD (sample) Parameters ($\mu_{\logEC50}$ and $\sigma_{\logEC50}$), Predicted Surface Water Concentrations (C), and Hazard Units (HUs; \textit{eq 4}) based on $\mu_{\logEC50}$ and C for the 13 Active Pharmaceutical Ingredients (APIs) in the Ruhr Area in Germany

<table>
<thead>
<tr>
<th>name</th>
<th>CAS RN</th>
<th>$n_{\text{species}}$</th>
<th>$\mu_{\logEC50}$</th>
<th>$\sigma_{\logEC50}$</th>
<th>C (mg-L$^{-1}$)</th>
<th>HU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>55268-75-2</td>
<td>2</td>
<td>0.79</td>
<td>1.70</td>
<td>$6.66 \times 10^{-8}$</td>
<td>$1.07 \times 10^{-4}$</td>
</tr>
<tr>
<td>Chlorotetracycline</td>
<td>57-62-5</td>
<td>9</td>
<td>$-0.23$</td>
<td>3.05</td>
<td>$3.26 \times 10^{-9}$</td>
<td>$5.59 \times 10^{-9}$</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>085721-33-1</td>
<td>8</td>
<td>$-0.29$</td>
<td>0.99</td>
<td>$9.70 \times 10^{-5}$</td>
<td>$1.89 \times 10^{-4}$</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>114-07-8</td>
<td>25</td>
<td>0.07</td>
<td>1.42</td>
<td>$2.08 \times 10^{-5}$</td>
<td>$1.77 \times 10^{-5}$</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>100986-85-4</td>
<td>6</td>
<td>$-0.35$</td>
<td>1.21</td>
<td>$1.08 \times 10^{-4}$</td>
<td>$2.39 \times 10^{-4}$</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>82419-36-1</td>
<td>13</td>
<td>$-0.15$</td>
<td>1.24</td>
<td>$3.55 \times 10^{-6}$</td>
<td>$5.05 \times 10^{-6}$</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>79-57-2</td>
<td>22</td>
<td>0.49</td>
<td>2.18</td>
<td>$3.99 \times 10^{-7}$</td>
<td>$1.30 \times 10^{-7}$</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>60-54-8</td>
<td>11</td>
<td>0.46</td>
<td>1.12</td>
<td>$5.09 \times 10^{-5}$</td>
<td>$1.77 \times 10^{-5}$</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>738-70-5</td>
<td>13</td>
<td>1.52</td>
<td>0.83</td>
<td>$1.92 \times 10^{-6}$</td>
<td>$5.75 \times 10^{-8}$</td>
</tr>
<tr>
<td>Anticancer Drugs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>50-18-0</td>
<td>2</td>
<td>3.03</td>
<td>0.09</td>
<td>$2.64 \times 10^{-7}$</td>
<td>$2.47 \times 10^{-10}$</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>51-21-8</td>
<td>7</td>
<td>$-0.55$</td>
<td>2.11</td>
<td>$9.12 \times 10^{-7}$</td>
<td>$3.22 \times 10^{-6}$</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>59-05-2</td>
<td>5</td>
<td>2.36</td>
<td>0.58</td>
<td>$9.56 \times 10^{-7}$</td>
<td>$4.13 \times 10^{-9}$</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>10540-29-1</td>
<td>5</td>
<td>$-1.75$</td>
<td>1.56</td>
<td>$4.51 \times 10^{-8}$</td>
<td>$2.51 \times 10^{-6}$</td>
</tr>
</tbody>
</table>
In eq 1, PAFi denotes the potentially affected fraction for chemical i. In eq 3, msPAFca,j denotes the msPAFca for chemicals sharing the same TMoA j. In eq 2, σ denotes the average spread in log-transformed toxic sensitivity between species over all chemicals in the mixture, and the toxic unit (TU) is calculated as the sum of the chemical-specific hazard units (HU):

\[ TU = \sum_i HU_i = \sum_i \frac{C_i}{10^{\mu_{\log EC50_i}}} \]  

where \( C_i \) is the surface water concentration of chemical i.

