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# Estimating the Impact of High-Production-Volume Chemicals on Remote Ecosystems by Toxic Pressure Calculation

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**Abstract:**

Although many chemicals are in use, the environmental impacts of only a few have been established, usually on per-chemical basis. Uncertainty remains about the overall impact of chemicals. This paper estimates combined toxic pressure on coastal North Sea ecosystems from 343 high-production-volume chemicals used within the catchment of rivers Rhine, Meuse, and Scheldt. Multimedia fate modeling and species sensitivity distribution-based effects estimation are applied. Calculations start from production volumes and emission rates and use physicochemical substance properties and aquatic ecotoxicity data. Parameter uncertainty is addressed by Monte Carlo simulations. Results suggest that the procedure is technically feasible. Combined toxic pressure of all 343 chemicals in coastal North Seawater is 0.025 (2.5% of the species are exposed to concentration levels above EC50 values), with a wide confidence interval of nearly 0-1. This uncertainty appears to be largely due to uncertainties in interspecies variances of aquatic toxicities and, to a lesser extent, to uncertainties in emissions and degradation rates. Due to these uncertainties, the results support gross ranking of chemicals in categories: negligible and possibly relevant contributions only. With 95% confidence, 283 of the 343 chemicals (83%) contribute negligibly (less than 0.1%) to overall toxic pressure, and only 60 (17%) need further consideration.

## Introduction

Large numbers of chemicals are used throughout the world. In the U.S.A., approximately 70 000 chemicals have been produced and used since 1976, with some 15 000 produced in significant amounts (*1*). The European inventory of existing commercial chemical substances (EINECS; ref *2*) lists 100 195 substances. Of these, 2465 are the so-called high-production-volume chemicals (HPVCs), i.e., substances produced in the EU in volumes exceeding 1000 tonnes

per year. Despite the great national and international efforts put into monitoring and risk assessment, impacts of only a few of these chemicals have been established, usually on a per-chemical basis. Uncertainty and even ignorance remains about the overall impact of chemicals on human health and ecosystems.

Since it is impossible to regulate mixtures on a regional scale, decision support systems have been developed to evaluate potential impacts of chemical releases and for chemical regulation, among other systems involving ranking methods. In the U.S., the chemical ranking and scoring model method "Chemical Hazard Evaluation for Management Strategies" or CHEMS-1 has been designed to select priority chemicals for the assessment of safer substitutes for major products and process uses; chemicals were selected from Toxics Release Inventory data (*1*). In Europe, the EU Ranking Method (EURAM; refs *3*, *4*) was developed to prioritize HPVCs for which data have been collected in the International Uniform Chemical Information Database (IUCLID; ref *5*). EU risk assessment on the prioritized substances is in progress (*6*). This risk assessment follows the standard procedures laid down in the Technical Guidance Documents (TGD; ref *7*). Commonly, the existing methods lead to the assessment of so-called risk quotients per chemical; the ratios of predicted environmental concentrations (PEC) and predicted no-effect concentrations (PNEC), with the absolute value serving as regulatory and ranking criterion. PECs and PNECs are obtained with a set of methods that vary between compounds as a consequence of data availability and scientific knowledge. Although the PEC and PNEC methods are conceptually simple and operationally needed in decisions processes, they do not sufficiently allow estimation of environmental impacts of mixtures in the ecosystem. Furthermore, although the parameters used in the quotients methods are known to be uncertain, the EU-TGD does not explicitly address uncertainty in PEC/PNEC, leaving an option to gain insight in relative parameter relevance and interpretation limitations aside.

The potentially affected fraction of species (PAF) has been proposed as an operational endpoint for estimating probable effects on species assemblages in ecological risk assessment of toxic substances. PAF can be considered as a conceptual improvement, since it takes the often-observed nonlinearity of species sensitivity distributions (SSDs), as well as SSD-slope differences in chemicals into account. PAF represents the fraction of species that are exposed to toxicologically significant concentration levels, like NOEC or EC50, and interprets this as a measure for "ecological risk" (*8*, *9*) or an index for "toxic stress" (*10*). In this paper we preferred to use "toxic pressure" as the most appropriate term representing the potentially affected fraction of species. PAF can represent the toxic pressure of a single substance (ssPAF), or of mixtures of chemicals (multi-substance PAF or msPAF) (*10*). This paper builds on these ideas and introduces the analysis of uncertainties in toxic pressure calculations. The combined toxic pressure of many chemicals is correlated to the occurrences and abundances of species in the field (*11*, *12*). Owing to this, PAF has been found useful as an assessment endpoint in life cycle impact assessment of products (*13*, *14*).

