

Spatiotemporal analysis of risks and their drivers posed by organic pollutants to aquatic ecosystems on macro scales

by

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- Wolfram, J., Stehle, S., Bub, S., Petschick, L. L., & Schulz, R. (2019). Insecticide risk in US Surface Waters: Drivers and Spatiotemporal Modeling. *Environmental Science & Technology*, 53(20), 12071-12080.
- Wolfram, J., Stehle, S., Bub, S., Petschick, L. L., & Schulz, R. (2021). Water Quality and Ecological Risks in European Surface Waters – Monitoring Improves While Water Quality Decreases. *Environment International*, 152, 106479.
- Wolfram, J., Bub, S., Petschick, L. L., Schemmer, A., Stehle, S. & Schulz, R. (2023). Pesticide occurrence in protected surface waters in nature conservation areas of Germany. *Science of The Total Environment*, 858, 160074.

Annotation

The cumulative dissertation is based on four publications written by multiple authors. For this reason, the first-person plural is used throughout this thesis.

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List of abbreviations

AAC	Annual aggregate concentration
AF	Assessment factor
CI	Confidence interval
DegT50x(y)	Degradation time 50% for compartment x with degradation pathway y
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
LfULG	Landesamt für Umwelt, Landwirtschaft und Geologie Sachsen
LOC	Level of concern
MLR	Multiple linear regression
M/R	Ratio of measured concentration to threshold level
MIC	Measured insecticide concentration
MQI	Monitoring Quality Index
MRAA	Most relevant annual aggregate
NAWQA	National Water Quality Assessment Program
NCA	Nature conservation area
OECD	Organization for Economic Co-operation and Development
PEC	Predicted environmental concentration
PCA	Principal component analysis
PIC	Predicted insecticide concentration
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
RO	Research objective
RTL _x	Regulatory threshold level for compartment or organism group x
SUR	Sustainable Use Regulation
TAT _x	Total applied toxicity for species group x
TSCA	Toxic Substances Control Act
US	United States
UPA	Unprotected area
US EPA	United States Environmental Protection Agency
WFD	Water Framework Directive

Abstract

Agricultural intensification has increased substantially in the last century to meet the globally growing demand for food, fodder, and bioenergy, thus agricultural cropland became the largest terrestrial biome globally. Pesticides became a central tool to this intensification strategy, thus pesticide application rose drastically over the last sixty years to secure or increase crop yields. However, pesticides are by design biologically active and known to contaminate non-target ecosystems, thereby adversely affecting their function or structure. Even though ecotoxicological knowledge about probable fate and effects has grown, little remains known about the spatiotemporal occurrence, potential effects, and risk drivers of pesticides on larger, i.e. macro, scales.

Consequently, the thesis gathered primarily pesticide exposure data via meta-analysis and from public monitoring databases to describe (i) detailed risks in aquatic ecosystems, (ii) the underlying risk drivers, (iii) associated spatiotemporal trends, (iv) the effect of land use and land-protection and (v) the protectiveness of regulatory frameworks. First, a meta-analysis of insecticides occurring in US surface waters ($n = 5,817$, 259 studies) revealed large-scale risks for aquatic ecosystems based on the exceedance of regulatory threshold levels (RTL) and identified high-risk substances, particularly pyrethroids, with increasing application trends (publication I). Following this, spatiotemporal factors driving insecticide risks were identified via model-building demonstrating that toxicity-weighted pesticide use was the primary driver in surface waters with subsequent model application generating a spatially comprehensive risk assessment for the United States (publication II). The toxicity-weighted pesticide use was subsequently expanded to an ongoing project covering additional species groups and all pesticides used in the US from 1992 – 2016, highlighting a drastic shift of toxic pressures from vertebrates to aquatic invertebrates. Large-scale monitoring data from European surface waters ($n > 8.3$ million) of 352 organic chemicals identified pesticides as the main class or organic contaminants causing risks in aquatic ecosystems. Additional analyses established links between agricultural intensity and resulting environmental risks for aquatic invertebrates and plants on this macro scale (publication III). Finally, high-resolution monitoring data from Saxony, Germany, provided, for the first time, detailed insights into the occurrence and resulting risks of organic contaminants (primarily pesticides) in protected surface waters of nature conservation areas (publication IV).

In summary, the thesis gathered and used large-scale datasets to analyze the impact of agricultural intensification – and later anthropogenic land use – on ecosystems to reduce knowledge deficits in ecotoxicology on macro scales. Insecticides were shown to be important and spatially extensive agents of impairments to surface water quality and being directly linked to their use in respective landscapes. Changes in the pesticide use composition over time shifted environmental risks from vertebrates to other central species groups (e.g. aquatic invertebrates), highlighting a new challenge to the integrity of aquatic environments. The thesis provided novel insights into contaminants' individual risk characteristics, their interaction with various spatiotemporal drivers and their relevance on various macro scales. Overall, a discrepancy remains evident between estimated environmental impacts of pesticides derived during regulatory approval processes contrasted by *a posteriori* field measurements detailing larger than assumed adverse exposures and effects. This discrepancy led to pesticides being the most impactful chemical stressor for aquatic ecosystems compared to other organic contaminants on a continental scale; a threat that even increased for some species groups. The extensive use of pesticides has reached levels where even strictly protected surface waters in Germany are regularly exposed adversely, hence threatening conservation areas' function as ecological refugia. Taken together, the thesis provides new macro-scale evidence regarding the contribution of pesticides (and associated drivers) to large-scale changes in biological systems evidenced over the last decades, underlining their likely contribution to the ongoing freshwater biodiversity crisis globally. Particularly agricultural systems will require substantial changes going forward to protect or reestablish the integrity of aquatic ecosystems and their provision of vital ecological services.

1 Introduction

1.1 Agricultural intensification

Agricultural land still constitutes the largest terrestrial biome on earth (Ramankutty et al., 2018). For instance, 17.2% of the US total land area is used for crops (excluding 29% used as rangeland and pastures), supporting a \$400 billion industry (Bigelow and Borchers, August 2017) and in the EU 39.1% of land is used for agriculture (including pastures) (Eurostat, 2021). However, the extensification of agricultural land reached spatial limits in most developed countries in the middle of the 20th century, unable to meet the globally growing demand for food, fodder, and biofuels (Popp et al., 2013). Other intensification strategies, such as increased chemical fertilization, improved crop breeds (including genetic modifications), growth stimulants, and pesticides, were thus applied in concert to increase crop yields to meet global demands, marking the start of the Green Revolution in the 1950s.

Pesticides became a central tool for this intensification strategy by combating a wide variety of target organisms and having low production costs, which made them a cost-effective tool for agricultural producers and led to pesticides' rapid adoption since the early 1960s (Popp et al., 2013; Sharma et al., 2019). In this context, a global assessment of potential crop losses to pests and pathogens estimated a relative loss of ca. 50% – 80% without the use of crop protection strategies (e.g. pesticides) (Oerke, 2006). Particularly, extensive and high-value crops (e.g. maize, soybeans, wheat), referred to as “cash crops”, were estimated to suffer the largest losses as a result of pest infestations. Pimentel et al. (2005) further quantified the economic benefits associated with the application of pesticides in US agriculture, estimating a gross return of approximately \$40 billion for the annually \$10 billion expended on pesticides. As such, pesticide application saw rapid adoption in all industrialized countries, resulting in ca. 412,000 t being applied in 2016 in the US and ca. 346,000 t being sold in 2018 in the EU (Baker and Stone, 2015; Eurostat, 2020). Despite pesticides' intensive use, pest organisms are estimated to have caused yield losses of up to 25% in the US each year (Pimentel, 2005). In part, (agro-)ecosystems have become increasingly under pressure from foreign pest organisms and alien invasive species but also experienced a drastic decline in beneficial insects able to suppress local pest outbreaks (Bebber et al., 2014). The intensive use of pesticides has furthermore resulted in a growing number of resistance developments in pest organisms, reducing the efficacy of pesticide applications in return increasing the frequency or amount of pesticides being applied (Fernandez-Cornejo et al., 2014; Gould et al., 2018; Pimentel, 2005). Taken together, a multitude of aforementioned factors has resulted in the increased and extensive use of chemicals in agricultural environments.

1.2 Pesticide exposure and effects

The chemization of environments, driven by various contaminants, saw the starkest increase in pesticide products (Bernhardt et al., 2017). Widespread and frequent pesticide use, partially in response to aforementioned resistance developments, resulted in surface waters, particularly those dominated by agriculture, being exposed to pesticides via various pathways (Gilliom et al., 2006a). For instance, the majority of applied pesticides do not reach target organisms but remain on plants or in top-layer soils (Pimentel and Burgess, 2012). Following rain-events or artificial irrigation can subsequently transport between 1 – 10% of the applied mass to edge-of-field environments, such as non-target aquatic ecosystems (Schulz, 2004; Skark et al., 2004). Other transport processes are also known to contribute to the diffuse entry of pesticides into non-target aquatic ecosystems, such as subsurface flows or drainage, spray-drift, volatilization with subsequent atmospheric deposition, stormwater overflows, and others (Tang et al., 2012; Zaller et al., 2022). Overall, the phenomenon of pesticide off-site transport into aquatic ecosystems is well described on various spatial scales (Gilliom et al., 2006b; Hladik et al., 2014a; Malaj et al., 2014; Schäfer et al., 2012; Schulz, 2004; Stehle and Schulz, 2015a). However, the spatiotemporal factors (e.g. land use, meteorological, geological) driving offsite transport have rarely been assessed on large spatiotemporal scales (Stone et al., 2013). Particularly quantitative links between detailed pesticide use data and resulting environmental risks are currently missing on macro scales.

Within the context of pesticides, insecticides represent the most toxic yet elusive substance class for most aquatic fauna, occurring only for short periods in surface waters in quantifiable concentrations (Stehle et al., 2013) and requiring highly targeted monitoring efforts to capture transient contamination peaks (Crawford, 2004; Schulz, 2004; Spycher et al., 2018; Stehle and Schulz, 2015a). Insecticides are also mostly applied reactively thereby contrasting the use patterns of herbicides or fungicides, which further adds to their temporally highly variable occurrence (Das, 2013; Simon, 2014). Most insecticides (excluding legacy, persistent organochlorines) generally possess physico-chemical properties that foster faster dissipation rates from the water phase compared to herbicides and fungicides, such as higher partitioning to nonpolar phases (e.g. k_{OC} or k_{OW}), lower environmental stability (e.g. $\text{DegT50}_{\text{aqua}}$ (photolysis), $\text{DegT50}_{\text{aqua}}$ (hydrolysis)) and lower solubilities (Lewis et al., 2016). The combination of punctual application patterns, fast modes of action (Devine and Furlong, 2007), and faster dissipation characteristics leads to highly transient occurrences of insecticides complicating their precise quantification in surface waters (Crawford, 2004; la Cecilia et al., 2021; Spycher et al., 2018). However, neonicotinoid insecticides, for the first time, contrast the aforementioned characteristics, being more soluble and environmentally stable, due to their systemic mode of action, which has produced increasingly chronic exposure profiles in

aquatic systems (Hladik and Kolpin, 2016; Hladik et al., 2014a; Hladik et al., 2014b; Morrissey et al., 2015). Despite their mostly transient occurrence, insecticides are ecologically relevant at very low concentrations (Deanovic et al., 2018) causing adverse effects at the organism, population, and ecosystem level, reducing aquatic biodiversity and adversely affecting associated ecological functions (Beketov and Liess, 2008; Domagalski et al., 2010; Schäfer et al., 2012; Walker et al., 2005). Even single short-term exposure pulses (<24 h) were shown to cause long-term alterations, such as delayed development in caddisfly larvae (Liess and Schulz, 1996; Schulz and Liess, 2000). Furthermore, pyrethroid insecticides are among the most transiently occurring insecticides in the water phase but have been shown to accumulate in aquatic biofilms in turn altering benthic community structures (Mahler et al., 2020) or initiating trophic cascades in invertebrate communities (Rogers et al., 2016). Insecticides, hence, require specific focus due to their risk of affecting pivotal ecosystem functions, such as detritus turnover (Schäfer et al., 2012). Taken together, the multitude of insecticide effects on non-target organisms has received attention so far. However, the spatial extent and particularly the underlying spatiotemporal risk drivers responsible for the observed adverse effects are currently sparse or missing completely on large scales.

In addition to insecticides, other organic contaminants, e.g. herbicides, fungicides, pharmaceuticals, or industrial chemicals, are known to occur in adverse concentrations in surface waters, although often affecting different ecosystem processes or species groups (Lozano et al., 2020; Rumschlag et al., 2020; Schäfer et al., 2011; Zubrod et al., 2019). For instance, Malaj et al. (2014) outlined large-scale risks for aquatic primary producers in Europe driven by herbicides and also showed that organotins and fungicides can contribute noteworthy to adverse effects in aquatic invertebrates. Hence, it is similarly important to again broaden the scope of considered chemicals during macro-scale environmental risk characterizations, particularly, if these data are readily available from governmental monitoring programs (see chapter 1.4). With such an approach a more comprehensive overview of potential impairments to multiple parts of aquatic ecosystems and their associated ecological functions can be achieved. Furthermore, contaminants can regularly co-occur, producing complex mixtures (Blackwell et al., 2018) that can additively or interactively affect exposed organisms (Backhaus et al., 2004; Loewe and Muischnek, 1926). Increasing the complexity of assessed mixtures also provides further insight into the protectiveness of enacted regulatory frameworks, which currently do not consider pesticides' or other organic contaminants' co-occurrence during their regulatory assessment (EFSA, 2013; U.S. EPA, 2018a). Taken together, combined analyses are necessary to identify those contaminants that most acutely impair aquatic ecosystems and to quantify the spatiotemporal drivers responsible for these processes.

1.3 Regulatory assessment of pesticides

1.3.1 Environmental risk assessment of pesticides in the EU and US

All agricultural pesticides, but also less extensively industrial chemicals and pharmaceuticals (c.f. Lahl and Hawxwell, 2006; TSCA, 1976), have to pass a prospective environmental risk assessment to be registered prior to their use to ensure that no “unreasonable” or “unacceptable” adverse effects can be expected in non-target ecosystems in the US and EU, respectively (EFSA, 2013; U.S. EPA, 2018a). The process should ensure that their widespread use does not adversely affect biodiversity or the structure and function of non-target ecosystems. Thus, for both the EU and the US, the overarching protection goals are similar, although the specificities of the underlying risk assessment procedure may differ as outlined in the Directive EC-1107/2009 and the Feder Insecticide, Fungicide and Rodenticide Act of 1947 (FIFRA, including subsequent amendments to the act), respectively.

Both environmental risk assessment procedures can be divided into two major assessment parts, exposure and effect assessments, which are subsequently combined for the risk assessment. Exposure assessments primarily use transport and fate modeling of single substances to estimate concentration profiles in edge-of-field surface waters under realistic worst-case conditions, yielding predicted environmental concentrations (PEC) (EFSA, 2013). However, the accuracy of PEC has been questioned following extensive comparisons with actual measured field concentrations gathered via comprehensive meta-analysis in European agricultural surface waters (Stehle and Schulz, 2015b). Effect assessments quantify adverse effect concentrations via standardized toxicity testing with varying levels of complexity in the underlying test systems, i.e. ranging from standardized test batteries to micro- and mesocosm experiments (EFSA, 2013; U.S. EPA, 2018a). Depending on the test system’s complexity, specific assessment factors (AF) or “levels of concern” (LOC) are applied to the effect endpoint in the EU and US, respectively (EFSA, 2013; U.S. EPA, 2018a). AF and LOC should represent the uncertainty associated with effect endpoints regarding their extrapolation of effects to natural ecosystems. The most sensitive effect endpoint applied with its AF or LOC is defined as a regulatory threshold level (RTL, see Stehle and Schulz (2015a)). RTL can be specific to aquatic environments in general, e.g. for freshwater organisms (RTL_{fw}), or for certain species groups within that environment, e.g. aquatic invertebrates (RTL_{inv}). PEC, derived during the exposure assessment, are subsequently compared to RTL, forming risk quotients, to quantify expected risks in aquatic environments and to evaluate if the intended uses of a specific pesticide pose aforementioned unacceptable or unreasonable risks to specific species groups or the aquatic environments in general. In case of unacceptable or unreasonable risks, additional risk mitigation measures can be applied to the proposed use of a pesticide (e.g. no-

spray buffer zones, use of drift-reducing nozzles) to reduce expected risks to acceptable levels (EFSA, 2013; U.S. EPA, 2018a). In addition, in the EU higher-tier testing (e.g. mesocosm studies) can be conducted, which is associated with lower AF, to derive higher RTL, in return reducing risk quotients, potentially indicating acceptable risk levels. Overall, the prospective environmental risk assessment defines expected worst-case environmental concentrations of pesticides (i.e. PEC) and effect threshold levels (i.e. RTL) that represent levels above which unacceptable effects in aquatic ecosystems are expected.

1.3.2 Suitability of RTL as assessment endpoints

In comparison to other organic chemicals (e.g. industrial chemicals, pharmaceuticals), which are regulated primarily via other instruments (e.g. REACH, TSCA), the pesticide authorization process can be regarded as one of the most scrutinous processes prospectively assessing potential environmental risks when comparing data requirements and associated testing costs (EFSA, 2013; TSCA, 1976; U.S. EPA, 2018a). Hence, the produced effect data constituting RTL are highly standardized and follow testing protocols as outlined in the technical guidelines in the US and respective OECD guidelines in the EU. RTL thus represent thresholds that had to pass manual validation by risk assessors and enable comparability between substances for specific species groups due to the standardization of testing protocols. However, Stehle and Schulz (2015a) have shown that significant negative effects on aquatic biodiversity, i.e. a 12% loss in family richness of freshwater invertebrates, occur at insecticide concentrations equaling $0.1 \times$ RTL based on analyses by Beketov et al. (2013). Furthermore, Schäfer et al. (2012) showed significant reductions of sensitive macroinvertebrate taxa below commonly used effect thresholds derived from the standard test species *D. magna* based on data from eight field studies. Liess and von der Ohe (2005) delineated clear effects in community structures at levels equaling below an AF of 100 to 1,000 based on acute *D. magna* toxicity tests. RTL consequently represent threshold concentrations above which unreasonable effects are expected to occur, leading to structural or functional alterations in valued nontarget ecosystems such as surface waters (Fowle and Dearfield, 2000; U.S. EPA, 1998, 2004). Ultimately, RTL denote concentrations that are assumed to not be exceeded in natural environments, as concluded during their registration, which would otherwise violate the established protection goals (EFSA, 2013). RTL can thus be leveraged for three principal analyses: First, RTL serve as an indicator regarding the protectiveness of environmental risk assessment procedures conducted during pesticides' registration by comparing measured field concentrations with RTL. Second, RTL are thresholds above which unacceptable ecological effects are likely to occur and at which a reduction of family richness of freshwater can be expected for aquatic invertebrates (Stehle and Schulz, 2015a). Third, RTL provide a standardized basis for ecotoxicological effects for various species groups that enables

comparisons of individual pesticide effects within a specific species group, e.g. by normalizing applied pesticide tonnages. Consequently, RTL have been used to evaluate insecticide and fungicide risks globally and the protectiveness of regulatory assumptions, due to their multifaceted use as reliable risk indicators (Stehle and Schulz, 2015a; Zubrod et al., 2019),

1.4 State of knowledge on macro scales

Characterization of environmental risks on macro scales is mostly available via two lines of evidence based on field measurements (i.e. excluding modeling approaches): meta-analytical work synthesizing peer-reviewed studies or evaluation of (trans-)national monitoring databases comprising primarily of governmental monitoring efforts. Meta-analytical work on pesticide occurrence in surface waters is available on multiple scales, e.g. for neonicotinoids globally (Morrissey et al., 2015), insecticides globally (Stehle and Schulz, 2015a), fungicides globally (Zubrod et al., 2019) or insecticides from non-agricultural sources in the US (Stehle et al., 2019). For instance, insecticides were found to acutely affect aquatic ecosystems globally by exceeding respective RTL frequently, and in particular, pyrethroids exceeded thresholds most frequently with 65.8% (Stehle and Schulz, 2015a). Morrissey et al. (2015) provided an initial review of neonicotinoids' potential risk to aquatic systems globally based on 29 studies, highlighting their particularly high chronic risk for aquatic insects. Zubrod et al. (2019) meta-analytically summarized the global exposure data available for >40 fungicides and their potential to adversely affect ecosystems. Although aforementioned studies provide valuable insights into potential continental or global issues, their broad scope came at the cost of lower specificity regarding spatiotemporal trends and underlying risk drivers. However, meta-data can offer leverageable advantages compared to most large-scale governmental monitoring data, namely more focused monitoring protocols (e.g. higher sampling intervals, higher spatial resolution on small scales, and more sensitive analytical limits, see chapter 4.1) that produce more reliable estimates about actual peak pesticide occurrence in the field, which are particularly valuable for the quantitation of spatiotemporal (e.g. meteorological, pesticide-application data) risk drivers. Yet, no comprehensive gathering of available literature has been conducted for the United States, despite the extensive and high-quality body of literature available there (see chapter 4.1) which can be combined with diverse publicly available spatiotemporal data to describe and quantify important drivers of insecticide risks on a macro scale.

In contrast to meta-data, large-scale governmental monitoring data excel by generating spatiotemporally larger data bases, surmounting meta-data by orders of magnitude, and providing wider substance spectra, i.e. multiple pesticide classes or organic chemicals in general (European Environment Agency, 2020; Landesamt für Umwelt, 2022). However,

monitoring protocols are often less focused, i.e. employing fixed-interval sampling with long intervals between sampling events, higher detection limits, etc. to meet legally defined monitoring objectives and balancing competing research goals whilst remaining fiscally efficient (National Research Council, 2012). Despite the aforementioned drawbacks, phase one of the Nation Water Quality Assessment Program (NAWQA, 1992 – 2001) identified insecticides as a main contributor causing aquatic life impairments at 57% of a total of 83 agricultural stream sites in the US (Stone et al., 2014a). Malaj et al. (2014) provided the first comprehensive overview of aquatic risks for Europe using data from 2006 – 2010 obtained via the Water Framework Directive (WFD) also identifying pesticides as the main group of chemicals impairing surface waters throughout Europe. Meso-scale monitoring programs, such as at 19 and 101 lower-order streams in Romania and Germany, respectively, have further underlined the widespread issue of pesticide occurrence in aquatic ecosystems (Halbach et al., 2021; Liess et al., 2021; Schreiner et al., 2021). Taken together, current meso- or macro-scale studies generally point towards pesticides as detrimental contaminants for aquatic ecosystems. In Europe, the WFD aims at achieving a good ecological status in surface waters by 2027 and gathered extensive chemical exposure data since 2000 to describe the contamination status of European surface waters (European Council, 2000). Although Malaj et al. (2014) provided an initial assessment for 2006 – 2010 using WFD data, it remains unclear how reliable these data are in describing environmental risks, how European water quality has changed over time, and how land use is connected to the occurrence of various groups of contaminants.

