

Occurrence and Toxicity of 331 Organic Pollutants in Large Rivers of North Germany over a Decade (1994 to 2004)

Ralf B. Schäfer,^{*,†} Peter Carsten von der Ohe,[‡] Ralph Kühne,[§] Gerrit Schüürmann,^{§,||} and Matthias Liess[⊥]

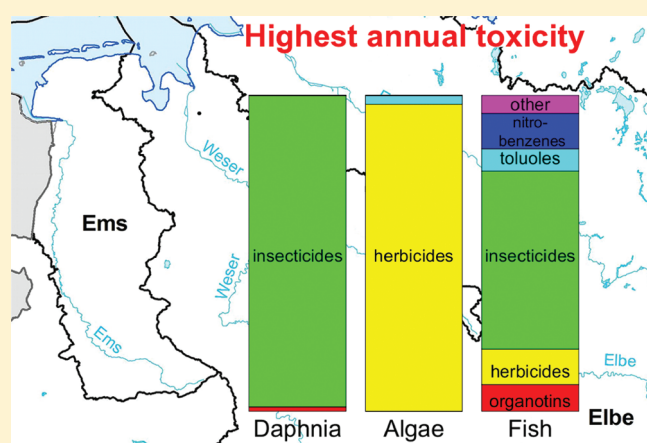
[†]Institute for Environmental Sciences, University Koblenz-Landau, Fortstrasse 7, 76829 Landau, Germany

[‡]Department of Effect-Directed Analysis, [§]Department of Ecological Chemistry, and [⊥]Department of System Ecotoxicology, UFZ, Helmholtz Centre for Environmental Research, Permoserstrasse 15, 04318 Leipzig, Germany

^{||}Institute for Organic Chemistry, Technical University Bergakademie Freiberg, Leipziger Strasse 29, 09596 Freiberg, Germany

S Supporting Information

ABSTRACT: We analyzed the detection frequencies and concentrations for 331 organic compounds measured between 1994 and 2004 in the four largest rivers of north Germany, the Elbe, Weser, Aller, and Ems Rivers, and we assessed the potential risk for aquatic fauna using experimental and predicted acute toxicity data for the green alga *Pseudokirchneriella subcapitata*, the crustacean *Daphnia magna*, and the fish *Pimephales promelas*. The detection frequency for most compounds decreased significantly from 1994 to 2004. Polycyclic aromatic hydrocarbons (PAHs) were most frequently detected, while pesticides were the most important chemical group concerning toxicity for the standard test organisms. The predicted toxicity for *D. magna* was significantly higher than for the other organisms and reached levels envisaging acute toxic effects on the invertebrate fauna, still in 2004. Most of the compounds responsible for potential acute effects on aquatic organisms are currently not considered as priority substances in the European Union, while only 2 of 25 priority substances that have been measured occurred at levels that may be relevant in terms of toxicity for the selected test organisms. We conclude that attenuation of pesticides and other organic toxicants should play an increased role in river basin management.



INTRODUCTION

A wide range of activities in agriculture, industry, and households is associated with the use of organic chemicals. Depending on the physicochemical properties and usage, these compounds may be released to the environment and can result in the pollution of freshwater ecosystems.¹ In the European Union (EU), the Water Framework Directive (WFD) was established in 2000 and aims at achieving good ecological and good chemical status of surface waters by 2015.² Besides water chemistry characteristics such as nutrient concentrations, the evaluation of chemical status relies on compliance with Environmental Quality Standards (EQS) for 33 so-called priority substances (PS), among them metals, pesticides, polycyclic aromatic hydrocarbons (PAH), and organotin compounds.³ Moreover, the EU member states are requested to identify further compounds of concern for the respective river basins, which could compromise good ecological status.

One approach for the identification of ecologically relevant compounds is long-term screening of the environment for a large set of chemicals in concert with an assessment of the potential toxicity of the observed concentrations, which can be done by use of measured or predicted effect concentrations for standard test

species.⁴ Given the high costs involved, long-term river basin monitoring studies on hundreds of organic contaminants in freshwater ecosystems, with a few exceptions,⁵ have been conducted only through governmental monitoring programs. However, there is a paucity of meta-analyses of such data concerning the toxicity of contaminants for freshwater ecosystems. This may be because the toxicity assessment of monitoring data is hampered by the scarcity of acute toxicity data for aquatic species for many compounds. For example, toxicity data for algae, invertebrates, and fish were available for only 16% of 500 compounds.⁴ However, quantitative structure–activity relationships (QSAR) and read-across methods can be used to estimate the toxicity of chemicals if experimental toxicity data for compounds of similar structure are available.

To our knowledge, this paper presents the most comprehensive long-term study on the concentrations and associated

Received: April 15, 2011

Accepted: June 13, 2011

Revised: June 9, 2011

Published: June 13, 2011

Table 1. Location of Sampling Sites with Discharge and Statistics on Measurement of Compounds

sampling site	river	basin	median discharge ^a (m ³ /s)	measurement frequency	total no. of samples measured	total no. of compds measured	compds ≥ LOQ ^b	total no. of measurements
Cuxhaven	Elbe	Elbe	^c	monthly ^d	138	238	167	21 185
Grauerort	Elbe	Elbe	^c	monthly ^d	143	319	225	25 013
Schnackenburg	Elbe	Elbe	493	monthly ^d	149	319	229	31 508
Herbrum	Ems	Ems	57	monthly ^{d,e}	123	284	183	22 631
Farge	Weser	Weser	223	monthly ^{d,e}	122	284	179	21 241
Hemeln	Weser	Weser	74	monthly ^f	189	272	152	23 552
Verden	Aller	Weser	72	monthly ^f	189	272	154	23 400

^aFor monthly average data of the years 1994–2001. Data were kindly provided by the Federal Institute for Hydrology (BFG), Koblenz, Germany.