In this paper, we use toxic pressure (ssPAF, msPAF) calculation to explore the possibilities of (i) estimating the overall impacts of chemical mixtures on ecosystems, (ii) quantification and ranking the relative importance of individual chemicals and (iii) establishing parameter uncertainty. Our calculation method involves estimations of concentration in the environment from known production volumes, impact assessments of chemicals on aquatic ecosystems, and ranking series of chemicals on the basis of this outcome, and essentially follows EURAM. Unlike EURAM, which yields semiquantitative "environmental scores", our procedure more closely follows the calculations of the EU-TGD, which yields

quantitative output. Our procedure differs from the EU-TGD in that it uses toxic pressure estimates (PAF) instead of the risk quotients (PEC/PNEC). We applied our calculations procedure to a set of 343 EU high-production-volume chemicals. We assessed the combined toxic pressure of these chemicals on the coastal seawater ecosystem of the Southern Bight of the North Sea, modeling the combined catchments of the rivers Rhine, Meuse, and Scheldt.

The overall toxic pressure on ecosystems and the relative contributions of individual chemicals to this can only be meaningfully evaluated on the basis of thorough evaluation of the uncertainties in the results obtained. Not only are the emission factors, from which the calculation starts, largely unknown, the physical, chemical, and ecotoxicological substance properties on which the calculation sequence rests are not known with great accuracy either. We deal with these uncertainties by treating the entire calculation probabilistically. We judge the end result in the light of the uncertainty associated with it.

## Methods

**Toxic Pressure Calculation.** Toxic pressure calculation was done according to descriptions given elsewhere (*8*, *9*, *13*, *14*). In brief, this calculation uses chemical-specific species sensitivity distributions (SSDs) to describe the variation in sensitivities for a set of species under acute or chronic exposure to a certain compound. SSDs can be described by various distribution curves, i.e., logistic, normal and triangular distributions. Several studies have demonstrated that the logistic distribution for log-transformed toxicity data is valid (*15*, *16*).

The ssPAF can be used as an approximation of the ecological risk of a single substance to the ecosystem at measured or predicted ambient concentration (*17*, *18*) and is calculated by

$$\mathrm{ssPAF} = \frac{1}{1 + e^{-(\log(C) - \alpha/\beta)}} \qquad (1)$$

where $C$ is the environmental concentration of the compound under consideration and $\alpha$ and $\beta$ reflect the logistic distribu tion of the SSD. Following De Zwart (*15*), the $\beta$ or the slope of the species sensitivity distributions was assumed to be equal for compounds with the same toxic mode of action (TMoA).

To aggregate ssPAF values to a single overall msPAF, an aggregation protocol was applied (*8*, *9*, *12*, *15*). This protocol is based on the application of two toxicological models: concentration addition (CA) and response addition (RA). CA was applied for compounds that have the same TMoA. The cumulative and potentially affected fraction of species for mixtures of chemicals with the same toxic mode of action ($PAF_{TMoA}$) is read by toxic unit (TU) addition for a single TMoA and is calculated by

$$\mathrm{PAF}_{\mathrm{TMoA}} = \frac{1}{1 + e^{-\log\sum TU_{TMoA}/\beta_{TMoA}}} \qquad (2)$$

where $\sum TU_{TMoA}$ is the sum of the toxic units for all chemicals with the same mode of action and $\beta_{TMoA}$ is the mode of action specific $\beta$.

The modes of action specific PAF or $PAF_{TMoA}$ values were finally aggregated to an overall msPAF by RA (see eq 3), assuming that the susceptibility of species for the (groups of) chemicals is statistically independent ($r = 0$).

$$\mathrm{msPAF} = 1 - \prod_{\mathrm{TMoA}} (1 - \mathrm{PAF}_{\mathrm{TMoA}}) \qquad (3)$$

**Relative Importance Assessment.** The relative contribu tion of a single substance to the overall toxic pressure ($R$) was defined by

$$R = 1 - \frac{\mathrm{msPAF}_{n-1}}{\mathrm{msPAF}} \qquad (4)$$

where msPAF represents the toxic pressure on ecosystems caused by the full set of chemicals and $msPAF_{n-1}$ is defined as the toxic pressure on ecosystems caused by the set of chemicals without the contribution of the chemical under consideration.

**Case Study.** A case study area was selected in order to inspect the technical feasibility and outcomes of the toxic pressure calculation. The study area, the Southern Bight of the North Sea, was chosen to investigate potentially typical output patterns for a remote system influenced by an array of human activities downstream of highly populated river catchments of the rivers Rhine, Meuse, and Scheldt. This part of the North Sea is the terminal station of these rivers, and the refreshment-time of the North Sea is relatively long (approximately 30 d) compared to the streaming-time of the three rivers from headwater to the North Sea (5-10 d). Relative to this unstable situation within rivers, we consider the Southern Bight of the North Sea as a "remote" and rather stable ecosystem.

**Substances.** Substances were selected from a list of nearly 2700 chemicals that were studied for priority setting of existing chemicals with EURAM. From this list, 343 well-defined single organic substances with ecotoxicity data available for at least three major taxonomic groups ($n$ = >3) were selected. Substance data were taken from IUCLID (*5*) and from Estimation Programs Interface for Windows (EPIWIN; ref *19*). Mainly log-normal distributions were used to quantify the uncertainty in physicochemical parameters (*20*). Ad ditional information on the uncertainty distributions and the physicochemical properties of all 343 HPVCs are provided in the Supporting Information.