Overall, detailed analyses of individual risks of substance(-classes), spatiotemporal drivers, mixture toxicity, and linkages to pesticide use are sparse on large spatial scales. Yet, the aforementioned aspects will be central indicators of the progress or success of current regulatory frameworks such as the Sustainable Use Regulation (SUR, (European Commission, 2021)) or the WFD, the former aiming at improving environmental conditions, e.g. via a 50% reduction of pesticide risks by 2030. Despite its likely central role, pesticide use data has not been comprehensively evaluated for its links to instream risks on large-scales for multiple species groups and neither its potential suitability for assessing current trends in environmental risks. Furthermore, while some work added to the understanding of potential macro-scale issues, the occurrence of pesticides in specifically protected surface waters, on the other hand, has received little attention globally. Available literature is currently restricted only to individual or very few surface waters specifically (e.g. Barakat et al., 2013; Bradley et al., 2021; Buah-Kwofie and Humphries, 2017; Forrester et al., 2017; Kaiser, 2011), and in most cases focusing on persistent, legacy-use pesticides. Thus, no comprehensive overview of the occurrence, risks, and spatial drivers exists for protected surface waters despite their

intended role as ecological refugia and recolonization sources in otherwise intensively used landscapes (Bundesamt für Naturschutz, 2022). Importantly, protection areas are also currently being considered a central tool to reach the risk reduction targets set out by the SUR and the Convention on Biological Diversity by prohibiting pesticide use therein (Ainsworth et al., 2022). Thus, deriving comprehensive risk analyses for protected surface waters is now urgently needed to provide context about their exposure to contaminants, resulting environmental risks, and underlying drivers and contaminant pathways.

The current macro scale knowledge of risks posed by organic contaminants (particularly pesticides) is characterized by data with low spatial density and lacking the identification of risk drivers. Hence, meta-analytical data derived from the data-rich environment of the United States will be used to provide detailed insights into the spatiotemporal risks and associated drivers that affect the complex issue of insecticide contamination of aquatic ecosystems. Following this, extensive long-term governmental monitoring data from the EU can be used to broaden the scope to all organic contaminants concerning their impact on aquatic ecosystems and assess how the underlying data quality affects the ability to characterize environmental risks. Finally, one currently envisioned way forward for safeguarding aquatic ecosystems and their biodiversity is the strict protection of sensitive areas (i.e. protection areas), yet, no comprehensive analysis of their current state, contamination, or potential exposure pathways has been conducted to this date. Hence, this dissertation aims at reducing the aforementioned knowledge deficits through the gathering and analysis of extensive, large-scale environmental data.

2 Research objectives and thesis outline

The thesis aims at adding to the domain ecotoxicology on macro scales by assessing environmental risks based on monitoring data obtained via two lines of evidence (Figure 1) and using these data to provide macro-scale insight into (i) detailed risks and their underlying risk drivers, (ii) associated spatiotemporal trends, (iii) the effect of land use and land-protection, and (iv) the protectiveness of regulatory frameworks. The thesis should outline past and current challenges to freshwater integrity as well as the main promoters of these adverse impairments such that future improvements can be reached.

The thesis addressed the following four research (**RO**; Figure 2):

1. Detailed analysis of insecticide risks in agricultural surface waters and the protectiveness of regulatory framework based on comprehensive meta-analysis (*Appendix I*)
2. Identification and quantification of large-scale insecticide risk drivers via modelbuilding and subsequent national mapping to estimate the current risks in US agricultural surface waters. (*Appendix II*)
3. Continental risk analysis of organic contaminants in European surface waters with quantitation of land-scape level drivers, temporal risk trends, and the reliability of large-scale monitoring data. (*Appendix III*)
4. Characterization of environmental risks in protected surface waters of nature conservation areas and the identification of contamination pathways for pesticides (*Appendix IV*)

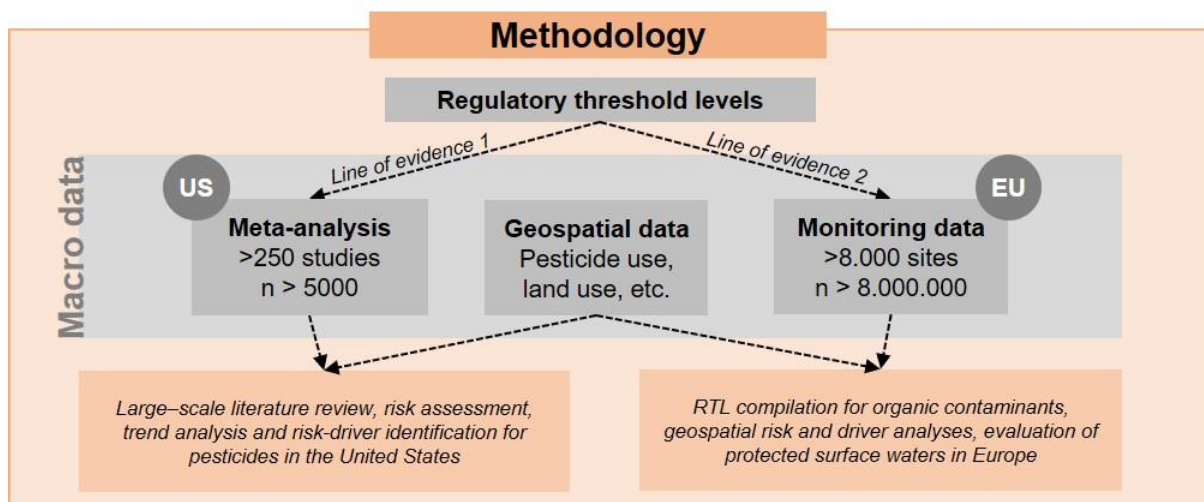
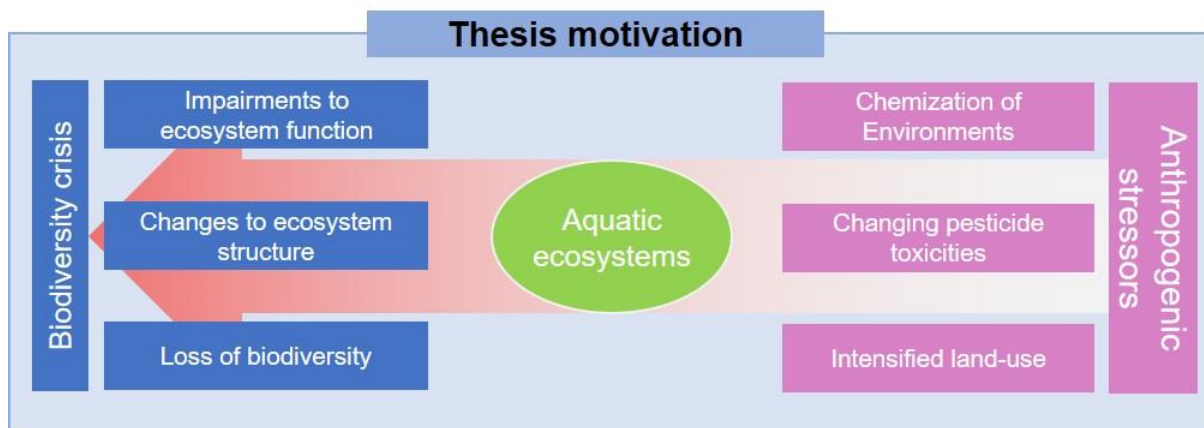


Figure 1: Overview of the general rationale and motivation for the thesis as well as a generalized overview of the applied methodologies. The rationale and motivation are outlined in the Introduction (chapter 1), which focusses on the effect of anthropogenic stressors (primarily pesticides) and their potential adverse effects in aquatic ecosystems on macro scales. More detailed methodological descriptions can be found in chapter 3 (Overview of methods and concepts).

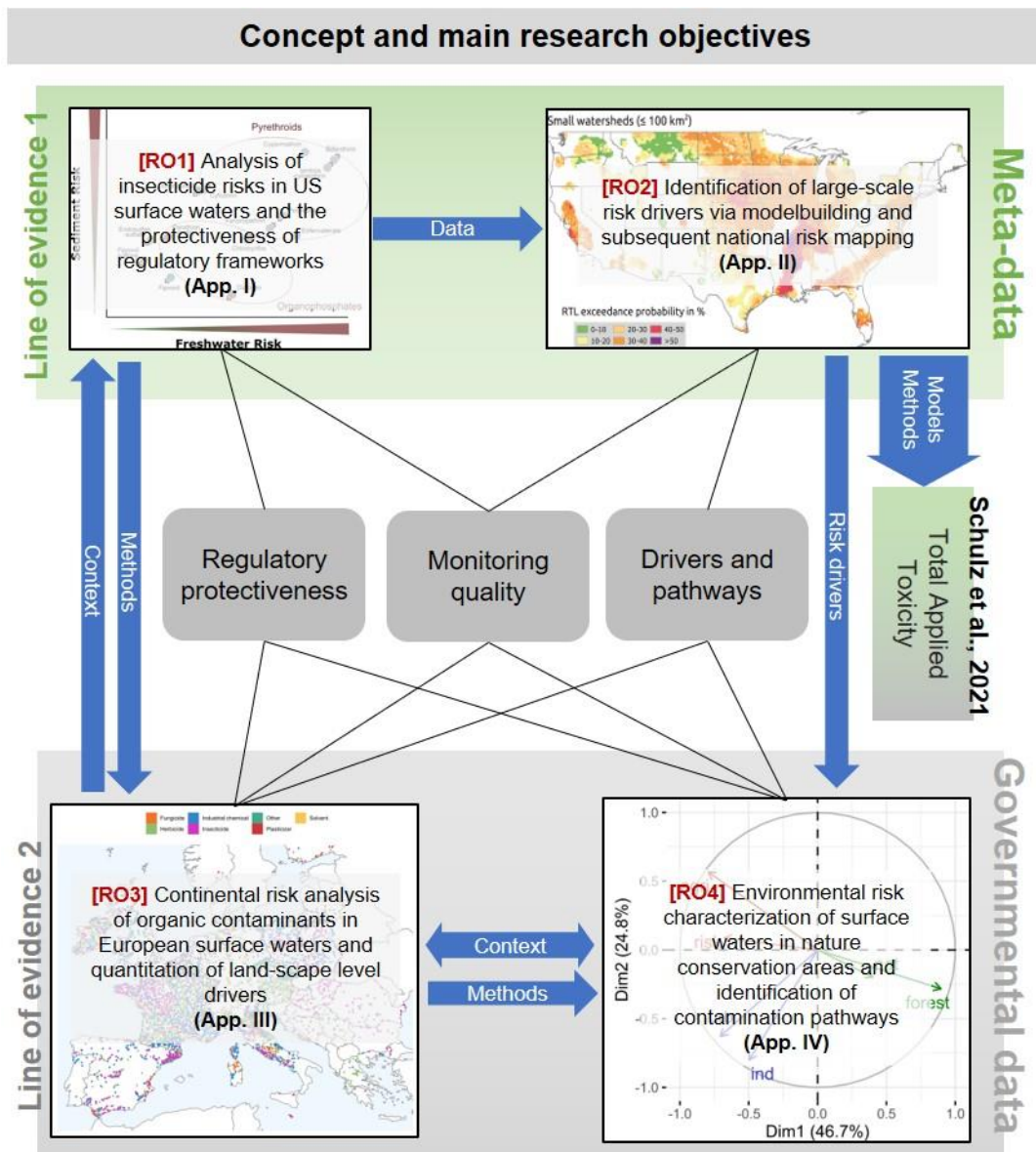


Figure 2: Conceptual overview and associated main research objectives (RO) of the present thesis derived from the rationale and motivation as well as methodological overview given in Figure 1. The connections of RO and respective *Appendices* are symbolized with named arrows indicating the nature of respective links. The main data sources are annotated. Meta-data refers to data obtained via comprehensive meta-analysis, whereas governmental data refers to monitoring data that is obtained and made available via governmental agencies.

The thesis is based on four RO which were addressed by the following four peer-reviewed publications. For details about the rationale and motivation see Figure 1 and for a conceptual overview of all four RO see Figure 2. All relevant peer-reviewed publications, on which this thesis is based, can be found in *Appendices I – IV*.

Appendix I: Wolfram, J., Stehle, S., Bub, S., Petschick, L. L., & Schulz, R. (2018). Meta-analysis of Insecticides in United States Surface Waters: Status and Future Implications. Environmental Science & Technology, 52(24), 14452-14460.

The publication provides an assessment of insecticide risks in agricultural surface waters in the US based on a comprehensive meta-analysis. It identifies high-risk substances in water and sediment phases regularly exceeding RTL and provides current application trends to derive substances' potential future implications (**RO1**). The obtained data provides the basis for risk driver identification and model application in *Appendix II* (**RO2**), whereas the observed trends, threshold derivation, and pesticide use data formed the basis for *Schulz et al. (2021)*.

Appendix II: Wolfram, J., Stehle, S., Bub, S., Petschick, L. L., & Schulz, R. (2019). Insecticide risk in US Surface Waters: Drivers and Spatiotemporal Modeling. Environmental Science & Technology, 53(20), 12071-12080.

The publication provides regression models based on meta-data from *Appendix I* to quantify relevant spatiotemporal factors that drive insecticide risks in surface waters and their sediments in the US (**RO2**). Particularly the importance of toxicity-normalized use, catchment sizes, and pesticide classes are highlighted as primary risk drivers (**RO2**). The model-building allowed predicting RTL exceedance probabilities for the digital stream network of the US, highlighting regions in which aquatic biodiversity and their function were at risk (**RO2**). Establishing the link between applied toxicities with risks in aquatic ecosystems formed a qualitative and methodological basis for *Schulz et al. (2021)*.

Appendix III: Wolfram, J., Stehle, S., Bub, S., Petschick, L. L., & Schulz, R. (2021). Water Quality and Ecological Risks in European Surface Waters – Monitoring Improves While Water Quality Decreases. Environment International, 152, 106479.

The publication provides an extensive overview of the environmental risks posed by 352 organic contaminants in European surface waters (8,213 sites) for the last 15 years, identifying pesticides as the primary class of organic contaminants impairing aquatic ecosystems on a continental scale (**RO3**). Geospatial analyses established a link between agricultural intensification and resulting risks for aquatic invertebrates and aquatic plants throughout Europe (**RO3**). The work also details how data heterogeneity negatively influences the accuracy of risk characterizations, adding to those identified in *Appendix I* and *Appendix II*, and provides recommendations for future improvements.

Appendix IV: Wolfram, J., Bub, S., Petschick, L. L., Schemmer, A., Stehle, S. & Schulz, R. (2023). Pesticide occurrence in protected surface waters in nature conservation areas of Germany. Science of The Total Environment, 858, 160074.

The publication provides detailed insight into the contamination of protected surface waters in nature conservation areas based on data from Saxony, Germany (**RO4**). The results highlight

comparable and frequent environmental risks in these protected ecosystems compared to unprotected areas (UPA). Fluvial inflows from unprotected upstream areas, particularly those characterized by intensive agriculture, were linked to instream risks (**RO4**). Thus, the work provides new macro-scale evidence regarding the impact of organic contaminants on protected ecosystems and their function as ecological refugia, thus contextualizing the results of *Appendix III* (**RO3**).

3 Overview of methods and concepts

Chapter 3 provides a condensed overview of the methods used to address the four RO outlined for this thesis (Figure 2). *Methods in Brief* sections are added for each RO when all relevant methods are introduced. Detailed descriptions of the used materials and methods can be found in *Appendices I – IV*.

3.1 Meta-analysis

Meta-analytical data formed the basis for *Appendices I and II*. For the present thesis, data from peer-reviewed studies for US agricultural surface waters (n = 215 studies containing 3,854 MIC for 22 insecticides) were taken from a global analysis covering the period from 1962 to 2012 (Stehle and Schulz, 2015a). These data were extended through a search in scientific databases (see Stehle and Schulz (2015a) and *Appendix I*) considering 40 insecticides and their degradation products, resulting in reported data up to the year 2018. Detailed descriptions of the literature review method and its validation can be found in (Stehle and Schulz, 2015a). In total, we identified 259 studies containing 5,817 MIC (including degradate concentrations) present in US surface waters (freshwater, sediment, or estuarine water) resulting from agricultural nonpoint source entries. MIC were attributed with various covariates and geospatial factors (for a full list see *Appendix I*). In total, 40 insecticides (i.e. active ingredients) were included in the meta-analysis covering two organochlorines, six organophosphates and three carbamates, 21 pyrethroids, seven neonicotinoids, and one phenylpyrazole insecticide (fipronil). In addition, insecticide degradates and metabolites (both referred to as degradates) of each insecticide were identified by using the US EPA OPP Pesticide Chemical Search (U.S. EPA, 2018b), the Pesticide Properties Database (Lewis et al., 2016), and publicly available US EPA registration documents.

3.2 Governmental monitoring data

3.2.1 European monitoring data

For *Appendix III*, European monitoring data were obtained from the WISE Water Quality database (WISE6) (European Environment Agency, 2020) and procured as part of the WFD (European Council, 2000) to characterize the chemical status of European waterbodies. In total, 352 organic contaminants, measured at 8,209 locations in 7,227 waterbodies for 31 countries between 2001 – 2015, were identified and listed along with their chemical class and other attributes in *Appendix III*. Organic contaminants were assigned to eleven major chemical classes: anti-fouling agents; flame retardants; fungicides; herbicides; industrial chemicals; insecticides; plasticizers; solvents; pharmaceuticals; hormones; and miscellaneous

chemicals. The dataset contains at least 8,383,640 samples, which were aggregated per chemical and location to average and maximum “annual aggregate concentrations” (=AAC; $n = 971,381$). The highest risk (i.e. concentration / RTL, see chapter 3.3.2), hence presumably most ecotoxicologically relevant (Wolfram et al., 2019) AAC per site, year, and organism group (=MRAA), was determined and used for most risk analyses. For a detailed description of the terms sample, AAC, and MRAA, see *Appendix III*. Furthermore, the quality of monitoring data was assessed, because nationally organized monitoring schemes may differ substantially in quantity and quality depending on how thoroughly the dimensions of space, time and chemicals are probed. To provide a basis for comparison among countries, we derived an indicative index, named here monitoring-quality-index (MQI), which incorporates the most important dimensions that could be derived from the present data (for details see *Appendix III*). The MQI was used in *Appendix III* to link monitoring quality with the programs’ capabilities of detecting organic contaminants and quantifying environmental risks.

3.2.2 Saxon monitoring data

For *Appendix IV*, chemical monitoring data from regular, long-term surface water monitoring were obtained from the Federal Agency for the Environment, Agriculture and Geology of Saxony (=LfULG, Landesamt für Umwelt, Landwirtschaft und Geologie). The monitoring data were used to gain detailed insight into the contamination of protected surface waters in nature conservation areas (NCA) compared to unprotected areas (UPA). In total, 208 organic pesticides measured at 1,371 locations between 1998 – 2020 ($n = 3,822,553$ measurements) were identified and attributed with their pesticide class (e.g. “herbicides”) and other attributes in addition to applying quality assurance protocols (see *Appendix IV*).

3.3 Effect data and regulatory threshold levels

3.3.1 US RTL

US RTL (see *Appendix I*) were used to provide a regulatory reference level for the ecological implications that may arise from an observed insecticide or degradate concentration in *Appendices I and II*. RTL are determined for each of the three environmental phases analyzed in this thesis (*Appendices I and II*), i.e. freshwater (RTL_{fw}), sediment (RTL_{sed}), or estuarine water (RTL_{est}), and represent threshold levels above which adverse effects in aquatic organisms are expected (U.S. EPA, 1998, 2004). However, Stehle and Schulz (2015a) have shown that significant negative effects on aquatic biodiversity, i.e. a 12% loss in family richness of freshwater invertebrates, occur at insecticide concentrations equaling $0.1 \times \text{RTL}$, a value that has also been used for evaluation in the thesis.

3.3.2 European RTL

European RTL were used for acute risk characterizations in *Appendices III and IV*. Acute ecotoxicological effect data for standard test species (for details see EFSA (2013) and Petschick et al. (2019)) were obtained from three sources to derive the most relevant and reliable endpoint for aquatic invertebrates, fish, and aquatic plants for all chemical classes in Europe. Additionally, a most sensitive endpoint representing all three organism groups was derived, representing the level at which one of those groups is acutely affected. Three databases, providing endpoints used during ecological risk assessments in the EU (e.g. Regulation (EC) No 1107/2009), were used hierarchically (see *Appendix III*). A uniform methodology for RTL derivation was applied to all organic chemicals (n = 352) for the WFD data to provide comparable risk estimates across various chemical classes. For *Appendix IV*, RTL derivation followed the same principles with slight alterations, i.e. the omission of EnviroTox data (Connors et al., 2019), due to the associated uncertainties which were supplemented by valid endpoints from the Pesticide Properties Database (Lewis et al., 2016). For a complete list of RTL and further details see *Appendix IV*. In *Appendix IV*, environmental risks of cooccurring contaminants were assessed as well, assuming concentration addition, yielding daily ($\text{sum}(M/R)$) or annual maximum ($\text{sum}(M/R)_{\text{max}}$) risk estimates per site.

3.4 Geospatial data

3.4.1 US spatial data

Annual county-level agricultural pesticide use estimates were obtained for the period from 1992 to 2014 (Baker, 2016). Temporal use trends from 1992 to 2014 were used to contextualize substance exceedance frequencies and median exceedance severity to help identify substances with potentially increasing risks to US surface waters (*Appendix I*).

Methods in Brief for RO1

US meta-data (chapter 3.1), US RTL (chapter 3.3.1) and pesticide use data (chapter 3.4.1) were used to analyze insecticide risks in agricultural surface waters, to evaluate the protectiveness of US regulatory frameworks and to contextualize environmental risks with current pesticide use trends to derive potential future implications of high-risk substances (RO1, Figure 2, *Appendix I*).