^bCompounds detected above the level of quantification (LOQ). ^cTidal zone. ^dOnly 9 or 10 measurements in 1995 and 1996. ^eNo measurements in 1994. ^fNo measurements in 1994 and 2004, only 9 or 10 measurements in 1995 and 2003.

ecological risks of organic toxicants in large rivers in central Europe. In detail, we describe the exposure of four large rivers of north Germany to 331 organic compounds over a period of 10 years, using the results of monthly monitoring programs from governmental agencies. The compounds mainly belonged to the following chemical groups: (A) pesticides and transformation products; (B) polychlorinated biphenyls (PCB); (C) polycyclic aromatic hydrocarbons (PAH) and derivatives; (D) halogenated benzenes and nitrobenzenes; (E) halogenated alkanes; (F) phenols and chlorophenols; (G) anilines, anisoles, and alkylated benzenes; (H) toluenes, and halogenated derivatives; and (I) organotins. A risk assessment of the measured compound concentrations for fish, invertebrates, and algae was conducted by use of experimental acute toxicity data for standard test organisms: fathead minnow *Pimephales promelas*, waterflea *Daphnia magna*, and green alga *Pseudokirchneriella subcapitata*. Where no experimental data were available we used estimates of a novel QSAR approach as an approximation. Finally, the identified compounds of concern were compared to the assessment of chemical status based on priority substances as outlined in the WFD.

EXPERIMENTAL SECTION

Description of Sampling Sites. Seven sampling sites in four of the five largest rivers of north Germany were sampled monthly as part of environmental monitoring programs from 1994 to 2004 (Table 1; Figure S1, Supporting Information). The median of the mean monthly discharge in the sites ranged from 57 to 493 m³/s (Table 1). In general, all rivers are heavily modified, were dredged for shipping, and receive inputs of inorganic and organic pollutants from industry, agriculture, households, and sewage treatment plants, although the magnitude of impact may differ between the rivers.

Data Acquisition and Quality. The results of the chemical water monitoring in the seven sampling sites were kindly provided by the Lower Saxony Water Management, Coastal Defense and Nature Conservation Agency (NLWKN) and comprised monthly observations of a total of 331 organic pollutants (see Table S1 in Supporting Information for complete compound list) measured from 1994 to 2004.^{6,7} Between 78 and 300 compounds were analyzed in each of the 1053 samples from all sampling sites (Table 1), except for five samples where only 15–61 compounds were analyzed. The monitoring program in the seven sites exhibited differences in the total numbers of measured compounds and samples per site and to a minor extent in the measurement frequency (Table 1).

All steps of the monitoring program, for example, sampling, sample storage, sample treatment, and chemical analysis, were carried out according to certified methods and in compliance with a quality assurance program to ensure reliability and compatibility of the results (see Table S1, Supporting Information, for details on the chemical analysis). Briefly, the sampling consisted of taking a nonfiltered whole water grab sample. In the laboratory the samples were handled according to the respective method and measured by gas chromatographic or high-performance liquid chromatographic methods. The chemical analysis was conducted solely in accredited laboratories. Due to the long observation time and different laboratories involved, the levels of quantification (LOQ) exhibited variation (Table 2; see Table S2, Supporting Information for full table).

Consideration of Compound Partitioning between Water and Suspended Particles. The concentrations of compounds in the monitoring data referred to the nonfiltered whole water sample. Therefore these concentrations could overestimate the concentrations in the water phase since a significant proportion of a hydrophobic substance may be adsorbed or bound to suspended organic particles, which can reduce the toxicity of that compound and should be accounted for in the risk assessment for aquatic organisms.⁸ We used a reformulation of the equilibrium partitioning approach to approximate the freely available concentration C_d (in micrograms per liter) of organic compounds:⁹

$$C_d = \frac{C_{\text{tot}}}{(f_{\text{OC}}K_{\text{OC}} + 1)}$$

where C_{tot} is the total concentration in the whole water sample in micrograms per liter, K_{OC} is the dimensionless soil organic carbon–water partitioning coefficient, and f_{OC} is the fraction of organic carbon that was approximated with the total organic carbon content (TOC).

Compilation and Estimation of Toxicity Data for Invertebrates, Algae, and Fish. If available, we used laboratory-derived acute toxicity data for standard test organisms [48-h median effect concentration (EC₅₀) for *D. magna*, 48-h to 96-h EC₅₀ for *P. subcapitata*, and 96-h median lethal effect concentration (LC₅₀) for *P. promelas*] to assess the risk of a compound. In the following, we do not distinguish between EC₅₀ and LC₅₀ and only use the term EC₅₀ to enhance readability. The toxicity data were compiled from peer-reviewed literature as well as available databases,⁴ and wherever possible peer-reviewed literature was consulted to confirm toxicity data from databases (see Table S2, Supporting Information, for further details).

Table 2. Compounds with Higher Than 40% Detection Frequencies with Their Minimum and Maximum Levels of Quantification, Maximum Concentration, and Number of Measurements in All Sites