Selection of Chemicals and Location. The msPAF was calculated for a set of 13 active pharmaceutical ingredients (APIs), as selected within the PHARMAS project (http://www.pharmas-eu.net). From these 13 APIs, 9 are antibiotics and 4 are anticancer drugs (Table 1). Experimental species-specific EC50 values on these APIs were derived from publicly available databases and are listed in Appendix A of the Supporting Information. When multiple data were available for a combination of API and species, the EC50 for the most sensitive end point was used. When multiple data were available for the same end point, their geometric mean was used. Table 1 contains the SSD sample mean (\( \bar{X}_{\log EC50} \)) and sample standard deviation (\( s_{\log EC50} \)) for all 13 APIs, based on the available EC50 data. Additionally, this table contains the surface water concentrations that are used in the calculations. These concentrations are taken from earlier work, in which they were estimated for 100 × 100 km grids covering Europe. A spatial grid in the Ruhr area in Western Germany was selected as the case study location (Figure 1), because (1) it is one of the most densely populated areas in Europe, and (2) only in Germany, all 13 APIs are actually being prescribed.

Uncertainty Analysis: Classical Approach. The classical approach in risk assessment of chemicals has been that each individual chemical is considered in isolation, i.e., it is treated as if it were the first chemical ever to be assessed. Uncertainty assessments are thus based upon observations on the one chemical of concern, considering observations on other chemicals irrelevant. Here, we focus on uncertainties in the SSD parameters \( \mu_{\log EC50} \) and \( \sigma_{\log EC50} \) as a result of limited data availability. The uncertainties in these parameters are propagated into msPAF values via Markov Chain Monte Carlo (MCMC) simulations with the program OpenBUGS. Two chains of 200,000 iterations were run, after which the first half of the iterations was discarded on the basis of the burn-in principle. To check for convergence, potential scale reduction factors (PSRFs) were calculated for the remaining 100,000 iterations. Appendix C of the Supporting Information contains the syntax of the model and a graphical representation in the form of a Directed Acyclic Graph (DAG).

Since it is required by OpenBUGS, the parametrization of (log-)normal distributions is done with precision \( \tau \), which is the reciprocal of variance \( \sigma^2 \). The SSD parameters of each individual chemical in the mixture are separately assigned noninformative prior distributions. Their \( \mu_{\log EC50} \) is assigned a normal prior distribution with mean \( \mu \) and precision \( \tau \), expressed as \( N(\mu, \tau) \), and their \( \tau_{\log EC50} \) is assigned a gamma distribution with shape \( \alpha \) and rate \( \beta \), expressed as \( \Gamma(\alpha, \beta) \). These noninformative prior distributions should reflect the complete absence of prior knowledge associated with the classical approach. Ideally, this would imply improper normal and gamma prior distributions like \( N(0, 0) \) and \( \Gamma(0, 0) \). However, since OpenBUGS does not accept improper prior distributions, we approach these in the model with proper prior distributions that are sufficiently wide to be considered noninformative, i.e., \( N(0, 1 \times 10^{-5}) \) and \( \Gamma(1 \times 10^{-5}, 1 \times 10^{-5}) \). Subsequently, these noninformative prior distributions are transformed into posterior distributions for every chemical in the mixture using their respective available toxicity data (Appendix A of the Supporting Information), which are then used in the calculation of msPAFca, msPAFca, and msPAFca.
Uncertainty Analysis: Hierarchical Approach. Contrary to the classical approach, the hierarchical approach that we propose here places the assessment of each individual chemical in a broader context, i.e., as part of a larger population of chemicals. Under the assumption that the variation in \( \mu_{\logEC50} \) and \( \tau_{\logEC50} \) within this population of chemicals can be described by a statistical distribution, each individual chemical can be considered a random draw from that distribution. Consequently, the noninformative prior distributions on \( \mu_{\logEC50} \) and \( \tau_{\logEC50} \) from the classical approach are replaced with distributions that reflect the potential range of values for \( \mu_{\logEC50} \) and \( \tau_{\logEC50} \) based on the larger population of chemicals. Here, we assume that the interchemical variation in \( \mu_{\logEC50} \) can be described by a normal distribution with mean \( \mu \) and precision \( \tau \), expressed as \( N(\mu, \tau) \), and that the interchemical variation in \( \tau_{\logEC50} \) can be described by a gamma distribution with shape \( \alpha \) and rate \( \beta \), expressed as \( \Gamma(\alpha, \beta) \). The validity of these assumptions is supported with quantile-quantile (Q-Q) plots based on the sample mean \( \bar{x}_{\logEC50} \) and sample precision \( s_{\logEC50} \) of data-rich chemicals from the larger population (i.e., chemicals with \( n_{\text{species}} \geq 30 \)) (Figure 2).