Pesticide use data was then normalized by pesticides' toxicities (i.e. RTL) for **RO2** (see *Appendix II*) to account for the highly variable toxicity of pesticides. The concept of toxicity-normalized use was subsequently expanded to (i) all pesticides, (ii) eight species groups, and

(iii) the years 2015 – 2016 for Schulz et al. (2021) and was renamed to Total Applied Toxicity (TAT).

Spatially explicit MIC (n = 4,391) were attributed with geospatial information derived from a 50-km radial buffer area around the sampling location based on convergence analysis (for details see *Appendix II*). Multiple spatial attributes were derived from these areas: annual county-level pesticide use estimates (Baker, 2016) and projected onto cropland estimates (U.S. Department of Agriculture, 2018), adjusted rainfall erosivity factors Biasutti and Seager (2015), estimates of soil restrictive layers Nakagaki (2012) and agriculturally irrigated areas Brown and Pervez (2014) amongst others (for details see *Appendix II*). The full US digital stream network (McKay et al., 2012) was processed as well for the subsequent model application of predicted environmental risks.

Methods in Brief for RO2

Geospatial data were used in combination with meta-data (chapter 3.1) and US RTL (chapter 3.3.1) to quantify significant spatial drivers of insecticide risks via model-building and subsequent model application for risk mapping of the US stream network (RO2, Figure 2, *Appendix II*). Thus, *Appendix II* aimed at quantifying significant insecticide risk drivers on the national scale and using the obtained models to provide a comprehensive, spatially explicit overview of current insecticide risks resulting from agricultural nonpoint source pollution in the US.

3.4.2 European spatial data

In *Appendix III*, spatial data were used to deduce watershed features upstream of WFD monitoring sites (European Environment Agency, 2012) and national or watershed-specific land cover characteristics (Copernicus Land Monitoring Service, 2020). Upstream catchment polygons could be derived for the majority of monitoring sites (88.3%, n = 7,249) based on functional elementary catchments using string-pattern matching (see *Appendix III*)

Methods in Brief for RO3

EU spatial data were used in concert with EU monitoring data (chapter 3.2.1) and EU-RTL (chapter 3.3.2) to provide a continental spatiotemporal risk analysis of organic contaminants in European surface waters with quantitation of land-scape level risk drivers (RO3, Figure 2, *Appendix III*). *Appendix III* furthermore assessed the impact of monitoring quality on the ability of accurately capturing the occurrence of organic contaminants in aquatic ecosystems and hence the reliability of large-scale monitoring data in describing current environmental risk trends.

In *Appendix IV*, for general land use or land cover characteristics (e.g. urban, natural), the Corine Land Cover layer 2012 was used as well in addition to detailed crop-distribution data (Blickensdörfer, 2021) for 24 crops in Germany (2017 – 2019). Pesticide use data for Saxony was obtained from GfK Kynetec (Kleffmann Group) for the years 2004 – 2020 detailing the application of 152 active ingredients in 31 crops and crop classes on the governmental district level (Kynetec, 2021). A geodata service of the LfULG Saxony was used to derive upstream catchment features (Landesamt für Umwelt, 2021) for all 1,371 monitoring sites based on sub-catchment polygons ($n = 6,606$, median area = 1.24 km²).

Methods in Brief for RO4

Geospatial data was used in concert with Saxon monitoring data (chapter 3.2.2) and adjusted EU-RTL (chapter 3.3.2) to characterize environmental risks in protected surface waters of NCA in comparison to UPA and to identify contamination pathways responsible for NCA contamination (RO4, Figure 2, *Appendix IV*).

3.5 Statistical and analytical techniques

Throughout the thesis for descriptive analyses mean (parametric) or median (nonparametric) 95% confidence intervals (95% CI) were used for comparisons of various grouping factors (Altman et al., 2013). Two sample tests, describing significant differences were performed using either Student's *t*-test (parametric) or Mann-Whitney's *U*-test (nonparametric). Generalized linear models were used to calculate all insecticide temporal use trends at the national level, which were then used to derive potential future implications of the respective substance (*Appendix I*). In *Appendix II*; multiple linear regressions (MLR) were used to test for significant relationships between insecticide risks (i.e. MIC to RTL ratio as dependent variable) and covariates (independent variables, *Appendix II*), here generally referred to as risk drivers.

MLR were built manually to describe individual MIC to RTL ratios (freshwater, sediments) or mixture MIC to RTL ratios (freshwater) and aimed at providing good explanatory power while remaining as parsimonious as possible. In *Appendix III*, correlations were assessed using Pearson product-moment correlation, linear regressions, generalized additive models, and LOESS regressions depending on the underlying data structure. The aforementioned methods were applied to derive significant correlations of environmental risks over time and the influence of monitoring quality on the detection probability of contaminants. In *Appendix IV*, the similarity of risk profiles between NCA and UPA groups was assessed based on the overlap of areas-under-the-curve of density distributions (Pastore and Calcagni, 2019). Principal component analysis (PCA, consensus approach, see Charrad et al. (2014)) and k-means clustering were applied to link catchment-specific land use characteristics with observed environmental risks to identify potential contamination pathways of pesticides into NCA.

4 Results and discussion

Chapter 4 provides a brief overview and discussion of the most important findings of this dissertation. For more specific results and discussion, please see *Appendices I – V*.

4.1 Insecticide risks in US surface waters and regulatory implications

In our analysis, we demonstrated high risks for US aquatic systems with predominant agricultural land use based on a comprehensive meta-analysis (see *Appendix I* for details). Overall, 49.2% of all MIC ($n = 5,817$) exceeded their RTL, with 69.7% of all sites ($n = 644$) experiencing at least one exceedance. Both MIC in the water phase (MIC_{sw}) and sediments (MIC_{sed}) were shown to exhibit similar exceedance frequencies, suggesting considerable risks for aquatic species groups in respective compartments. It is likely that the effects apply to a multitude of species in the wild, among them even more sensitive or endangered ones (Morrissey et al., 2015; Van den Brink et al., 2016; Weston and Lydy, 2014) and, as a consequence, adversely affecting aquatic biota via structural and functional alterations (Beketov et al., 2013; Schäfer et al., 2012; Stehle and Schulz, 2015a). More so, concentrations above one tenth of the RTL, which already lead to biodiversity effects (Stehle and Schulz, 2015a) were exceeded by 71.3% of the MIC_{sw} (Fig 1a, c). The results thus revealed noteworthy shortcomings of prospective US EPA regulatory risk assessment and management schemes for agricultural insecticides. More so, the findings suggested reevaluating currently applied regulatory methods and, if necessary, their subsequent refinement (**RO1**).

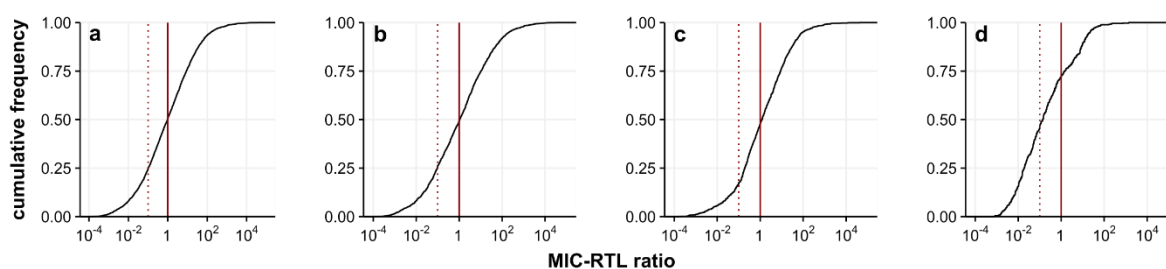


Figure 3 Cumulative frequency distributions of \log_{10} -transformed measured insecticide concentrations (MIC) in relation to their regulatory threshold levels (RTL, red solid line) for (a) all MIC ($n = 5,817$), (b) freshwater phase MIC ($n = 3,586$), (c) sediment MIC (freshwater and estuarine, $n = 1,779$), and (d) estuarine water phase MIC ($n = 452$). Dotted lines represent $0.1 \times$ RTL, at which a significant reduction in aquatic biodiversity was demonstrated (Stehle and Schulz, 2015a). MIC to RTL ratios larger than one represent threshold exceedances. Figure is taken from *Appendix I*.

Using this extensive dataset, our analyses also demonstrated that the phenomenon of excess agricultural insecticide concentrations was far more widespread in the US than previously anticipated by existing US large-scale monitoring studies from Gilliom et al. (2006b) and Stone et al. (2014b). Moreover, our data highlighted that even for the most recent years since 2002, 50.4% of MIC in freshwater and its sediments ($n = 3,099$) exceeded their RTL at 67.9% of the

sites (n = 369). Excess insecticide concentrations are therefore a phenomenon that may persist in the majority of US surface waters currently (**RO1**). Their functional integrity, which is critical to downstream consumers and beneficiaries of ecosystem services (Vannote et al., 1980; Wallace et al., 1997) hence appears to be at risk (Beketov et al., 2013; Schäfer et al., 2012). Additionally, small headwater streams (watershed size < 12.1 km²) are the predominant feature in agricultural landscapes (Lyon et al., 2015), which were shown to experience substantially higher RTL exceedances (61% in small headwater streams versus 49.2% in the total dataset) due to their size and proximity to agricultural fields. These findings highlighted a particularly concerning issue given that small headwater streams are estimated to constitute the largest riparian interface globally (Downing et al., 2012) and that they provide important refuges for both clean water ecosystems and biodiversity, allowing them to serve as areas for recolonization and support of endangered species (Biggs et al., 2017; Relyea, 2005; Scholz et al., 2000; Schulz, 2004). Hence, the thesis produced the most comprehensive evaluation of the widespread threat that insecticides pose to the integrity of US surface waters, particularly those pivotal headwater streams.

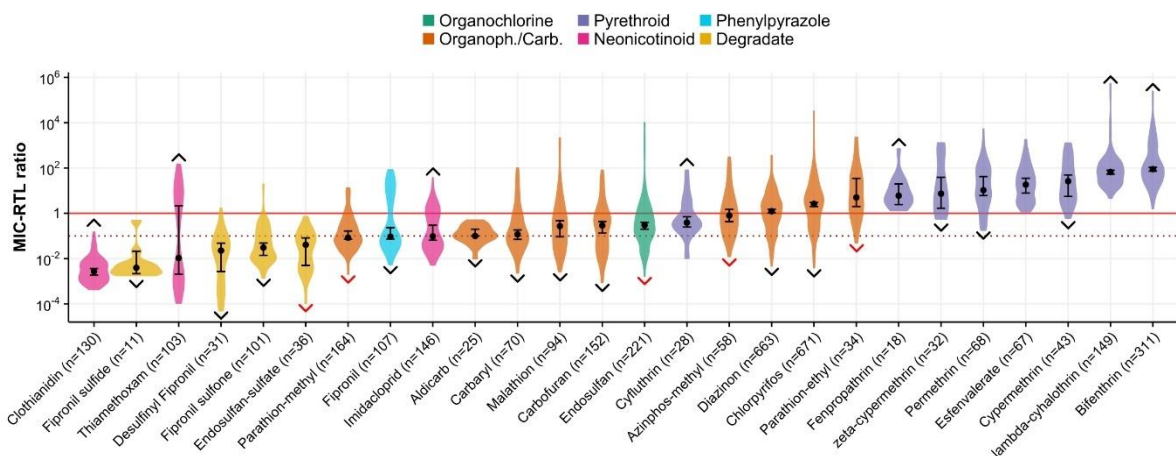


Figure 4: MIC to RTL ratios per substance for those with more than ten MIC for the freshwater phase with their median (dot), its corresponding 95% CI (black bars), and smoothed distributions (violin plots). RTL are depicted with a straight red line, and threshold concentrations of adverse effects on regional biodiversity (Beketov et al., 2013; Schulz, 2004) are represented by a red dotted line. Insecticide use trends (1992-2014) are depicted with arrowheads. Insecticides and their degradates that are no longer registered under FIFRA, Section 3 are marked with a red arrowhead. Figure is taken from *Appendix I*.

Considerable differences became apparent regarding the RTL exceedance frequency of individual substances in freshwaters (Figure 4), being lowest for organochlorines (24.7%, n = 230) and neonicotinoids (22.4%, n = 388), and highest for organophosphates and carbamates (48.7%, n = 1,931) and pyrethroids (92.8%, n = 737). Strikingly, seven pyrethroids significantly exceeded their RTL for freshwaters (Figure 4), with bifenthrin and lambda-cyhalothrin at the top of the exceedance profile. The composition of risk-defining insecticides was substantially different between the water and sediment phases (*Appendix I*). In sediments, exceedances

were driven by organochlorines (49.6%, n = 149) and again pyrethroids (72.0%, n = 1,023) likely due to their high persistence and sorptivity to sediment (Quinete et al., 2013; Scott et al., 2002). Pyrethroid insecticides were thus identified as the insecticide class currently posing the largest risks to aquatic ecosystems both for the organisms in the water phase and sediment phase (**RO1**, see *Appendix I*). Overall, these findings provided an integral line of evidence to the debate concerning the re-evaluation of the risk assessment schemes for pyrethroids (U.S. EPA, 2016) but also raised concerns regarding their protectiveness as these newer pyrethroids have increased environmental risks rather than alleviating the effects of older generation insecticides (e.g. organophosphates).

More detailed risk analyses were also conducted for neonicotinoids since neonicotinoids show prolonged and repeated exposure in surface waters, which stresses the importance of chronic toxicity (Morrissey et al., 2015; Tennekes and Sanchez-Bayo, 2011). Neonicotinoids have rapidly become the highly used insecticides in the US, with annual applications of approximately 4,000 t (Baker, 2016). However, adequately assessing neonicotinoid toxicity in aquatic systems remains challenging due to its variable toxicity between taxa (Morrissey et al., 2015), within taxa (Roessink et al., 2013), and uncertainties regarding sublethal (Beketov and Liess, 2008), cumulative (Liess et al., 2013) or food-mediated effects (Englert et al., 2012; Englert et al., 2017). The evaluation of MIC with chronic freshwater RTL and the chronic LLHC₅ (see Morrissey et al. (2015) and *Appendix I*) revealed considerably higher threshold exceedances of 57.6% and 52.3%, respectively, which was, however, still below the acute risk levels of nearly all pyrethroids and several organophosphates (c.f. Figure 4). Neonicotinoid risks in US surface waters had not yet been comprehensively evaluated on the national level using available literature, thus our results added a detailed perspective to their widespread occurrence and potential for chronic risks (**RO1**).

Annual insecticide use estimates were used to provide additional information for potential future developments of individual insecticides, reflecting a multitude of macro-agronomical factors. Regression analyses suggested that pesticide concentrations correlate significantly with annual insecticide use estimates on different spatial aggregation scales (see *Appendix I*). In summary, organochlorine and organophosphate use have been in decline (see Figure 4) due to regulatory actions (see *Appendix I*), partially in response to their high mammalian toxicity or their high persistence (Costa, 2006; U.S. EPA, 2011, 2012). However, in response to the aforementioned reduced use, the application of several pyrethroids (cyfluthrin, fenpropathrin, lambda-cyhalothrin, and bifenthrin) increased substantially between 1992 and 2014 (Figure 4). Particularly pyrethroids were associated with most regular and severe RTL exceedances, thus our use-trend analysis highlighted their continuing potential for ecologically adverse effects (**RO1**, Figure 4). Similarly, we showed that the adoption of neonicotinoids has

also increased drastically alongside several pyrethroids, a trend that is likely paralleled by more frequent RTL exceedances. Therefore, several pyrethroids and neonicotinoids likely continue to be highly problematic substances, impairing aquatic systems at the national scale and representing a large-scale risk shift from vertebrate to invertebrate species in aquatic ecosystems (RO1).

Overall, *Appendix I* demonstrated widespread and considerable risks for aquatic ecosystems in the US as a result of non-point source insecticide pollution. The increasing use of high-risk substances, particularly pyrethroids, revealed how the impairment of primarily aquatic invertebrate communities is currently being exacerbated. *Appendix I* also provided a first comprehensive assessment of neonicotinoids for the US suggesting lower acute risks compared to most other insecticides. Furthermore, small surface waters were demonstrated to experience substantially higher risks, highlighting elevated adverse effects in functionally important parts of aquatic ecosystems. Overall, the protectiveness of current US regulatory frameworks was shown to be deficient for several high-use substances applied in US agriculture.

4.2 Identification of insecticide risk drivers and national risk mapping

Building upon the overview of insecticide risks in the US (*Appendix I*), *Appendix II* provided further insight regarding the influence of underlying spatiotemporal drivers causing the aforementioned risks. In surface waters (n = 3,699), median risks expressed as MIC to RTL ratios decreased sharply with increasing sampling intervals (Figure 5). Daily and event-based sampling quantified MIC significantly above respective RTL (based on 95% CI) suggesting that MIC can be commonly detected above RTL in agricultural water bodies if temporal sampling resolution is sufficiently high, whereas risks were underestimated by more than one order of magnitude with larger sampling intervals, in line with results of Stehle and Schulz (2015a). In sediments, on the other hand, no significant difference was found between various sampling frequencies (Figure 5), likely a result of different transport and dissipation characteristics (*see Appendix II*). Therefore, assessing risks in sediment-dwelling communities appears less prone to errors regarding the magnitude of MIC resulting from different sampling strategies.

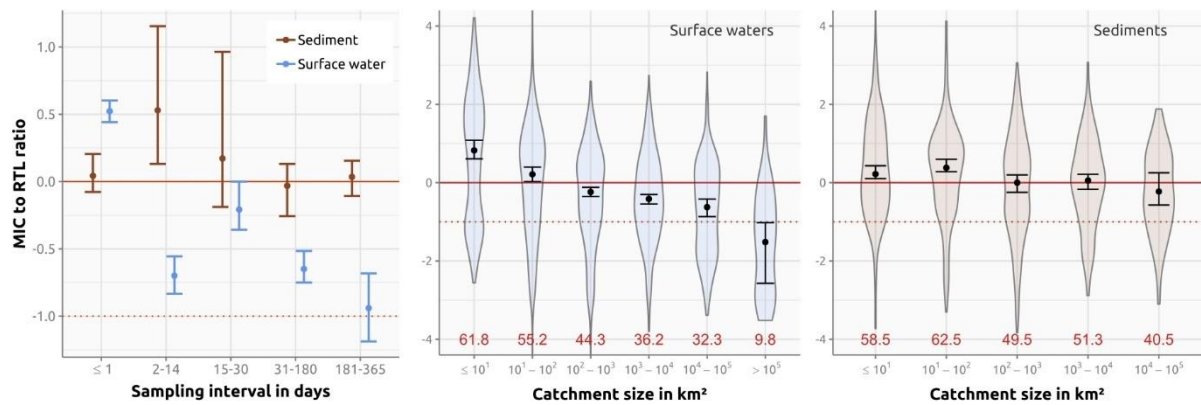


Figure 5: MIC to RTL ratios (\log_{10} -transformed) for surface waters (blue) and their sediments (brown) with their median (dot), their corresponding 95% CI (black error bars), and smoothed distributions (violin plots). RTL are depicted with a solid red line, and threshold concentrations of adverse effects on regional biodiversity are represented by dotted lines. Annotations (red) detail RTL exceedance frequencies (%) per respective group. Figure is taken from *Appendix II*.

Risks also differed significantly between waterbodies of varying catchment sizes (Figure 5), in line with assessments on the national (Spycher et al., 2018) and global scale (Stehle and Schulz, 2015a). Overall, water-phase risks decreased gradually with increasing catchment size (Figure 5) demonstrating that lower-order streams are disproportionately exposed to insecticides (Schulz, 2004), yet they represent pivotal ecosystems of high local biodiversity, providing critical functions such as recolonization refuges, spawning grounds for endangered species, and sources of organic matter breakdown crucial for downstream consumers (Biggs et al., 2017; Relyea, 2005; Vander Vorste et al., 2017; Vannote et al., 1980). Our results underscored that these important ecosystems across the US are exposed to various insecticides at ecologically relevant concentrations for decades, likely contributing to the ongoing biodiversity decline (Vörösmarty et al., 2010). In sediments, no relationship between the catchment size and risks was observed (Figure 5), hence sediment toxicity being more evenly spread compared to water-phase exposures, yet peak sediment exposures in headwaters being less pronounced. Decades of repeated insecticide applications may have resulted in widespread and long-term contamination of sediments along the entire longitudinal gradient of lotic systems (see *Appendix II*).

In *Appendix II*, risk drivers in the water phase (for sediments and mixture toxicity see *Appendix II*) were furthermore identified and weighted against each other using MLR (**RO2**, R^2 adj. = 57.69, $n = 2,031$, $p < 0.001$, see *Appendix II*). MIC to RTL ratios were by far most dependent on toxicity-normalized use, with a substantially lower influence of average burst rainfall erosivity. Pronounced use of high-risk substances combined with irregular erosive rainfall events (expressed by the burst factor, see Biasutti and Seager (2015)) facilitates a strong runoff potential with subsequent high transient toxicant loads (Weston et al., 2015). In addition to catchment sizes and sampling intervals, risks were furthermore positively related with areas

characterized by high degrees of irrigated agricultural land use, likely a result of higher drainage potentials, increased water to land interface area (e.g. discharge channels), or higher agricultural intensification indicated by irrigation practices (Howell, 2001; Kröger et al., 2009; Mize et al., 2008; Oster and Grattan, 2002; Rhoades, 1997). The drivers for water-phase and sediment-phase risks (see *Appendix II*) differed substantially, except for toxicity-normalized use, which was found to be the most important driver explaining aquatic risks in both compartments. The result hence had two crucial implications: First, to predict environmental risks in aquatic systems, pesticide use data must be assessed based on its ecotoxicological effect properties. Second, the established link between toxicity-normalized use was robust even for highly transiently occurring substances such as pyrethroids and shared between water-phase and sediment-phase predictions. This result addressed **RO2** and established the central link for the methodology of Schulz et al. (2021).

