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ARTICLE *in* ENVIRONMENTAL SCIENCE & TECHNOLOGY · JUNE 2013

Impact Factor: 5.33 · DOI: 10.1021/es4014954 · Source: PubMed

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How to Characterize Chemical Exposure to Predict Ecologic Effects on Aquatic Communities?

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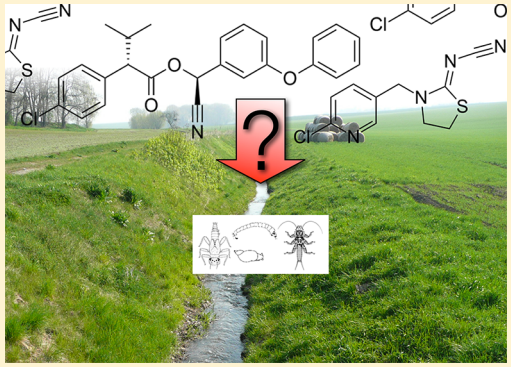
Supporting Information

ABSTRACT: Reliable characterization of exposure is indispensable for ecological risk assessment of chemicals. To deal with mixtures, several approaches have been developed, but their relevance for predicting ecological effects on communities in the field has not been elucidated. In the present study, we compared nine metrics designed for estimating the total toxicity of mixtures regarding their relationship with an effect metric for stream macroinvertebrates. This was done using monitoring data of biota and organic chemicals, mainly pesticides, from five studies comprising 102 streams in several regions of Europe and South-East Australia. Mixtures of less than 10 pesticides per water sample were most common for concurrent exposure. Exposure metrics based on the 5% fraction of a species sensitivity distribution performed best, closely followed by metrics based on the most sensitive species and *Daphnia magna* as benchmark. Considering only the compound with the highest toxicity and ignoring mixture toxicity was sufficient to estimate toxicity in predominantly agricultural regions with pesticide exposure. The multisubstance Potentially Affected Fraction (msPAF) that combines concentration and response addition was advantageous in the study where further organic toxicants occurred. We give recommendations on exposure metric selection depending on data availability and the involved compounds.



INTRODUCTION

The characterization of chemical exposure in freshwater ecosystems is a crucial prerequisite for ecological risk assessments, but is hampered by practical and theoretical issues. Practically, it remains a challenge to sample and analyze all ecotoxicologically relevant substances that enter a water body.[1] Theoretically, even if a complete characterization of exposure would be obtained for a certain site, potential mixture effects and the limited availability of effects data for species in the target system render the assessment difficult.[2−4] Different approaches to (1) assess the risk from observed concentrations of chemicals and (2) deal with chemical mixtures have been developed. Toxic Units (TU)[5] are a relatively simple method to assess the risks from toxicant exposure for a group of organisms (e.g., invertebrates, plants, fish) and have been widely applied to standardize observed toxicant concentrations based on acute and/or chronic toxicity data from standard test organisms (e.g., *Daphnia magna* and *Hyalella azteca* for invertebrates, *Pimephales promelas* for fish). While TUs are often calculated relative to one species only, they provide a benchmark for the toxicity to other parts of the community as long as the relative sensitivity of these organisms to a chemical remains similar.[6] However, the use of TU for *D. magna* ($TU_{D.\ magna}$) has been criticized, as this species is not always the most sensitive species.[7] Equally relevant, cases exist where the relative sensitivity between *D. magna* and other aquatic invertebrates differs substantially among compounds. For example, compared to insects, *D. magna* exhibits much lower sensitivity to neonicotinoids and insect growth inhibitors,[8] while in general

Received: April 10, 2013
Revised: June 12, 2013
Accepted: June 13, 2013
Published: June 13, 2013

dx.doi.org/10.1021/es4014954 | Environ. Sci. Technol. 2013, 47, 7996−8004

D. magna and other cladocerans tend to be more or similarly sensitive to other organic toxicants than many but not all freshwater insects.[6]

Furthermore, species sensitivity distributions (SSDs) have been introduced to integrate acute and/or chronic toxicity data for several species into a concentration−effect relationship from which hazardous concentrations (HC_p) leading to effects on p% of species can be derived.[9] This HC_p can be used in the TU approach to standardize the observed concentrations to a defined fraction of potentially affected species (p) instead of a particular species and could therefore provide a benchmark that is more robust to variations in the relative sensitivity of species. However, due to the scarcity of toxicity data, SSDs for different chemicals rely on differing sets of species, which may compromise the suitability of the derived HC_p to provide a consistent benchmark. Moreover, SSDs have been criticized because they rely on a number of assumptions that are generally not met, e.g., that the set of species used in a SSD is an unbiased sample of the target group of species or that the loss of any species is of equal ecological relevance.[10] Consequently, for a given concentration the fraction of affected taxa in an ecosystem can differ from the estimated p%.[11]

