Environmental Chemistry

Effect Modeling Quantifies the Difference Between the Toxicity of Average Pesticide Concentrations and Time-Variable Exposures from Water Quality Monitoring

Roman Ashauer,[a,*] Roland Kuhl,[b] Elke Zimmer,[b] and Marion Junghans[c]

[a]Environment Department, University of York, Heslington, York, United Kingdom

[b]ibacon, Rossdorf, Germany

[c]Oekotoxzentrum, Eawag-EPFL, Dübendorf, Switzerland

Abstract: Synthetic chemicals are frequently detected in water bodies, and their concentrations vary over time. Water monitoring programs typically employ either a sequence of grab samples or continuous sampling, followed by chemical analysis. Continuous time-proportional sampling yields the time-weighted average concentration, which is taken as proxy for the real, time-variable exposure. However, we do not know how much the toxicity of the average concentration differs from the toxicity of the corresponding fluctuating exposure profile. We used toxicokinetic–toxicodynamic models (invertebrates, fish) and population growth models (algae, duckweed) to calculate the margin of safety in moving time windows across measured aquatic concentration time series (7 pesticides) in 5 streams. A longer sampling period (14 d) for time-proportional sampling leads to more deviations from the real chemical stress than shorter sampling durations (3 d). The associated error is a factor of 4 or less in the margin of safety value toward underestimating and an error of factor 9 toward overestimating chemical stress in the most toxic time windows. Under- and overestimations occur with approximate equal frequency and are very small compared with the overall variation, which ranged from 0.027 to 2.4×10^{10} (margin of safety values). We conclude that continuous, time-proportional sampling for a period of 3 and 14 d for acute and chronic assessment, respectively, yields sufficiently accurate average concentrations to assess ecotoxicological effects. *Environ Toxicol Chem* 2020;39:2158–2168. © 2020 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Effect modeling; Risk assessment; Time-weighted average; Ecotoxicology; Aquatic toxicology; Sampling strategy

INTRODUCTION

Synthetic chemicals such as pesticides, pharmaceuticals, biocides, industrial chemicals, and veterinary medicines either are released directly into the environment or reach the environment after their intended use and disposal. They are often termed "micropollutants" because they occur in the range of micrograms per liter (Schwarzenbach et al. 2006). The interplay of intermittent use patterns and environmental fate processes results in highly time-variable exposure concentrations in water bodies, in particular for pesticides in small streams

(Kreuger 1998; Leu et al. 2004; Gilliom et al. 2007; Wittmer et al. 2010; Spycher et al. 2018; Willkommen et al. 2019). Assessing the risk to the environment of these chemicals requires measuring their presence in water bodies and establishing their temporal concentration profile. Many countries maintain regular water quality monitoring programs for this purpose, their practical implementation often delegated to regional or local authorities. In theory, one could propose to automatically take hourly samples to achieve high temporal resolution; however, in practice this would quickly result in too many samples (e.g., 720 samples per site per month). The analytical chemistry procedures required to extract micropollutants are costly and not feasible for thousands of samples. Therefore, monitoring strategies typically either use a sequence of grab samples (e.g., European Community 2000; Baas et al. 2016) or continuous sampling where samples are pooled over a certain time period, followed by chemical analysis of the pooled sample for the whole sampling duration (e.g., Moschet et al. 2014;

* Address correspondence to roman.ashauer@syngenta.com

Spycher et al. 2018). Continuous sampling (e.g., with on-site autosamplers) is more likely to capture peak concentrations of short duration than other methods, and these short peaks can be important (Ashauer and Brown 2013; Stehle et al. 2013).

Continuous time-proportional sampling of water bodies has been proposed as one strategy to capture the highly time-variable exposure of aquatic organisms to micropollutants (Spycher et al. 2018). Assuming that the chemicals do not degrade in the sampler and the concentration of exposure peaks is not diluted to concentrations below the limit of quantification, this method will yield the time-weighted average (TWA) concentration over the sampling duration. If sampling times are equally spaced, this corresponds simply to the average concentration. This average concentration is then compared with ecotoxicity data to approximate the chemical stress posed by such exposures (e.g., Spycher et al. 2018). The average concentration measured in the pooled sample is taken as a representative proxy for the real, time-variable exposure. However, we do not know how much the toxicity of the average concentration differs from the toxicity of the corresponding fluctuating exposure profile. In other words, is the realistic, time-variable exposure more or less stressful for aquatic organisms than the exposure to the corresponding average concentration? We aim to answer that question by quantifying how much the toxicity of the average concentration differs from the toxicity of the corresponding fluctuating exposure profile for a range of substances, species, and water bodies.

MATERIALS AND METHODS

Toxicity modeling approach

To answer this question, we carried out computer-based toxicity modeling. We used toxicokinetic–toxicodynamic (TKTD) models (Ashauer and Escher 2010; Jager et al. 2014) for *Gammarus pulex*, *Daphnia magna*, and *Pimephales promelas* as well as simple exponential population growth models for different species of *Lemna* and algae (see Supplemental Data, Appendix C). These models account for the time course of toxicity and were selected because suitable models and data for parameterizing them were available. The models were calibrated using toxicity test data, and all models included processes which allowed the organisms to recover during periods of lower exposure concentrations. This approach enables modeling the time course of toxicity with better accuracy than

using average concentrations. The toxicity of an average concentration only equals the toxicity of the corresponding fluctuating concentration when the organism's toxicokinetics and toxicodynamics are much faster than the timescale of the exposure fluctuations (Rozman and Doull 2000; Ashauer and Brown 2013). This situation is called "Haber's law" or "time effect reciprocity" (Figure 1). At the population level the same principle holds if population dynamics are much faster than the fluctuations of the exposure profile (Ashauer and Brown 2013).

We assume that the effect models used in the present study represent the real time course of toxicity with sufficient accuracy (Ashauer and Brown 2013; Ashauer et al. 2015; Focks et al. 2018; Jager and Ashauer 2018b; Ockleford et al. 2018; Zimmer et al. 2018). Based on that, we simulate the toxic effects of the fluctuating exposure profile and its corresponding average concentration and compare the 2. This predicts how the toxicity of the average concentration (representing time-proportional sampling) might differ from the real toxicity resulting from fluctuating concentrations (based on real monitoring data from Swiss water bodies; see *Results*). In other words, we provide an evaluation of time-proportional water pollution sampling by approximating the systematic error due to divergence from Haber's law. By doing these simulations and comparisons for different durations (3 and 14 d), we can also assess the consequences of shorter compared with longer sampling durations.

Margin of safety

When simulating the toxicity of exposure profiles from monitoring data, the resulting effects are often 0%, when the concentrations are below the toxic range, or 100%, when the concentrations are above the toxic range (see *Results*). This is because real concentrations in water bodies vary across several orders of magnitude (Moschet et al. 2014; Spycher et al. 2018). Nothing could be learned about the difference in toxicity caused by different exposure profiles from such all-or-nothing simulation results because we need to assess the differences in toxicity when comparing average versus fluctuating concentrations. To overcome this problem, we use the margin of safety concept (Ashauer et al. 2013). This is built on the idea that every concentration profile can be multiplied with a factor, the margin of safety, to bring it into the toxic range. This factor is larger than 1 if the concentration profile was originally below toxic levels, or
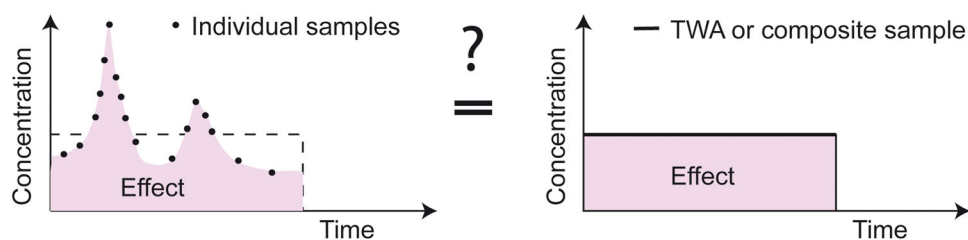
FIGURE 1: Illustration of time-proportional sampling (left) and the corresponding average concentration in the pooled sample (right). If both result in the same toxic effect, then this is a special case called "time-concentration reciprocity" or "Haber's law." It occurs when toxicokinetics and toxicodynamics operate on much faster timescales than the concentration fluctuations (Rozman and Doull 2000; Ashauer and Brown 2013). TWA = time-weighted average.