CAS no.	English name	chemical group ^a	min LOQ ^b ($\mu\text{g/L}$)	max LOQ ^b ($\mu\text{g/L}$)	max concn ($\mu\text{g/L}$)	<i>n</i> ^c	detection frequency ^d (%)	priority substance no. ^e
206-44-0	fluoranthene	C	0.002	0.002	0.053	270	99	15
129-00-0	pyrene	C	0.002	0.002	0.046	224	99	
58-89-9	γ -hexachlorocyclohexane	A	0.000 08	0.000 08	0.03	795	98	18
60-00-4	EDTA	J	0.1	0.3	35	387	93	
85-01-8	phenanthrene	C	0.002	0.002	0.038	224	91	
205-99-2	benzo[<i>b</i>]fluoranthene	C	0.002	0.005	0.025	270	89	28
50-32-8	benzo[<i>a</i>]pyrene	C	0.002	0.005	0.024	270	88	28
193-39-5	indeno[1,2,3- <i>c,d</i>]pyrene	C	0.002	0.002	0.033	268	88	28
127-18-4	tetrachloroethylene	E	0.0002	0.002	1.2	697	88	29a
191-24-2	benzo[<i>ghi</i>]perylene	C	0.002	0.018	0.2	270	87	28
218-01-9	chrysene	C	0.002	0.002	0.024	224	85	
56-55-3	benz[<i>a</i>]anthracene	C	0.002	0.002	0.023	224	78	
139-13-9	nitrilotriacetic acid	J	0.1	0.5	10	421	77	
319-84-6	α -hexachlorocyclohexane	A	0.000 07	0.000 07	0.2	719	67	
207-08-9	benzo[<i>k</i>]fluoranthene	C	0.002	0.014	0.012	269	65	28
1912-24-9	atrazine	A	0.001	0.006	0.6	1017	62	3
118-74-1	hexachlorobenzene	A	0.000 04	0.000 08	0.03	673	58	16
75-25-2	bromoform	E	0.002	0.008	0.3	696	56	
56-23-5	carbon tetrachloride	E	0.0002	0.004	0.05	697	52	6a
118-96-7	2,4,6-trinitrotoluene	H	0.000 09	0.02	3.5	104	52	
67-66-3	trichloromethane	E	0.004	0.02	1.5	696	48	32
79-01-6	trichloroethylene	E	0.001	0.006	0.5	697	46	29b
1007-28-9	desisopropylatrazine	A	0.003	0.09	0.6	1037	44	
5915-41-3	terbutylazine	A	0.003	0.006	0.5	1037	43	

^a A, pesticides and transformation products; B, polychlorinated biphenyls; C, polycyclic aromatic hydrocarbons and derivatives; D, halogenated benzenes and nitrobenzenes; E, halogenated alkanes; F, phenols and chlorophenols; G, anilines, anisoles, and alkylated benzenes; H, toluoles, toluenes, and halogenated derivatives; I, organotin compounds; J, miscellaneous. ^b LOQ = limit of quantification. ^c *n* = total number of measurements. ^d Ratio for observations above LOQ. ^e As outlined in ref 3.

However, for only 207, 107, and 100 of the 331 compounds were experimental toxicity data found for *D. magna*, *P. subcapitata*, and *P. promelas*, respectively. Missing toxicity data were estimated from experimental values for similar compounds as described in Schüürmann et al.¹⁰ Initially, a data set containing available experimental toxicity values for each of the above-mentioned standard test species, containing about 1000, 550, and 700 experimental toxicity values, respectively, together with the respective chemical structures was recorded. To predict the toxicity of a compound not part of this set, the arithmetic average of the experimental values for the three most similar compounds from the data set was calculated. The similarity of compounds was evaluated by employing a atom-centered fragments (ACF) based approach.¹¹ Actually, the results of two different levels of ACF determination were combined and filtered by thresholds regarding similarity and number of similar compounds. The particular weights for the combination of these levels as well as the respective thresholds were fitted individually for each species by means of cross-validation with the training set. The averaged prediction error of the model ranged from 0.5 to 1.0 logarithmic unit for data where experimental values were available for reasonably similar compounds, that is, a similarity ≥ 0.75 on a scale from 0 to 1¹⁰ (see Table S2, Supporting Information, for the categories of similarity of the read-across compounds). In case sufficiently similar compounds were not available, baseline toxicity estimated

from the octanol–water partitioning coefficient (K_{ow}) was used, employing established QSAR models for the three standard test organisms.^{12,13} The structural alerts of compounds for which the baseline toxicity was estimated did not indicate enhanced toxicity.¹³ Compounds with a predicted toxicity 10 times higher than the estimated water solubility¹² and a melting point of more than 100 °C were excluded from the assessment.¹⁴

Toxicity Assessment. The toxicity of the dissolved water concentrations C_d for each of the three trophic groups was predicted by the toxic unit approach,¹⁵ where the toxic unit (TU) for a compound is the compound concentration divided by the respective 48-h or 96-h EC_{50} for the standard test species. We used the maximum TU (mTU), which is the highest TU of all observed individual compound concentrations in each sample, as an indicator for the minimal expected toxicity of the respective sample. We did not use the sum of all TUs (sumTU) in a sample because the sumTU exhibits a stronger dependency on the number of compounds measured and could overestimate toxicity of compounds with a dissimilar mode of action. The mTU accounted for >50% of the sumTU of a sample for 80%, 61%, and 40% of samples ($n = 1052$, one sample with no detection of compounds excluded from analysis) for *D. magna*, *P. subcapitata*, and *P. promelas*, respectively. This means that, for the majority of samples in the risk assessment for invertebrates and primary producers and a considerable fraction of the samples for the risk assessment of

fish, the most toxic individual compound as represented by the mTU would also dominate the toxicity of all compounds in terms of sumTU.

Chemical Status Assessment with Regard to Priority Substances. According to the WFD, a good chemical status requires compliance with either the maximum allowable concentration (MAC) EQS and the so-called annual average (AA) EQS values for the set of 33 priority substances. Of the 33 priority substances, a total of 25 organic compounds were measured in the basins of the study (see Table S2, Supporting Information). The number of measured priority substances varied between years and sites, with 6–18 and 9–22 measured compounds per site in the years 1994–1997 and 1998–2004, respectively (see Table S3, Supporting Information, for details). The latest available EQS values were used for compliance checking.³

Data Analysis. For the analysis of differences in the detection of chemicals between sites, years, and months, the compounds were split into 10 chemical groups (Table 2). Since the number of total measured compounds varied between sites, years, and months (Table 1), we used the relative detection frequency per sample for comparisons among these variables. Generalized linear models (GLM) with logit link were used to identify which of the variables (i.e., year, site, month, basin) and their interactions are relevant to explain variation in the response variable detection frequency, which was assumed to be binomial distributed.¹⁶ Stepwise model selection with the Akaike information criterion (AIC) as goodness-of-fit measure and starting with the intercept-only null model was used in order to identify the best-fit model. Since the best-fit model for the full data set [AIC 14 610, deviance 4807, degrees of freedom (df) 3828] contained several two-way interaction terms with the variable chemical groups, the analysis was conducted separately for each of the chemical groups in order to ease interpretation. In case of over- or underdispersion of a GLM, a quasi-binomial model was employed to verify the results of the binomial model.¹⁶ Significant differences between factor levels of sites and months were identified by a multiple comparison procedure with Tukey's all-pairwise comparison contrasts (TALC) as described in ref 17.