By neglecting potentially synergistic effects between chemicals, concentration addition (CA) represents a conservative approach to deal with chemical mixtures.[3] For TUs, CA corresponds to the sum of the TU (sumTU) of each chemical detected in a sample. By contrast, considering only the potential effects of the compound with the maximum toxicity and ignoring potential effects from all other compounds results in the so-called maximum TU (maxTU) indicating the minimum estimated toxicity of the most potent component of the mixture. This maxTU has successfully been applied to evaluate pesticide effects on stream macroinvertebrates and compared to the sumTU showed a similarly high association with macroinvertebrate-based effect metrics.[12,13] While CA relies on the theoretical assumption of the same mode of action (MOA), a second model of mixture toxicity, independent action, also called response addition (RA) and used here for consistency with De Zwart and Posthuma,[14] integrates effects from compounds with different MOAs.[3] RA requires a concentration−response model, which is not available for the TU approach, but exists for SSDs. On theoretical grounds, a combination of CA and RA has been suggested for complex mixtures,[15] and the multisubstance Potentially Affected Fraction (msPAF) was introduced.[14] The msPAF approach first applies CA to compounds with the same MOA and subsequently uses RA to aggregate the different MOAs. Previous studies examined effects of mixtures from different groups of organic and inorganic chemicals and found statistically significant associations with the abundances of 50% to 74% of taxa in communities.[16,17]

The aim of this study was to compare the relationship of different exposure metrics for summarizing the total toxicity of mixtures of organic chemicals (mostly pesticides) with an effect metric for stream macroinvertebrate communities. We used $SPEAR_{pesticides}$,[12] which indicates the fraction of pesticide-sensitive invertebrate taxa in a community based on their physiological sensitivity and biological traits such as generation time and dispersal capacity, as effect metric because it displayed a close relationship and high specificity to pesticide exposure in previous field studies from different regions of the world.[18] We hypothesized that, due to the limited availability of ecotoxicological data, using $TU_{D.\ magna}$ would outperform SSD-based methods relying on toxicity data from differing sets of species. Moreover, based on previous studies we did not expect a relevant increase in predictive power for community effects from the consideration of mixture effects.

MATERIAL AND METHODS

Study Selection and Description. We selected five studies for which data were available on an organic toxicant exposure gradient for multiple streams (>5) with concurrent macroinvertebrate community data that indicated effects of this exposure. Four of the five studies including their data have been described in a recent meta-analysis on thresholds for the effects of pesticides[18] and were complemented by a further study reporting effects of organic toxicants on macroinvertebrate communities.[19] The selected studies encompassed 14 to 28 streams in predominantly agricultural areas in different regions of Europe and in South-East Australia (Table 1). In the following we refer to the individual studies with the country name, while the related freshwater ecoregions are given in Table 1. Between 10 and 153 individual organic compounds were measured and across all data sets 107 different compounds (Supporting Information (SI) Table S1) were detected. The toxicant exposure was expressed as $maxTU_{D.\ magna}$ (eq 2) in these studies. The relationships between $maxTU_{D.\ magna}$ of the original studies and potential effects in terms of $SPEAR_{pesticides}$ were relatively high ($0.61 < r^2 < 0.89$) (Table 1) and was in some of the studies moderated by the availability of forested reaches upstream that may serve as landscape recolonization pools.[12,20] The values for the effect metric ($SPEAR_{pesticides}$) were taken from the meta-analysis[18] and von der Ohe et al.,[19] and details on the calculation of this metric can be found therein. Furthermore, the chemical concentrations from the included studies were compiled and corrected for bioavailability by the

Table 1. Included Studies with Information on Number (No.) of Sites and Measured Compounds, Relationship between Exposure, and Effect ($SPEAR_{pesticides}$) Metrics for Models Reported in the Original and in This Study

				r^2 for relationship between exposure and effect metric			
Region	No. of sites	Ecoregion[a]	No. of compounds measured	Model original study[b]	Best-fit model this study[c]	Model original/this study contained FUS[d]?	ref.
Brittany, France	16	Central and Western Europe	10	0.72	0.77	yes/yes	20
Central Germany	20	Central and Western Europe	21	0.75	0.73	yes/yes	12
Victoria, Australia	24	Bass Strait Drainages	97	0.68[e]	0.81[e]	no/yes	13
Denmark	14	Central and Western Europe	31	0.61	0.68	no/no	23
Catalonia, Spain	28	Eastern Iberia	153	0.89	0.90	yes/no	19

[a]According to Abell et al.[24] [b]$maxTU_{D.\ magna}$ was exposure metric. [c]Exposure metric of best-fit model given in Table S3. [d]Forested upstream sections. [e]After removal of one outlier.