it can be smaller than 1 if the original concentrations were higher than the toxic range. We define the margin of safety as the factor that, when used to multiply the exposure concentrations, results in 50% effects at the end of the simulated exposure profile. This factor is also known as the "multiplication factor" to an entire specific exposure profile that causes 50% lethality (LP50; Ockleford et al. 2018). In simulations where the endpoint is survival (mortality, acute toxicity), the margin of safety would be the factor to multiply concentrations in a monitoring time series with so that after simulating, for example, 14 d of exposure 50% of *G. pulex* (or *P. promelas*) would be dead. Similarly, we used 50% reduction in growth or reproductive output of *D. magna* as well as 50% reduction in biomass produced by *Lemna* or algae populations to calculate the margin of safety. The margin of safety is calculated for simulations with the average concentration (representing time-proportional sampling) and simulations with the fluctuating exposure profile (representing real exposures). Then, the 2 margin of safety values were compared to assess if the time-proportional sampling over- or underestimates the chemical stress and by how much. Note that the margin of safety is similar to the toxicity–exposure ratios traditionally used in plant protection product authorization and can be interpreted the same way.

Monitoring data

The aquatic monitoring data originate from a monitoring campaign conducted in 2015 in 5 small water bodies (Table 1) situated in intensively used agricultural areas in Switzerland. Details on the water bodies and the monitoring can be found in Doppler et al. (2017) and Spycher et al. (2018). In short, samples were taken from March to August 2015, and automated samplers took half-day time-proportional composite samples. To reduce the effort of chemical analysis, samples during periods of dry weather were combined before analysis, assuming that concentration peaks are rainfall-driven. This results in samples with a maximum temporal resolution of 12 h. Chemical analysis was performed by liquid chromatography mass spectrometry (high-resolution tandem mass spectrometry; Orbitrap-Technology) after enrichment via online solid-phase extraction (Spycher et al. 2018).

Simulated time windows

For each monitoring data set, we simulated moving time windows of 14 or 3 d duration because these have been proposed as durations of time-proportional water sampling in Switzerland (Wittmer et al. 2014). An analysis by Spycher et al. (2018) further showed that averaging times should not exceed 3 d for acute risk assessments for pesticides in small streams because peak exposure might be diluted to concentrations below the limit of quantification or well below acute water quality criteria like the maximum acceptable concentration under the European Union Water Framework Directive. The margin of safety was calculated for the resulting effects at the end of each time window. Each sampling time is the starting point of a single time window. Therefore, 2 time windows were created for each day because the exposure data have a time step of 12 h.

Substances studied

Although the monitoring comprised more than 100 substances, the concentration profiles of 7 pesticides were selected for the effect modeling (Table 2) based on the availability of ecotoxicity data suitable for TKTD model calibration. They cover 5 different chemical classes and modes of action (Table 2) as well as 5 different monitoring sites and 5 ecotoxicological endpoints (Table 3).

Endpoint mortality in G. pulex *and* P. promelas

For *G. pulex* and *P. promelas*, we simulated toxic effects on survival using the TKTD model general unified threshold model of survival (GUTS; Jager et al. 2011). More specifically, we used the limit cases GUTS scaled internal concentration (SIC) with stochastic death and individual tolerance (Nyman et al. 2012; Ashauer et al. 2016; Jager and Ashauer 2018b). Because the model calibration to toxicity data for GUTS-SIC-individual tolerance resulted in much poorer quality of fit than for GUTS-SIC-stochastic death in *G. pulex* for chlorpyrifos (–log-likelihood stochastic death = 942, individual tolerance = 1004) and carbendazim (–log-likelihood stochastic death = 737, individual tolerance = 1006), we used only GUTS-SIC-stochastic death for this species. For *P. promelas*, we used GUTS-SIC-stochastic death and GUTS-SIC-individual tolerance and averaged the percentage of time data from the 2 models. Five substances (carbendazim, chlorpyrifos, diazinon, dimethoate, and imidacloprid) were assessed for *G. pulex* in 3 locations (Table 3), depending on the availability of monitoring data. Two substances were assessed for *P. promelas* in 2 locations (Table 3). Two durations of the time window (3 and 14 d) were assessed.

TABLE 1: Description of the sampling sites

Name of water body	Area arable farming (%)	Area orchards (%)	Area viticulture (%)	Area settlement (%)	Catchment size (km^2)
Canale Piano di Magadino	15	0	3	17	9.0
Eschelisbach	29	13	0	0	2.0
La Tsatonire	0	1	22	11	2.4
Mooskanal	65	0	0	8	3.4
Weierbach	52	3	0	14	1.6

TABLE 2: Selected substances, their modes of action, and the most sensitive taxonomic groups[a]

Substance (CAS number)	Chemical class	Mode of action (Tomlin 2009)	Most sensitive taxonomic group
Carbendazim (10605-21-7)	Benzimidazole fungicide	Inhibition of beta-tubulin synthesis	Arthropods and fish
Diuron (330-54-1)	Phenylurea herbicide	Inhibition of photosynthesis at photosystem II	Cyanobacteria and algae
Metazachlor (67129-08-2)	Chloroacetamide herbicide	Inhibition of very long chain fatty acids	Green algae and higher plants
Chlorpyrifos (2921-88-2)	Organophosphate insecticide	Inhibition of cholinesterase	Arthropods
Diazinon (333-41-5)	Organophosphate insecticide	Inhibition of cholinesterase	Arthropods
Dimethoate (60-51-5)	Organophosphate insecticide	Inhibition of cholinesterase	Arthropods
Imidacloprid (138261-41-3)	Neonicotinoid insecticide	Binding to postsynaptic nicotinoid receptors in the insect central nervous system (antagonist)	Arthropods

[a]Based on the environmental quality standard dossiers by the Swiss Centre for Applied Ecotoxicology (Korkaric et al. 2019).
CAS = Chemical Abstracts Service.

The toxicity data for calibration of GUTS for *G. pulex* originated from previously published studies (chlorpyrifos, Ashauer et al. 2007; diazinon, Ashauer et al. 2010; imidacloprid, Nyman et al. 2013) and previously unpublished acute toxicity experiments (carbendazim, dimethoate) which were carried out following established protocols (Ashauer et al. 2011). The toxicity data for calibration of GUTS for *P. promelas* also originated from the scientific literature (Geiger et al. 1988, 1990). See more details on the GUTS model calibration and use in Supplemental Data, Appendix A, and previously unpublished toxicity data (*G. pulex*, carbendazim, dimethoate) in Supplemental Data, Appendix D.

Endpoints growth and reproduction in D. magna

For *D. magna*, we simulated the effects of imidacloprid on 2 sublethal endpoints: organism growth ("length") and reproductive output ("offspring") using a dynamic energy budget model with a toxicity module (DEBtox; Jager and Zimmer 2012). The model was calibrated based on published data (Agatz et al. 2013), and we simulated moving 14-d time windows in 3 locations (Eschelisbach, La Tsatonire, and Weierbach). For *D. magna* only the simulations with the 14-d time window are discussed because that duration is more relevant for the chronic endpoints modeled in *D. magna* than the 3-d time window. See more details on the DEBtox model parameterization and simulations in Supplemental Data, Appendix B.