The model incorporating risk drivers was subsequently applied to the entire digital stream network of the US (Figure 6), yielding predicted insecticide concentration (PIC) to RTL ratios in freshwaters (**RO2**, $R^2 = 62.17$, $p < 0.001$, *Appendix II*). In multiple regions, the interaction of risk drivers suggested acute effects for nontarget aquatic organisms. Half of the total stream length in the US was estimated to experience at least 28.9% of RTL exceedances in small watersheds, increasing even to 60.5% in some regions (Figure 6). The presented model thus provided, for the first time, a comprehensive aquatic risk characterization for a wide range of relevant insecticides at the national level of the US, highlighting the most vulnerable regions for watersheds of various sizes, such as the Mississippi Portal, a hydrologically and biologically diverse river ecosystem that provides habitat for several endangered or threatened species (U.S. Fish & Wildlife Service, 2015).

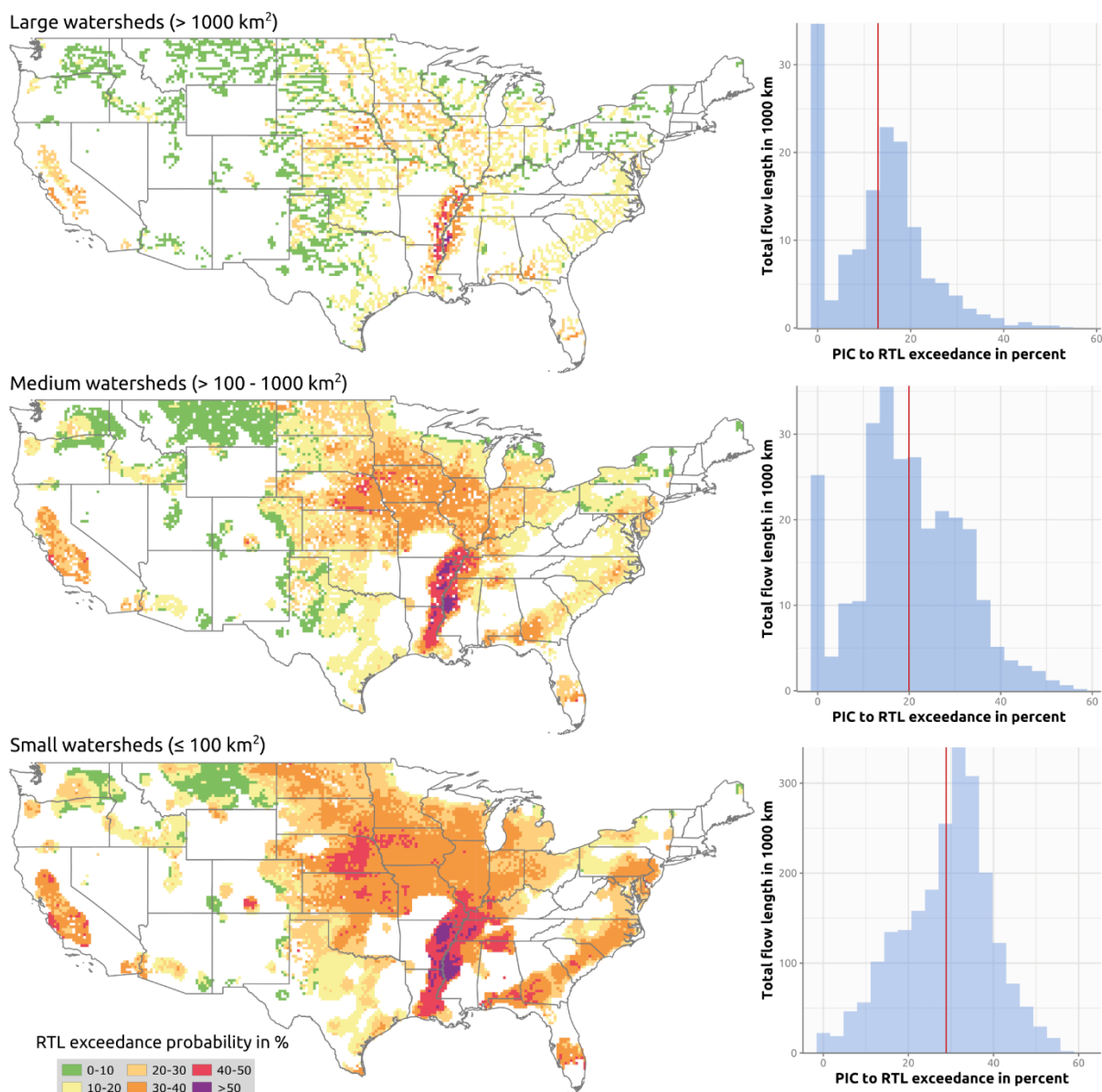


Figure 6: National model application of PIC to RTL ratios in freshwaters for small (≤ 100 km², bottom), medium ($>100 - 1000$ km², center), and large (>1000 km², top) watersheds in the United States. Histograms show the total flow lengths of streams or rivers relative to their RTL exceedance probability and the red line depicts the average RTL exceedance probability weighted by flow length. Simulation results are based on MIC (i.e. quantifications) and thus, refer to respective exceedance probabilities of quantified insecticide concentrations. Figure is taken from *Appendix II*.

Finally, *Appendix II* formed the basis and methodological links for a new concept of assessing the potential large-scale environmental risks for multiple species groups using ecotoxicity-normalized pesticide use data Schulz et al. (2021). The concept of toxicity-normalized use was rephrased as Total Applied Toxicity (TAT) and demonstrated how regulatory changes resulted in drastic shifts of environmental risks from vertebrate to invertebrate species in the US, which was thus far overlooked by mass-based approaches (Osteen and Fernandez-Cornejo, 2013; Perry et al., 2016) and was in line with the large-scale results of *Appendix II*. Importantly, it also unraveled that previously assumed environmental benefits of genetically

modified crops (e.g. insect resistant variants) did not result in lower applied toxicities, on the contrary, it led to drastic increases for aquatic invertebrates and pollinator species (Schulz et al., 2021). The concept, therefore, broke with the spurious assumption that the decreasing amounts of applied pesticides had resulted in improved environmental conditions. Thus, *Appendix II* combined with work by Schulz et al. (2021) provided a new approach to assessing large-scale pesticide risks that are highly valuable for regulatory bodies and scientists alike, further aiding in understanding the global chemical boundary (Steffen et al., 2015).

Overall, *Appendix II* identified and quantified multiple risk drivers relevant to the assessment of insecticide risks in aquatic ecosystems and underlined the substantial differences between water-phase and sediment-phase exposures. Toxicity-normalized pesticide use was shown to form the central link between agricultural land use and resulting environmental risks in aquatic ecosystems, furthermore forming the methodological basis for Schulz et al. (2021). The derived risk models enabled, for the first time, a large-scale prediction about the extent of surface water impairments in the US.

4.3 Continental risk characterization of organic pollutants in European surface waters

In *Appendix III*, the scope of the dissertation was further expanded from insecticides (*Appendices I and II*) or pesticides (Schulz et al., 2021) to multiple groups of organic pollutants to provide a comprehensive assessment of environmental risks in European surface waters. The main goal of this study was to address all four core dimensions of large-scale ecotoxicology, namely the chemical, organismic, spatial, and temporal dimensions, as well as identifying organic contaminants defining risks on a continental scale.

Monitoring quality was highly variable among European countries, expressed in differences in MQI (see *Appendix III*). Addressing and quantifying the heterogeneity of the underlying data was a central aspect of *Appendix III* because it can lead to substantial underestimation of risk estimates (Crawford, 2004; Stehle and Schulz, 2015a; Wolfram et al., 2019). This significant relationship was established between the MQI and the fraction of monitoring stations that did not quantify any organic contaminants (*Appendix III*). Thereby, the importance of proper monitoring strategies for finding contaminants in the aquatic environment was quantified but it also underlined how scarce and qualitatively lacking the data basis is in Europe regarding the macro scale contamination of surface waters. With such caveats, it remains questionable if the WFD goals of reaching a good chemical status in surface waters (European Council, 2000) can truly be achieved if the underlying data shows such qualitative issues. Adding to this, a general lack of pharmaceutical monitoring data was uncovered, despite pharmaceuticals detection frequencies of 58.3%, which was substantially higher than those of any other chemical class currently monitored (see *Appendix III*). Particularly non-steroidal anti-inflammatory drugs and antibiotics were frequently detected and have been linked to

detrimental effects in aquatic ecosystems (Geissen et al., 2015; Godoy et al., 2015; Jonsson et al., 2015; Katipoglu-Yazan et al., 2015; Roose-Amsaleg and Laverman, 2016) and can cross the aquatic-terrestrial interface (Richmond et al., 2018), suggesting that continued monitoring of these substances is critical and yet mostly lacking in the EU.

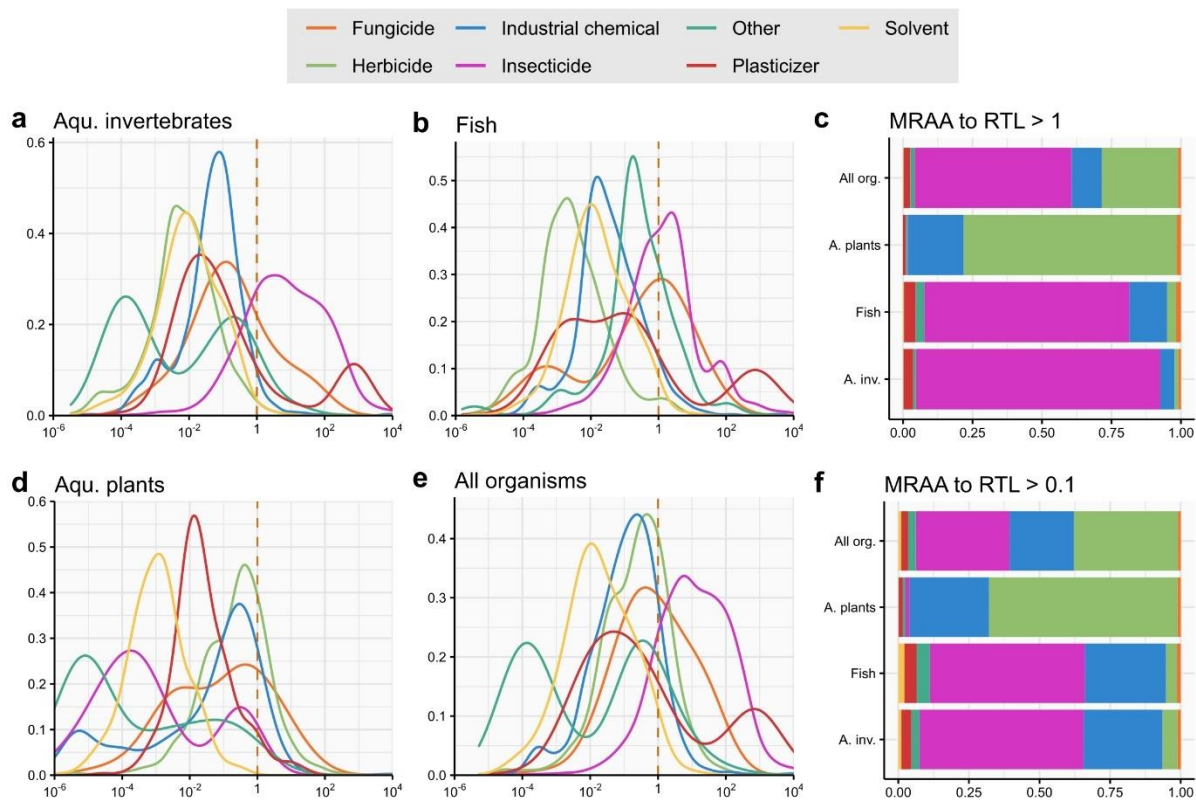


Figure 7: Density distributions of MRAA to RTL ratios per chemical class and organism group (a, b, d, e). Density distributions depict how MRAA of certain chemical classes compare to their respective RTL (dashed line: equality of MRAA and RTL) but do not provide any information about the absolute number of concentrations that occur in these ranges. In (c), the fraction of all MRAA to RTL exceedances is provided per chemical class to deduce their relative impact on water quality. In (f), the fraction of MRAA to $(0.1 \times \text{RTL})$ exceedances is depicted. Figure is taken from *Appendix III*.

In general, detection frequencies of organic contaminants increased since 2007 with a continuous upward trend (*Appendix III*) for nearly all chemicals. As such, many organic contaminants appear to be transported more frequently into surface waters throughout Europe, adding to the debate about the advancing macro-scale chemization of environments (Bernhardt et al., 2017) (**RO3**). Contrasting the comparatively low detection frequencies of most chemical classes (e.g. 1.7% for insecticides, *Appendix III*), single organic contaminants still posed substantial risks to non-target aquatic species. We found that 23.9% ($n = 17,162$), 18.9% ($n = 16,588$), and 17.8% ($n = 17,053$) of MRAA exceeded their respective $\text{RTL}_{\text{aqua-inverts}}$, RTL_{fish} , and $\text{RTL}_{\text{aqua-plants}}$ (Figure 7a) and more than one third (35.4%, $n = 17,358$) of MRAA exceeded their ecological threshold (RTL_{sw}), putting at least one of the organism groups at significant risk once per year (Figure 7). *Appendix III*, thus demonstrated widespread risks for

aquatic ecosystems on the macro scale of Europe, likely being one key factor in the freshwater biodiversity crisis (Lemm et al., 2021; Vörösmarty et al., 2010). Pesticides were responsible for 85.0% of all RTL_{sw} exceedances (Figure 7c) in European surface waters, highlighting that agricultural pollution remained the dominant threat to freshwater integrity (Lemm et al., 2021; Malaj et al., 2014). The results thus provided timely evidence for current debates in the EU aiming to substantially reduce pesticide use and its associated environmental impact by 2030 (European Commission, 2020). Furthermore, the concept of the TAT (Schulz et al., 2021) could be applied in a European context to further identify current high-risk substances which require special focus by monitoring programs, such as poorly monitored pyrethroids, which are likely an unperceived issue in Europe (see *Appendix I*). From an ecosystem perspective, we showed that insecticides and herbicides were the primary risk drivers in European surface waters (**RO3**), followed by industrial chemicals (Figure 7e), in total explaining more than 95% of RTL exceedances.

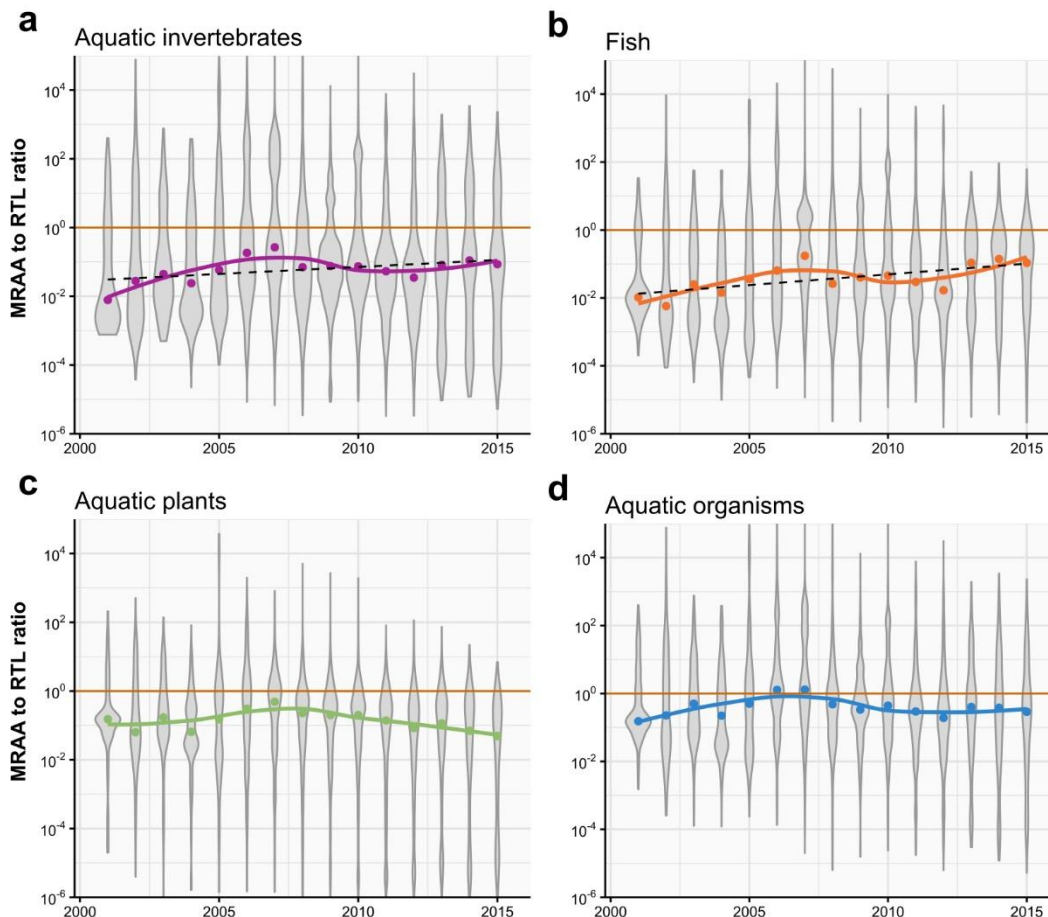


Figure 8: Median MRAA (dots) to (a) $RTL_{aqua-inverts}$, (b) RTL_{fish} , (c) $RTL_{aqua-plants}$, and (d) RTL_{sw} ratios over time with the underlying data distribution per year depicted with violin plots (grey). Temporal trends are indicated by LOESS regression. Significant monotonic temporal trends (Mann-Kendall, $p < 0.05$) are depicted with dashed lines (black). Specific temporal trend analyses of the most impactful chemical classes (insecticides, herbicides, and industrial chemicals) can be found in *Appendix IV*. Figure is taken from *Appendix III*.

Temporal risk analyses showed that further changes to current management and regulatory strategies are needed to improve the chemical quality of European water bodies. Trend analysis suggested that risks remained unchanged ($p > 0.05$) for aquatic plants and all organism groups combined (Figure 8), whereas risks significantly increased over time for aquatic vertebrates ($\tau = 0.498$, $p = 0.01$; Figure 8a) and invertebrates ($\tau = 0.429$, $p = 0.03$; Figure 8b). A substantial fraction of European waterbodies thus frequently and recently experienced chemical exposures that adversely impact biota, posing unacceptable risks to the environment (**RO3**). Currently applied regulatory methods and management strategies appear insufficient in meaningfully decreasing risks in most European waterbodies, therefore achieving the goals set out by the WFD and SUR remains a challenge without any substantial changes to agricultural production systems. Recent studies' conclusions that the health of aquatic invertebrate communities is improving globally and in Europe, would initially appear to be in opposition to our results (Outhwaite et al., 2020; van Klink et al., 2020), as we found that aquatic invertebrates were the organism group most acutely at risk. However, we found that pesticides are the main driver of observed aquatic risks but Outhwaite et al. (2020) as well as van Klink et al. (2020) could not derive results that are directly attributable to agricultural practices, which furthermore highlights the present ecotoxicological knowledge gap concerning large-scale and comprehensive links between agriculture and aquatic invertebrate health.

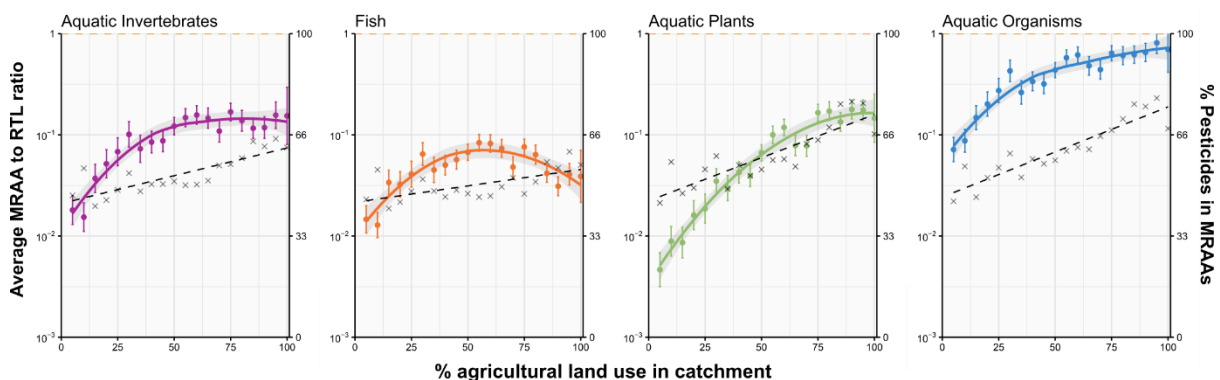


Figure 9: Average aquatic risks ($\pm 95\%$ CI, MRAA to RTL ratios, primary y-axis) as a function of agricultural land use (aggregated into evenly-spaced 5% intervals) for (a) invertebrates, (b) fish, (c) aquatic plants, and (d) all organism groups combined (RTL_{sw}). LOESS regressions depict the approximate relationship between the degree of agricultural intensification within respective catchments and observed average risks for each organism group (a-d). Pesticides (i.e. herbicides, insecticides, fungicides) percentage contribution to the MRAA is depicted with black crosses, and corresponding linear trends are depicted with black dashed lines (secondary y-axis). Figure is taken from *Appendix III*.

Only a few spatial clusters did not show at least sporadic average RTL exceedances for MRAA (*Appendix III*) underscoring that ecosystem impairments are prevalent throughout Europe. Still, the thesis was able to highlight multiple regions (e.g. Lombardy, Andalusia) in which toxic pressures differed substantially between organism groups, resulting from differing agricultural land use types and the associated use of pesticides (see *Appendix III*). As such, risk reduction

strategies need to be tailored according to land use characteristics, which vary regionally, producing discernible differences in aquatic risks for organism groups on continental scales (RO3). *Appendix III* concluded by establishing a clear link between agricultural land use and environmental risks in surface waters throughout Europe (RO3, Figure 9) with average risks differing by approximately an order of magnitude between sites with low (<15%) versus high (>80%) agricultural land use. However, pesticides commonly defined risks even in surface waters with minimal agricultural land use (Figure 9), primarily caused by herbicides, which is in accord with their diverse commercial and private application patterns (Heap and Duke, 2018). Landscape-level management schemes should therefore prioritize reducing the use of pesticides or prevent their subsequent transport into non-target aquatic systems curbing environmental risks in highly intensified regions. Nonetheless, more holistic approaches will be necessary, i.e. also addressing smaller-scale industrial and urban sources, reducing the vast complexity of organic contaminants currently introduced into aquatic ecosystems (van Gils et al., 2020). The contamination of European surface waters remains a complex, multi-dimensional issue for which *Appendix III* provided extensive insights. However, in light of flaws and heterogeneity of current monitoring programs (see MQI, *Appendix III*) and the underrepresentation of critical substances (e.g. pyrethroids), both of which are crucial for precise risk estimations (see *Appendix I and II*), suggests that the true environmental risks in Europe remain underestimated.