dx.doi.org/10.1021/es4014954 | Environ. Sci. Technol. 2013, 47, 7996−8004

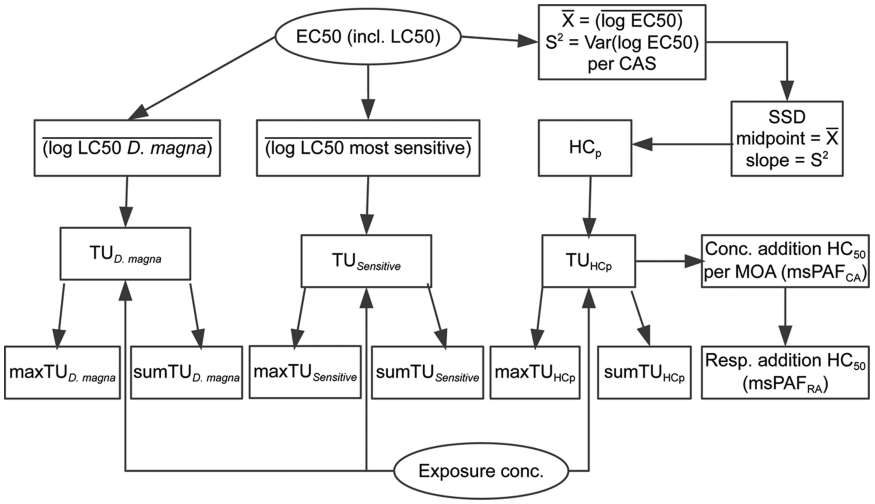


Figure 1. Schematic overview on the calculation of the exposure metrics. See paragraph "Calculation of exposure metrics" for explanation of the acronyms. An overline indicates the mean for the respective variable, Var refers to the variance. Conc. = Concentration; Resp. = Response.

total organic carbon (TOC) content based on the partitioning between water and organic carbon according to DiToro et al.[21]

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

where C_d approximates the dissolved, bioavailable concentration, C_{tot} is the total concentration in the whole water sample, 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 TOC content. We note that this correction may underestimate the ecotoxicologically active concentration since particle-adsorbed compounds can still exert toxic effects.[22] Finally, the chemical concentrations were employed to calculate the different exposure metrics.

Calculation of Exposure Metrics. Four different metrics were employed to assess the exposure to organic toxicants: the $TU_{D.magna}$, the TU for the most sensitive organism for which toxicity data was available ($TU_{Sensitive}$), the TU based on the HC_p from a SSD (TU_{HCp}) and the msPAF, also based on SSDs. The schematic calculation of metrics is displayed in Figure 1 and data and computer code for computation is given in the SI. Briefly, the TU for a chemical i is calculated as

$$TU_i = \frac{c_i}{LC50_{i,j}} \quad (2)$$

where c is the concentration measured in the environment and $LC50_{i,j}$ the median lethal concentration for species j, which is *D. magna* in case of $TU_{D. magna}$ and the most sensitive species in the case of $TU_{Sensitive}$, i.e., the species with lowest available LC50. To compute TU_{HCp}, the LC50 was substituted by HC_p in eq 2. We calculated the HC_5 and HC_{50} based on a SSD for each compound, which represent the concentrations potentially affecting 5% and 50% of the tested species, respectively. The SSDs were computed for individual compounds with a minimum of 6 data points (i.e., species; see below for rationale) assuming a log-normal model. Note that we did not examine other model types (e.g., Weibull, Probit) for the individual SSDs[25] due to (1) the large number of compounds in our data set and (2) the statistical properties of the log-normal distribution that simplified computation of msPAF.[14] The slopes of the resulting SSDs were averaged per MOA for msPAF calculation, after assigning each compound to one of four MOAs (SI Table S1), depending on whether it affected (1) acetylcholinesterase, (2) the sodium channel, or (3) the electron transport chain or acted as (4) narcotic. We separated (1) and (2) following Stenersen[26] and since the slopes were statistically significantly different (Welch two sample t test, $p = 0.014$). Four (cyanide, propargite, spinosyn d, tebufenozide) of the 107 compounds that could not be assigned to any of these MOAs and that were ecotoxicologically negligible with respect to their concentrations were omitted in the calculation of all exposure metrics. The minimum requirement of 6 data points for SSD calculation was selected to provide robust estimates of the mean slope per MOA (see SI Figure S1), which is in agreement with results from another study.[27] If less than 6 data points were available for a compound, which was the case for 72 of 103 compounds (SI Table S1), the average of the available data points was used as SSD midpoint (= HC_{50}) and combined with the mean slope of the related MOA to derive the HC_5. Note that the requirement of a minimum of 6 data points represented a compromise between the uncertainty related to the construction of individual SSDs from few data points and the uncertainty related to assigning the mean slope, but more data points are usually required to derive precise estimates of the HC_p for regulatory risk assessment.[28]

For all exposure metrics different end points regarding chemical mixtures were computed including the sumTUs based on the CA approach and the maxTU based only on the single compound exhibiting the maximum expected toxicity as outlined above (Figure 1). Note that calculation of the sumTU for $TU_{Sensitive}$ and HC_p can lead to summation of effects related to different species (or sets of species), but was done for sake of completeness. Moreover, for 85 of 103 compounds a crustacean was the most sensitive species, thus related species would be pooled in most cases. For calculation of msPAF, in the first step, the TU_{HC50} of all compounds with the same MOA k were added up based on the CA approach. Subsequently, the estimated response ($msPAF_{CA,k}$) was derived using the mean slope related to k. These $msPAF_{CA,k}$ were then employed to compute $msPAF_{RA}$ based on RA:

$$msPAF_{RA} = 1 - \prod (1 - msPAF_{CA,k}) \quad (3)$$

dx.doi.org/10.1021/es4014954 | Environ. Sci. Technol. 2013, 47, 7996−8004

where k = 1 to n different MOAs. In our study n = 4 since there were 4 different MOAs (SI Table S1). Note two limitations in our study with respect to the original protocol by De Zwart and Posthuma[14] for calculation of $msPAF_{RA}$. Their protocol suggested assigning a new MOA when slopes of compounds with the same MOA would deviate by >10%. We classified compounds into 4 broad MOAs to guarantee availability of a sufficient number of compounds per MOA. Strict application of the protocol would have resulted in several MOAs with only one or a few compounds to compute the slope of the related MOA. Given that for approximately 70% compounds no SSD could be computed and a mean slope of the related MOA was assigned, we decided to estimate this slope based on a high number of compounds to yield more robust estimates.[27] Still only 13, 10, 7, and 1 compounds were available for calculation of the mean slope for the MOAs narcotic, acetylcholinesterase, sodium channel, and electron transport chain, respectively. Moreover, in the case of non-narcotic MOA, the protocol suggested to include in the SSD for a compound only taxa, which are known to respond to the specific MOA of this compound. Again, this rule was not adapted due to the low number of available data points (i.e., species) per compound for SSD calculation. However, we restricted the input data for SSDs to freshwater invertebrates, and since the effect metric was related to the freshwater macroinvertebrate community, this should be a minor issue.

The exposure metrics were calculated per site for each of the included studies (Table 1). As for the original studies, if different sampling methods or sampling dates for a site were available, the maximum exposure metric for this site was used in later analysis, based on the rationale that the highest toxic event determines the community effect.[13] For 8 of 102 sites without quantifiable detections, no exposure metrics could be calculated and they were set to 1/10 of the minimum value for the related metric in that study.

Processing of Acute Toxicity Data. Acute toxicity data for 48, 72, and 96 h exposure periods (for sources, see SI Text S1) were restricted to the taxonomic phyla of invertebrates in freshwater ecosystems. Only studies with LC50 as well as the median effect concentration (EC50) for the end points mortality or immobility were selected. All effect concentrations were converted into μg/L and the median was calculated for replicates (being defined as same species + compound + exposure duration + reference). Subsequently, this preprocessed toxicity data was limited to the 103 included compounds (SI Table S1), and was complemented by baseline calculations for compounds with missing toxicity data (SI Text S1). The whole data set was further processed applying the following rules: data for the shortest exposure period were selected if data from different exposure periods for the same species−compound combination were available; data for the same species−compound−exposure period combination were excluded as outliers if they differed by a factor of >30 from the group mean.

In addition, the water solubility, the baseline toxicity, and toxicity data for closely related taxa were considered to check the plausibility of individual toxicity values. Due to the inherent variability in the data, no correction for differences in the exposure periods was applied. If multiple data points per species−compound combination were available, the mean was calculated after log-transformation. Before TU calculation (eq 2), the data was back-transformed using the antilogarithm.

Data Analysis. Before data analysis, all exposure metrics were log-transformed. The intercorrelation and the relationship between the newly calculated and the original $maxTU_{D.\ magna}$ were checked using Pearson's correlation coefficient r. The performance of the exposure metrics when employed to explain ecological effects was examined by establishing separate linear models with $SPEAR_{pesticides}$ as response variable. Given that the selected studies reported mediation of toxicant effects by forested upstream sections (FUS) as defined in the original studies (Table 1), for each exposure metric two models with and without the variable FUS were built. This yielded a total of 90 models (5 countries × 9 exposure metrics × 2 levels for FUS), which were evaluated based on r^2 and Bayesian information criterion (BIC). No indication of a nonlinear relationship was found during visual checking of all models. The Wilcoxon rank-sum test on the BIC was used to decide on the inclusion of the variable FUS in the final model separately over all models from each study. Based on the Wilcoxon rank-sum tests, the final models with or without FUS were selected and the models ranked per country based on the BIC. Subsequently, the ranks for each exposure metric were summed across countries irrespective of whether the model included the variable FUS. The lowest rank indicated the exposure metric with the lowest BICs across all countries. Furthermore, in accordance with Burnham and Anderson,[29] all models with a difference of ≤2 to the BIC of the best-fit model in terms of lowest BIC per country were selected and counted across all countries, again ignoring the variable FUS. Finally, we selected the best-fit model in terms of BIC for each country and compared the explained variance (r^2) to that of the original model of the respective study to explore potential improvements in the relationship between the exposure and effect metric. All computations and graphics were done using R[30] and we provide the full code and all data except for the Australian study (SI) to enable reproducible research.[31]

RESULTS

Fifty percent of the samples contained 2 to 6 individual compounds at quantifiable concentrations in Australia and Denmark, 4 to 7 in France, 2 to 4 in Germany, and 1 to 8 in Spain (Figure 2). The TU-based exposure metrics exhibited