Endpoint population biomass in duckweed and algae

To assess responses to different exposure profiles of herbicides, we simulated effects on biomass growth for metazachlor and *Lemna gibba*, diuron and *Lemna minor*, metazachlor and *Scenedesmus subspicatus*, as well as diuron and the blue-green alga *Synechococcus* sp. Simulations for metazachlor used exposure data from Mooskanal, La Tsatonire, and Weierbach, whereas simulations for diuron used exposure data from Eschelisbach, La Tsatonire, and Weierbach. The effect model was a simple exponential population model for biomass growth as is also used to analyze standard toxicity tests with algae and *Lemna*. We assessed 3- and 14-d moving time windows.

The simulations were based on published growth rate data. For metazachlor the data were taken from the draft assessment report (European Food Safety Authority 2005). The 7-d growth rates for *L. gibba* derive from Scheerbaum (2000a). The 3-d growth data for the alga *Scenedesmus subspicatus* derive from Scheerbaum (2000b). The 3-d growth rates for diuron and *L. minor* were published by Drost (2011). And the 3-d growth rates for the blue-green alga *Synechococcus* sp. were taken from Devilla et al. (2005).

Detailed documentation of modeling

The modeling work is described in more detail in 3 appendices available as Supplemental Data: A (*G. pulex* and *P. promelas*), B (*D. magna*), and C (*Lemna* sp. and algae).

TABLE 3: Overview of the organism, exposure profile, and substances, showing all combinations of available monitoring data and suitable ecotoxicity models and data[a]

Substance	Canale Piano di Magadino		Eschelisbach		La Tsatonire		Mooskanal		Weierbach	
Carbendazim			1		1				1	
Chlorpyrifos			1	2	1	2				
Diazinon			1	2					1	2
Dimethoate					1				1	
Imidacloprid			1	3	1	3			1	3
Diuron			4	5	4	5			4	5
Metazachlor	4	5					4	5	4	5

[a]Numbers indicate the modeled effect endpoint: 1 = mortality of crustacean Gammarus pulex; 2 = mortality of fish (Pimephales promelas); 3 = growth and reproduction of crustacean Daphnia magna; 4 = growth of duckweed (Lemna gibba or Lemna minor; 5 = growth of algae populations (various species, see section Endpoint population biomass in duckweed and algae).

RESULTS

By how much does time-proportional sampling over- or underestimate chemical stress?

To answer this question, we analyzed the margin of safety values from the most toxic time windows, that is, those with the smallest margin of safety values (Figure 2). The most toxic time window varied depending on sampling site, compound, and species (see Supplemental Data. Appendices A–C).

Generally, TWA and fluctuating concentration-based margin of safety values correlate well across orders of magnitude (Figure 2). For *G. pulex* and *P. promelas* (Figure 2A), we observe that during the most toxic exposure periods the TWA method generally predicts higher margin of safety values; that is, it underestimates chemical stress. On average, the margin of safety is a factor of 1.4 higher with the TWA method during the most toxic periods, with the difference being more pronounced for 14 d (factor 1.7 higher) than for 3 d (factor 1.1 higher). This means that a time-proportional sampling strategy would underestimate the chemical stress by a factor of 1.4 on average (factor 1.7 when sampling 14 d).

For *D. magna* (Figure 2B), we observe that during the most toxic exposure periods the TWA method predicts higher margin of safety values for "offspring" but lower margin of safety values for "length"; that is, it underestimates chemical stress for effects on *D. magna* offspring but overestimates chemical stress for effects on *D. magna* length. On average, the margin of safety calculated with the TWA method for length is 0.51 times the value calculated for fluctuating exposure. For effects on offspring, the TWA method calculates 1.1 times the value of the fluctuating exposure (14-d time window). Hence, time-proportional sampling overestimates chemical stress for effects on growth and slightly underestimates chemical stress for effects on offspring.

For *Lemna* (Figure 2C) and algae (Figure 2D), we observe that the margin of safety values calculated with the TWA method are mostly equal to those calculated with the fluctuating concentrations (outlier: Weierbach and diuron), and sometimes the TWA method overestimates chemical stress (i.e., it errs on the conservative side). On average, the margin of safety is a factor of 0.8 lower with the TWA method during the most toxic periods for *Lemna* with the 3-d time window and equal with the fluctuating concentrations for the 14-d time
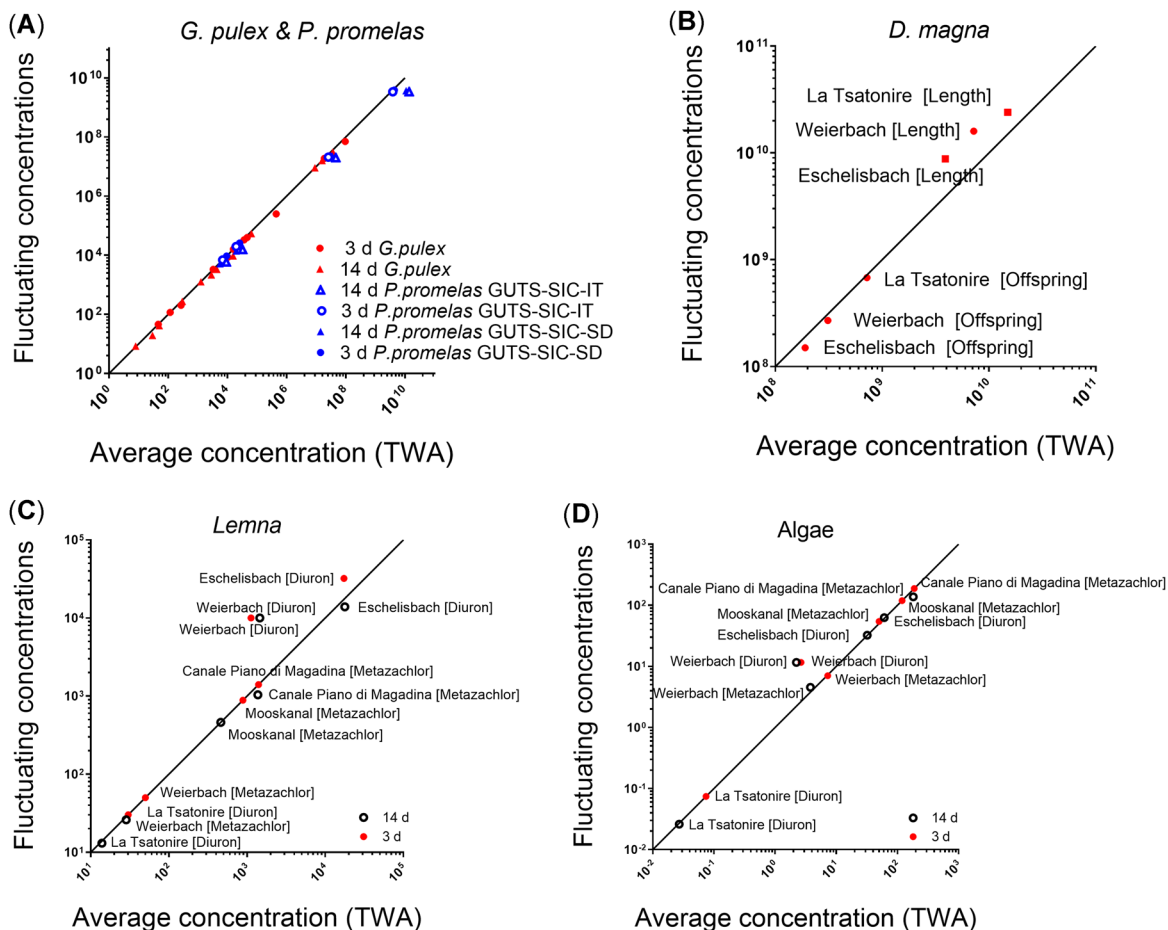
FIGURE 2: Comparison of margin of safety values based on time-weighted average concentrations (as proxy for time-proportional sampling) and based on fluctuating concentrations. General unified threshold model of survival -scaled internal concentration-individual tolerance/-stochastic death = general unified threshold model of survival with scaled internal concentration using the assumptions of individual tolerance or stochastic death, respectively; GUTS-SIC-IT = general unified threshold model of survival-scaled internal concentration-individual tolerance; SD = stochastic death; TWA = time-weighted average.

window. For algae, the margin of safety is a factor of 0.9 lower on average with the TWA method during the most toxic periods with the 3- and the 14-d time windows.