**Emissions.** Amounts produced and used in the Rhine-Meuse-Scheldt catchments were derived from EU-wide production volumes. Since the surface area of the Rhine-Meuse-Scheldt region is approximately 1/16 of the EU and the population density is about three times above average, 3/16 of the EU production volume was assigned to the region modeled. Emissions into the environment in the Rhine-Meuse-Scheldt catchments were estimated on the basis of EU-TGD emission tables (A- and B-tables; ref *7*). These tables were simplified considerably to allow emission estimation on the basis of the industrial category only. The condensed emission tables used are provided in the Supporting Information, as well as additional information on the procedure to estimate the emission. The obtained emissions need to be viewed as rough estimates only. Further to this, it is known that TGD emission tables are useful only as estimates of upper limits; TGD tables were found to overestimate real emissions by up to 3-4 orders of magnitude (*20*). This leads to a very great, however unknown, uncertainty in the estimated emissions. We have dealt with this by treating the emission as a stochastic variable with a uniform distribution between the

estimated value and a factor 1000 lower than the estimated value. Deterministic emission amounts to air, water, and soil on the continental scale are given in the Supporting Information.

**Concentrations.** The multimedia fate model SimpleBox3.0 (*21*) was used to predict steady-state chemical concentrations see Figure 1). SimpleBox3.0 is a nested multimedia fate model of the "Mackay type" (*22*, *23*). It uses substance-specific parameters and emission profiles to air, water, and soil as input to calculate steady-state concentrations of chemicals in the various environmental compartments. The environ ment is modeled as a set of 40 well-mixed, homogeneous compartments (air, freshwater and marine water compart ments, sediment, soil, and vegetation compartments) on a regional, continental, and global (arctic, moderate, tropic) scale. Default settings of the regional scale are set to match the Rhine-Meuse-Scheldt basin. The continental scale is set to represent Western Europe.

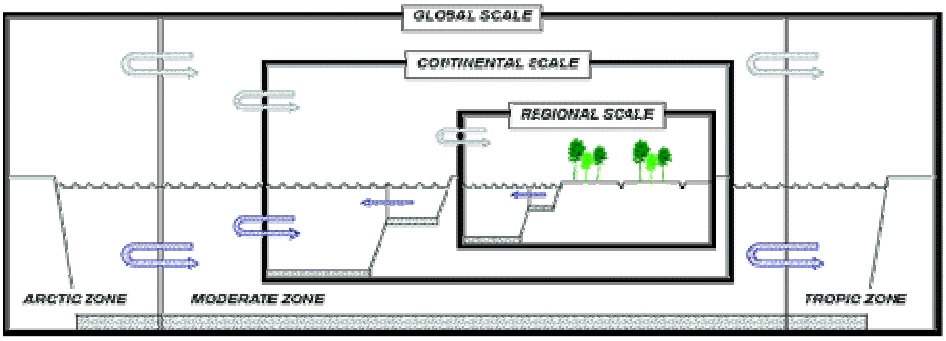


Figure 1 Schematic representation of the multimedia fate model SimpleBox3.0.

The SimpleBox version used in the present study was modified according to the description given by Hollander et al. (*24*), to correct mass fluxes from soil by volatilization, leaching, run off, and drainage by depth-specific soil concentrations.

**Ecotoxicity Data.** Log-transformed species-averaged EC50 values of chemicals, $\alpha$, and interspecies variances in toxicities, $\beta$, were derived from the RIVM e-toxBase (*25*) and EURAM (*3*). The RIVM e-toxBase is a database on ecotoxicity data comprising the data from U.S. Environmental Protection Agency's Ecotox (*26*) database (aquatic, terrestrial, plants) combined with data from ongoing projects at RIVM, with a test-duration of at most 20 days. The EC50-data refer to both lethal and sublethal effects. Data pertain not only to ecotoxicity test endpoints (NOECs, EC50s) but also to a primary toxic mode of action and the taxonomical clas sification of the tested species. Data are grouped per species to prevent the species with a large number of test-data to be over-represented in the SSD curve. In our study, we confined ourselves to HPVCs with test data for at least three major taxonomic groups (fish, algae, daphnia). In Table 1, toxic modes of action and corresponding number of chemicals included in the case study are listed. The $\beta$ values are assumed to be mode of action-specific (*15*) and substances in e-toxBase acutely tested with 10 or more species are selected for the estimation of $\beta$. Uncertainty in $\alpha$ is characterized by a student's t-distribution and uncertainty in $\beta$ by a log-normal distribution. Detailed information is provided in the Sup porting Information.