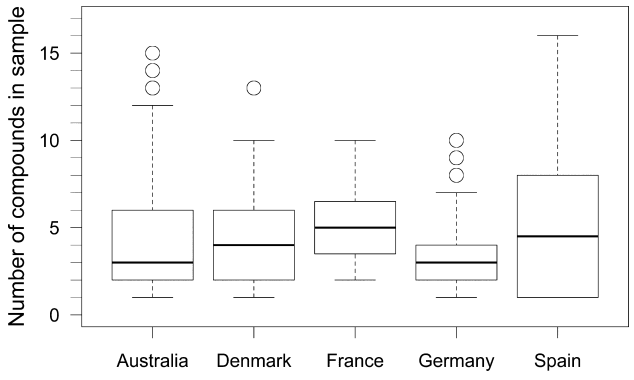


Figure 2. Box-and-whisker plot[49] of the number of compounds above the limit of quantification found in the different studies per water sample. Note the different number of sampled sites, sampling techniques, limits of quantification, and measured compounds per study (Table 1).

a high intercorrelation ($0.9 \leq r \leq 0.99$), whereas r was slightly lower for the correlation of $msPAF_{RA}$ with these metrics and ranged from 0.82 to 0.94 (SI Table S2). Similarly, there was a very high correlation (all $r \geq 0.96$) between the newly

dx.doi.org/10.1021/es4014954 | Environ. Sci. Technol. 2013, 47, 7996−8004

calculated $maxTU_{D.\ magna}$ and the $maxTU_{D.\ magna}$ reported in the original studies (SI Figure S2).

The relationship between the exposure metrics and the effect metric $SPEAR_{pesticides}$ exhibited the lowest BIC when FUS were included as variables for Germany, France, Australia, and Spain (Table 3). Hypothesis testing indicated statistical significance of the inclusion of FUS for France and Australia and of the exclusion for Denmark (Wilcoxon rank sum test, all $p < 0.05$), whereas no statistical differences between the ranks of models with and without FUS were found for Germany ($p = 0.11$) and Spain ($p = 0.86$). Nevertheless, 6 of the 8 models with the lowest BIC for Germany contained the variable FUS (Table 3) and we therefore included this variable in the final models. Across all countries, the two metrics $TU_{Sensitive}$ and TU_{HC5} had the lowest ranks for the BICs and accounted for the best-fit models for all countries except Spain, where $msPAF_{RA}$ performed best (Table 2, Table 3). For Australia and Denmark the best-fit models involved sumTU, for Germany and France maxTU and for Spain msPAF (Table 3). The models within a BIC range of 2 to the best-fit model exhibited a maximum reduction in r^2 of 0.04 for Denmark, and ≤0.02 for all other countries. The $TU_{Sensitive}$, TU_{HC5}, and $sumTU_{D.\ magna}$ were among these models for ≥3 of the 5 countries (Table 2). The best-fit model for the newly calculated metrics improved the relationship with the effect metric $SPEAR_{pesticides}$ by 1% to 13% in terms of explained variance, except for Germany with a 2% reduction in r^2 (Table 1).

Table 2. Rank Sums Across Countries and Selection for Set of Best Models for the Different Exposure Metrics[a]

Exposure metric	Rank sum of metric across countries	Number of times metric was among models within a range of 2 to BIC of best-fit model
$sumTU_{HC5}$	14	4
$sumTU_{Sensitive}$	18	3
$maxTU_{HC5}$	21	3
$maxTU_{Sensitive}$	21	3
$maxTU_{D.magna}$	22	2
$sumTU_{D.magna}$	26	3
$msPAF_{RA}$	32	1
$maxTU_{HC50}$	35	1
$sumTU_{HC50}$	36	1

[a]Results for the same exposure metric were pooled irrespective of whether the model included the variable "forested upstream sections".

DISCUSSION

Composition of Toxicant Mixtures. The present results showed that, despite several hundreds of currently used pesticides in agriculture, mixtures of less than 10 pesticides for a water sample seem most common for the concurrent exposure of freshwater ecosystems in agricultural regions. Thus, although the studies were very different in terms of sampling, number of measured compounds, limits of quantification, and sampling intervals (Table 1), they yielded a remarkably similar number of compounds per water sample with 75% of samples having ≤7 compounds detected at quantifiable concentrations (Figure 2), except for Spain. However, the Spanish study involved further organic toxicants in addition to pesticides because the sites were not limited to mainly agricultural influences as for the other studies. Our results are in agreement with a study on 83 pesticides in agricultural streams of the US that found 2 to 10 compounds in most water samples.[32] Less information is available for tropical regions, but a study on 11 streams in tropical northeast Australia similarly detected up to 10 and an average of 4 pesticides in event-driven water samplers.[33,34]