In summary, these findings suggest that time-proportional sampling would underestimate chemical stress for *G. pulex*, *D. magna*, and *P. promelas* and slightly overestimate chemical stress for *Lemna* and algae. The largest differences, during the most toxic time windows, were found for the chemical stress caused by diazinon on *P. promelas* in Weierbach, where the margin of safety for the 14-d TWA was 4 times higher than the margin of safety for the corresponding fluctuating concentration profile and for chemical stress of diuron in *Lemna* in Weierbach, where the margin of safety for the 3-d TWA was 9 times lower than the margin of safety of the corresponding fluctuating concentration profile. However, the chemical stress, approximated as margin of safety values, ranges over 12 orders of magnitude, whereas the differences between the time-proportional sampling method and the real, fluctuating exposure are comparatively small. In other words, a difference in the margin of safety value of <1 order of magnitude is small when margin of safety values range over 12 orders of magnitude (from 0.027 to 2.4×10^{10}; see also Supplemental Data). The margin of safety value is a proxy for chemical stress. Our analysis shows that the chemical stress in small Swiss streams can vary over 12 orders of magnitude depending on location, compound, and species. In this context the calculated error of the time-proportional sampling method (i.e., the difference in chemical stress from the real exposure profile) is relatively small.
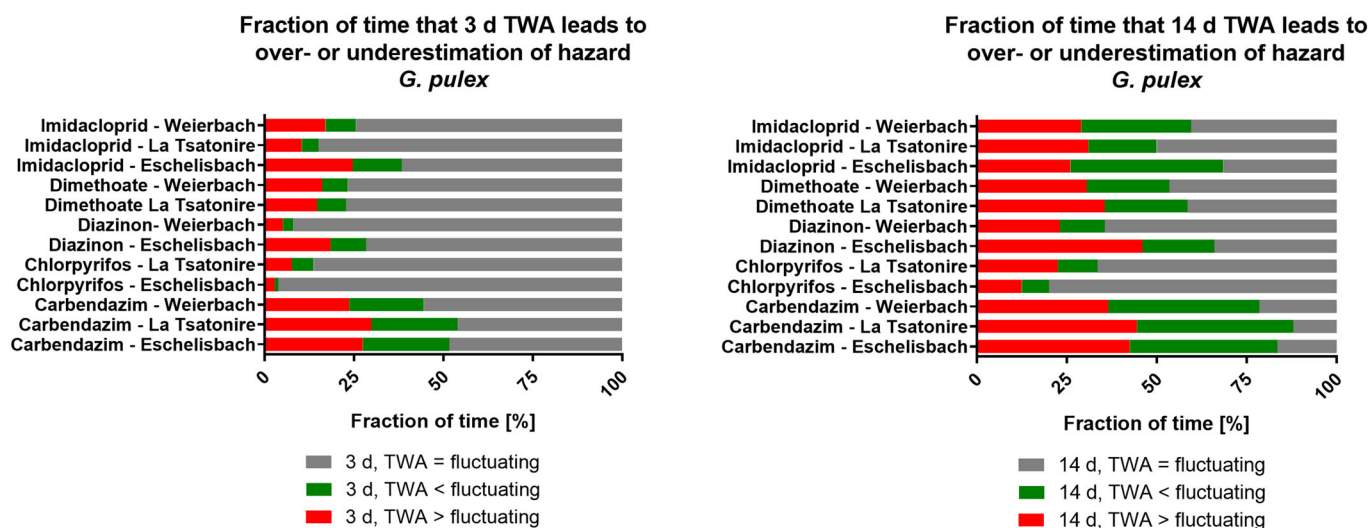
How often does time-proportional sampling over- or underestimate chemical stress?

To answer this question, we analyzed the margin of safety values from all time windows over the whole length of the monitoring profiles.
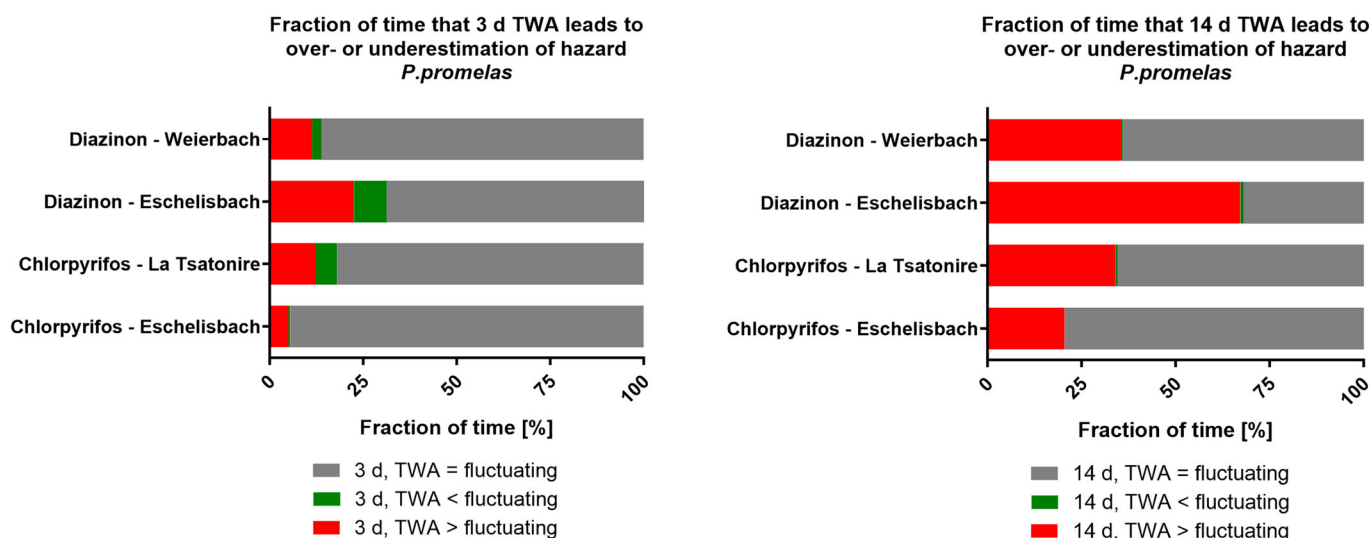
The simulations with gammarids and fish show that during a 14-d period the TWA method deviates more often from the fluctuating exposure assessment than during a 3-d window (Figures 3 and 4). Both the 3- and the 14-d duration simulations show the time-proportional sampling more frequently underestimating chemical stress for *G. pulex* (17 and 32% of the time, respectively) than overestimating chemical stress (11 and 26% of the time, respectively; Figure 3). For *P. promelas*, the 3-d simulations underestimate chemical stress 13% of the time and overestimate chemical stress 4% of the time, and the 14-d simulations underestimate chemical stress 39% of the time and overestimate chemical stress 0% of the time (Figure 4). This is because peak exposures are driving the toxicity for diazinon and chlorpyrifos in *P. promelas*. Averaging out the peaks to lower TWA concentrations almost always reduces the toxicity for these 2 compounds in *P. promelas*; however, the absolute error is a factor of 2.1 or less (see margin of safety values, previous section).