Given that the chemical status with regard to priority substances is based on annual values, we calculated maximum annual mTUs for each site for the risk assessment based on the mTU. Analysis of variance was employed to identify significant differences between basins, test species, and years for the response variable annual mTU, by the same model selection and multiple comparison procedures as described for the GLMs. In order to detect differences in the sensitivity of the three standard test organisms to the 331 organic compounds, the logarithmic ratio of their acute toxicity data (ECR) was calculated:

$$ECR_{x,y}(c) = \log \frac{EC_{50_x}(c)}{EC_{50_y}(c)}$$

for each compound c and for each of two species x and y . A similar sensitivity of two organisms to the compounds in the data set would translate to a median of 0 and an even spread toward positive and negative values for the ECR values.

All statistical computations and graphics were created with the open-source software package R (www.r-project.org) using version 2.8.0 (for Mac OS X, 10.5.5).

RESULTS AND DISCUSSION

Spatiotemporal Patterns of Occurrence of Organic Pollutants. Of the 331 compounds, 257 compounds were detected at equal or higher levels than the LOQ in the water samples of all rivers, whereas 74 compounds were detected only below the level of quantification (see Table S2, Supporting Information). Twenty-four compounds were detected in more than 40% of the water samples (Table 2). With the exception of one sample (March 2003 in Cuxhaven), at least one substance was detected in each water sample. On average, 14% of the measured compounds were found in a sample, while this ratio varied strongly between samples [53% relative standard deviation (RSD)]. The detection frequency was not correlated with the number of measurements or the limit of quantification (all Pearson r between -0.04 and -0.09 ; all $p > 0.17$; $n = 249$; compounds without concentrations above the LOQ were excluded; see Table S2, Supporting Information, for values). The GLMs for detection frequency as response variable indicated significant differences between the sampling sites for all chemical groups, between years for all chemical groups except for polychlorinated biphenyls, and between months for 6 of the 10 chemical groups (Table S4, Supporting Information). By contrast, any chemical group exhibited significant differences in the detection frequencies between basins (Table S4, Supporting Information).

The chemical group with the highest total detection frequency (43%) was the polycyclic aromatic hydrocarbons (PAH); 10 of the 15 most frequently detected compounds belonged to this group (Table 2). Our results are in accordance with other studies that highlighted the high frequency of detection of PAHs in aquatic environments due to their diffusive input pathway.^{5,18} Significantly higher detection frequencies of PAHs were observed in the months January–March compared to the rest of the year, especially to the months July–September (all $p < 0.01$, TALC). Similar patterns were observed in a study on PAHs in the Seine River in France and may result from higher combustion activities, remobilization due to flooding, or decreased photodecomposition in winter.¹⁹

Except for Cuxhaven, where the tidal influence presumably led to dilution by seawater, the sites in the Elbe exhibited significantly higher detection frequencies compared to the sites in the Ems and Weser basin for some groups of chemicals such as pesticides and halogenated alkanes ($p < 0.05$, TALC, see Table S4, Supporting Information, for details). This is in line with the fact that, in the 20th century, the Elbe was among the most polluted rivers in Germany.²⁰

Except for pesticides and PAHs (see above), no patterns in the detection frequency for months were observed. Pesticides showed significantly higher detection frequencies from June to July compared to the months October–April (all $p < 0.02$, TALC) with the exception of January (all $p > 0.05$, TALC). This period of higher detection frequencies matches the application period of pesticides in central Europe. The elevated detection frequency at the beginning of the year was also observed for several pesticides in a 1-year study in the Humble River in northeast England¹⁸ and may result from field runoff associated with flooding as suggested for PAHs (see above).

From 1994 to 2004, several chemical groups showed a reduction in detection frequencies over all sampling sites (Table S4, Supporting Information). Pesticides and halogenated alkanes exhibited a continuous decline, while the groups of (B) polychlorinated biphenyls, (D) halogenated benzenes and nitrobenzenes, (H)

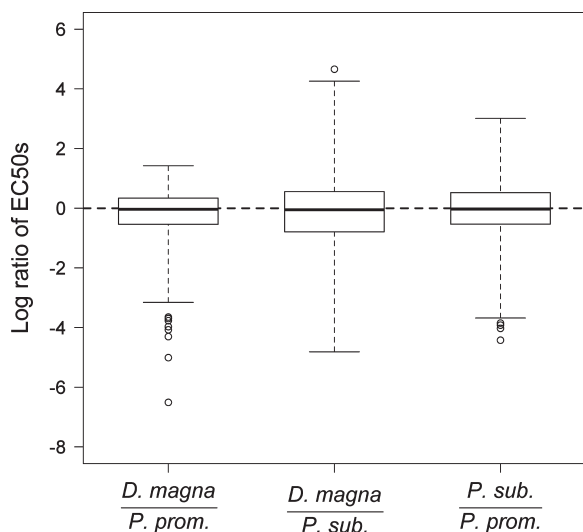


Figure 1. Box-and-whisker plots for relative toxicity of monitored compounds for the test organisms *D. magna*, *P. subcapitata*, and *P. promelas*. The whiskers extend out from the box to a maximum of 3 times the interquartile range.

toluoles, toluenes, and halogenated derivatives showed significantly lower detection frequencies in the period 1998–2004 compared to years 1994–1997 (Table S4, Supporting Information). These observations match with long-term sediment quality studies in the river Elbe from 1991 to 2001 that reported a general decline for several inorganic and organic pollutants.²⁰