Is *Daphnia magna* a Sufficiently Good Benchmark for Toxicity? The results refuted our hypothesis that $TU_{D.\ magna}$ would outperform methods based on SSDs, because TU_{HC5} was ranked as best metric and was selected most frequently among the best-fit models (Table 2). The results of individual countries showed that either TU_{HC5} (in Australia), $TU_{Sensitive}$ (Denmark, France and Germany), or $msPAF_{RA}$ (Spain) performed best in terms of BIC (Table 3). Given that the SSDs were not checked individually for more appropriate models than the log-normal model,[25] the SSD-based exposure metrics might still be enhanced.[28] Nevertheless, compared to the best $TU_{D.magna}$ model, i.e., irrespective of max or sum and FUS, the best fit model improved the explained variance (r^2) only by 1% to 4% (Table 3), except for France (+8%). Moreover, the low performance of the TU_{HC50}, which was calculated as the mean of all toxicity data for a compound, demonstrates that indeed different sets of compounds used in SSD calculation can increase the noise. Finally, SSDs require model fitting and are often limited by the available toxicity data, whereas the $TU_{D.magna}$ relies on much simpler calculus and is less restricted by data limitations, since *D. magna* belongs to the most tested species. However, these characteristics hold as well for the $TU_{Sensitive}$, which outperformed $TU_{D.magna}$ (Table 2), despite the fact that for 58% of the compounds *D. magna* was also the most sensitive species (SI Table S3). This was largely because for 46% of compounds *D. magna* was the only freshwater invertebrate tested. For the 56 compounds where multiple freshwater invertebrates were tested, in more than 75% and 30% of these cases (43 and 18 compounds) another species was more sensitive and >1 log unit more sensitive than *D. magna*, respectively (SI Figure S3, Table S3). Hence, our results support the criticism[7] regarding $TU_{D.magna}$ that depending on the mode of action of the compound *D. magna* is not always the most sensitive species. Nevertheless, the differences in r^2 between the best $TU_{D.magna}$ and $TU_{Sensitive}$ model were <4%, except for France and Spain with 8% higher $TU_{Sensitive}$ and $TU_{D.magna}$ models, respectively (Table 3). This ambiguous result is probably due to individual compounds, for which either *D. magna* is not sensitive, e.g., neonicotinoids or insect growth regulators,[8] or for which the most sensitive species differ too much in their sensitivity to be a reliable benchmark for community effects. Moreover, if more toxicity data became available, this might increase the differences between effect metrics, which currently all heavily rely on *D. magna*. Overall, our study shows that the TU_{HC5} provides the most reliable exposure metric for streams draining agricultural catchments, but under data or resource constraints, both $TU_{Sensitive}$ and $TU_{D.magna}$ could be applied.

How Should Mixture Toxicity Be Considered? The differences between maxTU and sumTU were negligible both in terms of explained variance between best maxTU and sumTU models per country (<2% for all, Table 3) and in terms of counts, where sumTU and maxTU were 11 and 9 times among the best models across countries (Table 2). Moreover, for all metrics the according sumTU and maxTU were extremely highly correlated (all $r = 0.99$, SI Table S2). Furthermore, in 25 of 34 sites where acutely toxic concentrations occurred ($TU_{Sensitive} > 0.1$, cf.[35]), the $maxTU_{Sensitive}$ accounted for ≥87% of

Table 3. Goodness of Fit Measures (r^2 and BIC) for the Different Exposure Metrics Used in the Linear Models with $SPEAR_{pesticides}$ as Response Variable for the Different Countries Sorted by BIC[a]