**Uncertainty Propagation.** Monte Carlo simulations were performed with Latin Hypercube sampling (LHS) using Crystal Ball 5.0 (*30*) to quantify the degree of uncertainty in the overall toxic pressure (msPAF) and the relative importance of an individual chemical (*R*). Due to technical limitations, the Monte Carlo experiments consisted of two consecutive simulations. The uncertainty in chemical concentrations in the marine water was quantified by performing a Monte Carlo experiment in SimpleBox3.0. Output values of the concentrations were fitted well to log-normal distributions. In the second Monte Carlo experiment, the log-normal distribution functions of the concentrations and uncertainty distributions of the ecotoxicological parameters ( and ) were used to determine the uncertainty in ssPAF, msPAF, and *R*. The Monte Carlo simulations consisted of 10 000 iterations. A contribu tion to variance analysis, based on rank correlation, was performed to determine which uncertain input parameters had the greatest impact on the relative importance of a single substance.

## Results

**Overall Toxic Pressure.** The overall EC50-based toxic pres sure of the 343 EU high-production-volume chemicals considered appears to amount 2.5% (median value), with an uncertainty range from 0.1% (5th percentile) to 100% (95th percentile), as shown in Figure 2. The result implies that (with a reliability of 95%) at least 0.1% of all species in the regional marine water compartment are possibly exposed to concentrations above their EC50 as a result of the use of the 343 HPVCs in the combined catchments of Rhine, Meuse, and Scheldt.

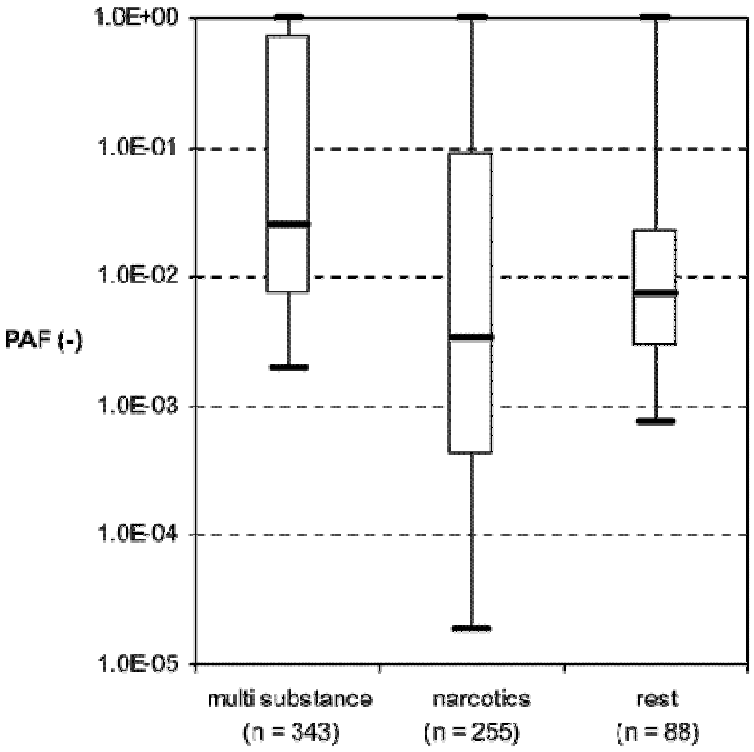


Figure 2 Boxplots of the overall toxic pressure in the North Sea coastal waters, influenced by the Rhine-Meuse-Scheldt basin, for all 343 high-production-volume chemicals (msPAF; $n$ = 343), toxic pressure caused by narcotics only ($PAF_{\sum narcotics}$; $n$ = 255), and toxic pressure caused by the remaining set of chemicals

($PAF_{rest}$; $n$ = 88). The center of the box represents the median value, the edges of the box indicates the 25th and 75th percentiles, and the whiskers represent the 5th and 95th percentiles of the uncertainty distributions. Toxic pressures were calculated assuming concentration addition within toxic modes of action (TMoA) and response addition between TMoA.

As the majority of the 343 HPVCs have a narcotic TMoA (i.e., nonpolar narcosis, polar narcosis, or ester narcosis; $n$ = 255), the msPAF is divided in the toxic pressure caused by narcotics only ($PAF_{\sum narcotics}$) and the toxic pressure caused by the remaining chemicals ($PAF_{rest}$). The results indicate that, although narcotics dominate the data set regarding numbers of chemicals, narcotics do not dominate the toxic pressure in the regional marine water compartment.

**Relative Importance.** Contributions of individual chemicals to overall toxic pressure ranged without exception from 0 to nearly 1. All the chemicals considered had extremely low 5th percentile and even median $R$ values, indicating a lack of evidence of substantial contributions to overall toxic stress for any of the individual chemicals (Figure 3). The median $R$ values of single substances ranges from $1 \times 10^{-2}$ to $1 \times 10^{-15}$, with most chemicals $R = 1 \times 10^{-8}$. These assessments do show, however, that there are upper limits to what individual chemicals have contributed to overall toxic pressure. The results indicate that with a reliability of 95%, the great majority of the HPVCs (283 of the 343; 83%) contribute insignificantly ($R < 1 \times 10^{-3}$) to the total toxic pressure. With complementary confidence (5%), a small minority (60 of the 343; 17%) contributed more than $1 \times 10^{-3}$. An overview of the 5th percentile, median, and 95th percentile of the relative importance ($R$) of each chemical is given in the Supporting Information.