Toxicity Assessment for Standard Test Species and Comparison with Biological Monitoring Results. The three standard test organisms *D. magna*, *P. promelas*, and *P. subcapitata* were relatively equal in their sensitivity to most of the 331 organic compounds in terms of the logarithmic ratio of EC₅₀ values (ECR). This was indicated by medians between -0.05 and -0.03 and a relatively even spread of the ECR values between the upper and lower quartile around the median (Figure 1). The compounds related to these ECR values were presumably narcotics, that is, they exhibited baseline toxicity.¹³ The spread of ECR values was less even toward the maximum and minimum ECR values (Figure 1). *P. promelas* was only a maximum of 26-fold and 1024-fold more sensitive than *D. magna* and *P. subcapitata*, respectively, while both *D. magna* and *P. subcapitata* showed a 10 000-fold higher sensitivity than *P. promelas* for several compounds (Figure 1). A total of 72 different compounds exhibited at least 100-fold lower or higher EC₅₀ values (i.e., $ECR \geq 2$ or $ECR \leq -2$) for a test species in relation to another test species. Interestingly, 58 of the 63 compounds for *D. magna*, 55 of the 59 compounds for *P. subcapitata*, and 5 of the 7 compounds for *P. promelas* with ECR values ≥ 2 or ≤ -2 were pesticides. This can be explained by the fact that the majority of current-use pesticides, especially herbicides and insecticides, are specifically designed to eliminate certain groups of target organisms and therefore exhibit excess toxicity to these groups while being relatively nontoxic to other groups of organisms.²¹

The annual mTU values for *D. magna* were significantly higher than for *P. subcapitata* and *P. promelas* (both $p < 0.001$, TALC for best-fit model for annual mTU, $AIC = -746$, $n = 213$), whereas they were not significantly different between the latter two species ($p = 0.44$) (Figure 2). By contrast, a study on the toxicity of 83 pesticides in 17 streams reported 5–10-fold higher median

mTU values for primary producers (*P. subcapitata* and *Lemna gibba*) compared to *D. magna*,²² though the fish species (*Lepomis macrochirus*) was also at lowest risk to be acutely affected by toxicants.²² This suggests that the group of organisms at highest risk from organic toxicants varies between basins and the risk assessment should therefore always include organisms from all different trophic levels.

Predominantly, herbicides and organophosphate insecticides were accountable for the highest annual risk of toxicity for *P. subcapitata* and *D. magna*, respectively (Table 3; Table S5, Supporting Information). For *P. promelas*, four nonpesticides were responsible for the highest annual mTUs, but pesticides were still accountable for 48 of the 71 annual mTUs (Table S5, Supporting Information). Although several studies have highlighted the ecotoxicological relevance of pesticide input for small streams in agricultural areas^{24,25} and that pesticide concentrations decrease with the size of surface water bodies,²⁶ this shows that pesticides can be the most potent toxicants even in large rivers.

The concentrations of several compounds reached levels that were within 1 order of magnitude of the EC₅₀ values for *D. magna* and *P. subcapitata* and were even higher than the acute EC₅₀ for *D. magna* for the insecticide dichlorvos for one site in 1994 and four sites in 1996 (Figure 2; Table S6, Supporting Information). Meta-analyses of results from freshwater mesocosm studies suggest that effects of insecticide and herbicide contamination on the invertebrate community can, depending on the mode of action of the respective substance, occur above a TU of 0.01 for *D. magna*, while this threshold is higher for phytoplankton (algae) and fish with a TU of 0.1.^{27,28} Though these thresholds should be interpreted with caution, they clearly indicate a risk of acute toxic effects when exceeded. Based on these thresholds, the risk of acute effects on fish and algae was minor as 0% and 8% of the annual mTU exceeded 0.1, respectively (Figure 2; Table S6, Supporting Information). By contrast, the measured concentrations of organic compounds were related to a high risk of acute toxic effects on the invertebrate community for the majority of sites and years (89% of observations with annual mTU for *D. magna* > 0.01 ; Figure 2; Table S6, Supporting Information). Annual mTU values for *D. magna* below 0.01 were only observed for single sites in the Elbe and Weser basin between 2000 and 2003, except for Cuxhaven in 1998. Nevertheless, in 2004 the concentrations exceeded the threshold again at all sites and thus it remains unclear if there is an ongoing decrease. The best-fit linear models for the annual mTU values of the three species indicated significant differences between years for *D. magna*, while there were significant differences between sites for *P. subcapitata* and *P. promelas* ($AIC = -205$, -371 , and -757 , respectively; all $p < 0.002$; $n = 71$ for each test species). Overall, our results are in accordance with ecotoxicity tests for several sites along the Elbe between 1992 and 2001 that also found serious mortality for daphnids with no clear temporal trend.²⁰

Although our results suggest a high risk for acute toxic effects on the aquatic fauna and especially invertebrates, the risk assessment approach used here, relying on the mTU for the bioavailable water concentrations and mesocosm thresholds, may still underestimate real acute toxic effects. First of all, the concentrations used were derived from monthly point water samples that are not suitable to assess the peak water concentrations, especially for compounds with varying exposure patterns such as pesticides.²⁹ Hence, the actual TUs will be higher than the ones based on the measured concentrations. In addition, we do not consider (1) additive or synergistic