Country	Explanatory variables in model	r^2	BIC
Australia	$sumTU_{HC5}$ + FUS	0.69	−31.4
Australia	$maxTU_{HC5}$ + FUS	0.69	−31.2
Australia	$maxTU_{D.magna}$ + FUS	0.67	−29.9
Australia	$sumTU_{D.magna}$ + FUS	0.67	−29.7
Australia	$sumTU_{Sensitive}$ + FUS	0.63	−26.8
Australia	$maxTU_{Sensitive}$ + FUS	0.62	−26.5
Australia	$maxTU_{HC50}$ + FUS	0.62	−26.4
Australia	$msPAF_{RA}$ + FUS	0.61	−25.9
Australia	$sumTU_{HC50}$ + FUS	0.61	−25.5
Australia	$sumTU_{HC5}$	0.53	−24.6
Australia	$maxTU_{HC5}$	0.53	−24.5
Australia	$maxTU_{D.magna}$	0.47	−21.5
Australia	$sumTU_{D.magna}$	0.45	−20.6
Australia	$sumTU_{Sensitive}$	0.42	−19.2
Australia	$maxTU_{Sensitive}$	0.40	−18.4
Australia	$maxTU_{HC50}$	0.30	−14.9
Australia	$msPAF_{RA}$	0.30	−14.8
Australia	$sumTU_{HC50}$	0.24	−13.0
Denmark	$sumTU_{Sensitive}$	0.68	−40.5
Denmark	$sumTU_{HC5}$	0.67	−40.3
Denmark	$sumTU_{HC50}$	0.66	−39.9
Denmark	$maxTU_{Sensitive}$	0.66	−39.7
Denmark	$sumTU_{Sensitive}$ + FUS	0.71	−39.2
Denmark	$maxTU_{HC5}$	0.64	−39.1
Denmark	$sumTU_{D.magna}$	0.64	−38.9
Denmark	$maxTU_{HC50}$	0.63	−38.7
Denmark	$sumTU_{HC50}$ + FUS	0.69	−38.3
Denmark	$maxTU_{Sensitive}$ + FUS	0.68	−38.2
Denmark	$sumTU_{HC5}$ + FUS	0.68	−38.0
Denmark	$maxTU_{D.magna}$	0.61	−37.9
Denmark	$sumTU_{D.magna}$ + FUS	0.66	−37.0
Denmark	$maxTU_{HC5}$ + FUS	0.64	−36.5
Denmark	$maxTU_{HC50}$ + FUS	0.64	−36.4
Denmark	$maxTU_{D.magna}$ + FUS	0.62	−35.5
Denmark	$msPAF_{RA}$	0.49	−34.1
Denmark	$msPAF_{RA}$ + FUS	0.57	−33.9
France	$maxTU_{Sensitive}$ + FUS	0.77	−23.2
France	$sumTU_{Sensitive}$ + FUS	0.75	−22.4
France	$maxTU_{Sensitive}$	0.68	−21.2
France	$sumTU_{Sensitive}$	0.66	−20.1
France	$maxTU_{D.magna}$ + FUS	0.69	−18.5
France	$sumTU_{D.magna}$ + FUS	0.68	−18.4
France	$msPAF_{RA}$ + FUS	0.66	−17.5
France	$maxTU_{HC5}$ + FUS	0.63	−15.7
France	$sumTU_{HC5}$ + FUS	0.62	−15.6
France	$msPAF_{RA}$	0.53	−14.9
France	$sumTU_{HC50}$ + FUS	0.60	−14.7
France	$maxTU_{HC50}$ + FUS	0.60	−14.6
France	$maxTU_{D.magna}$	0.49	−13.5
France	$maxTU_{HC5}$	0.48	−13.4
France	$sumTU_{D.magna}$	0.48	−13.3
France	$sumTU_{HC5}$	0.48	−13.1
France	$sumTU_{HC50}$	0.44	−12.1
France	$maxTU_{HC50}$	0.44	−12.1
Germany	$maxTU_{Sensitive}$ + FUS	0.73	−34.6
Germany	$sumTU_{HC5}$ + FUS	0.72	−34.2
Germany	$sumTU_{Sensitive}$ + FUS	0.72	−34.1
Germany	$maxTU_{D.magna}$ + FUS	0.72	−33.8
Germany	$maxTU_{Sensitive}$	0.67	−33.7
Germany	$sumTU_{HC5}$	0.67	−33.7
Germany	$maxTU_{HC5}$ + FUS	0.71	−33.5
Germany	$sumTU_{D.magna}$ + FUS	0.71	−33.5
Germany	$sumTU_{Sensitive}$	0.66	−33.1
Germany	$maxTU_{D.magna}$	0.66	−33.1
Germany	$sumTU_{D.magna}$	0.66	−32.9
Germany	$maxTU_{HC5}$	0.65	−32.8
Germany	$maxTU_{HC50}$ + FUS	0.67	−30.9
Germany	$sumTU_{HC50}$ + FUS	0.67	−30.5
Germany	$msPAF_{RA}$ + FUS	0.67	−30.5
Germany	$maxTU_{HC50}$	0.59	−29.5
Germany	$sumTU_{HC50}$	0.59	−29.3
Germany	$msPAF_{RA}$	0.57	−28.3
Spain	$msPAF_{RA}$ + FUS	0.92	−75.7
Spain	$msPAF_{RA}$	0.90	−73.0
Spain	$sumTU_{HC5}$	0.90	−72.2
Spain	$sumTU_{HC5}$ + FUS	0.90	−69.7
Spain	$maxTU_{HC5}$	0.89	−68.4
Spain	$maxTU_{HC5}$ + FUS	0.89	−67.2
Spain	$maxTU_{D.magna}$	0.86	−61.9
Spain	$maxTU_{D.magna}$ + FUS	0.86	−59.0
Spain	$maxTU_{HC50}$	0.83	−57.8
Spain	$maxTU_{HC50}$ + FUS	0.84	−55.4
Spain	$sumTU_{D.magna}$	0.81	−54.1
Spain	$sumTU_{D.magna}$ + FUS	0.81	−51.6
Spain	$sumTU_{Sensitive}$	0.78	−49.6
Spain	$sumTU_{Sensitive}$ + FUS	0.80	−49.1
Spain	$maxTU_{Sensitive}$ + FUS	0.76	−44.9
Spain	$sumTU_{HC50}$	0.73	−44.4
Spain	$maxTU_{Sensitive}$	0.72	−43.1
Spain	$sumTU_{HC50}$ + FUS	0.75	−43.0

[a]FUS = forested upstream sections.

toxicity in terms of $sumTU_{Sensitive}$ (Figure S4). This is in agreement with a recent review of ecotoxicological mesocosm studies concluding that "the effects are mostly no larger than those of the most toxic substance".[36] Our results are not in contrast with previous reviews highlighting the applicability of mixture toxicity models (i.e., CA and RA) for prediction of pesticide toxicity.[3,37,38] They rather show that in agricultural regions the toxic effects are mainly driven by a single compound and consequently maxTU is often sufficient to predict toxicity on stream macroinvertebrate communities.