Overall, *Appendix III* identified pesticides as the primary class of organic contaminants impairing aquatic ecosystems on the continental scale as well as uncovering increasing risks over time for aquatic invertebrates and fish (RO3). RTL were frequently exceeded throughout Europe, signaling that the regulatory risk assessment requires noteworthy improvements to safeguard aquatic ecosystems. Agricultural land use was the most central risk driver, being linked to the observed environmental risks and able to explain smaller-scale (regional) risk clusters as well. Hence, future application of the TAT (Schulz et al., 2021) could help identify high-risk pesticides or regions and further the understanding of the complex issue that is the contamination of aquatic ecosystems.

4.4 Environmental risks in protected surface waters

Appendix IV concludes the thesis with a specific focus on surface waters in NCA. NCA represent some of the few remaining natural environments in Germany that should experience only minimal or no adverse anthropogenic impact, as stipulated in BNatSchG §1. *Appendix IV* focused on pesticides as they were identified as the primary risks driver throughout Europe (*Appendix III*). Pesticides were detected less frequently in NCA than in UPA, however, temporally very similar to those of UPA (see *Appendix IV*, $R^2 = 0.70 - 0.92$). We found that agricultural land use is substantially lower or virtually absent in NCA (<3.2%, *Appendix IV*), hence pesticide sources were primarily located outside the actual protection area, i.e. most of

NCA catchment areas were found to be unprotected (*Appendix IV*). Taken together, detection frequencies in NCA appeared to be driven at two scales: the local scale (i.e. the protection area itself) reducing detection frequency due to its predominantly natural land cover, and the catchment scale, being largely unprotected and contributing to the transport of pesticides into NCA (**RO4**).

The average concentrations between NCA and UPA (n = 276,362 detections, *Appendix IV*) were mostly similar for all pesticide classes except herbicides, differing only by a factor of 1.29 – 1.77. Pesticide exposure in NCA thus exhibited two differing characteristics: On the one hand, detection frequencies were lower, whereas, on the other hand, average concentrations did not show large differences, which means that contamination events can pose similar risks as observed in UPA (see below). In addition, metabolites, such as metazachlor sulfonic acid, were shown to occur virtually permanently in protected surface waters, which underscored that unprotected upstream areas likely served as pesticide sources (i.e. via fluvial inflows) and explained the high degree of similarities between NCA and UPA (Bradley et al., 2021; Gerber et al., 2016). This also raised the issue of adverse chronic effects in various species groups due to the continuous or repeated occurrence of multiple contaminants. Whilst previous works already highlighted the contamination of individual protection areas (e.g. national parks) by primarily legacy contaminants (Buah-Kwofie and Humphries, 2017; Camacho-Munoz et al., 2010; Elliott and VanderMeulen, 2017; Hageman et al., 2006; LeNoir et al., 1999), no comprehensive assessment on a large spatial scale thus far detailed the contamination of protected surface waters by organic contaminants (**RO4**). Based on these results, it appears highly questionable if the integrity of these ecologically vital systems is safeguarded as required under BNatSchG §1 given the similar contamination characteristics with UPA, in which adverse effects on ecosystem function and structure are already known (Halbach et al., 2021; Liess et al., 2021; Schäfer et al., 2012).

Acute ecotoxicological risk profiles were highly similar between NCA and UPA for all pesticide classes, with aquatic plants and invertebrates being the most affected. Here, both the threshold exceedance frequencies (*Appendix IV*) as well as the complete risk distributions (e.g. sum(M/R) distributions, Figure 10, **RO4**) demonstrated that the observed risks were highly similar for NCA (84.38 – 88.27%). Infrequently occurring substances often defined risks (e.g. chlorpyrifos-ethyl, cypermethrin), which highlighted the discrepancy between regular occurrences and environmental relevance. This discrepancy remains a central challenge for precise risk characterizations in ecotoxicology yet also underscores the importance of appropriate designs of monitoring schemes (see also *Appendices I and III*, Spycher et al. (2018)). Furthermore, high-use pyrethroids were still severely underrepresented (e.g. lambda-cyhalothrin, deltamethrin) in *Appendix IV*, with 10 – 40 times fewer samples compared to other

insecticides, or missing completely (beta-cyfluthrin). As such, risks for aquatic invertebrates did not fully reflect the potential effects in aquatic ecosystems (Mahler et al., 2020; Rogers et al., 2016) in both UPA and NCA, revealing a critical blind-spot in current monitoring strategies, which was also observed on the European scale (*Appendix III*). The thesis thus suggests that one of the most impactful chemical stressors (c.f. *Appendix I*) in Europe is nearly completely overlooked in Germany and Europe. For aquatic plants, herbicide compounds primarily defined risks, and the major risk contributors (e.g. diflufenican, metazachlor) also showed the highest applied toxicities towards aquatic plants in Germany. This link underscored the benefit of ecotoxicity-normalized risk indicators (Schulz et al., 2021) for assessing pesticide application data and their potential link to adverse effects in non-target aquatic ecosystems (*Appendix II*), which could also bridge the gap between oftentimes mismatching biological and chemical monitoring data in the future.

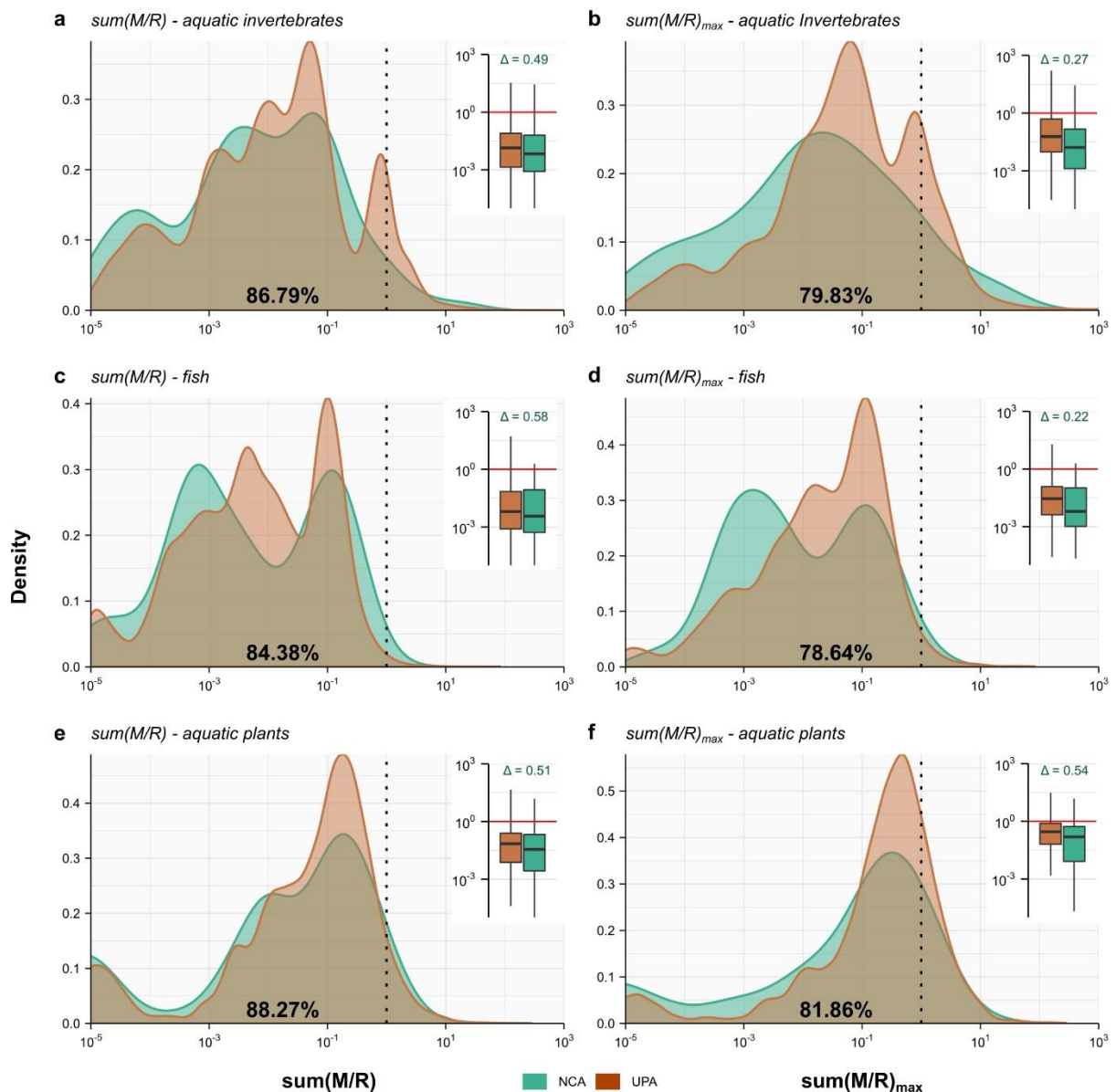


Figure 10: Density distributions of $\text{sum}(M/R)$ are provided for (a) aquatic invertebrates, (c) fish, and (e) aquatic plants. Density distributions of $\text{sum}(M/R)_{\max}$ per site are provided for (b) aquatic invertebrates, (d) fish, and (f) aquatic plants. Similarities of risk profiles can be deduced from the overlap (brown) of density distributions for NCA (green) and UPA (orange), annotated in bold (black). Instances of acute risk can be assumed in the case of $\text{sum}(M/R)$ (left) or $\text{sum}(M/R)_{\max}$ (right) being larger than 1 (black, dotted vertical line). $\text{sum}(M/R)$ and $\text{sum}(M/R)_{\max}$ are also depicted as boxplots in the upper right corner of the respective panel. The median $\text{sum}(M/R)$ or $\text{sum}(M/R)_{\max}$ of NCA compared to UPA (quotient, untransformed) is annotated with Δ . Figure is taken from *Appendix IV*.

The size of NCA in Saxony (2.52 km² average size) is comparably small with minimal agricultural land use (<2.5%) whereas directly surrounding areas (1000 m, see *Appendix IV*) were intensively used agricultural areas (>30%) similar to the rest of Saxony ($R^2 = 0.92$, *Appendix IV*). Therefore, potential agricultural sources of pesticides can be spatially close to NCA, resulting in direct inflows or even drift-ins of pesticides (Gerber et al., 2016; Zaller et al., 2022). *Appendix IV* supported this assumption by linking upstream land use characteristics to instream risks for all species groups via PCA (**RO4**, Figure 11). We found that environmental

risks in NCA surface waters were primarily driven by upstream agricultural activity and further analyses also showed that NCA do not function as significant pesticide sources (see *Appendix V*). Environmental risks per site were strongly correlated with agricultural land use in their catchments for aquatic plants (82.4%) and aquatic invertebrates (80.5%), and were strongly negatively correlated with natural or forested land cover (90.1 – 96.4% for invertebrates, 91.2 – 97.3% for aquatic plants). Therefore, the areal intensity of agriculture in each catchment directly defined the extent to which aquatic plants and invertebrates were at risk. Consequently, pesticides' major pathway into NCA appears to be fluvial inflows, underscoring that contamination of NCA is driven on the catchment scale (Bradley et al., 2021; Gerber et al., 2016; Roche et al., 2009). Conversely, protection or expansion of natural land cover in upstream watersheds, e.g. by establishing wide riparian buffers (Bradley et al., 2020), could be future tools to reduce the ecotoxicological risks for these protected surface waters.

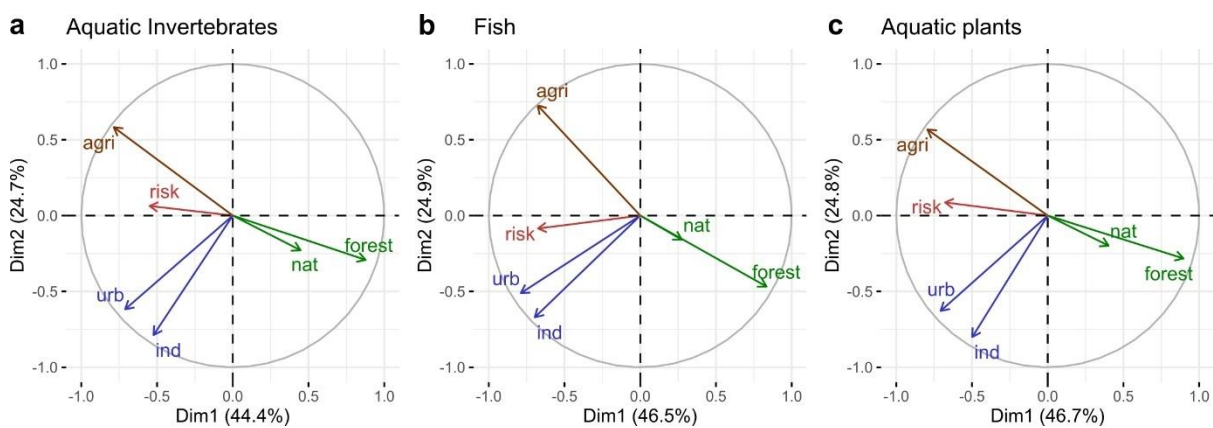


Figure 11: Principal component analysis of land use compositions within upstream catchments of NCA with their respective influence on average risks over time (=risk, red, mean $\sum(M/R)_{max}$) for (a) aquatic invertebrates, (b) fish and (c) aquatic plants. Variable plots use type-II scaling of principal components, as such, angles between vectors are indicative of variable correlation and the orthogonal length of variable vectors denotes their relative influence in building respective principal components. Land use or land cover was aggregated into five groups: urban and industrial (blue), natural and forested (green), and agricultural (brown). Figure is taken from *Appendix IV*.

NCA are among the most strictly protected areas in Germany and represent some of the few remaining natural refuges in otherwise intensively used landscapes (Bundesamt für Naturschutz, 2022), yet *Appendix IV* showed that aquatic systems in these protected areas are subject to similar risk levels than UPA, jeopardized established protection goals (**RO4**). Particularly aforementioned impairments to aquatic invertebrate and plant assemblages (Lozano et al., 2020; Schäfer et al., 2011) can adversely affect stream productivity or the provision of ecological services (Rumschlag et al., 2020). Pesticides can also transfer from the aquatic phase of NCA into the terrestrial phase (Graf et al., 2020; Roodt et al., 2022; Wickramasinghe et al., 2004) establishing a contaminant pathway through trophic links. Whilst NCA should function as key recovery refuges and recolonization sources (Bundestag, 2009), it appears questionable if they can serve this role in light of the present aquatic risks. Thus, if

surface waters in even the most protected areas in Germany cannot fulfill this role, it becomes unclear how pesticides can be registered under the assumption of recolonization and recovery in the first place (EFSA, 2013). Therefore, *Appendix IV* provided timely evidence in support of the current goals of the European Commission to reduce pesticide risks in sensitive areas, such as NCA (European Commission, 2021). However, as shown in *Appendix IV*, it remains critical to consider and manage the whole watershed context, otherwise, protection goals cannot be reached due to the strong hydrological and potentially trophic connection between UPA and NCA.

Overall, *Appendix IV* demonstrated substantial environmental risks in protected surface waters, resulting from similar exposure frequencies and concentrations as evidenced in unprotected areas (RO4). A clear link was established between intensified upstream agricultural land use transporting contaminants from unprotected areas into protected downstream areas via fluvial inflows (RO4). Thus far, no large-scale evaluation of NCA surface waters was conducted in Germany or Europe, hence *Appendix IV* provided timely insight into the contamination dynamics within these pivotal ecosystems, adding to the ongoing debate surrounding the Sustainable Use Regulation.

5 Conclusion

The chemization of aquatic environments is increasing (Bernhardt et al., 2017), as supported in this thesis for pesticides and other organic contaminants in a national and continental context (see *Appendices I, III, and IV*). As a result, the impairment of multiple aquatic species groups, detailed for various large spatiotemporal scales, has persisted or even progressed. Particularly pesticides were responsible for these expected adverse effects in ecosystem function or structure and were shown to frequently violate the assumption under which they were registered. The envisioned risk reduction outlined by the SUR (European Commission, 2021), is thus urgently needed, however, the core assumptions of the regulatory process for pesticides will also require refinements in light of their frequent and substantial unacceptable risks outlined here. Several works provided initial insights into this large-scale challenge to freshwater integrity (Liess et al., 2021; Malaj et al., 2014; Schäfer et al., 2011; Stehle and Schulz, 2015a; Stehle and Schulz, 2015b; Stone et al., 2013; Zubrod et al., 2019). Therefore, the present thesis added to their work by deriving risk trends and spatial drivers, outlining how environmental risks have evolved temporally and are influenced spatiotemporally in the US (*Appendix I and II*) and the EU (*Appendix III and IV*). However, it became clear how data heterogeneity significantly impairs the ability to characterize environmental risks on large scales; an issue most prevalent in the EU. Pyrethroid insecticides exemplified this issue by being particularly detrimental substances (*Appendix I*), requiring highly targeted monitoring efforts that are (*Appendix II*), however, in most cases not conducted (see *Appendix III and IV*). Hence, their large-scale impact is likely severely underestimated in Europe, supported by recent analyses of the applied toxicity in Germany (Bub et al., 2023). In addition, the thesis revealed how the contamination of aquatic ecosystems has even reached into strictly protected areas, which provide pivotal refuges for freshwater integrity (Biggs et al., 2017), demonstrating how widespread diffuse chemical pollution has become (*Appendix IV*). At this point, it becomes evident that current regulatory assumptions and established frameworks do not intersect with actual ecotoxicological phenomena and suggesting the necessity of substantial changes to the way that environments are anthropogenically used. In the future, combining risk drivers (*Appendix II*), estimates of applied toxicities (Bub et al., 2023; Schulz et al., 2021), and additional geospatial data (*Appendix II – IV*) can help quantify pesticides' impact on aquatic ecosystems on macro scales and potentially provide new avenues of linking heterogeneous monitoring data with biological data to understand the current state of aquatic ecosystems. Such efforts will be vital if any significant progress towards the current goals of international efforts (e.g. SUR, WFD) is to be achieved. In summary, the thesis gave new insights into macro-scale risk through the use and combination of large datasets as well as providing new approaches to assessing the impact of organic contaminants in these contexts.

6 References

- Ainsworth, D., Collins, T., d'Amico, F., 2022. Nations adopt four goals, 23 targets for 2030 in Landmark UN Biodiversity Agreement, Convention on Biological Diversity December, pp. 2022-2012.
- Altman, D., Machin, D., Bryant, T., Gardner, M., 2013. *Statistics with confidence: confidence intervals and statistical guidelines*. John Wiley & Sons, New York.
- Backhaus, T., Arrhenius, Å., Blanck, H., 2004. Toxicity of a Mixture of Dissimilarly Acting Substances to Natural Algal Communities: Predictive Power and Limitations of Independent Action and Concentration Addition. *Environ. Sci. Technol.* 38 (23), 6363-6370.
- Baker, N.T., 2016. Agricultural Pesticide Use Estimates for the USGS National Water Quality Network, 1992-2014. U.S. Geological Survey data release.
- Baker, N.T., Stone, W.W., 2015. Estimated annual agricultural pesticide use for counties of the conterminous United States, 2008-12, Data Series, Reston, VA, p. 18, <http://pubs.er.usgs.gov/publication/ds907>.
- Barakat, A.O., Khairy, M., Aukaily, I., 2013. Persistent organochlorine pesticide and PCB residues in surface sediments of Lake Qarun, a protected area of Egypt. *Chemosphere* 90 (9), 2467-2476.
- Bebber, D.P., Holmes, T., Gurr, S.J., 2014. The global spread of crop pests and pathogens. *Global Ecol. Biogeogr.* 23 (12), 1398-1407.
- Beketov, M.A., Kefford, B.J., Schäfer, R.B., Liess, M., 2013. Pesticides reduce regional biodiversity of stream invertebrates. *Proc. Natl. Acad. Sci. USA* 110 (27), 11039-11043.
- Beketov, M.A., Liess, M., 2008. Potential of 11 Pesticides to Initiate Downstream Drift of Stream Macroinvertebrates. *Arch. Environ. Contam. Toxicol.* 55 (2), 247-253.
- Bernhardt, E.S., Rosi, E.J., Gessner, M.O., 2017. Synthetic chemicals as agents of global change. *Front. Ecol. Environ.* 15 (2), 84-90.
- Biasutti, M., Seager, R., 2015. Projected changes in US rainfall erosivity.
- Bigelow, D.P., Borchers, A., August 2017. Major Uses of Land in the United States, 2012. United States, Department of Agriculture, Economic Research Service.
- Biggs, J., Von Fumetti, S., Kelly-Quinn, M., 2017. The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers. *Hydrobiologia* 793 (1), 3-39.
- Blackwell, B.R., Ankley, G.T., Bradley, P.M., Houck, K.A., Makarov, S.S., Medvedev, A.V., Swintek, J., Villeneuve, D.L., 2018. Potential toxicity of complex mixtures in surface waters from a nationwide survey of United States streams: Identifying in vitro bioactivities and causative chemicals. *Environ. Sci. Technol.* 53 (2), 973-983.
- Blickensdörfer, L., Schwieder, Marcel, Pflugmacher, Dirk, Nendel, Claas, Erasmi, Stefan, & Hostert, Patrick, 2021. National-scale crop type maps for Germany from combined time series of Sentinel-1, Sentinel-2 and Landsat 8 data (2017, 2018 and 2019), <https://doi.org/10.5281/zenodo.5153047>.
- Bradley, P.M., Kulp, M.A., Huffman, B.J., Romanok, K.M., Smalling, K.L., Breitmeyer, S.E., Clark, J.M., Journey, C.A., 2021. Reconnaissance of cumulative risk of pesticides and pharmaceuticals in Great Smoky Mountains National Park streams. *Sci. Total Environ.* 781 146711.
- Bradley, P.M., Romanok, K.M., Duncan, J.R., Battaglin, W.A., Clark, J.M., Hladik, M.L., Huffman, B.J., Iwanowicz, L.R., Journey, C.A., Smalling, K.L., 2020. Exposure and potential effects of