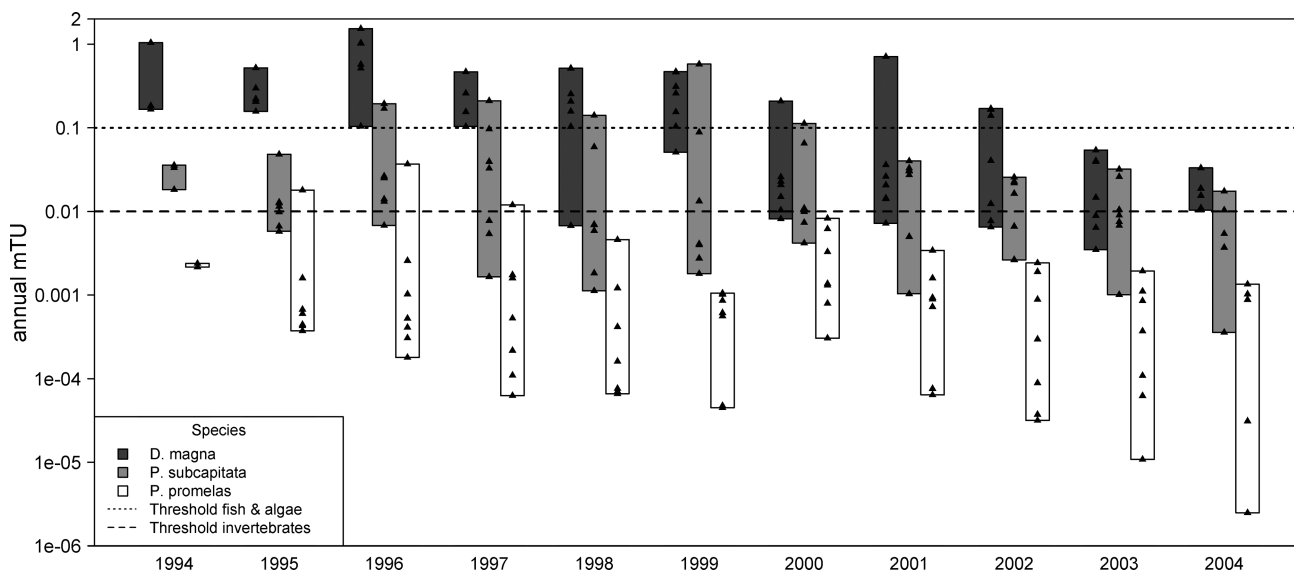


Figure 2. Min–max bar plot of annual maximum toxicity (mTU) for *D. magna*, *P. subcapitata*, and *P. promelas* for all sites from 1994 to 2004 and the respective thresholds for aquatic organisms (see text for details). Triangles display the variation in mTU values for the seven sampling sites.

Table 3. Compounds Accountable for Threshold Annual mTU with EC₅₀, Number of Times Accountable for Annual mTU, Pesticide Group, and Highest Annual mTU^a

compd ^b	EC ₅₀ (μg/L)	source EC ₅₀ ^c	no. annual mTU ^d	pesticide group ^e	highest annual mTU
Annual mTU > 0.01 for <i>D. magna</i>					
dichlorvos	0.19	E	35	insecticide (OP)	1.5
diazinon	1.00	E	6	insecticide (OP)	0.71
pirimiphos-ethyl	0.03	P/3	13	insecticide (OP)	0.57
azinphos-ethyl	0.20	E	2	insecticide (OP)	0.21
pirimiphos-methyl	0.21	E	5	insecticide (OP)	0.18
chlorpyrifos-methyl	0.60	E	1	insecticide (OP)	0.033
fonofos	2.30	E	1	insecticide (OP)	0.021
fenclorfos	0.73	P/2	1	insecticide (OP)	0.019
ethion	0.06	E	1	insecticide (OP)	0.015
malathion	0.7	E	2	insecticide (OP)	0.011
Annual mTU > 0.1 for <i>P. subcapitata</i>					
diuron	2	E	17	herbicide	0.58
alachlor	5	E	17	herbicide	0.19

^a See Table S5, Supporting Information, for all compounds accountable for annual mTU. ^b For all compounds listed, chemical group is A = pesticide. ^c E = experimental data from literature (see Table S2, Supporting Information, for details); P = predicted data from read across, together with the level of similarity (los) to compounds with experimental data (1, los ≤ 0.5; 2, 0.5 < los ≤ 0.7; 3, 0.7 < los ≤ 0.85; and 4, los > 0.85). ^d Number of times accountable for annual mTU for all seven sampling sites and for all years (*n* = 71). ^e Pesticide group as given in the FOOTPRINT pesticide properties database;²³ OP = organophosphate.

effects between compounds, (2) chronic effects, or (3) recurring pulses of toxicants, all of which may increase the toxicity. Furthermore, not all existing organic toxicants were measured, and relevant compounds in terms of toxicity may have been missed.⁵ Moreover, the annual mTU values were predominantly associated with compounds for which experimental EC₅₀ values were available, whereas compounds with predicted EC₅₀ values accounted only to a minor extent for annual mTU values (Table 3; Table S5, Supporting Information). With respect to prediction errors, an underestimation of the real EC₅₀ (i.e., higher real EC₅₀ values) would only lead to minor changes in the toxicity assessment using annual mTUs. By contrast, overestimation of the real EC₅₀ values (i.e., lower real EC₅₀

values) would result in even higher annual mTU values. Finally, uncertainties remain whether the effect thresholds derived from existing mesocosm studies are protective for aquatic communities since field studies demonstrated effects at lower concentrations^{24,30} and these effects may not have been detected in previous mesocosm studies due to the low proportion of sensitive long-living taxa in mesocosm communities.³¹ However, a similar study in a Spanish river basin also indicated significant effects of organic pollutants on the macroinvertebrate community for mTU > 0.01.³¹

Significance of Results for River Basin Management. In the European Union, current management of the chemical pollution of freshwater ecosystems focuses on the assessment of 33 priority

substances that are assumed to present a specific risk for the environment when they exceed the environmental quality standards (EQS).³² Indeed, 13 of the 24 compounds most frequently detected in the rivers of north Germany were priority substances (Table 2). Moreover, the chemical status indicated potential effects of the organic priority substances for several years and sites in terms of exceedance of EQS values (Table S3, Supporting Information). Nevertheless, only five of the 25 organic priority substances that have been measured exceeded the EQS values in the years 1994–2004 for the seven sampling sites: alachlor, trifluralin, and tributyltin as well as the PAHs benzo[ghi]perylene and indeno[1,2,3-c,d]pyrene. However, not all organic priority substances were measured in the monitoring programs, so there may be other priority substances that exceed their respective EQS values. This possibility is supported by the fact that we found a significant correlation between the number of measured priority substances and the number of exceedances of EQS values for our data (Pearson $r = 0.75$, $p < 0.001$, $n = 71$).