The simulations with *D. magna* and imidacloprid show that over- and underestimations of chemical stress are frequent (Figure 5). Overestimation of chemical stress in the time-proportional sampling occurs more frequently overall (44% of the time overestimated vs 24% underestimated). This is due to overestimation of chemical stress for effects on growth (58% of the time overestimated vs 12% underestimated), whereas effects on reproduction are generally underestimated by the time-proportional sampling (30% of the time overestimated vs 36% underestimated).

Simulations of biomass growth with duckweed and algae show that during a 14-d period the TWA method deviates more often from the fluctuating exposure assessment than during a 3-d window (Figure 6). For duckweed (*Lemna*), we observe that the 3-d simulations overestimate chemical stress but that the 14-d simulations seem to underestimate chemical stress (28% of the time) more often than overestimating it

Fraction of time that 3 d TWA leads to over- or underestimation of hazard
G. pulex

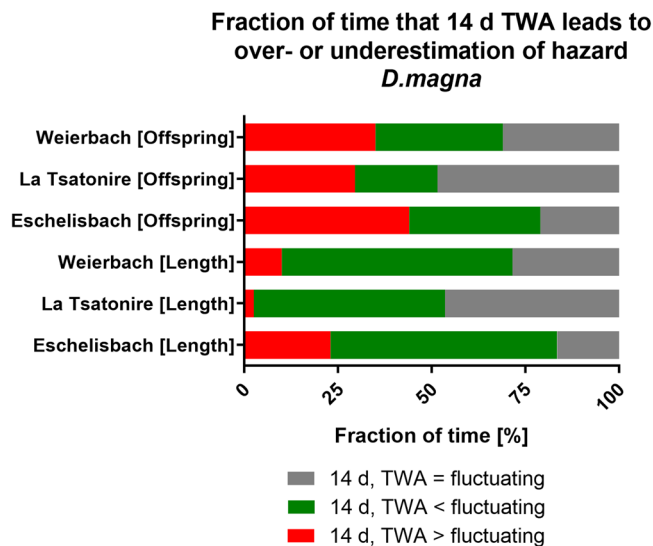**Fraction of time that 14 d TWA leads to over- or underestimation of hazard**
G. pulex

FIGURE 3: Fraction of time windows during which time-proportional sampling (TWA) would over- or underestimate the margin of safety for gammarids: TWA > fluctuating means the hazard is underestimated (red), and TWA < fluctuating means that time-proportional sampling would overestimate (green). Comparison of 3 d (left) versus 14 d (right). The fraction of equal hazard (gray) generally corresponds to very low exposure, which approaches infinitely large margin of safety values when exposure approaches zero (cutoff value in practice).

Fraction of time that 3 d TWA leads to over- or underestimation of hazard
P.promelas

Fraction of time that 14 d TWA leads to over- or underestimation of hazard
P.promelas

FIGURE 4: Fraction of time windows during which time-proportional sampling (TWA) would over- or underestimate the margin of safety for fish: TWA > fluctuating means the hazard is underestimated (red), and TWA < fluctuating means that time-proportional sampling would overestimate (green). Comparison of 3 d (left) versus 14 d (right). The fraction of equal hazard (gray) generally corresponds to very low exposure, which approaches infinitely large margin of safety values when exposure approaches zero (cutoff value in practice).

(17% of the time). For algae, we observe that it depends more strongly on the location and substance sampled whether the 3- or 14-d duration over- or underestimates chemical stress compared with the other species. However, the time-proportional sampling would generally overestimate chemical stress for algae on average (overestimation, 12% [3 d] and 36% [14 d]; underestimation, 11% [3 d] and 15% [14 d]).

Fraction of time that 14 d TWA leads to over- or underestimation of hazard
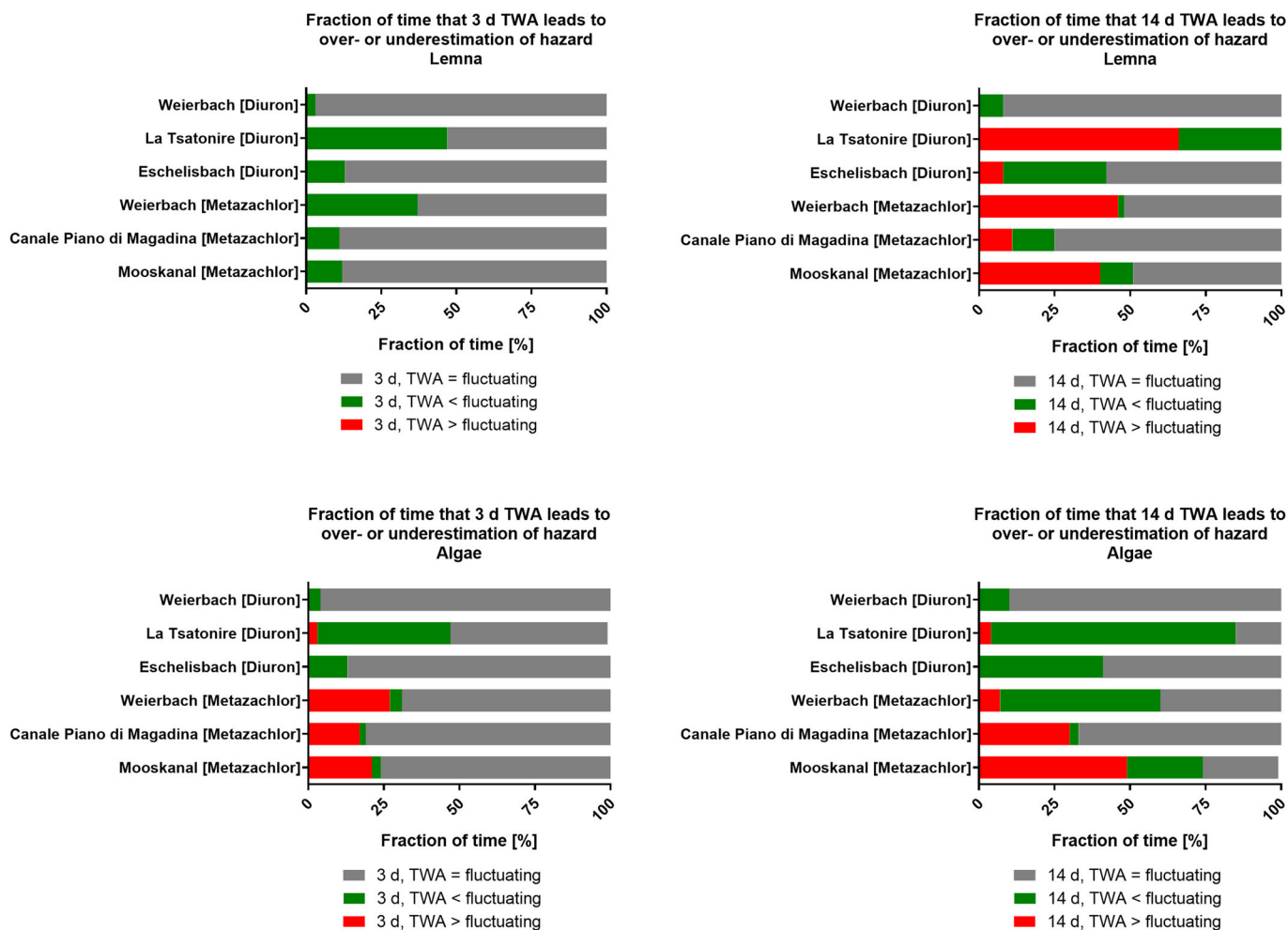### *D.magna*

FIGURE 5: Fraction of time windows during which time-proportional sampling (time-weighted average [TWA]) would over- or underestimate the margin of safety for *Daphnia magna* and imidacloprid: TWA > fluctuating means the chemical stress is underestimated (red), and TWA < fluctuating means that time-proportional sampling would overestimate (green). Comparison of 3 d (left) versus 14 d (right). The fraction of equal hazard (gray) generally corresponds to very low exposure, which approaches infinitely large margin of safety values when exposure approaches zero (cutoff value in practice).