- pesticides and pharmaceuticals in protected streams of the US National park Service southeast region. *Sci. Total Environ.* 704 135431.
- Brown, J., Pervez, M.S., 2014. Merging remote sensing data and national agricultural statistics to model change in irrigated agriculture. *Agricultural Systems* 127 28-40.
- Buah-Kwofie, A., Humphries, M.S., 2017. The distribution of organochlorine pesticides in sediments from iSimangaliso Wetland Park: ecological risks and implications for conservation in a biodiversity hotspot. *Environ. Pollut.* 229 715-723.
- Bub, S., Wolfram, J., Petschick, L.L., Stehle, S., Schulz, R., 2023. Trends of Total Applied Pesticide Toxicity in German Agriculture. *Environ. Sci. Technol.* 57 (1), 852-861.
- Bundesamt für Naturschutz, 2022. Naturschutzgebiete, <https://www.bfn.de/naturschutzgebiete>.
- Bundestag, 2009. Gesetz über Naturschutz und Landschaftspflege (Bundesnaturschutzgesetz - BNatSchG) vom 29. Juli 2009 (BGBl. I S. 2542), das zuletzt durch Artikel 1 des Gesetzes vom 20. Juli 2022 (BGBl. I S. 1362) geändert worden ist, in: Bundestag (Ed.), BGBl. I S. 2542.
- Camacho-Munoz, M., Santos, J., Aparicio, I., Alonso, E., 2010. Presence of pharmaceutically active compounds in Donana Park (Spain) main watersheds. *J. Hazard. Mater.* 177 (1-3), 1159-1162.
- Charrad, M., Ghazzali, N., Boiteau, V., Niknafs, A., 2014. NbClust: an R package for determining the relevant number of clusters in a data set. *Journal of statistical software* 61 1-36.
- Connors, K.A., Beasley, A., Barron, M.G., Belanger, S.E., Bonnell, M., Brill, J.L., de Zwart, D., Kienzler, A., Krailler, J., Otter, R., 2019. Creation of a curated aquatic toxicology database: EnviroTox. *Environ. Toxicol. Chem.* 38 (5), 1062-1073.
- Copernicus Land Monitoring Service, 2020. Corine Land Cover - 2012 - 100 m resolution, <https://bit.ly/2KhEat1>.
- Costa, L.G., 2006. Current issues in organophosphate toxicology. *Clinica chimica acta* 366 (1-2), 1-13.
- Crawford, C.G., 2004. Sampling strategies for estimating acute and chronic exposures of pesticides in streams. *JAWRA Journal of the American Water Resources Association* 40 (2), 485-502.
- Das, S.K., 2013. Mode of action of pesticides and the novel trends – A critical review. *International Research Journal of Agricultural Science and soil Science* 3 393-401.
- Deanovic, L.A., Stillway, M., Hammock, B.G., Fong, S., Werner, I., 2018. Tracking pyrethroid toxicity in surface water samples: Exposure dynamics and toxicity identification tools for laboratory tests with *Hyalella azteca* (Amphipoda). *Environ. Toxicol. Chem.* 37 (2), 462-472.
- Devine, G.J., Furlong, M.J., 2007. Insecticide use: Contexts and ecological consequences. *Agriculture and Human Values* 24 (3), 281-306.
- Domagalski, J.L., Weston, D.P., Zhang, M., Hladik, M., 2010. Pyrethroid insecticide concentrations and toxicity in streambed sediments and loads in surface waters of the San Joaquin Valley, California, USA. *Environ. Toxicol. Chem.* 29 (4), 813-823.
- Downing, J.A., Cole, J.J., Duarte, C., Middelburg, J.J., Melack, J.M., Prairie, Y.T., Kortelainen, P., Striegl, R.G., McDowell, W.H., Tranvik, L.J., 2012. Global abundance and size distribution of streams and rivers. *Inland waters* 2 (4), 229-236.
- EFSA, 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. *EFSA Journal* 11 (7), 3290.

- Elliott, S.M., VanderMeulen, D.D., 2017. A regional assessment of chemicals of concern in surface waters of four Midwestern United States national parks. *Sci. Total Environ.* 579 1726-1735.
- Englert, D., Bundschuh, M., Schulz, R., 2012. Thiacloprid affects trophic interaction between gammarids and mayflies. *Environ. Pollut.* 167 41-46.
- Englert, D., Zubrod, J.P., Pietz, S., Stefani, S., Krauss, M., Schulz, R., Bundschuh, M., 2017. Relative importance of dietary uptake and waterborne exposure for a leaf-shredding amphipod exposed to thiacloprid-contaminated leaves. *Scientific Reports* 7 (1), 16182.
- European Commission, 2020. Farm to fork strategy: for a fair, healthy and environmentally-friendly food system. DG SANTE/Unit 'Food information and composition, food waste'".
- European Commission, 2021. Regulation on the Sustainable Use of Plant Protection Products - EC 2021/2115 - COM/2022/305 final, <https://bit.ly/3RpGk7q>.
- European Council, 2000. Water Framework Directive - A Framework for Community Action in the Field of Water Policy. Official Journal of the European Communities, <https://bit.ly/3asirt1>.
- European Environment Agency, 2012. European Catchments and Rivers Network System (Ecrins, version 1.2), <https://bit.ly/3nCTleY>.
- European Environment Agency, 2020. Waterbase - Water Quality ICM (WISE6, version 6), <https://bit.ly/3r8lqvA>.
- Eurostat, 2020. Agri-environmental indicator - consumption of pesticides, <https://bit.ly/3sLkwZ1>.
- Eurostat, 2021. Land use statistics, EU 2018, <https://bit.ly/3XAvvmi>.
- Fernandez-Cornejo, J., Nehring, R.F., Osteen, C., Wechsler, S., Martin, A., Vialou, A., 2014. Pesticide use in US agriculture: 21 selected crops, 1960-2008. U.S. Government Printing Office, Washington, DC.
- Forrester, H., Clow, D., Roche, J., Heyvaert, A., Battaglin, W., 2017. Effects of backpacker use, pack stock trail use, and pack stock grazing on water-quality indicators, including nutrients, E. coli, hormones, and pharmaceuticals, in Yosemite National Park, USA. *Environ. Manage.* 60 (3), 526-543.
- Fowle, J.R., Dearfield, K.L., 2000. Risk characterization handbook. Science Policy Council, U.S. Environmental Protection Agency, Washington, DC.
- Geissen, V., Mol, H., Klumpp, E., Umlauf, G., Nadal, M., van der Ploeg, M., van de Zee, S.E., Ritsema, C.J., 2015. Emerging pollutants in the environment: a challenge for water resource management. *International soil and water conservation research* 3 (1), 57-65.
- Gerber, R., Smit, N.J., Van Vuren, J.H., Nakayama, S.M., Yohannes, Y.B., Ikenaka, Y., Ishizuka, M., Wepener, V., 2016. Bioaccumulation and human health risk assessment of DDT and other organochlorine pesticides in an apex aquatic predator from a premier conservation area. *Sci. Total Environ.* 550 522-533.
- Gilliom, R.J., Barbash, J.E., Crawford, C.G., Hamilton, P.A., Martin, J.D., Nakagaki, N., Nowell, L.H., Scott, J.C., Stackelberg, P.E., Thelin, G.P., 2006a. Pesticides in the nation's streams and ground water, 1992-2001. US Geological Survey.
- Gilliom, R.J., Barbash, J.E., Crawford, C.G., Hamilton, P.A., Martin, J.D., Nakagaki, N., Nowell, L.H., Scott, J.C., Stackelberg, P.E., Thelin, G.P., Wolock, D.M., 2006b. Pesticides in the Nation's Streams and Ground Water, 1992-2001, Circular, Revised Feb 2007 ed, Reston, VA, p. 180, <http://pubs.er.usgs.gov/publication/cir1291>.

- Godoy, A.A., Kummrow, F., Pamplin, P.A.Z., 2015. Occurrence, ecotoxicological effects and risk assessment of antihypertensive pharmaceutical residues in the aquatic environment-A review. *Chemosphere* 138 281-291.
- Gould, F., Brown, Z.S., Kuzma, J., 2018. Wicked evolution: Can we address the sociobiological dilemma of pesticide resistance? *Science* 360 (6390), 728-732.
- Graf, N., Battes, K.P., Cimpean, M., Entling, M.H., Frisch, K., Link, M., Scharmüller, A., Schreiner, V.C., Szöcs, E., Zubrod, J.P., Schäfer, R.B., 2020. Relationship between agricultural pesticides and the diet of riparian spiders in the field. *Environmental Sciences Europe* 32 (1), 1.
- Hageman, K.J., Simonich, S.L., Campbell, D.H., Wilson, G.R., Landers, D.H., 2006. Atmospheric deposition of current-use and historic-use pesticides in snow at national parks in the western United States. *Environ. Sci. Technol.* 40 (10), 3174-3180.
- Halbach, K., Möder, M., Schrader, S., Liebmann, L., Schäfer, R.B., Schneeweiss, A., Schreiner, V.C., Vormeier, P., Weisner, O., Liess, M., Reemtsma, T., 2021. Small streams—large concentrations? Pesticide monitoring in small agricultural streams in Germany during dry weather and rainfall. *Water Res.* 203 117535.
- Heap, I., Duke, S.O., 2018. Overview of glyphosate-resistant weeds worldwide. *Pest Manage. Sci.* 74 (5), 1040-1049.
- Hladik, M.L., Kolpin, D.W., 2016. First national-scale reconnaissance of neonicotinoid insecticides in streams across the USA. *Environ. Chem.* 13 (1), 12-20.
- Hladik, M.L., Kolpin, D.W., Kuivila, K.M., 2014a. Neonicotinoid insecticide occurrence in agricultural and urban streams. *Abstracts of Papers American Chemical Society* 248 492-AGRO.
- Hladik, M.L., Kolpin, D.W., Kuivila, K.M., 2014b. Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA. *Environ. Pollut.* 193 189-196.
- Howell, T.A., 2001. Enhancing water use efficiency in irrigated agriculture. *Agron. J.* 93 (2), 281-289.
- Jonsson, M., Ershammar, E., Fick, J., Brodin, T., Klaminder, J., 2015. Effects of an antihistamine on carbon and nutrient recycling in streams. *Sci. Total Environ.* 538 240-245.
- Kaiser, K., 2011. Preliminary study of pesticide drift into the Maya Mountain protected areas of Belize. *Bull. Environ. Contam. Toxicol.* 86 (1), 56-59.
- Katipoglu-Yazan, T., Merlin, C., Pons, M.-N., Ubay-Cokgor, E., Orhon, D., 2015. Chronic impact of tetracycline on nitrification kinetics and the activity of enriched nitrifying microbial culture. *Water Res.* 72 227-238.
- Kröger, R., Moore, M., Locke, M., Cullum, R., Steinriede Jr, R., Testa Iii, S., Bryant, C., Cooper, C., 2009. Evaluating the influence of wetland vegetation on chemical residence time in Mississippi Delta drainage ditches. *Agric. Water Manage.* 96 (7), 1175-1179.
- Kynetec, 2021. FarmTrak - Pesticide active ingredient tonnages applied in Saxon agriculture from 2004 - 2020, <https://www.kynetec.com/farmtrak-agriculture-tracking-studies>.
- la Cecilia, D., Dax, A., Ehmann, H., Koster, M., Singer, H., Stamm, C., 2021. Continuous high-frequency pesticide monitoring to observe the unexpected and the overlooked. *Water research X* 13 100125.
- Lahl, U., Hawxwell, K.A., 2006. REACH—the new European chemicals law. ACS Publications.
- Landesamt für Umwelt, Landwirtschaft und Geologie,, 2021. Geodatendownload des Fachbereichs Wasser, <https://bit.ly/3FHcNz9>.

- Landesamt für Umwelt, L.u.G., 2022. Geodatendownload des Fachbereichs Wasser - Gewässernetz Sachsen, <https://bit.ly/3cyOWt1>.
- Lemm, J.U., Venohr, M., Globevnik, L., Stefanidis, K., Panagopoulos, Y., van Gils, J., Posthuma, L., Kristensen, P., Feld, C.K., Mahnkopf, J., 2021. Multiple stressors determine river ecological status at the European scale: Towards an integrated understanding of river status deterioration. *Global Change Biol.* 27 (9), 1962-1975.
- LeNoir, J.S., McConnell, L.L., Fellers, G.M., Cahill, T.M., Seiber, J.N., 1999. Summertime transport of current-use pesticides from California's Central Valley to the Sierra Nevada Mountain Range, USA. *Environmental Toxicology and Chemistry: An International Journal* 18 (12), 2715-2722.
- Lewis, K.A., Tzilivakis, J., Warner, D.J., Green, A., 2016. An international database for pesticide risk assessments and management. *Human and Ecological Risk Assessment: An International Journal* 22 (4), 1050-1064.
- Liess, M., Foit, K., Becker, A., Hassold, E., Dolciotti, I., Kattwinkel, M., Duquesne, S., 2013. Culmination of Low-Dose Pesticide Effects. *Environ. Sci. Technol.* 47 (15), 8862-8868.
- Liess, M., Liebmann, L., Vormeier, P., Weisner, O., Altenburger, R., Borchardt, D., Brack, W., Chatzinotas, A., Escher, B., Foit, K., Gunold, R., Henz, S., Hitzfeld, K.L., Schmitt-Jansen, M., Kamjunke, N., Kaske, O., Knillmann, S., Krauss, M., Küster, E., Link, M., Lück, M., Möder, M., Müller, A., Paschke, A., Schäfer, R.B., Schneeweiss, A., Schreiner, V.C., Schulze, T., Schüürmann, G., von Tümpling, W., Weitere, M., Wogram, J., Reemtsma, T., 2021. Pesticides are the dominant stressors for vulnerable insects in lowland streams. *Water Res.* 201 117262.
- Liess, M., Schulz, R., 1996. Chronic effects of short-term contamination with the pyrethroid insecticide fenvalerate on the caddisfly *Limnephilus lunatus*. *Hydrobiologia* 324 (2), 99-106.
- Liess, M., von der Ohe, P.C., 2005. Analyzing effects of pesticides on invertebrate communities in streams. *Environ. Toxicol. Chem.* 24 (4), 954-965.
- Loewe, S., Muischnek, H., 1926. Über Kombinationswirkungen. *Naunyn-Schmiedebergs Archiv für experimentelle Pathologie und Pharmakologie* 114 (5), 313-326.
- Lozano, V.L., Allen Dohle, S., Vera, M.S., Torremorell, A., Pizarro, H.N., 2020. Primary production of freshwater microbial communities is affected by a cocktail of herbicides in an outdoor experiment. *Ecotoxicol. Environ. Saf.* 201 110821.
- Lyon, J.G., Trimble, S.W., Ward, A.D., Burckhard, S.R., 2015. *Environmental hydrology*. CRC Press, Boca Raton, p. 695.
- Mahler, B.J., Schmidt, T.S., Nowell, L.H., Qi, S.L., Van Metre, P.C., Hladik, M.L., Carlisle, D.M., Munn, M.D., May, J., 2020. Biofilms Provide New Insight into Pesticide Occurrence in Streams and Links to Aquatic Ecological Communities. *Environ. Sci. Technol.* 54 (9), 5509-5519.
- Malaj, E., Peter, C., Grote, M., Kühne, R., Mondy, C.P., Usseglio-Polatera, P., Brack, W., Schäfer, R.B., 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proc. Natl. Acad. Sci. USA* 111 (26), 9549-9554.
- McKay, L., Bondelid, T., Dewald, T., Johnston, J., Moore, R., Rea, A., 2012. *NHDPlus Version 2: user guide*. US Environmental Protection Agency.
- Mize, S.V., Porter, S.D., Demcheck, D.K., 2008. Influence of fipronil compounds and rice-cultivation land-use intensity on macroinvertebrate communities in streams of southwestern Louisiana, USA. *Environ. Pollut.* 152 (2), 491-503.
- Morrissey, C.A., Mineau, P., Devries, J.H., Sanchez-Bayo, F., Liess, M., Cavallaro, M.C., Liber, K., 2015. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: a review. *Environ. Int.* 74 291-303.

- Nakagaki, N.S., Wesley W. ; Wieczorek, Michael E. , 2012. Estimates of the Soil Restrictive Layer in the Upper 25,35,45, and 55 centimeters of agricultural land in the conterminous United States https://water.usgs.gov/GIS/metadata/usgswrd/XML/ssurgo_srlag.xml.
- National Research Council, 2012. Preparing for the Third Decade of the National Water-Quality Assessment Program. National Academies Press, Washington, DC.
- Oerke, E.-C., 2006. Crop losses to pests. *The Journal of Agricultural Science* 144 (1), 31-43.
- Osteen, C.D., Fernandez-Cornejo, J., 2013. Economic and policy issues of US agricultural pesticide use trends. *Pest Manage. Sci.* 69 (9), 1001-1025.
- Oster, J., Grattan, S., 2002. Drainage water reuse. *Irrigation and Drainage Systems* 16 (4), 297-310.
- Outhwaite, C.L., Gregory, R.D., Chandler, R.E., Collen, B., Isaac, N.J.B., 2020. Complex long-term biodiversity change among invertebrates, bryophytes and lichens. *Nature Ecology & Evolution* 4 (3), 384-392.
- Pastore, M., Calcagni, A., 2019. Measuring distribution similarities between samples: A distribution-free overlapping index. *Frontiers in psychology* 10 1089.
- Perry, E.D., Ciliberto, F., Hennessy, D.A., Moschini, G., 2016. Genetically engineered crops and pesticide use in US maize and soybeans. *Science advances* 2 (8), e1600850.
- Petschick, L.L., Bub, S., Wolfram, J., Stehle, S., Schulz, R., 2019. Modeling Regulatory Threshold Levels for Pesticides in Surface Waters from Effect Databases. *Data* 4 (4), 150.
- Pimentel, D., 2005. Environmental and economic costs of the application of pesticides primarily in the United States. *Environ. Dev. Sustainability* 7 (2), 229-252.
- Pimentel, D., Burgess, M., 2012. Small amounts of pesticides reaching target insects. Springer, pp. 1-2.
- Pimentel, D., Zuniga, R., Morrison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econ.* 52 (3), 273-288.
- Popp, J., Pető, K., Nagy, J., 2013. Pesticide productivity and food security. A review. *Agron. Sustainable Dev.* 33 (1), 243-255.
- Quinete, N., Castro, J., Fernandez, A., Zamora-Ley, I.M., Rand, G.M., Gardinali, P.R., 2013. Occurrence and Distribution of Endosulfan in Water, Sediment, and Fish Tissue: An Ecological Assessment of Protected Lands in South Florida. *J. Agric. Food Chem.* 61 (49), 11881-11892.
- Ramankutty, N., Mehrabi, Z., Waha, K., Jarvis, L., Kremen, C., Herrero, M., Rieseberg, L.H., 2018. Trends in global agricultural land use: implications for environmental health and food security. *Annu. Rev. Plant Biol.* 69 (1), 789-815.
- Relyea, R.A., 2005. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecol. Appl.* 15 (2), 618-627.
- Rhoades, J., 1997. Sustainability of irrigation: An overview of salinity problems and control strategies. US Salinity Laboratory, Riverside, California.
- Richmond, E.K., Rosi, E.J., Walters, D.M., Fick, J., Hamilton, S.K., Brodin, T., Sundelin, A., Grace, M.R., 2018. A diverse suite of pharmaceuticals contaminates stream and riparian food webs. *Nature communications* 9 (1), 1-9.

- Roche, H., Vollaire, Y., Martin, E., Rouer, C., Coulet, E., Grillas, P., Banas, D., 2009. Rice fields regulate organochlorine pesticides and PCBs in lagoons of the Nature Reserve of Camargue. *Chemosphere* 75 (4), 526-533.
- Roessink, I., Merga, L.B., Zweers, H.J., Van den Brink, P.J., 2013. The neonicotinoid imidacloprid shows high chronic toxicity to mayfly nymphs. *Environ. Toxicol. Chem.* 32 (5), 1096-1100.
- Rogers, H.A., Schmidt, T.S., Dabney, B.L., Hladik, M.L., Mahler, B.J., Van Metre, P.C., 2016. Bifenthrin causes trophic cascade and altered insect emergence in mesocosms: implications for small streams. *Environ. Sci. Technol.* 50 (21), 11974-11983.
- Roodt, A.P., Röder, N., Pietz, S., Kolbensschlag, S., Manfrin, A., Schwenk, K., Bundschuh, M., Schulz, R., 2022. Emerging Midges Transport Pesticides from Aquatic to Terrestrial Ecosystems: Importance of Compound-and Organism-Specific Parameters. *Environ. Sci. Technol.* 56 (9), 5478-5488.
- Roose-Amsaleg, C., Laverman, A.M., 2016. Do antibiotics have environmental side-effects? Impact of synthetic antibiotics on biogeochemical processes. *Environmental Science and Pollution Research* 23 (5), 4000-4012.
- Rumschlag, S.L., Mahon, M.B., Hoverman, J.T., Raffel, T.R., Carrick, H.J., Hudson, P.J., Rohr, J.R., 2020. Consistent effects of pesticides on community structure and ecosystem function in freshwater systems. *Nature Communications* 11 (1), 6333.
- Schäfer, R.B., van den Brink, P.J., Liess, M., 2011. Impacts of pesticides on freshwater ecosystems. *Ecological impacts of toxic chemicals 2011* 111-137.
- Schäfer, R.B., von der Ohe, P.C., Rasmussen, J., Kefford, B.J., Beketov, M.A., Schulz, R., Liess, M., 2012. Thresholds for the effects of pesticides on invertebrate communities and leaf breakdown in stream ecosystems. *Environ. Sci. Technol.* 46 (9), 5134-5142.
- Scholz, N.L., Truelove, N.K., French, B.L., Berejikian, B.A., Quinn, T.P., Casillas, E., Collier, T.K., 2000. Diazinon disrupts antipredator and homing behaviors in chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.* 57 (9), 1911-1918.
- Schreiner, V.C., Link, M., Kunz, S., Szöcs, E., Scharmüller, A., Vogler, B., Beck, B., Battes, K.P., Cimpean, M., Singer, H.P., Hollender, J., Schäfer, R.B., 2021. Paradise lost? Pesticide pollution in a European region with considerable amount of traditional agriculture. *Water Res.* 188 116528.
- Schulz, R., 2004. Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution. *J. Environ. Qual.* 33 (2), 419-448.
- Schulz, R., Bub, S., Petschick, L.L., Stehle, S., Wolfram, J., 2021. Applied pesticide toxicity shifts toward plants and invertebrates, even in GM crops. *Science* 372 (6537), 81-84.
- Schulz, R., Liess, M., 2000. Toxicity of fenvalerate to caddisfly larvae: chronic effects of 1- vs 10-h pulse-exposure with constant doses. *Chemosphere* 41 (10), 1511-1517.
- Scott, G., Fulton, M., Wirth, E., Chandler, G., Key, P., Daugomah, J., Bearden, D., Chung, K., Strozier, E., DeLorenzo, M., 2002. Toxicological studies in tropical ecosystems: an ecotoxicological risk assessment of pesticide runoff in south Florida estuarine ecosystems. *J. Agric. Food Chem.* 50 (15), 4400-4408.
- Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G.P.S., Handa, N., Kohli, S.K., Yadav, P., Bali, A.S., Parihar, R.D., 2019. Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences* 1 (11), 1-16.
- Simon, J.Y., 2014. *The toxicology and biochemistry of insecticides*. CRC press.