Since EQS values integrate protection goals that are not related to ecotoxicological effects (e.g., fish consumption, drinking water production), an exceedance of EQS values does not necessarily indicate ecotoxicological risk. Indeed, of the priority substances that exceeded the EQS values, only two (alachlor and trifluralin) occurred in concentrations that exceeded a TU of 0.1 for *P. subcapitata* and none reached a TU of 0.01 for *D. magna* or of 0.1 for *P. promelas* (Table S2, Supporting Information). Conversely, although diuron accounted 17 times for the highest annual toxicity to *P. subcapitata*, with concentrations up to 58% of the EC₅₀ value (Table 3), this did not lead to an exceedance of the respective EQS value (1.8 µg/L). Given that mesocosm studies demonstrated effects for concentrations of this order of magnitude, the current EQS value may not be protective for phytoplankton communities.^{27,28} We suggest that the EQS values should be revised to consider the ecotoxicological risk of priority substances to all trophic groups.

Only two of the substances most relevant for the risk of acute toxic effect to the standard test organisms were priority substances (alachlor and diuron) (Table 3). Hence, priority substances were of only minor importance for the risk assessment for primary producers, invertebrates, and fish. Our study is in accordance with a review by Brack et al.,³³ which highlighted that in several investigations compounds other than priority substances were relevant for toxicity to the aquatic biocenosis. This result is especially important for river conservation and restoration measures. In the case that the current practice, relying on the assessment of priority substances, assumes a good chemical status, this may lead to measures to improve the ecological quality that may not be successful since nonpriority organic substances can still have acute toxic effects. For example, some studies reported that conservation and restoration measures showed only minor improvement of the ecological quality of the stream, though this is not necessarily due to organic toxicants.^{34,35} Overall, our study highlights that organic toxicants and especially pesticides may play a more important role for the ecological conditions in river systems than is currently acknowledged. Therefore, we suggest (1) to include these compounds in the list of river basin specific pollutants and (2) in general to use approaches such as the one outlined here to identify, based on chemical monitoring data, ecotoxicologically relevant compounds in other river basins.

■ ASSOCIATED CONTENT

● **Supporting Information.** One figure of the sampling sites and six tables giving a full list of measured compounds with

methods used in chemical analysis and toxicity data, overview of priority substance measurements and the respective exceedances of EQS values, a description of the GLM models, and a list of compounds accountable for highest annual toxicity per site and per organism. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: ++49 (0) 6341 28031536; e-mail: senator@ecotoxicology.de.

■ ACKNOWLEDGMENT

We thank Dieter Steffen for support with the study and data compilation. In addition, we are grateful to several staff members of the local agencies of the Lower Saxony Water Management, Coastal Defence and Nature Conservation Agency (NLWKN) for providing the invertebrate biomonitoring data. Special thanks to Jan Kirchmeyer for creating the map with sampling sites. Ben Kefford gave valuable suggestions that improved the quality of the manuscript. R.B.S. received funding from the Deutsche Forschungsgemeinschaft (SCHA 1580/1-1). P.C.v.d.O. was supported by the European Commission through the Integrated Project MODELKEY (Contract 511237-GOCE) and by the Deutsche Forschungsgemeinschaft (PAK 406-1). G.S and R.K. appreciate financial support from the EU via the Integrated Project OSIRIS (Contract 037017).

■ REFERENCES

- (1) Schwarzenbach, R. P.; Escher, B. I.; Fenner, K.; Hofstetter, T. B.; Johnson, C. A.; von Gunten, U.; Wehrli, B. The challenge of micro-pollutants in aquatic systems. *Science* **2006**, *313* (5790), 1072–1077.
- (2) European Commission Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000, establishing a framework for Community action in the field of water policy.
- (3) European Commission Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy.
- (4) von der Ohe, P. C.; Dulio, V.; Slobodnik, J.; De Deckere, E.; Kühne, R.; Ebert, R.-U.; Ginebreda, A.; De Cooman, W.; Schüürmann, G.; Brack, W. A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European Water Framework Directive. *Sci. Total Environ.* **2011**, *409* (11), 2064–2077.
- (5) Franke, S.; Hildebrandt, S.; Schwarzbauer, J.; Link, M.; Francke, W. Organic compounds as contaminants of the Elbe river and its tributaries 2. GC/MS screening for contaminants of the Elbe water. *Fresenius' J. Anal. Chem.* **1995**, *353* (1), 39–49.
- (6) Schäfer, R. B.; Palm, W.-U.; Steffen, D.; Ruck, W. Pflanzenbehandlungs- und Schädlingsbekämpfungsmittel in niedersächsischen Fließgewässern von 1994 bis 2001. *Hydrol. Wasserbewirtsch.* **2004**, *48* (3), 117–125.
- (7) Niedersächsisches Landesamt für Ökologie, *Gewässergütebericht 2000*.
- (8) Droge, S. T. J.; Postma, J. F.; Hermens, J. L. M. Sediment toxicity of a rapidly biodegrading nonionic surfactant: Comparing the equilibrium partitioning approach with measurements in pore water. *Environ. Sci. Technol.* **2008**, *42* (11), 4215–4221.
- (9) DiToro, D. M.; Zarba, C. S.; Hansen, D. J.; Berry, W. J.; Swartz, R. C.; Cowan, C. E.; Pavlou, S. P.; Allen, H. E.; Thomas, N. E.; Paquin, P. R. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ. Toxicol. Chem.* **1991**, *10*, 1541–1583.