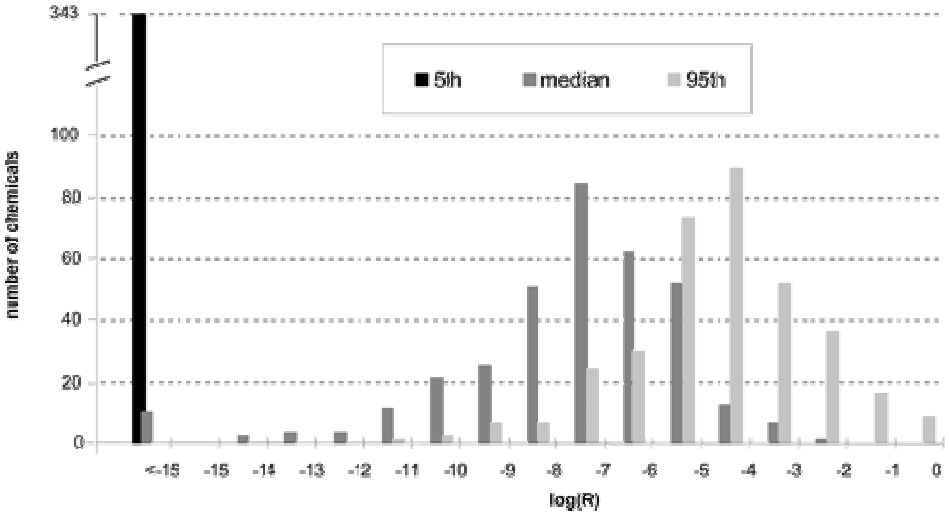


Figure 3 Uncertainty distributions (5th percentile, median, and 95th percentile) of the relative importance ($R$) of each of the 343 substances to the overall toxic pressure (msPAF) in relationship with the corresponding number of chemicals. The relative importance is calculated from the ratio of the toxic pressure on ecosystems caused by the set of chemicals without the contribution of the chemical under consideration ($msPAF_{n-1}$) and the toxic pressure on ecosystems caused by the full set of chemicals (msPAF).

The gross ranking results from this study were compared with calculated aquatic priority scores (APS) for each of the 343 HPVCs from EURAM. In Table 2,

numbers of chemicals are shown that are (not) part of the top 60 chemicals based on the $R_{95}$ and are (not) part of the top 60 chemicals based on APS.

Outcomes were only in part similar. Approximately half (27 out of 60) of the chemicals that belong to the top 60 following the msPAF-concept also belong to the top 60, based on EURAM scores.

**Contribution to Variance.** A contribution to variance analysis was performed to identify which uncertain input parameters contributes most to variance in the relative importance of a single substance. In Table 3, results are shown as a minimum, median, and maximum contribution to variance of each uncertain parameter, based on a random selection of chemicals ($n$ = 10) out of the 343 HPVCs.

The results show that the mode of action specific slope ( ) of the species sensitivity distributions had the highest contribution to variance (median = 49.4%). The uncertainty in degradation half-lives in water (5.7%), emissions (3.8%) and (1.7%) apparently also contributes probably meaning fully to variance in the relative importance of a single substance. All the substance-specific parameters have a cumulative contribution to variance of approximately 60 to 80%. As uncertainty in the relative importance of one chemical depends also on uncertainty distributions of the remaining 342 HPVCs (following equation), 20 to 40% of the contribution to variance is due to uncertainties in substance-specific parameters of the remaining 342 high-production-volume chemicals.

## Discussion

This paper used toxic pressure (msPAF) calculation for a case study to explore the issues of overall impacts of complex mixtures of chemicals on aquatic ecosystems in coastal waters as a result of chemicals emission scenarios in associated river basins and of impact-based ranking. The procedure appeared technically feasible and is organized in such a way that it can be adapted to serve for various applications and situations, e.g., looking at different toxicological endpoints (e.g., EC50, NOEC, human toxicity), different environmental compartments (e.g., coastal water, freshwater, soil) and different sets of chemicals (e.g., metals, pesticides, pharmaceuticals). Method results can support gross ranking, like in the categories of negligible and possibly relevant contributions to overall toxic pressure, which, in turn, may be of help in designing monitoring programs. Uncertainty assess ment could identify the model parameters that were most uncertain. Given current uncertainties, the method primarily provides insight in what we do not know but should know, and what should thus be subject of priority research for regional risk assessment problems.