- Skark, C., Zullei-Seibert, N., Willme, U., Gatzemann, U., Schlett, C., 2004. Contribution of non-agricultural pesticides to pesticide load in surface water. *Pest Management Science: formerly Pesticide Science* 60 (6), 525-530.
- 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 (8), 4526-4535.
- Steffen, W., Richardson, K., Rockström, J., Cornell, S.E., Fetzer, I., Bennett, E.M., Biggs, R., Carpenter, S.R., De Vries, W., De Wit, C.A., 2015. Planetary boundaries: Guiding human development on a changing planet. *Science* 347 (6223), 1259855.
- Stehle, S., Blin, A., Bub, S., Petschick, L.L., Wolfram, J., Schulz, R., 2019. Aquatic pesticide exposure in the US as a result of non-agricultural uses. *Environ. Int.* 133 105234.
- Stehle, S., Knäbel, A., Schulz, R., 2013. Probabilistic risk assessment of insecticide concentrations in agricultural surface waters: a critical appraisal. *Environ. Monit. Assess.* 185 (8), 6295-6310.
- Stehle, S., Schulz, R., 2015a. Agricultural insecticides threaten surface waters at the global scale. *Proc. Natl. Acad. Sci. USA* 112 (18), 5750-5755.
- Stehle, S., Schulz, R., 2015b. Pesticide authorization in the EU—environment unprotected? *Environmental Science and Pollution Research* 22 (24), 19632-19647.
- Stone, W.W., Crawford, C.G., Gilliom, R.J., 2013. Watershed Regressions for Pesticides (WARP) Models for Predicting Stream Concentrations of Multiple Pesticides. *J. Environ. Qual.* 42 (6), 1838-1851.
- Stone, W.W., Gilliom, R.J., Martin, J.D., 2014a. An overview comparing results from two decades of monitoring for pesticides in the Nation's streams and rivers, 1992-2001 and 2002-2011, Scientific Investigations Report, Reston, VA, p. 32, <http://pubs.er.usgs.gov/publication/sir20145154>.
- Stone, W.W., Gilliom, R.J., Ryberg, K.R., 2014b. Pesticides in U.S. Streams and Rivers: Occurrence and Trends during 1992-2011. *Environ. Sci. Technol.* 48 (19), 11025-11030.
- Tang, X., Zhu, B., Katou, H., 2012. A review of rapid transport of pesticides from sloping farmland to surface waters: Processes and mitigation strategies. *Journal of Environmental Sciences* 24 (3), 351-361.
- Tennekes, H.A., Sanchez-Bayo, F., 2011. Time-dependent toxicity of neonicotinoids and other toxicants: implications for a new approach to risk assessment. *Journal of Environmental & Analytical Toxicology* (4), 1-8.
- TSCA, 1976. Toxic Substances Control Act, 15 U.S.C. §2601 et seq.
- U.S. Department of Agriculture, 2018. National Agricultural Statistics Service, 2018 Crop Frequency Layers, <https://tinyurl.com/y47armyz>.
- U.S. EPA, 1998. Guidelines for Ecological Risk Assessment. *Fed. Regist.* 63 (93), 26846 - 26924.
- U.S. EPA, 2004. Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs, U.S. Environmental Protection Agency - Endangered and Threatened Species Effects Determinations. U.S. Government Printing Office, Washington, DC.
- U.S. EPA, 2011. Notice of Receipt of Request to Voluntarily Cancel Certain Pesticide Registrations. *Fed. Regist.* 76 (120), 36535-36537.

- U.S. EPA, 2012. Notice of Receipt of Request to Voluntarily Cancel Certain Pesticide Registrations. Fed. Regist. 77 (120), 12299-12301.
- U.S. EPA, 2016. Preliminary Comparative Environmental Fate and Ecological Risk Assessment for the Registration Review of Eight Synthetic Pyrethroids and Pyrethrins. U.S. EPA, Washington, DC.
- U.S. EPA, 2018a. Ecological Risk Assessment for Pesticides: Technical Overview, <https://bit.ly/3q6y9OF>.
- U.S. EPA, 2018b. Pesticide Chemical Search, <https://bit.ly/3bQGDMU>.
- U.S. Fish & Wildlife Service, 2015. Listed species believed or known to occur in Mississippi, <https://tinyurl.com/y25vlqco>.
- Van den Brink, P.J., Van Smeden, J.M., Bekele, R.S., Dierick, W., De Gelder, D.M., Noteboom, M., Roessink, I., 2016. Acute and chronic toxicity of neonicotinoids to nymphs of a mayfly species and some notes on seasonal differences. Environ. Toxicol. Chem. 35 (1), 128-133.
- van Gils, J., Posthuma, L., Cousins, I.T., Brack, W., Altenburger, R., Baveco, H., Focks, A., Greskowiak, J., Kühne, R., Kutsarova, S., 2020. Computational material flow analysis for thousands of chemicals of emerging concern in European waters. J. Hazard. Mater. 397 122655.
- van Klink, R., Bowler, D.E., Gongalsky, K.B., Swengel, A.B., Gentile, A., Chase, J.M., 2020. Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances. Science 368 (6489), 417-420.
- Vander Vorste, R., McElmurray, P., Bell, S., Eliason, K., Brown, B., 2017. Does stream size really explain biodiversity patterns in lotic systems? A call for mechanistic explanations. Diversity 9 (3), 26.
- Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., Cushing, C.E., 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37 (1), 130-137.
- Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R., 2010. Global threats to human water security and river biodiversity. Nature 467 (7315), 555-561.
- Walker, C.H., Sibly, R., Hopkin, S., Peakall, D.B., 2005. Principles of ecotoxicology. CRC press.
- Wallace, J.B., Eggert, S.L., Meyer, J.L., Webster, J.R., 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277 (5322), 102-104.
- Weston, D.P., Chen, D., Lydy, M.J., 2015. Stormwater-related transport of the insecticides bifenthrin, fipronil, imidacloprid, and chlorpyrifos into a tidal wetland, San Francisco Bay, California. Sci. Total Environ. 527 18-25.
- Weston, D.P., Lydy, M.J., 2014. Toxicity of the Insecticide Fipronil and Its Degradates to Benthic Macroinvertebrates of Urban Streams. Environ. Sci. Technol. 48 (2), 1290-1297.
- Wickramasinghe, L.P., Harris, S., Jones, G., Vaughan Jennings, N., 2004. Abundance and Species Richness of Nocturnal Insects on Organic and Conventional Farms: Effects of Agricultural Intensification on Bat Foraging. Conserv. Biol. 18 (5), 1283-1292.
- Wolfram, J., Stehle, S., Bub, S., Petschick, L.L., Schulz, R., 2019. Insecticide Risk in US Surface Waters: Drivers and Spatiotemporal Modeling. Environ. Sci. Technol. 53 (20), 12071-12080.
- Zaller, J.G., Kruse-Plaus, M., Schlechtriemen, U., Gruber, E., Peer, M., Nadeem, I., Formayer, H., Hutter, H.-P., Landler, L., 2022. Pesticides in ambient air, influenced by surrounding land use and weather, pose a potential threat to biodiversity and humans. Sci. Total Environ. 156012.

Zubrod, J.P., Bundschuh, M., Arts, G., Brühl, C.A., Imfeld, G., Knäbel, A., Payraudeau, S., Rasmussen, J.J., Rohr, J., Scharmüller, A., Smalling, K., Stehle, S., Schulz, R., Schäfer, R.B., 2019. Fungicides: An Overlooked Pesticide Class? *Environ. Sci. Technol.* 53 (7), 3347-3365.

7 Declaration

I, the undersigned, author of this work, declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education.

Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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Date

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Signature

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9 Curriculum vitae

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Scientific Education and Career

2020 – now Study advisor for the international Masters of Environmental Pollution Management (Ecotoxicology) at the University of Koblenz-Landau, Campus Landau (teaching obligations 8 SWS)

2017 – 2023 Ph.D. study, Institute for Environmental Sciences, University of Koblenz-Landau, Campus Landau (Working group: “Ecotoxicology and Environment”). Ph.D. study conducted partially in the DFG Research Project: “Makro-Ökotoxikologie: Räumliche und zeitliche Aspekte von Insektizid Belastungen in Gewässern”. Ph.D. study conducted partially in the DBU Research Projekt “Pestizide in Schutzgebieten: Bewertung, Analyse und Maßnahmen”.

2016 Diploma thesis entitled “Combined and chronic effects of a fungicide mixture on the population integrity of the key shredder *Gammarus fossarum*”

2008 – 2016 Study of Environmental Sciences (Diploma, focus Aquatic Ecotoxicology), University of Koblenz-Landau, Campus Landau

10 List of publications

Bub, S.; **Wolfram, J.**; Petschick, L. L.; Stehle, S.; Schulz, R., Trends of Total Applied Pesticide Toxicity in German Agriculture. *Environ. Sci. Technol.* **2023**, *57*, (1), 852-861.

Bub, S.; **Wolfram, J.**; Stehle, S.; Petschick, L. L.; Schulz, R., Graphing Ecotoxicology: The MAGIC Graph for Linking Environmental Data on Chemicals. *Data* **2019**, *4*, (1), 34.

Petschick, L. L.; Bub, S.; **Wolfram, J.**; Stehle, S.; Schulz, R., Modeling Regulatory Threshold Levels for Pesticides in Surface Waters from Effect Databases. *Data* **2019**, *4*, (4), 150.

Schulz, R.; Bub, S.; Petschick, L. L.; Stehle, S.; **Wolfram, J.**, Applied pesticide toxicity shifts toward plants and invertebrates, even in GM crops. *Science* **2021**, *372*, (6537), 81-84.

Stehle, S.; Bline, A.; Bub, S.; Petschick, L. L.; **Wolfram, J.**; Schulz, R., Aquatic pesticide exposure in the US as a result of non-agricultural uses. *Environ. Int.* **2019**, *133*, 105234.

Stehle, S.; Ovcharova, V.; **Wolfram, J.**; Bub, S.; Herrmann, L. Z.; Petschick, L. L.; Schulz, R., Neonicotinoid insecticides in global agricultural surface waters – Exposure, risks and regulatory challenges. *Sci. Total Environ.* **2023**, *867*, 161383.

Wolfram, J.; Bub, S.; Petschick, L. L.; Schemmer, A.; Stehle, S.; Schulz, R., Pesticide occurrence in protected surface waters in nature conservation areas of Germany. *Sci. Total Environ.* **2023**, *858*, 160074.

Wolfram, J.; Stehle, S.; Bub, S.; Petschick, L. L.; Schulz, R., Meta-Analysis of Insecticides in United States Surface Waters: Status and Future Implications. *Environ. Sci. Technol.* **2018**, *52*, (24), 14452-14460.

Wolfram, J.; Stehle, S.; Bub, S.; Petschick, L. L.; Schulz, R., Insecticide Risk in US Surface Waters: Drivers and Spatiotemporal Modeling. *Environ. Sci. Technol.* **2019**, *53*, (20), 12071-12080.

Wolfram, J.; Stehle, S.; Bub, S.; Petschick, L. L.; Schulz, R., Water quality and ecological risks in European surface waters—Monitoring improves while water quality decreases. *Environ. Int.* **2021**, *152*, 106479.

Zubrod, J.; Englert, D.; Rosenfeldt, R.; **Wolfram, J.**; Lüderwald, S.; Wallace, D.; Schnetzer, N.; Schulz, R.; Bundschuh, M., The relative importance of diet-related and waterborne effects of copper for a leaf-shredding invertebrate. *Environ. Pollut.* **2015**, *205*, 16-22.

Zubrod, J. P.; Englert, D.; **Wolfram, J.**; Rosenfeldt, R. R.; Feckler, A.; Bundschuh, R.; Seitz, F.; Konschak, M.; Baudy, P.; Lüderwald, S., Long-term effects of fungicides on leaf-associated

microorganisms and shredder populations—an artificial stream study. *Environ. Toxicol. Chem.* **2017**, 36, (8), 2178-2189.

Zubrod, J. P.; Englert, D.; **Wolfram, J.**; Wallace, D.; Schnetzer, N.; Baudy, P.; Kanschak, M.; Schulz, R.; Bundschuh, M., Waterborne toxicity and diet-related effects of fungicides in the key leaf shredder *Gammarus fossarum* (Crustacea: Amphipoda). *Aquat. Toxicol.* **2015**, 169, 105-112.

11 Appendices

Appendix I

Wolfram, J., Stehle, S., Bub, S., Petschick, L. L., & Schulz, R. (2018). *Meta-analysis of Insecticides in United States Surface Waters: Status and Future Implications*. *Environmental Science & Technology*, 52(24), 14452-14460.

Appendix II

Wolfram, J., Stehle, S., Bub, S., Petschick, L. L., & Schulz, R. (2019). *Insecticide risk in US Surface Waters: Drivers and Spatiotemporal Modeling*. *Environmental Science & Technology*, 53(20), 12071-12080.

Appendix III

Wolfram, J., Stehle, S., Bub, S., Petschick, L. L., & Schulz, R. (2021). *Water Quality and Ecological Risks in European Surface Waters – Monitoring Improves While Water Quality Decreases*. *Environment International*, 152, 106479.

Appendix IV

Wolfram, J., Bub, S., Petschick, L. L., Schemmer, A., Stehle, S. & Schulz, R. (2023). *Pesticide occurrence in protected surface waters in nature conservation areas of Germany*. *Science of The Total Environment*, 858, 160074.

Meta-Analysis of Insecticides in United States Surface Waters: Status and Future Implications

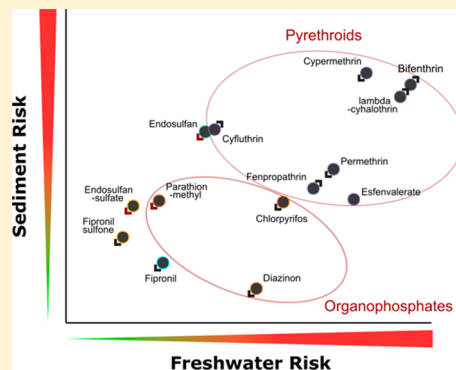
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Supporting Information

ABSTRACT: Agricultural insecticides occur in U.S. surface waters, yet our knowledge of their current and potential future large-scale risks for biodiversity is restricted. Here, we conducted a meta-analysis of measured insecticide concentrations (MICs; $n = 5817$; 1962–2017) in U.S. surface waters and sediments reported in 259 peer-reviewed scientific studies for 32 important insecticide compounds and their degradation products ($n = 6$). To assess overall and substance-specific ecological risks and future implications, MICs were compared with official U.S. Environmental Protection Agency regulatory threshold levels (RTLs) and insecticide use trends. Approximately half of the MICs, i.e., 49.4% (at 69.7% of the 644 sites covered), exceeded their RTLs, indicating substantial risks to the integrity of U.S. aquatic ecosystems and potential shortcomings of regulatory risk assessment procedures. Overall, pyrethroids had the highest exceedance rate (80.7%; $n = 1808$), followed by organophosphates and carbamates (42.2%, $n = 2618$), and organochlorines (33.3%, $n = 468$). Pronounced increasing use trends were found for neonicotinoids, which exceeded their chronic RTLs, i.e., those of high relevance due to neonicotinoids' persistence in surface waters, for 56.8% of MICs (22.2% for acute RTLs). A regression analysis of insecticide use trends, although to be interpreted with care, indicated a future increase in applied amounts of several high risk insecticides such as pyrethroids and neonicotinoids, suggesting elevated prospective risks for U.S. surface waters, biodiversity, and endangered species.



INTRODUCTION

In the United States, 17.2% of the total land area is used for agricultural crops, constituting one of the largest terrestrial biomes and supporting a \$400 billion dollar industry.^{1,2} Despite pesticide management practices, pest organisms cause yield losses of up to 25% in the U.S. each year.^{3,4} In part, U.S. ecosystems have become increasingly under pressure from foreign pest organisms⁵ and alien invasive species,⁶ causing estimated losses of up to \$58 billion/year.⁷ Consequently, pesticide use remains crucial for the protection of crops against pathogens and pests and for remaining competitive in an increasingly globalized market.^{8,9}

Based on annual expenditures,¹⁰ insecticides are an important component of the U.S. pesticide market, which is experiencing fundamental shifts as organochlorines, organophosphates, and carbamates are used less or phased out,^{11,12} whereas newer generations of insecticides, such as pyrethroids and neonicotinoids, have gained substantial market shares within the past few decades.¹³ Shifts in insecticide use trends are a result of more cost-efficient products entering the market, regulatory changes taking effect, shifting crop profitability, invasive pest species, a warming global climate that is more favorable to insects, and evolving pest resistances, among other factors.^{10,14–16} Therefore, use trends combine several regulatory and macroagronomical factors that provide ample

opportunities to estimate the future importance of individual insecticides. In this context, neonicotinoids represent the most recent, major insecticide class to be available in the United States, and they have been adopted rapidly with annual applications in agriculture reaching approximately 4000 tons.¹³ However, neonicotinoids' environmental effects have been heavily debated in the European Union (EU), resulting in a recent ban of non-greenhouse uses of three previously registered neonicotinoids.¹⁷ Comparative regulatory actions are likely to take effect in Canada, yet neonicotinoid use is expected to remain a central pest control measure globally.^{18,19}

In the U.S., all insecticides produced, distributed, or sold have to be registered by the U.S. Environmental Protection Agency (EPA) as required under the Federal Insecticide, Fungicide and Rodenticide Act of 1947 (FIFRA²⁰). As part of the registration process, an ecological risk assessment is conducted that should ensure that pesticide use has no unreasonable adverse effects on the environment.^{21,22} In this process, regulatory threshold levels (RTLs, see Stehle and Schulz²³), which are based on the most sensitive end point of a

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variety of tested species that is multiplied with the so-called “levels of concern”,²² are derived. RTLs represent threshold concentrations above which unreasonable effects are expected to occur, leading to structural or functional alterations in valued nontarget ecosystems such as surface waters.^{21,22,24} Thus, despite rarely being utilized in this context, RTLs denote elaborated thresholds highly applicable to assess insecticides’ environmental risks in the field.

Despite advanced prospective assessment protocols and as a result of the frequent and widespread use across the United States, agricultural surface waters are exposed to insecticides via various pathways (e.g., runoff, spray drift, and leaching).^{25–27} Indeed, extensive monitoring efforts (1992–2001) by the U.S. Geological Survey, as a part of an effort to assess U.S. aquatic systems, identified insecticides as a main contributor causing aquatic life impairments at 57% of a total of 83 agricultural stream sites.²⁵ In the subsequent observation period (2002–2011) at a total of 36 agricultural sites, the impacts of insecticides appear to have greatly lessened, although methodological changes of the sampling design limit comparability between both studies.²⁶

Insecticides represent the most toxic yet elusive substance class among all pesticides, occurring only for short periods of time in surface waters in quantifiable concentrations²⁸ and requiring highly targeted monitoring efforts to capture transient contamination peaks.^{23,27} Despite their transient occurrence, insecticides are ecologically relevant at very low concentrations,²⁹ causing adverse effects at the organism, population, and ecosystem level, reducing aquatic biodiversity, and adversely affecting associated ecological functions.^{30–33}

Therefore, this study aims to conduct a comprehensive evaluation of field studies reporting measured insecticide concentrations (MICs) for U.S. surface waters, covering the most recent data, important current-use substance classes such as pyrethroids and neonicotinoids, and a substantially larger number of stream sites (cf. Gilliom et al.,²⁵ Stone et al.²⁶). Through a comparison of MICs with RTLs, additional lines of evidence regarding potential risks from transient nonpoint source pollution in agricultural streams can be provided to existing work.^{25,26} Stehle and Schulz²³ compiled MICs at the global level and assessed them for the first time through a comparison with RTLs. However, their study covered a time span only until 2012, and due to its global scope it did not provide detailed insights specific to the U.S. In the present study, existing data relevant to the U.S. from Stehle and Schulz²³ have been included ($n = 3854$ MICs for 22 insecticides) and extended to the year 2017 as well as to a larger amount of compounds ($n = 5817$ MICs for 32 insecticides and 6 degradates, covering 644 sampling sites). The present study reviews MICs for individual compounds, assesses substance-specific RTL exceedance rates, and provides for the first time a large-scale comprehensive analysis of peer-reviewed insecticide occurrence in the U.S. Additionally, an online tool termed “Insecticide classification calculator U.S.” (ICC-US) was implemented to compare and interactively engage with the results presented here (<https://static.magic.eco/icc-us/v1>). Overall, this study aims to provide further insights regarding insecticides’ impacts on U.S. surface waters, which is crucial to informing policy decisions that can include the development of robust guidance and protect water resources at peril.³⁴ To move regulations and environmental management forward,³⁵ our present ex post analysis of insecticide contamination is combined with prospective

estimations of substances’ future implications, based on temporal use trends.