- (10) Schüürmann, G.; Ebert, R. U.; Kühne, R. Quantitative read-across for predicting the acute fish toxicity of organic compounds. *Environ. Sci. Technol.* **2011**, *45*, 4616–4622.
- (11) Kühne, R.; Kleint, F.; Ebert, R.-U.; Schüürmann, G. Calculation of compound properties using experimental data from sufficiently similar chemicals. In *Software Development in Chemistry 10*; Gasteiger, J., Ed.; Springer: Berlin, 1996.
- (12) U.S. Environmental Protection Agency. Ecological structure activity relationships; <http://www.epa.gov/oppt/newchems/tools/21ecosar.htm> (accessed 15 April 2011).
- (13) von der Ohe, P. C.; Kühne, R.; Ebert, R. U.; Altenburger, R.; Liess, M.; Schüürmann, G. Structural alerts—A new classification model to discriminate excess toxicity from narcotic effect levels of organic compounds in the acute daphnid assay. *Chem. Res. Toxicol.* **2005**, *18* (3), 536–555.
- (14) Mayer, P.; Reichenberg, F. Can highly hydrophobic organic substances cause aquatic baseline toxicity and can they contribute to mixture toxicity? *Environ. Toxicol. Chem.* **2006**, *25* (10), 2639–2644.
- (15) Sprague, J. B. Measurement of pollutant toxicity to fish, II: Utilizing and applying bioassay results. *Water Res.* **1970**, *4* (1), 3–32.
- (16) Fox, J., *Applied regression analysis and generalized linear models*; SAGE: Los Angeles, 2008.
- (17) Hothorn, T.; Bretz, F.; Westfall, P. Simultaneous inference in general parametric models. *Biom. J.* **2008**, *50* (3), 346–363.
- (18) Long, J. L. A.; House, W. A.; Parker, A.; Rae, J. E. Micro-organic compounds associated with sediments in the Humber rivers. *Sci. Total Environ.* **1998**, *210* (1–6), 229–253.
- (19) Cailleaud, K.; Forget-Leray, J.; Souissi, S.; Hilde, D.; LeMenach, K.; Budzinski, H. Seasonal variations of hydrophobic organic contaminant concentrations in the water-column of the Seine Estuary and their transfer to a planktonic species *Eurytemora affinis* (Calanoida, copepoda). Part 1: PCBs and PAHs. *Chemosphere* **2007**, *70* (2), 270–280.
- (20) Heininger, P.; Pelzer, J.; Claus, E.; Pfitzner, S. Results of long-term sediment quality studies on the river Elbe. *Acta Hydrochim. Hydrobiol.* **2004**, *31* (4–5), 356–367.
- (21) Stenersen, J., *Chemical Pesticides: Mode of action and toxicology*; CRC: Boca Raton, FL, 2004.
- (22) Belden, J. B.; Gilliom, R. J.; Martin, J. D.; Lydy, M. J. Relative toxicity and occurrence patterns of pesticide mixtures in streams draining agricultural watersheds dominated by corn and soybean production. *Integr. Environ. Assess. Manage.* **2007**, *3* (1), 90–100.
- (23) The FOOTPRINT Pesticide Properties DataBase. Database collated by the University of Hertfordshire as part of the EU-funded FOOTPRINT project (FP6-SSP-022704); <http://www.eu-footprint.org/ppdb.html> (accessed 15 April 2011).
- (24) Liess, M.; Schäfer, R. B.; Schriever, C. A. The footprint of pesticide stress in communities: species traits reveal community effects of toxicants. *Sci. Total Environ.* **2008**, *406* (3), 484–490.
- (25) Schäfer, R. B.; Pettigrove, V.; Rose, G.; Allinson, G.; Wightwick, A.; von der Ohe, P. C.; Shimeta, J.; Kühne, R.; Kefford, B. J. Effects of pesticides monitored with three sampling methods in 24 sites on macroinvertebrates and microorganisms. *Environ. Sci. Technol.* **2011**, *45* (4), 1665–1672.
- (26) Schulz, R. Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution: A review. *J. Environ. Qual.* **2004**, *33* (2), 419–448.
- (27) Van Wijngaarden, R. P. A.; Brock, T. C. M.; Van Den Brink, P. J. Threshold levels for effects of insecticides in freshwater ecosystems: A review. *Ecotoxicology* **2005**, *14* (3), 355–380.
- (28) Brock, T. C. M.; Lahr, J.; Van den Brink, P. J. *Ecological risks of pesticides in freshwater ecosystems, Part 1: Herbicides*; Alterra: Wageningen, The Netherlands, 2000.
- (29) Liess, M.; Schulz, R.; Liess, M. H.-D.; Rother, B.; Kreuzig, R. Determination of insecticide contamination in agricultural headwater streams. *Water Res.* **1999**, *33* (1), 239–247.
- (30) Schäfer, R. B.; Caquet, T.; Siimes, K.; Mueller, R.; Lagadic, L.; Liess, M. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. *Sci. Total Environ.* **2007**, *382* (2–3), 272–285.
- (31) Beketov, M.; Schäfer, R. B.; Marwitz, A.; Paschke, A.; Liess, M. Long-term stream invertebrate community alterations induced by the insecticide thiacloprid: Effect concentrations and recovery dynamics. *Sci. Total Environ.* **2008**, *405*, 96–108.
- (32) von der Ohe, P. C.; de Deckere, E.; Prüss, A.; Munoz, I.; Wolfram, G.; Villagrasa, M.; Ginebreda, A.; Hein, M.; Brack, W. Toward an integrated assessment of the ecological and chemical status of European river basins. *Integr. Environ. Assess. Manage.* **2009**, *5* (1), 50–61.
- (33) Brack, W.; Klamer, H. J. C.; De Alda, M. L.; Barcelo, D. Effect-directed analysis of key toxicants in European river basins: a review. *Environ. Sci. Pollut. Res.* **2007**, *14* (1), 30–38.
- (34) Suren, A. M.; Riis, T.; Biggs, B. J. F.; McMurtrie, S.; Barker, R. Assessing the effectiveness of enhancement activities in urban streams: I. Habitat responses. *River Res. Appl.* **2005**, *21* (4), 381–401.
- (35) Harrison, S. S. C.; Pretty, J. L.; Shepherd, D.; Hildrew, A. G.; Smith, C.; Hey, R. D. The effect of instream rehabilitation structures on macroinvertebrates in lowland rivers. *J. Appl. Ecol.* **2004**, *41* (6), 1140–1154.