Despite $msPAF_{RA}$ relying on the most sophisticated theoretical grounds, it was only superior for the Spanish data with 2% and 0.3% gain in r^2 with and without FUS (Table S3). Although the improvement was minimal, it may result from mixtures of both pesticides and nonagricultural organic toxicants occurring in Spain, whereas in the other countries pesticides were the only relevant organic toxicants. Given that the SSD-based TU_{HC5} was the most reliable exposure metric, the lower performance of $msPAF_{RA}$ in the other countries seems not due to our simplified SSD approach, but can be explained by the higher noise associated with the HC_{50} on which msPAF relied and by accounting for specific MOAs. The msPAF is advantageous if inorganic and organic toxicants have to be considered, and future studies should investigate whether

dx.doi.org/10.1021/es4014954 | Environ. Sci. Technol. 2013, 47, 7996−8004

msPAF outperforms other methods under exposure of different classes of organic toxicants.

Potential Limitations and Outlook. *(1). Calculation of Exposure Metrics.* There were only minor differences between the $maxTU_{D.\ magna}$ calculated in the current study and those calculated in the original studies (SI Figure S2). These differences are due to newer data for this study and the fact that the original studies often used data sources such as the pesticide manual[39] or the Pesticide Properties Database,[40] which give only one acute toxicity value per compound based on data quality considerations. By contrast, we calculated the mean in the case of multiple values per species−compound combination owing to the large number of compounds and toxicity data, though the plausibility of individual values was checked. Since our best-fit models in most cases improved the relationship with the effect metrics (Table 1), this justifies the automated approach we employed. Nevertheless, for individual compounds a more thorough quality check of the input toxicity data might still lead to improvements.

(2). Measuring Effects with $SPEAR_{pesticides}$. It could be argued that our results are restricted to the effect metric $SPEAR_{pesticides}$, which may be biased and not truly represent community change. However, several studies found $SPEAR_{pesticides}$ more indicative of pesticide-induced community change than other commonly used metrics.[41,42] Moreover, $SPEAR_{pesticides}$ showed high discriminatory power to nontoxicity gradients[13] and to our knowledge is the only metric that has been successfully validated for detecting pesticide stress across ecoregions.[18] Finally, the effects detected by this metric have been shown to translate into losses of regional biodiversity.[43]

An alternative approach to effect metrics is multivariate statistical methods for biotic community data, but these entail the risk that a high association of an exposure metric with one of the many nontoxicity gradients present in community data, would be falsely interpreted as reliable exposure metric. In fact, the variation in communities due to toxicants can be very low compared to other nontoxicity gradients.[44] Thus, a simulation model of toxicant-impaired ecological communities with a strong and known toxicity gradient would be needed, but such models are scarce.[45] We thus argue that $SPEAR_{pesticides}$ is currently the most suitable effect metric to evaluate toxicity exposure metrics, but we provide data and computer code so that the results can be scrutinized using other approaches.

(3). Data Availability and Effect Thresholds. Our findings depend on the available toxicity data for freshwater invertebrates, and if more toxicity data for species other than *D. magna* become available, the superiority of exposure metrics such as $TU_{Sensitive}$ or TU_{HCp} could increase. Moreover, the msPAF approach could benefit from a finer consideration of different MOA and limiting SSDs to taxa specifically affected. Despite criticism on the over-reliance of ecotoxicology on a few test species over several decades,[46] *D. magna* was the only tested freshwater invertebrate for almost half of the compounds in this study. This situation is not likely to change soon, unless testing methods are adopted that are specifically designed to accelerate the testing of many species[11] and access to existing toxicity data is improved.[47]

If exposure metrics based on species other than *D. magna* were more widely employed, this would pose the question of adaptation of effect thresholds. Currently, regulatory effect thresholds such as the first tier of the European Union Uniform Principles for the authorization of pesticides are partly defined with respect to standard toxicity tests with *D. magna*.[48] Furthermore, reviews and meta-analyses have suggested effect thresholds for freshwater ecosystems with a strong focus on *D. magna* as benchmark organism.[18,35] Hence, future studies should examine whether the formerly derived effect thresholds still apply for exposure metrics based on SSDs or other species than *D. magna*.

■ ASSOCIATED CONTENT

Supporting Information

Figures for mean slope of SSDs for different MOAs, for the correlation between exposure metrics and for differences of acute toxicity for *D. magna* and more sensitive species. Tables for the detected compounds, for intercorrelation of exposure metrics and for available toxicity data. *R* code for computation and most data to reproduce analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

Study design: RBS; provision of data: RBS, PCO, JR, ML; calculation of exposure metrics: NG, PCO, RBS, DdZ; data analysis: RBS; discussion and interpretation of results: all; drafting of manuscript: RBS, BJK, MB; revising manuscript: all.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank all persons and funding bodies, especially Melbourne Water, involved in the field studies analyzed in this paper. Eduard Szöcs is thanked for checking the computer code. Three anonymous reviewers are thanked for helpful comments. PCO was supported through a Deutsche Forschungsgemeinschaft (DFG) postdoctoral fellowship (PAK 406/1).

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