**Overall Toxic Pressure.** Application of the method to the North Sea coastal waters influenced by the Rhine-Meuse-Scheldt basin, for 343 high-production-volume chemicals, showed that the median toxic pressure is relatively low (2.5%). The confidence intervals of this estimated median are, however, wide, which implies that the $msPAF_{EC50}$ can in fact range from 0.1 to 100% of the species, suggesting possible but less probable degrees of impact in the tails. The predicted value is probably underestimated as only 343 single organic chemicals out of the nearly 2700 chemicals, that were studied for priority setting of existing chemicals with EURAM, were included in this study. The sub-selection of 343 compounds is thus a limitation, related to the availability of relevant data for all steps in the modeling process. Among others, emissions of heavy metals, pesticides, and metabolites are also not included in the calculations. Including

these chemicals will definitely result in an increase in the predicted toxic pressure.

The estimates are distantly supported by information on the general "toxicity" status of the North Sea because the predicted toxic pressure distribution is in line with the apparent lack of regular mass mortality for the considered area. Validation of the method by looking at measured concentrations would be helpful, but this is not feasible within the current context, due to lack of monitoring data for the high-production-volume chemicals included in this study (*29*). The median value for overall toxic pressure is further in line with other msPAF values estimated for field conditions, where the latter are partly based on measured concentrations. De Zwart (*30*) reported a median $msPAF_{EC50}$ in river stretches in Ohio of approximately 6% (range in local rivers stretches from 0 to 55%) for a set of household chemicals and metals. Like in the coastal waters, the msPAF variability was high, but the median values were also relatively low. The Ohio case study suggested further that (on average) 10% EC50 msPAF is associated to, at maximum, a 10% loss of species. Mulder et al. (*12*) found statistically significant evidence for a cascade effect of contaminants on the local butterfly community through effects on host plant species. Considering the relative methods used in the cited studies (msPAF correlated to impacts), it can be concluded that msPAF can be used as a relative measure of likely impacts, but not yet as an absolute predictor of toxic effects on species as semblages.

**Relative Importance.** The relative contributions of the separate compounds would conceptually allow for ranking within sites among compound relevance (this paper), ranking across sites (*31*), and ranking of most impacted species types (*32*). The relative importance of a single chemical in the overall toxic pressure ranges from 0 (5th percentile) to almost 100% (95th percentile). Despite the variation and although the sets of tested species for each of the 343 compounds are not necessarily representative for the species assemblages in coastal waters, the results strongly suggest that impacts larger than 50% likely occur due to a minority of the considered compounds. The results thus support ranking by gross classification only, the most prominent one being the distinction between compounds that are unlikely to contribute to total toxic pressure ($R_{95}$ < 0.1%; approximately 83% of the chemicals, $n$ = 283) and those that might contribute ($R_{95}$ > 0.1%; approximately 17% of the chemicals, $n$ = 60), with 0.1% chosen as an example. The design of monitoring programs, or risk management decisions could, fully or in part, be based on information from toxic pressure calculations to help focus on the compounds that may contribute most to the toxic pressure in a regional compart ment. When uncertainties appear large for a case, the approach, at least, identifies the issues of which we know least.

Differences between our gross ranking results and the aquatic priority scores from EURAM are attributable to differences in the specifications of the exposure scenario (generic chemical analysis versus specific site analysis), and handling parameters and uncertainty. Uncertainty is explicitly addressed in the msPAF concept, so that similar compounds can show overlap and the ranking is no more absolute. Related to this, the msPAF concept removes the "hidden assump tions" of some PNECs, for example in the cases where the PNEC is determined by applying a safety factor on the lowest toxicity data point. When safety factors would occur regularly, the outcomes of ratio-based rankings would be compromised by this, as well as the ecological interpretation of the probable impact. By taking into account the often-observed nonlinearity of species sensitivity distributions, as well as SSD-slope differences in chemicals (*15*), the results provide a conceptually more realistic approach to estimate ecotoxicological effects. This is further supported by some initial validation studies (*11*, *12*, *30*).

As our results support only gross ranking of chemicals and comparison with aquatic priority scores from EURAM showed partial similarities, our study indicates that priority assessment of chemicals based on their impact on ecosystems should be interpreted with care as a result of various uncertainties. Variances should be taken into account to derive scientifically justified conclu sions.

**Model Uncertainty.** Concentration addition (CA) and response addition (RA) are regarded as the two biologically plausible reference concepts suitable to calculate an expected combined effect (msPAF) based on effect information from the components of the mixture under consideration. The appropriate selection of the reference concept to be applied is believed to depend on the similarity of the TMoA, because the TMoA of a compound is codetermined by the receptor (*9*). A potentially better concept is to consider "mechanisms" of action instead of "modes" of action. Mechanism of action denotes the molecular sequence of events leading from the absorption of an effective dose of a chemical to the production of a specific biological response in the target organ, whereas mode of action is a more general description of chemical action (*33*). However, there is almost a full lack of operational knowledge to use mechanisms of action. It was shown that databases are available to assign primary TMoA to a compound (*25-27*). In contrast, to evaluate whether mixture toxicity can be predicted by mechanism of action of its component chemicals, a detailed characterization of pharmacodynamics, pharmacokinetics, and slope of dose response curves may be necessary. Although we thus neglect secondary and higher order effects, there is reason to interpret the outcomes with confidence. This relates to the *numerical behavior* of the msPAF approach. With slope values of the SSDs as given here, the numerical outcomes of the alternative models is often indistinguishable. This was shown by example calculations from Traas et al. (*8*) and Deneer (*34*), while Drescher and Boedeker (*35*) provides an in-depth model behavioral analysis to support the findings of the example calculations.