The parameters of these distributions, i.e., \( \mu \), \( \tau \), \( \alpha \), and \( \beta \), are themselves assigned noninformative prior distributions which should reflect the initial absence of knowledge: \( N(0, 1 \times 10^{-5}) \) for \( \mu \) and \( \Gamma(1 \times 10^{-3}, 1 \times 10^{-3}) \) for \( \tau \), \( \alpha \), and \( \beta \). Subsequently, these noninformative prior distributions are updated with toxicity data from a chemical inventory. This chemical inventory contains EC50, LC50, and IC50 values gathered from e-toxBae4 in earlier studies, \(^8,47\) supplemented with toxicity data for APIs from publicly accessible data-bases \(^33,34,36,48\) and the ECHA chemicals registry.\(^49\) When multiple data were available for a combination of chemical and species, the value for the most sensitive end point was used. When multiple data were available for the most sensitive end point, their geometric mean was used.\(^38\) All chemicals with \( n_{\text{species}} > 1 \), for which \( x_{\logEC50} \) and \( \tau_{\logEC50} \) (i.e., sample mean and sample precision) could be calculated, were included in the inventory. The resulting inventory consists of a total of 2043 chemicals, including 106 APIs, of which 24 are ABs and 9 are ACs (Appendix B of the Supporting Information).

Since the sample mean \( \bar{x}_{\logEC50} \) and sample precision \( s_{\logEC50} \) in the chemical inventory are approximations of the population mean \( \mu_{\logEC50} \) and population precision \( \tau_{\logEC50} \), they cannot be used directly to update the prior distributions. Instead, the accuracy of these approximations should be taken into account first. This accuracy depends on the amount of data used for the calculation of \( \bar{x}_{\logEC50} \) and \( s_{\logEC50} \). To account for this, the \( \bar{x}_{\logEC50} \) values in the inventory were first expressed as sample variance \( s_{\logEC50}^2 \) (i.e., the reciprocal of \( \bar{x}_{\logEC50} \)). These \( s_{\logEC50}^2 \) values follow a chi-square sampling distribution with \( n_{\text{species}} - 1 \) degrees of freedom. The \( \bar{x}_{\logEC50} \) values in the inventory follow a normal distribution with a sampling precision of the sampling mean based on \( n_{\text{species}} \) and the value drawn from this chi-square distribution.\(^47\)

We formulate three hypotheses on the toxicity of the individual chemicals in the mixture. The hypotheses are based on the assumption that the SSD parameters estimated for (a subset of) substances, with sufficient available toxicity data, are representative for the range of possible SSD parameters of the chemical of concern. For each toxicity hypothesis, the prior distributions are updated with a different (sub)dataset from the chemical inventory, resulting in different posterior distributions and subsequent distributions of msPAF, msPAFca, and msPAFhyb. Increasing in their level of specificity, these hypotheses are

1. The SSD parameters for all 2043 chemicals in the chemical inventory are representative for the range of possible SSD parameters for the 13 active pharmaceutical ingredients (APIs) considered.
2. The SSD parameters for the 106 APIs in the chemical inventory are representative for the range of possible SSD parameters for the 13 APIs considered.
3. The SSD parameters for the 24 antibiotics (ABs) in the chemical inventory are representative for the range of possible SSD parameters for the ABs in the set of 13 APIs considered; the SSD parameters for the 9 anticancer drugs (ACs) in the chemical inventory are representative for the range of possible SSD parameters for the ACs in the set of 13 APIs considered.

Similar to the classical approach, MCMC simulations with two chains of 100,000 iterations after convergence are performed with the program OpenBUGS,\(^40,42\) propagating the uncertainties in the SSD parameters of the individual chemicals in the mixture into the msPAF values. The syntaxes and DAGs of the hierarchical models can be found in Appendix C of the Supporting Information.