DISCUSSION

Insights from the margin of safety analysis and its limitations

To our knowledge, this is the first time that margin of safety (Ashauer et al. 2013) or LP50 (Ockleford et al. 2018) values were calculated for real exposure time series from environmental monitoring. A margin of safety value is a proxy for risk of toxicity and has a similar interpretation as the toxicity–exposure ratio or risk quotient (van Leeuwen and Vermeire 2007). Small margin of safety values, near 1 or below, indicate chemical stress; and large values indicate little or no chemical stress. Our analysis, using TKTD modeling to quantify effects on invertebrates and fish and population modeling for effects on algae and aquatic plants, confirmed the findings of Spycher et al. (2018), who calculated risk quotients for the same monitoring data and found several instances of potential risk to aquatic organisms. In the most toxic time windows, we calculated margin of safety values far below 1 for algae (La Tsatonire, diuron; Figure 2D), which indicates a potential risk to primary producers. We also found several instances of margin of safety values above but within an order of magnitude of 1. These indicate that for the effect endpoints and species combinations modeled, we would not expect effects from exposure to the modeled substances alone. Interestingly, we found a difference in margin of safety values for *D. magna* when calculated for effects on growth or reproduction, and we observed outlier data points for algae and *Lemna* with diuron in the Weierbach. The endpoint-specific results for *D. magna* suggest that the physiological mode of action is important (see also Ashauer and Jager 2018), and it should also be noted that *D. magna* is less sensitive to imidacloprid than other crustacean species (Ashauer et al. 2011; Roessink et al. 2013). Further, we found

FIGURE 6: Fraction of time windows during which time-proportional sampling (time-weighted average [TWA]) would over- or underestimate the margin of safety for *Lemna* and algae: TWA > fluctuating means the chemical stress is underestimated (red), and TWA < fluctuating means that time-proportional sampling would overestimate (green). Comparison of 3 d (left) versus 14 d (right). The fraction of equal hazard (gray) generally corresponds to very low exposure, which approaches infinitely large margin of safety values when exposure approaches zero (cutoff value in practice).

that either under- or overestimation of risk for *D. magna* occurred frequently. We know already that TKTD model results are sensitive to changes in exposure patterns (Ashauer et al. 2013; Van den Brink et al. 2019), but we do not know if our findings would be observed for a wider range of exposure profiles too. Understanding the causes of these observations warrants further research.

It is important to bear in mind that we simulated only a small selection of sublethal effects in a very small number of species and locations. A more comprehensive understanding will become possible in the near future because more and more TKTD models parameterized for additional species are becoming available (e.g., Focks et al. 2018; Zimmer et al. 2018; Vighi et al. 2019; Dalhoff et al. 2020) as well as a better understanding of how species traits determine TK and TD (Van Den Berg et al. 2019; Dalhoff et al. 2020). Since our study, conducted mostly in 2017, new software for TKTD modeling with GUTS became available (Openguts 2019), which means that the problem with poor fits

for GUTS-SIC-individual tolerance for 2 of our data sets could probably be overcome in the future. Also, we do not know how accurate our models are because TKTD model validation studies are rare (Augusiak et al. 2014; Jager and Ashauer 2018a). Most of our analysis was based on acute toxicity data, and all of our analyses concerned only exposure to a single substance at a time. Other possible effects of micropollutants on aquatic life, such as those on the endocrine system (Ankley and Villeneuve 2015), community-level impacts (Baert et al. 2016; Rohr et al. 2016), and epigenetic effects or behavioral changes (Nyman et al. 2013; Pedersen et al. 2013; Ford et al. 2018), might be ecologically relevant for chronic risk assessment of micropollutants as well as mixture toxicity (Ashauer et al. 2017; Escher et al. 2020) and interactions with other, nonchemical stressors (Holmstrup et al. 2010; Woodward et al. 2012). We analyzed models for each species separately. In addition, one could model the effects on species sensitivity distributions (Van den Brink et al. 2019) and communities (De Laender et al. 2016; Strauss

et al. 2017). Our species were selected based on the availability of appropriate TKTD models and the data required to parameterize these and do not necessarily include the most sensitive species for a given substance (Van Den Berg et al. 2019). However, these aspects were not considered because our aim was to compare TWA concentrations with time-proportional sampling.

Choosing a time-proportional sampling duration

Our aim was to evaluate water sampling strategies (Robertson and Roerish 1999; Ort et al. 2010), specifically from an ecotoxicological effects perspective, because previous research has focused only on comparing average concentrations derived with different sampling methods such as grab samples and passive sampling (Hyne et al. 2004; Fernández et al. 2014) or on theoretical (eco)toxicological considerations (Rozman and Doull 2000; Ashauer and Brown 2013). We found that when using a 3-d sampling duration our simulations showed considerably fewer time periods when time-proportional sampling either under- or overestimates chemical stress compared to 14-d sampling duration. Therefore, it is reasonable to use a 3-d sampling duration, or a similarly short sampling duration, for assessment of acute effects. For chronic effects assessment, a longer sampling duration is more realistic. With the exception of fish, the 14-d sampling duration simulated in the present study shows a balanced frequency of over- or underestimations of chemical stress and a small overall error compared with the magnitude of variation in chemical stress. Hence, using 14-d time-proportional sampling to measure micropollutants for assessment of chronic effects appears reasonable from the perspective of time-variable exposure, but more simulations with different compounds would be desirable to strengthen the evidence base, especially in the case of fish. Furthermore, the question answered in the present study is not the only consideration when setting the duration of the water sampling for the assessment of chronic risk—other aspects need to be considered too, for example, the duration of ecotoxicity studies (Baas et al. 2010; Ashauer and Brown 2013), the ecology of the relevant species (Calow et al. 1997; Rohr et al. 2016), and the risk-management context of the assessment (van Leeuwen and Vermeire 2007).

Generally, the model simulations show that a longer sampling period (14 d) for time-proportional water sampling leads to more deviations from the real chemical stress than shorter sampling durations (3 d). However, the overall picture also shows that under- and overestimations of chemical stress occur with approximately equal frequency, although for some locations, compounds, and species we observed clear under- or overestimation of chemical stress (e.g., 14-d fish and 3-d *Lemna*). When under- or overestimations of chemical stress occur, they are very small compared with the overall variation in chemical stress observed. In this data set, the chemical stress to aquatic organisms from pesticides in Swiss streams ranged over 12 orders of magnitude, from 0.027 to 2.4×10^{10} (margin of safety values, where small values indicate a chemical stress and large values indicate safety).

CONCLUSIONS

We found that the time-proportional sampling method is associated with an error of factor 4 or less in the margin of safety value toward underestimating chemical stress and an error of factor of 9 toward overestimating chemical stress. This error margin is small compared to 12 orders of magnitude variation in chemical stress across locations, compounds, and species. We conclude that time-proportional sampling is suitable for chemical water quality monitoring because it characterizes the concentration time series sufficiently well. Continuous time-proportional sampling for a period of 3 and 14 d for acute and chronic assessment, respectively, yields sufficiently accurate average concentrations to assess ecotoxicological effects.

REFERENCES

Agatz A, Cole TA, Preuss TG, Zimmer E, Brown CD. 2013. Feeding inhibition explains effects of imidacloprid on the growth, maturation, reproduction, and survival of daphnia magna. *Environ Sci Technol* 47:2909–2917.