Besides these conceptual assumptions, a number of other simplifications and assumptions have been included in our study. The regional marine water in the Rhine-Meuse-Scheldt basin is modeled in SimpleBox 3.0 as a homogeneous compartment, implying that the chemical stress and the chemical ranking are based on averaged regional environmental and meteorological parameters. Chemical behavior and stress, however, can differ between various locations within a region, resulting in site-specific risks of chemicals (*31*). The species sensitivity distributions in the case study are based on acute toxicity data (EC50) only. Application of chronic toxicity data (NOECs) may change the results, as the chronic influences of specific chemicals on growth and reproduction may be different from those of acute exposure. The toxic pressure prediction and the calculation of the relative importance are based on 22 different toxic modes of action, mainly narcotics. Highly specific TMoA (like endocrine disruption and imposex effects) are not represented in the databases for constructing SSDs, and are thus not represented in the predictions and rankings. Further, the representativity of the test species for the assessed system needs be scrutinized, since test species are usually selected for their manageability under laboratory conditions, their sensitivity to toxicants and an assumed (general) ecological relevance. As typical test data sets mainly consist of fish, daphnia, and algae, effect data of terrestrial organisms, top predators, or microorganisms may also affect the calculated relative importance of an individual chemical (*3*). Ecological interac tions, habitat factors, or specific importance of keystone species and functional groups are also not accounted for in SSDs. The habitat (freshwater, seawater) of the species assemblages used to construct SSDs, however, does not have a significant influence on the assessment of a hazard (*36*).

**Parameter Uncertainty.** Two main assumptions have been made for the present study. First, uncertainty distribu tions for each substance-specific parameter were assumed to be the same for all chemicals, with no distinction between chemicals with more or less experimental data available. Second, although correlation in uncertainty distributions of substance-specific parameters within chemicals was taken into account (e.g., solubility and $K_{ow}$), no correlation is assumed between chemicals, whereas it is reasonable to think that some parameters may be correlated (e.g., emission estimates). Uncertainties in msPAF and *R* may be reduced by taking into account these issues in future applications.

Analysis of the contribution to variance showed that the SSD-parameters ($\alpha$ and $\beta$), the emissions, and degradation half-lives in water are the most important uncertainties in determining the relative contribution of an individual chemical to the overall toxic pressure in the case study. A number of issues could help in the further development and refinement of the uncertainty analysis in this study. First, focus could be put on the most uncertain parameter, here $\beta$. Uncertainty in $\beta$ may be reduced by further refinement and assuming a mode of action specific uncertainty distribution. Second, chemical releases in the Rhine-Meuse-Scheldt basin were predicted by means of the production volumes and emission factors by assuming that the use intensity for all chemicals in this region is 3 times greater than the average of the entire EU, thus 3/16 of the EU production volume was assigned to the region modeled. This assumption is rather uncertain, however, as we assumed that the emissions are overestimated by a factor 1000, a large uncertainty is already addressed to the emission estimates. This method to estimate chemical releases is also applied in EUSES (*37*), as better procedures to estimate emissions profiles are lacking. Investigations toward a more detailed and accurate estimation of chemical emissions should be realized to improve the reliability of the results. Third, for most chemicals, experimental degradation rates in air are used and, if not, degradation half-lives in air were estimated from structure reactivity relationships. Degradation rates in water, soil, and sediment were estimated from primary aerobic biodegradation half-lives. Investigations to improve the quality of degradation half-lives and to reduce the uncertainty are required. Fourth, the uncertainty in the sample mean of the log-transformed toxicity data ($\mu$) influences the model outcomes. A dominant factor in the uncertainty distribution of $\mu$ is the number of species tested (*n*) for each chemical. Our calculations indicated that chemicals with $n = <4$ have a relatively large uncertainty distribution, resulting in a large probability distribution of msPAF and *R*. In future applications, quantitative structure-activity relationships may be used to derive extra ecotoxicological input for species sensitivity distributions (*38-40*).