■ RESULTS

The classical and the hierarchical models all show convergence after 100,000 iterations, with potential scale reduction factors (PSRFs) close to 1 for the SSD parameters of all APIs (i.e., PSRF < 1.1). Additionally, Figure 3 contains the posterior

Figure 2. Q-Q plots for the normal distribution on sample mean \( \bar{x}_{\logEC50} \) (A) and gamma distribution on sample precision \( s_{\logEC50} \) (B), based on a set of 115 chemicals with \( n_{\text{species}} \geq 30 \).
secondary probability distributions of $\mu_{\log EC_{50}}$ and $\tau_{\log EC_{50}}$, i.e., the distributions of the interchemical distributions of $\mu_{\log EC_{50}}$ and $\tau_{\log EC_{50}}$. They describe the interchemical variation in the larger population of chemicals for each of the toxicity hypotheses and are derived according to Aldenberg and Jaworska. The MCMC simulation produces 100,000 possible posterior distributions of the interchemical variation in $\mu_{\log EC_{50}}$ and $\tau_{\log EC_{50}}$. At a fixed value for $\mu_{\log EC_{50}}$ or $\tau_{\log EC_{50}}$, these

Figure 3. Posterior secondary distributions of $\mu_{\log EC_{50}}$ (1) and $\tau_{\log EC_{50}}$ (2) describing the interchemical variation in the larger population of chemicals, based on (A) the total chemical inventory, (B) all APIs in the chemical inventory, (C) all ABs in the chemical inventory, and (D) all ACs in the chemical inventory. Solid line: 50th percentile; dashed lines: 5th and 95th percentiles; dots: chemical-specific sample mean $\bar{x}_{\log EC_{50}}$ and sample precision $t_{\log EC_{50}}$ data.
distributions each return one specific probability density value. From these 100,000 probability density values, the 5th and 95th percentiles as well as the median are derived at a range of $\mu_{\log EC50}$ and $\tau_{\log EC50}$. These outer curves are not probability density functions, since they do not integrate to one. Figure 3 shows that the interchemical variation tends to decrease with increasing specificity of the toxicity hypothesis. Simultaneously, the estimation of this interchemical variation becomes less accurate with increasing specificity of the toxicity hypothesis, due to lower data availability to populate the hierarchical model.

Furthermore, Appendix D contains the cumulative density functions (CDFs) of the posterior distributions of the SSD for all 13 chemicals in the mixture, after inference via the classical approach and via the hierarchical approach for each of the three toxicity hypotheses. More specifically, Figure 4 shows how the inclusion of information on the larger population of chemicals influences the posterior distribution of SSDs and the subsequent single substance PAF for the antibiotic cefuroxime. Cefuroxime was chosen as an example because of its low data availability (only two largely differing EC50 values; Table 1, Appendix A of the Supporting Information). The figure contains the CDF of the posterior distribution of SSDs for cefuroxime, derived via the classical approach (Figure 4A) as well as via the hierarchical approach with the third hypothesis (Figure 4D). Figure 4 clearly shows that the inclusion of information on the larger population of chemicals might significantly reduce both the credibility interval on the PAF and its median value.

The calculations of the msPAF, based on the principles of response addition (RA), concentration addition (CA), and a hybrid form of the two, result in PDFs as shown in Figure 5. Regardless which principles are followed, the classical approach always results in very wide 90% credibility intervals, i.e., 0.02–0.67 for RA, 0.01–0.94 for CA, and 0.01–0.91 for the hybrid form. When a hierarchical approach is taken, both median values and credibility intervals decrease. This decrease is largest when concentration addition or a hybrid form of concentration and response addition is applied. Concentration addition assumes the same interspecies variation in sensitivity for all chemicals in the mixture; i.e., the individual values are averaged into one generally applicable value (eq 2). Therefore, less uncertainty in $\tau_{\log EC50}$ for one chemical affects the estimations for all other chemicals in the mixture, increasing the possibility of an msPAF of 1.

**DISCUSSION**

The case study with a realistic mixture of 13 APIs in the aquatic environment in the Ruhr area in Western Germany (Figure 1) showed that the use of a hierarchical model results in a median potentially affected fraction of $\sim 0.01$ when concentration addition principles are assumed and of $\sim 0.01–0.02$ when response addition principles are assumed or a hybrid form of...
these two is applied. Contrary to this hierarchical approach, the classical approach leads to high msPAF estimations with much wider credibility intervals (Figure 5). This will become even more relevant for environmentally realistic mixtures, generally consisting of large numbers of chemicals with often scarcely available data.71,72 When studying realistic mixtures consisting of a large number of chemicals, however, the hybrid form of concentration and response addition applied here could become unfeasible since it requires chemical-specific knowledge of the TMoA for all chemicals present in the mixture.