Ankley GT, Villeneuve DL. 2015. Temporal changes in biological responses and uncertainty in assessing risks of endocrine-disrupting chemicals: Insights from intensive time-course studies with fish. *Toxicol Sci* 144:259–275.

Ashauer R, Albert C, Augustine S, Cedergreen N, Charles S, Ducrot V, Focks A, Gabsi F, Gergs A, Goussen B, Jager T, Kramer NI, Nyman A-M, Poulsen V, Reichenberger S, Schafer RB, Van den Brink PJ, Veltman K, Vogel S, Zimmer EI, Preuss TG. 2016. Modelling survival: Exposure pattern, species sensitivity and uncertainty. *Sci Rep* 6:29178.

Ashauer R, Boxall ABA, Brown CD. 2007. New ecotoxicological model to simulate survival of aquatic invertebrates after exposure to fluctuating and sequential pulses of pesticides. *Environ Sci Technol* 41:1480–1486.

Ashauer R, Brown CD. 2013. Highly time-variable exposure to chemicals—Towards an assessment strategy. *Integr Environ Assess Manag* 9:27–33.

Ashauer R, Escher BI. 2010. Advantages of toxicokinetic and toxicodynamic modelling in aquatic ecotoxicology and risk assessment. *J Environ Monit* 12:2056–2061.

Ashauer R, Hintermeister A, Caravatti I, Kretschmann A, Escher BI. 2010. Toxicokinetic–toxicodynamic modeling explains carry-over toxicity from exposure to diazinon by slow organism recovery. *Environ Sci Technol* 44:3963–3971.

Ashauer R, Hintermeister A, Potthoff E, Escher BI. 2011. Acute toxicity of organic chemicals to *Gammarus pulex* correlates with sensitivity of *Daphnia magna* across most modes of action. *Aquat Toxicol* 103:38–45.

Ashauer R, Jager T. 2018. Physiological modes of action across species and toxicants: The key to predictive ecotoxicology. *Environ Sci Process Impacts* 20:48–57.

Ashauer R, O'Connor I, Escher BI. 2017. Toxic mixtures in time—The sequence makes the poison. *Environ Sci Technol* 51:3084–3092.

Ashauer R, O'Connor I, Hintermeister A, Escher BI. 2015. Death dilemma and organism recovery in ecotoxicology. *Environ Sci Technol* 49:10136–10146.

Ashauer R, Thorbek P, Warinton JS, Wheeler JR, Maund S. 2013. A method to predict and understand fish survival under dynamic chemical stress using standard ecotoxicity data. *Environ Toxicol Chem* 23:954–965.

Augusiak J, Van den Brink PJ, Grimm V. 2014. Merging validation and evaluation of ecological models to "evaludation": A review of terminology and a practical approach. *Ecol Modell* 280:117–128.

Baas J, Jager T, Kooijman B. 2010. Understanding toxicity as processes in time. *Sci Total Environ* 408:3735–3739.

Baas J, Vijver M, Rambohul J, Dunbar M, van 't Zelfde M, Svendsen C, Spurgeon D. 2016. Comparison and evaluation of pesticide monitoring programs using a process-based mixture model. *Environ Toxicol Chem* 35:3113–3123.

Baert JM, Janssen CR, Sabbe K, De Laender F. 2016. Per capita interactions and stress tolerance drive stress-induced changes in biodiversity effects on ecosystem functions. *Nat Commun* 7:12486.

Calow P, Sibly RM, Forbes V. 1997. Risk assessment on the basis of simplified life-history scenarios. *Environ Toxicol Chem* 16:1983–1989.

Dalhoff K, Hansen AMB, Rasmussen JJ, Focks A, Strobel BW, Cedergreen N. 2020. Linking morphology, toxicokinetic, and toxicodynamic traits of aquatic invertebrates to pyrethroid sensitivity. *Environ Sci Technol* 54:5687–5699.

De Laender F, Rohr JR, Ashauer R, Baird DJ, Berger U, Eisenhauer N, Grimm V, Hommen U, Maltby L, Meliàn CJ, Pomati F, Roessink I, Radchuk V, Van den Brink PJ. 2016. Reintroducing environmental change drivers in biodiversity–ecosystem functioning research. *Trends Ecol Evol* 31:905–915.

Devilla RA, Brown MT, Donkin M, Tarran GA, Aiken J, Readman JW. 2005. Impact of antifouling booster biocides on single microalgal species and on a natural marine phytoplankton community. *Mar Ecol Prog Ser* 286:1–12.

Doppler T, Mangold S, Wittmer I, Spycher S, Comte R, Stamm C, Singer H, Junghans M, Kunz M. 2017. Hohe psm-belastung in schweizer bächen. *AQUA & GAS* 4:46–56.

Drost W. 2011. The factor time in assessing ecotoxicity—Studies with herbicides and metals applied singly and in combination to *Lemna minor* in simple and complex patterns. PhD dissertation. University of Bremen, Bremen, Germany.

Escher BI, Stapleton HM, Schymanski EL. 2020. Tracking complex mixtures of chemicals in our changing environment. *Science* 367:388.

European Community. 2000. 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. *Official Journal of the European Community* L327:1–72.

European Food Safety Authority. 2005. Metazachlor—Report and proposed decision of the United Kingdom made to the European Commission under article 8(1) of 91/414/eec. Parma, Italy.

Fernández D, Vermeirssen ELM, Bandow N, Muñoz K, Schäfer RB. 2014. Calibration and field application of passive sampling for episodic exposure to polar organic pesticides in streams. *Environ Pollut* 194:196–202.

Focks A, Belgers D, Boerwinkel M-C, Buijse L, Roessink I, Van den Brink PJ. 2018. Calibration and validation of toxicokinetic–toxicodynamic models for three neonicotinoids and some aquatic macroinvertebrates. *Ecotoxicology* 27:992–1007.

Ford AT, Hyett B, Cassidy D, Malyon G. 2018. The effects of fluoxetine on attachment and righting behaviours in marine (*Gibbula unbilicalis*) and freshwater (*Lymnea stagnalis*) gastropods. *Ecotoxicology* 27:477–484.

Geiger DL, Brooke LT, Call DJ. 1990. Acute toxicities of organic chemicals to fathead minnow (*Pimephales promelas*), Vol 5. University of Wisconsin-Superior, Superior, WI, USA.

Geiger DL, Call DJ, Brooke LT. 1988. Acute toxicities of organic chemicals to fathead minnow (*Pimephales promelas*), Vol 4. University of Wisconsin-Superior, Superior, WI, USA.

Gilliom RJ, Barbash Ej, Crawford CG, Hamilton PA, Martin D, Nakagaki N, Nowell LH, Scott JC, Stackelber PE, Thelin GP, Wolock DM. 2007. The quality of our nation`s waters—Pesticides in the nation`s streams and ground water 1992–2001. US Geological Survey, Reston, VA.

Holmstrup M, Bindesbøl A-M, Oostingh GJ, Duschl A, Scheil V, Köhler H-R, Loureiro S, Soares AMVM, Ferreira ALG, Kienle C, Gerhardt A, Laskowski R, Kramarz PE, Bayley M, Svendsen C, Spurgeon DJ. 2010. Interactions between effects of environmental chemicals and natural stressors: A review. *Sci Total Environ* 408:3746–3762.

Hyne RV, Pablo F, Aistrope M, Leonard AW, Ahmad N. 2004. Comparison of time-integrated pesticide concentrations determined from field-deployed passive samplers with daily river-water extractions. *Environ Toxicol Chem* 23:2090–2098.

Jager T, Albert C, Preuss TG, Ashauer R. 2011. General unified threshold model of survival—A toxicokinetic–toxicodynamic framework for ecotoxicology. *Environ Sci Technol* 45:2529–2540.

Jager T, Ashauer R. 2018a. How to evaluate the quality of toxicokinetic–toxicodynamic models in the context of environmental risk assessment. *Integr Environ Assess Manag* 14:604–614.