**Practical Applications.** Conceptually, the toxic pressure calculation method can serve for ranking purposes in environmental management, e.g., ranking across sites, rank ing of compounds, and of affected species groups. Practically, uncertainties may prohibit absolute interpretation of all these rankings. Given the current degree of uncertainties, the case study results suggest that the method may primarily serve to identify the most uncertain parameters, with as consequence of the focusing of further scientific research. For decision purposes, absolute outcomes are often needed in current practice, like decisions based on PEC/PNEC ratios. On the other hand, calculation of toxic pressure estimates (and their variance) can be relevant considering the upcoming use of weight-of-evidence approaches for site-specific as sessments in which it is considered that no single method provides a complete view on the facts, and the application of various methods can help to make responsible decisions. Regulatory agencies commonly have the responsibility to design and apply methods that provide sufficient protection of humans and the environment. They have to solve the

problem that many compounds are produced at different locations in different amounts, and that mixture regulation on the regional scale is not possible. The toxic pressure calculation method does not solve the problem that, in practice, this task boils down to regulating on a per-compound basis.

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**Supporting Information Available**

Species sensitivity distributions, emission estimates, substance-specific parameters, and their emission uncertainty distributions as well as a table with parameter settings and the uncertainty distribution (5th percentile, median, 95th percentile) of the relative importance of each chemical. This material is available free of charge via the Internet at http://pubs.acs.org.

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Table 1. Primary Toxic Modes of Action and Corresponding Number of Chemicals (*n*) Included in the Study

| toxic mode of action[a] | abbreviation | n | | toxic mode of action[a] | abbreviation | n |
|---|---|---|---|---|---|---|
| nonpolar narcosis | NN | 185 | | uncoupler of oxidative phosphorylation | UOP | 3 |
| polar narcosis | PN | 51 | | plant growth inhibition | PGI | 3 |
| alkylation/arylation based reactivity | AABR | 19 | | reactive dinitro group | RDG | 2 |
| ester narcosis | EN | 19 | | reactive | R | 2 |
| diesters | D | 15 | | inhibition of acetylcholinesterase (carbamates) | IAC | 1 |
| reactions with carbonyl compounds | RCC | 9 | | inhibition of cell division | ICD | 1 |
| unknown[b] | UN | 8 | | reactions with sulfhydryl groups | RSG | 1 |
| acrylate toxicity | AT | 6 | | aromatic | A | 1 |
| reactive diketones | RD | 6 | | inhibition of chlorophyll synthesis | ICS | 1 |
| inhibition of photosynthesis | IP | 5 | | quaternary ammonium compounds | QAC | 1 |
| inhibition of Acetylcholinesterase (organophosphates) | IAO | 3 | | wetting agent | WA | 1 |

[a] The terminology for modes of actions is equal to the terminology used in De Zwart (*15*) and ASTER (*27*).[b] Toxic modes of action for eight chemicals are not established in e-toxBase (*25*) or in ASTER (*27*) and are, therefore, classified as "unknown".

Table 2. Comparison of Gross Ranking Results Based on the 95th Percentile Relative Contribution of an Individual Chemical ($R_{95}$) to Overall Toxic Pressure (msPAF) with Calculated Aquatic Priority Scores (APS) from EURAM

| | APS (1-60) | APS (60-343) | |
|---|---|---|---|
| $R_{95} > 1 \times 10^{-3}$ | 27 | 33 | 60 |
| $R_{95} < 1 \times 10^{-3}$ | 33 | 250 | 283 |
| | 60 | 283 | 343 |

The gross ranking exists of a distinction in negligible chemicals (chosen criteria: $R_{95} < 1 \times 10^{-3}$; $n = 283$) and possibly relevant substances ($R_{95} > 1 \times 10^{-3}$; $n = 60$). *APS* are also divided in two categories with the upper 60 chemicals as chosen cutoff criterion to identify outcome similarities between the two procedures.

Table 3. Results of the Contribution to Variance Analysis for the Relative Importance ($R$) of 10 Randomly Selected Chemicals out of the Set of 343 Considered[a]

| | contribution to variance | | |
|---|---|---|---|
| substance-specific parameter | minimum | median | maximum |
| mode of action specific slope of the SSD ( ) | 29.1 | 49.4 | 72.9 |
| degradation half-life in water | 3.3 | 5.7 | 14.0 |
| emissions | 2.3 | 3.8 | 8.0 |
| sample mean of the log- transformed EC50-values ( ) | 0.8 | 1.7 | 27.9 |
| octanol-water partitioning coefficient ($K_{ow}$) | 0.0 | 0.1 | 1.1 |
| solubility | 0.0 | 0.1 | 0.9 |
| degradation half-life in soil | 0.0 | 0.0 | 1.3 |
| degradation half-life in sediment | 0.0 | 0.0 | 0.1 |
| vapor pressure | 0.0 | 0.0 | 0.1 |
| degradation half-life in air | 0.0 | 0.0 | 0.0 |
| melting point | 0.0 | 0.0 | 0.0 |
| substance-specific parameters other chemicals | 20.6 | 32.1 | 41.9 |

[a] Contribution to variance of each uncertain substance-specific parameter is shown as corresponding minimum, median, and maximum percentages based on the random selection.