At the basis of the hierarchical model lies the assumption that all chemicals in the mixture are part of a larger population of chemicals. The chemical inventory used to populate the hierarchical model should thus consist of a representative sample of that population. General practice in chemical risk assessment, however, implies that more toxic chemicals are tested more often than chemicals that show little initial toxicity. Consequently, relatively nontoxic chemicals might be under-represented in our chemical inventory, leading to a potential overestimation of the actual msPAF.

Although uncertainties due to limited $n_{\text{species}}$ were taken into account in the hierarchical model via the inclusion of sampling distributions on $\bar{\log EC}_{50}$ and $\log EC_{50}$, interspecies variability was not included as a source of uncertainty.56 When multiple toxicity data were available for a combination of chemical, species, and end point, we used their geometric mean as input. However, Craig57 showed that the difference between two separate measurements of the same chemical-species combination is approximately a factor of 0.3, with a considerable amount of cases where this factor exceeds 1. Moreover, we implicitly assume that all species are a priori exchangeable. Each toxicity value is thus considered a random sample from the SSD, regardless of the species measured.15,16 However, evidence shows that nonexchangeability is a reality for at least one standard test species.18

Finally, model structure uncertainty51 plays a role in our assessment, mainly in the selection and parametrization of the (hyper)distributions at different levels of the hierarchical model. First, at the level of the individual chemicals in the mixture, it relates to the choice for the log-normal species sensitivity distribution. Since there seems to be no good reason to prefer one distribution type over another based on theoretical grounds,14,17 and since it is impossible to statistically differentiate between different distributions at small sample size,52 this choice is difficult to justify based on the data available for the chemicals in the mixture. However, the CDFs of the posterior distribution of SSDs for all chemicals in the mixture (Appendix D of the Supporting Information) do show relative agreement between model and data. Future analysis could include other distribution types to assess the importance of this source of model structure uncertainty. Second, at the level of the larger population of chemicals, model structure uncertainty relates to the choice for the log-normal and gamma distributions to describe the interchemical variation in $\mu_{\log EC_{50}}$ and $t_{\log EC_{50}}$, respectively. However, Q-Q plots based on $\bar{\log EC}_{50}$ and $\log EC_{50}$ values of 115 data-rich chemicals ($n_{\text{species}} \geq 30$) support this choice (Figure 2). Third, at the hierarchical model’s highest level, model structure uncertainty relates to the parametrization of the noninformative prior distributions. Especially when data to populate the hierarchical model are scarcely available, these distributions can be more informative than desired, with posterior distributions dependent on the hyperparameter choices.53 To assess the relevance of this in our study, we ran the hierarchical model with three different sets of hyperparameters: one set as described, one set of smaller hyperparameters, i.e., $N(0, 1 \times 10^{-5})$ and $F(1 \times 10^{-6}, 1 \times 10^{-6})$, and one set of larger hyperparameters, i.e., $N(0, 1 \times 10^{-5})$ and $F(1 \times 10^{-2}, 1 \times 10^{-2})$. The model simulations show stable posterior distributions of $\mu_{\log EC_{50}}$ and $t_{\log EC_{50}}$ for all chemicals in the mixture (Appendix E of the Supporting Information), and the chemical inventory thus seems extensive enough for posterior distributions to not depend on hyperparameter choices.

In this paper, we have proposed a hierarchical Bayesian approach for the derivation of probabilistic msPAFs. We have
shown that such an approach could be a suitable method for probabilistic multisubstance aquatic effect assessments. While the classical approach may result in a counterintuitive representation of the actual uncertainty in msPAF of large but realistic mixtures, we feel that a hierarchical approach incorporating information on the larger population of chemicals addresses this uncertainty in a more realistic way. However, whether this conclusion remains valid when a larger mixture of compounds is assessed at higher water concentrations, for example in the effluent of sewage treatment plants, requires further investigation. Additionally, if this approach would be applied in the context of aquatic risk assessment, uncertainty in the exposure concentrations should also be addressed in order to get a complete view of the influence of uncertainty.

**ASSOCIATED CONTENT**

**Supporting Information**
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b02651.

Appendix A: Toxicity data for the 13 APIs in the mixture.
Appendix B: Toxicity data for the chemicals included in the chemical inventory. Appendix C: Description and syntax of the OpenBUGS model. Appendix D: Posterior distributions of the SSDs of the chemicals in the mixture.
Appendix E: Posterior distributions of the SSD parameters at different sets of hyperparameters. (PDF)

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**Notes**
The authors declare no competing financial interest.

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