Jager T, Ashauer R. 2018b. Modelling survival under chemical stress. A comprehensive guide to the guts framework. Toxicodynamics, York, UK. [cited 2018, January 18]. Available from: https://leanpub.com/guts_book

Jager T, Barsi A, Hamda NT, Martin BT, Zimmer EI, Ducrot V. 2014. Dynamic energy budgets in population ecotoxicology: Applications and outlook. *Ecol Modell* 280:140–147.

Jager T, Zimmer EI. 2012. Simplified dynamic energy budget model for analysing ecotoxicity data. *Ecol Modell* 225:74–81.

Korkaric M, Junghans M, Pasanen-Kase R, Werner I. 2019. Revising environmental quality standards: Lessons learned. *Integr Environ Assess Manag* 15:948–960.

Kreuger J. 1998. Pesticides in stream water within an agricultural catchment in southern Sweden, 1990–1996. *Sci Total Environ* 216:227–251.

Leu C, Singer H, Stamm C, Muller SR, Schwarzenbach RP. 2004. Variability of herbicide losses from 13 fields to surface water within a small catchment after a controlled herbicide application. *Environ Sci Technol* 38:3835–3841.

Moschet C, Wittmer I, Simovic J, Junghans M, Piazzoli A, Singer H, Stamm C, Leu C, Hollender J. 2014. How a complete pesticide screening changes the assessment of surface water quality. *Environ Sci Technol* 48:5423–5432.

Nyman A-M, Hintermeister A, Schirmer K, Ashauer R. 2013. The insecticide imidacloprid causes mortality of the freshwater amphipod *Gammarus pulex* by interfering with feeding behavior. *PLoS One* 8:e62472.

Nyman A-M, Schirmer K, Ashauer R. 2012. Toxicokinetic–toxicodynamic modelling of survival of *Gammarus pulex* in multiple pulse exposures to propiconazole: Model assumptions, calibration data requirements and predictive power. *Ecotoxicology* 21:1828–1840.

Ockleford C, Adriaanse P, Berny P, Brock T, Duquesne S, Grilli S, Hernandez-Jerez AF, Bennekou SH, Klein M, Kuhl T, Laskowski R, Machera K, Pelkonen O, Pieper S, Smith RH, Stemmer M, Sundh I, Tiktak A, Topping CJ, Wolterink G, Cedergreen N, Charles S, Focks A, Reed M, Arena M, Ippolito A, Byers H, Tedorovic I. 2018. Scientific opinion on the state of the art of toxicokinetic/toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms. *EFSA J* 16:e05377.

Openguts. 2019. Openguts software, Ver 1.0. [cited 2020 July 8]. Available from: http://openguts.info/

Ort C, Lawrence MG, Reungoat J, Mueller JF. 2010. Sampling for PPCPs in wastewater systems: Comparison of different sampling modes and optimization strategies. *Environ Sci Technol* 44:6289–6296.

Pedersen S, Palmqvist A, Thorbek P, Hamer M, Forbes V. 2013. Pairing behavior and reproduction in *Hyalella azteca* as sensitive endpoints for detecting long-term consequences of pesticide pulses. *Aquat Toxicol* 144–145:59–65.

Robertson DM, Roerish ED. 1999. Influence of various water quality sampling strategies on load estimates for small streams. *Water Resour Res* 35:3747–3759.

Roessink I, Merga LB, Zweers HJ, Van den Brink PJ. 2013. The neonicotinoid imidacloprid shows high chronic toxicity to mayfly nymphs. *Environ Toxicol Chem* 32:1096–1100.

Rohr JR, Salice CJ, Nisbet RM. 2016. The pros and cons of ecological risk assessment based on data from different levels of biological organization. *Crit Rev Toxicol* 46:756–784.

Rozman KK, Doull J. 2000. Dose and time as variables of toxicity. *Toxicology* 144:169–178.

Scheerbaum D. 2000a. Metazachlor—Aquatic plant toxicity test using *Lemna gibba*. Report Tla68811. Laboratorium für angewandte Biologie, Sarstedt, Germany.

Scheerbaum D. 2000b. Metazachlor—Alga, growth inhibition test with *Scenedesmus subspicatus*, 72 h. ReportSso68811. Laboratorium für angewandte Biologie, Sarstedt, Germany.

Schwarzenbach RP, Escher BI, Fenner K, Hofstetter TB, Johnson CA, von Gunten U, Wehrli B. 2006. The challenge of micropollutants in aquatic systems. *Science* 313:1072–1077.

Spycher S, Mangold S, Doppler T, Junghans M, Wittmer I, Stamm C, Singer H. 2018. Pesticide risks in small streams—How to get as close as possible to the stress imposed on aquatic organisms. *Environ Sci Technol* 52:4526–4535.

Stehle S, Knäbel A, Schulz R. 2013. Probabilistic risk assessment of insecticide concentrations in agricultural surface waters: A critical appraisal. *Environ Monit Assess* 185:6295–6310.

Strauss T, Gabsi F, Hammers-Wirtz M, Thorbek P, Preuss TG. 2017. The power of hybrid modelling: An example from aquatic ecosystems. *Ecol Modell* 364:77–88.

Tomlin CDS. 2009. *The Pesticide Manual*, 15th ed. British Crop Protection Council, Alton, UK.

Van Den Berg SJP, Baveco H, Butler E, De Laender F, Focks A, Franco A, Rendal C, Van Den Brink PJ. 2019. Modeling the sensitivity of aquatic macroinvertebrates to chemicals using traits. *Environ Sci Technol* 53:6025–6034.

Van den Brink PJ, Buijert-de Gelder DM, Brock TCM, Roessink I, Focks A. 2019. Exposure pattern–specific species sensitivity distributions for the ecological risk assessments of insecticides. *Ecotoxicol Environ Saf* 180:252–258.

van Leeuwen CJ, Vermeire TG. 2007. *Risk Assessment of Chemicals—An Introduction*, 2nd ed. Springer, Dordrecht, The Netherlands.

Vighi M, Barsi A, Focks A, Grisoni F. 2019. Predictive models in ecotoxicology: Bridging the gap between scientific progress and regulatory applicability—Remarks and research needs. *Integr Environ Assess Manag* 15:345–351.

Willkommen S, Pfannerstill M, Ulrich U, Guse B, Fohrer N. 2019. How weather conditions and physico-chemical properties control the leaching of flufenacet, diflufenican, and pendimethalin in a tile-drained landscape. *Agric Ecosyst Environ* 278:107–116.

Wittmer I, Junghans M, Singer H, Stamm C. 2014. Mikroverunreinigungen—Beurteilungskonzept für organische spurenstoffe aus diffusen einträgen. Eawag, Dübendorf, Switzerland.

Wittmer IK, Bader HP, Scheidegger R, Singer H, Lück A, Hanke I, Carlsson C, Stamm C. 2010. Significance of urban and agricultural land use for biocide and pesticide dynamics in surface waters. *Water Res* 44:2850–2862.

Woodward G, Gessner MO, Giller PS, Gulis V, Hladyz S, Lecerf A, Malmqvist B, McKie BG, Tiegs SD, Cariss H, Dobson M, Elosegi A, Ferreira V, Graca MAS, Fleituch T, Lacoursiere JO, Nistorescu M, Pozo J, Risnoveanu G, Schindler M, Vadineanu A, Vought LB-M, Chauvet E. 2012. Continental-scale effects of nutrient pollution on stream ecosystem functioning. *Science* 336:1438–1440.

Zimmer EI, Preuss TG, Norman S, Minten B, Ducrot V. 2018. Modelling effects of time-variable exposure to the pyrethroid beta-cyfluthrin on rainbow trout early life stages. *Environ Sci Eur* 30:36.