■ MATERIALS AND METHODS

Literature Review. For the present study, data from peer-reviewed studies for U.S. agricultural surface waters ($n = 215$ studies containing 3854 MICs for 22 insecticides) were taken from a global analysis covering the time period 1962 to 2012.²³ These data were extended through a search in scientific databases (see Stehle and Schulz²³ and [Supporting Information](#) (SI) for search methods and data selection criteria) considering 40 insecticides (of which 32 were found) and their degradation products (40 of which six were found), resulting in data up to the year 2017. Briefly, the Web of Science Citation Index Expanded (1945 to October 2017), BIOSIS Previews (1926 to October 2017), Zoological Record (1984–2007), CABI Direct Full Access (1910 to October 2017), and subsidiary databases therein were used to search relevant publications (see SI Methods). Furthermore, we applied footnote chasing to identify additional studies that could be relevant for the present meta-analysis.³⁶

In total, we identified 259 studies containing 5817 MICs (including degradate concentrations) present in U.S. surface waters (freshwater, sediment or estuarine water) resulting from agricultural nonpoint source entries. Estuarine sediment concentrations were deemed too scarce ($n = 132$) to allow for substantiated conclusions; therefore, freshwater and estuarine sediment MICs (MIC_{sed}) were assessed in total. Except for Tennessee, MICs were found for all U.S. states ($n = 37$) in which the selected insecticides are applied in considerable quantities (>1 kg/acre from 1992–2012) or considerable areas are used for permanent crops ($>600,000$ acres). In total, MICs were attributable to at least 644 different surface water sampling sites, which in many cases are representative of individual water bodies (53.4%, $n = 344$) rather than specific locations (36.6%, $n = 236$). In a small subset of cases (9.9%, $n = 64$), locations were deemed “unspecific” (e.g., “agricultural canals, Florida Bay, Florida, USA”); however, their impact on the overall analyses appears to be minimal (see SI, [Table S1](#)). Further information regarding use of hydrologic covariates can be found in the Supporting Information ([Table S2](#)).

Insecticides. In total, 40 insecticides (i.e., active ingredients) were included in this meta-analysis covering two organochlorines, six organophosphates and three carbamates, 21 pyrethroids, seven neonicotinoids, and one phenylpyrazole insecticide (fipronil) (see [Table S3](#)). In addition, insecticide degradates and metabolites (both referred to as degradates, see SI Methods) of each insecticide were identified by using the U.S. EPA OPP Pesticide Chemical Search,³⁷ the Pesticide Properties Database,³⁸ and publicly available U.S. EPA registration documents. Then, degradates had to fulfill U.S. EPA criteria on toxicity and relative abundance to be considered in the literature search (see the SI).²²

Agricultural Use Estimates. Annual county-level agricultural insecticide use estimates were obtained for the period from 1992 to 2014.¹³ Briefly, use data consisted of two separate estimation techniques, resulting in relatively higher (“EPest-high”) and lower estimations (“EPest-low”) at the county level with the former being used for the present analysis to minimize data gaps (for details, see Baker¹³). Preliminary use estimates for seed treatment applications were discontinued beginning in 2015; thus, neonicotinoid data are not

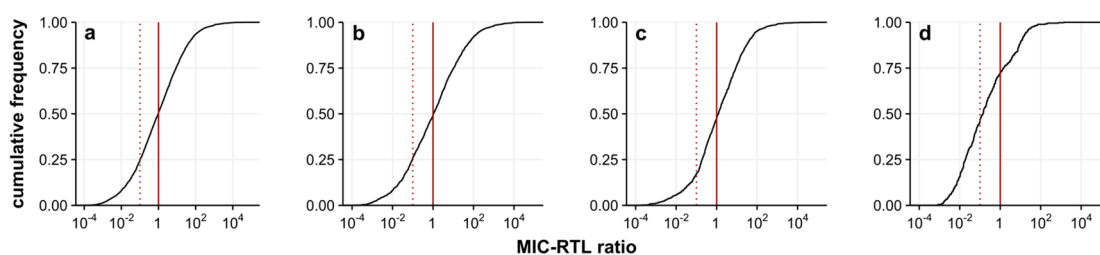


Figure 1. Cumulative frequency distributions of \log_{10} -transformed measured insecticide concentrations (MIC) in relation to their regulatory threshold levels (RTL, red solid line) for (a) all MICs ($n = 5817$), (b) freshwater phase MICs ($n = 3586$), (c) sediment MICs (freshwater and estuarine, $n = 1779$), and (d) estuarine water phase MICs ($n = 452$). Dotted lines represent $0.1 \times \text{RTL}$, at which a significant reduction in aquatic biodiversity was demonstrated.²³ MIC to RTL ratios larger than one represent threshold exceedances.

likely not comparable to previous estimates. Due to these methodological differences, preliminary insecticide use estimates for the year 2015 were not included in this analysis because neonicotinoid seed treatment is likely an important entry route into surface waters.³⁹ Additionally, due to a considerable time lag among the sampling, analysis and peer-reviewed publication, MIC data after 2014 are likely incomplete. Temporal use trends from 1992 to 2014 were used to contextualize substance exceedance frequencies and median exceedance severity to help identify substances with potentially increasing risks to U.S. surface waters.

Regulatory Threshold Levels. RTLs (Table S3; see also Stehle and Schulz²³) were used to provide a regulatory reference level for the ecological implications that may arise from an observed insecticide or degradate concentration. RTLs are derived during the registration process for pesticides as required under the FIFRA and are taken from official U.S. EPA registration documents.³⁷ A more detailed description can be found in Stehle and Schulz.²³ Briefly, RTLs are determined for each of the three environmental phases analyzed in this study, i.e., freshwater (RTL_{fw}), sediment (RTL_{sed}), or estuarine water (RTL_{est}), and represent threshold levels above which adverse effects in aquatic organisms are expected.^{21,22} However, Stehle and Schulz²³ have shown that significant negative effects on aquatic biodiversity, i.e., a 12% loss in family richness of freshwater invertebrates, occur at insecticide concentrations equaling $0.1 \times \text{RTL}$, a value that has also been used for evaluation in the present study. In several cases, RTL_{sed} was not available from regulatory documents (see SI Methods). In these cases, threshold effect benchmarks (TEBs) taken from Nowell et al.⁴⁰ or adjusted maximum permissible concentrations (MPCs) from Crommentuijn et al.⁴¹ were used to derive the respective RTL_{sed} (see SI Methods).

Analysis. Prior to all statistical analyses, MIC to RTL ratios were transformed using the decadal logarithm and an alpha level of 0.05 was used for all statistical tests. In addition to descriptive analyses (e.g., exceedance frequency), mean (parametric) or median (nonparametric) 95% confidence intervals (95% CI) of MIC-RTL distributions of individual substances were compared with their RTL.⁴² Two sample tests, describing significant differences between catchment size distributions (i.e., median size) or MIC-RTL distributions (i.e., insecticide risk), were performed using either Student's *t* test (parametric) or Mann-Whitney's U-test (nonparametric).

Annual insecticide use estimates¹³ were used in multiple linear regressions and modeled at three different spatial aggregation levels (i.e., national use, state-level use, state-level use intensity) to test for significant relationships between insecticide use (independent variable) and MIC to RTL ratios

(dependent variable) in the field (see SI Methods). Additionally, generalized linear models were used to calculate all insecticide temporal use trends at the national level, which were then used to derive potential future implications of the respective substance (see SI Methods).

All data were checked for normal distribution visually (QQ-plots), while homoscedasticity was assessed with Levene's test in combination with residual plots. Breusch-Pagan's test was used to check for the homoscedasticity assumption of linear regressions, and in the case of potential violations, White's heteroscedasticity-corrected covariance matrices were used to test for the significance of the parameter estimates.⁴² Pearson-adjusted R^2 was used to describe the correlation of the linear regressions. Several analyses can also be accessed online via the ICC-US (<https://static.magic.eco/icc-us/v1>), allowing comparisons and interactive engagement with our results. All calculations and figures were produced using R (R base: Ver. 3.3.2, 64-bit, Windows 10).⁴³

RESULTS AND DISCUSSION

MICs in Surface Waters and Sediments. In our analysis, we observed high risks for aquatic systems as 49.2% of all MICs ($n = 5817$, Figure 1a) exceeded their RTL, with 69.7% of all sites ($n = 644$) experiencing at least one exceedance. Multiple RTL exceedances occurred at 63.5% of the sites where at least two MICs were quantified ($n = 444$), and 47.5% of these sites experienced multiple exceedances in the same year. In the water phase, risks were only slightly lower compared to the overall data, amounting to 47.9% of the MICs in surface waters (MIC_{sw}) exceeding their RTL_{sw} ($n = 4038$, Figure 1b), whereas sediments were characterized by a slightly higher threshold exceedance frequency (52.2%, $n = 1779$, Figure 1c). Approximately 26% of MIC_{sw} and 19% of MICs in sediments (MIC_{sed}) exceeded their RTL by at least 1 order of magnitude, with maximum exceedances of more than five (water phase) and four (sediment) orders of magnitude (Figure 1b, c). RTL exceedances represent contamination events where surrogate test species are likely to experience significant adverse effects, such as, but not limited to, mortality, immobility, reduced physiological fitness or behavioral alterations.²² It is likely that the resulting effects apply to a multitude of species in the wild, among them even more sensitive or endangered ones^{44–46} and, as a consequence, adversely affecting aquatic biota via structural and functional alterations.^{23,32,47} More so, concentrations above one tenth of the RTL, which already lead to biodiversity effects,²³ were exceeded by 71.3% of the MIC_{sw} (Figure 1a, c). RTL exceedance rates observed in the U.S. compare well with data presented at the global level²³ (52.4%, $n = 11300$),

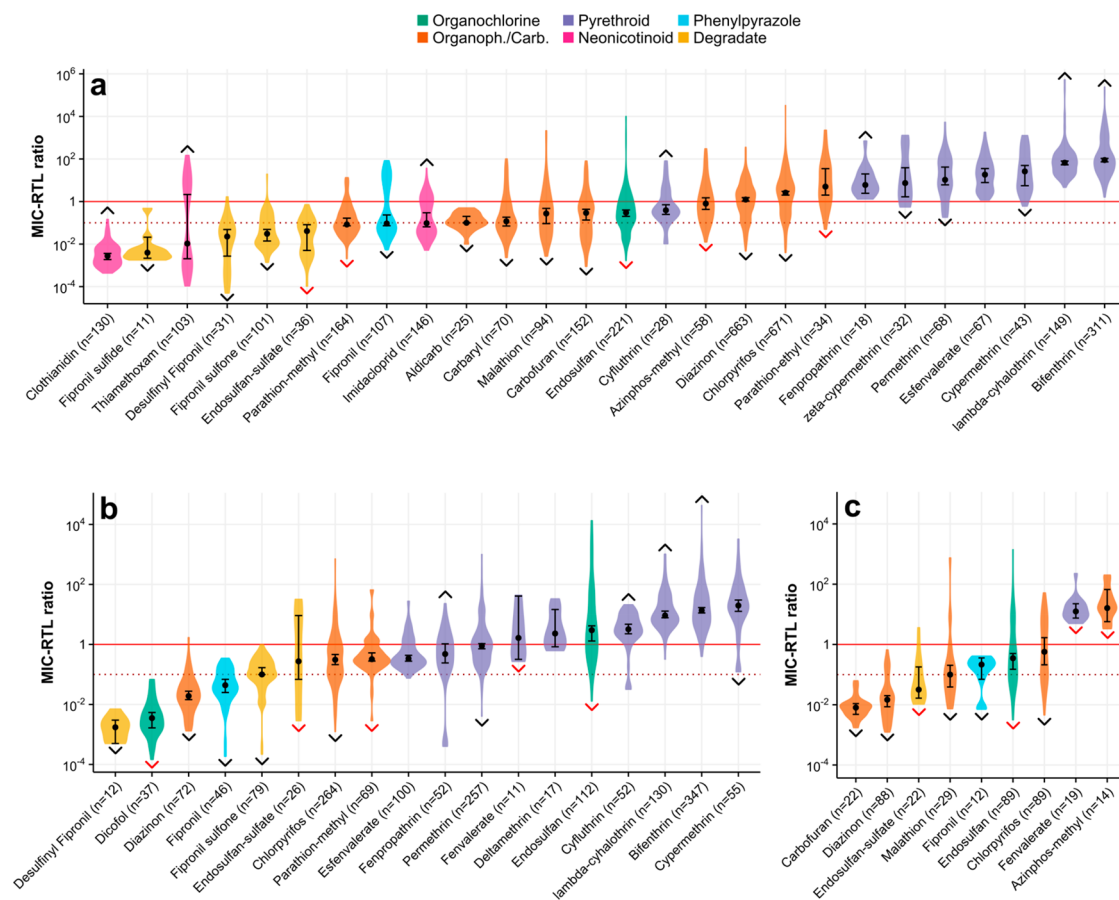


Figure 2. MIC to RTL ratios per substance for those with more than ten MICs for (a) the freshwater phase, (b) sediments and (c) the estuarine water phase with their median (dot), its corresponding 95% CIs (black bars) and smoothed distributions (violin plots). RTLs are depicted with a straight red line, and threshold concentrations of adverse effects on regional biodiversity^{23,47} are represented by a red dotted line. Insecticide use trends (1992–2014; see below) are depicted with arrow heads. Insecticides and their degradates that are no longer registered under FIFRA, Section 3 are marked with a red arrowhead. Organophosphates and carbamates (“Organoph./Carb.”) are grouped together due to their toxic mode of action.

suggesting that insecticide exposure levels in the U.S. do not substantially deviate from those in many other countries.

Overall, our data reveal potential shortcomings of prospective U.S. EPA regulatory risk assessment and management schemes for agricultural insecticides. This finding facilitates the re-evaluation of currently applied methods and, if necessary, their subsequent refinement.

In this context, Gilliom et al.²⁵ reported that, throughout the U.S. (1992–2001), pesticides exceeded aquatic life benchmarks (ALB) at 57% of the surveyed agricultural stream sites ($n = 83$), with insecticides being the main pesticide group causing ALB exceedances. While Stone et al.⁴⁸ also reported frequent ALB exceedances of pesticides for the subsequent observation period (2002–2011) at 61% of agricultural stream sites ($n = 36$), insecticides, except for malathion, exceeded benchmarks considerably less frequently. In part, these findings may be the result of fundamental changes made to the sampling design that is less suitable for detecting transient insecticide exposure.^{22,26,28,49} While these studies^{25,26} generally covered and identified excess insecticide concentrations in major U.S. watersheds but only at comparably few agricultural sites (i.e., 83²⁵ and 36²⁶ sites), for the first time, the present study shows that at least 449 sites out of the total of 644 sites covered experienced RTL exceedances. Thus, the phenomenon of excess agricultural insecticide concentrations seems to be far

more widespread than previously anticipated by existing U.S. monitoring studies. Moreover, our data highlight that even for the most recent years since 2002, 50.4% of MICs in freshwater and its sediments ($n = 3099$) exceeded their RTLs at 67.9% of the sites ($n = 369$). Excess insecticide concentrations are therefore a phenomenon that may still persist in the majority of U.S. surface water sites with published exposure information. As a consequence, their functional integrity, which is critical to downstream consumers and beneficiaries of ecosystem services,^{50,51} appears to be at risk.^{32,47}

Primarily small headwater streams (watershed size < 12.1 km²), which constitute up to 30% of the data considered here, are the predominant feature in agricultural landscapes.⁵² Due to their size and proximity to agricultural fields, RTL exceedances were more frequent (61% in small headwater streams versus 49.2% in the total data set). This scenario is concerning, given that small headwater streams are estimated to constitute the largest riparian interface globally⁵³ and that they provide important refuges for both clean water ecosystems and biodiversity, allowing them to serve as areas for recolonization and support of endangered species.^{27,54–56}

Significantly higher risks ($p < 0.001$) were observed for freshwater (51.2% MIC exceedances, $n = 5233$, Figure 1b) compared to estuaries (31.3%, $n = 584$, Figure 1d). Differing species sensitivity (data not shown) is unlikely to explain the

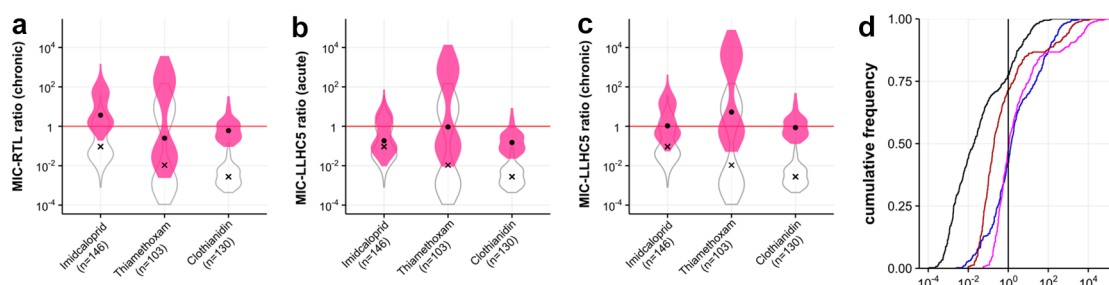


Figure 3. MIC distributions (smoothed violin plots) and their median (black dot) of neonicotinoids detected in the freshwater phase in relation to their (a) chronic RTL_{fw} , (b) the acute lower 95% confidence limit and 5% hazardous concentration ($LLHC_5$), and (c) the chronic $LLHC_5$. $LLHC_5$ are taken from Morrissey et al.⁴⁴ For comparison, gray violin plots and their median (cross) are added (a–c) depicting MIC- RTL_{fw} distributions based on the acute RTL_{fw} as shown in Figure 2. Cumulative frequency distributions (d) summarize all neonicotinoid detections based on their acute RTL_{fw} (black), chronic RTL_{fw} (blue), acute $LLHC_5$ (red), and chronic $LLHC_5$ (magenta) with the black solid line representing the respective threshold.

observed differences. Alternatively, longer transport distances between agricultural nonpoint sources and receiving estuarine waters appear to be a contributing factor fostering dissipation (e.g., adsorption, degradation) and dilution to take effect.²⁷ Catchment sizes could serve as a proxy for transport distances, which were significantly larger ($p < 0.001$) for estuarine waters compared to freshwater (Figure S3) supporting the aforementioned explanation.

Analysis of Individual Compounds. Substance-specific MIC distributions were assessed separately for substances with more than ten MICs available ($n = 29$ insecticides or degradates) by calculating their median with 95% confidence intervals (95% CI, Figure 2). These results can also be accessed and interacted with via the ICC-US (<https://static.magic.eco/icc-us/v1>).

In the freshwater phase, considerable differences became apparent regarding the RTL exceedance frequency of organochlorines (24.7%, $n = 230$), organophosphates and carbamates (48.7%, $n = 1931$), pyrethroids (92.8%, $n = 737$), neonicotinoids (22.4%, $n = 388$), and the phenylpyrazole fipronil (35.7%, $n = 109$). It is important to note that currently established RTL s of fipronil and its degradates likely underestimate actual toxicity by orders of magnitude;⁴⁶ which restricts the comparison of fipronil RTL exceedance rates to other classes (Figure 2). However, increasing importance of the “newer” pyrethroids vs “older” organochlorine or organophosphate insecticides has already been observed in a global data set,²³ which has not yet provided substance-specific data. Seven pyrethroids significantly exceeded their RTL_{fw} (Figure 2a), with bifenthrin and lambda-cyhalothrin at the top of the exceedance profile. It is noteworthy that due to pyrethroids’ high hydrophobicity water-phase concentrations can be associated with considerable uncertainty regarding the fraction that is freely dissolved.^{38,57} Nevertheless, pyrethroids significantly exceed their respective RTL s, and field concentrations are higher than partitioning coefficients generally indicate,⁵⁸ suggesting pronounced risks for aquatic ecosystems; this finding is also in line with preliminary risk characterizations by the U.S. EPA.⁵⁹ Except for aldicarb, potentially due to its tendency to move vertically into ground waters,⁶⁰ all organophosphates and carbamates exceeded their RTL_{fw} in 18.6% (carbaryl) to 76.5% (parathion-ethyl) of MIC_{fw} . Diazinon and chlorpyrifos represent the most extensively studied insecticides considered here and occurred frequently above their RTL_{fw} in 54.1% ($n = 662$) and 65% ($n = 670$), respectively, of MIC_{fw} . It is

noteworthy that chlorpyrifos exceeds its RTL_{fw} significantly (based on 95% CIs) and is used for a wide variety of crops (e.g., soy, corn, wheat) across the U.S.;¹³ thus, the associated risk for nontarget aquatic ecosystems appears to be most widespread compared to other organophosphates and carbamates.

In sediments, RTL exceedance frequencies were also considerably different (Figure 2b) among organochlorines (49.6%, $n = 149$), organophosphates and carbamates (24.5%, $n = 433$), pyrethroids (72.0%, $n = 1023$) and the phenylpyrazole fipronil (0%, $n = 46$). Endosulfan, which represents one of last actively used organochlorines in the U.S., significantly (based on 95% CIs) exceeded sediment thresholds in 66.1% of the cases ($n = 112$), likely due to its high persistence and sorptivity to sediment, resulting in accumulation of particle-bound runoff into adjacent water bodies.^{61,62} In comparison to the freshwater phase, fewer organophosphates and carbamates were detected in sediments and exceeded respective RTL_{sed} (24.4%, $n = 433$, Figure 2b); only chlorpyrifos and parathion-methyl exceeded their RTL_{sed} in more than 20% of the cases. In comparison to highly sorptive insecticides such as pyrethroids, organophosphates and carbamates have a lower tendency to bind to sediments, which appears to reduce their potential impact on benthic organisms. In contrast, pyrethroids exert also highest risks for sediments, with 72% of MIC_{sed} ($n = 1023$, Figure 2b) exceeding their RTL_{sed} . Lambda-cyhalothrin and bifenthrin are again among the most frequently detected pyrethroids and show together with cypermethrin the highest RTL_{sed} exceedances. Generally, all pyrethroids ($n > 10$) exceeded their RTL s, and 97.8% of pyrethroid MIC_{sed} ($n = 1023$) were quantified at the $RTL \times 0.1$ threshold, suggesting biodiversity effects.²³ Thus, as shown here, excess pyrethroid concentrations in sediments might contribute substantially to aquatic life impairments in many surface waters on the national scale. These findings are consistent with conclusions drawn at both the state and global levels,^{46,63} supporting the need for a re-evaluation of the risk assessment scheme for pyrethroids.⁵⁹

In estuaries, only nine substances occurred frequently enough in the water phase such that 95% CIs were calculated (Figure 2c). Median MICs of two insecticides (fenvalerate and azinphos-methyl, Figure 2c) significantly exceeded their RTL_{est} (based on 95% CIs); however, overall detections were rather rare (<20 MICs). In comparison to other stressors (e.g., persistent organic pollutants and excess nutrient loading), insecticides appear to be less important in terms of their

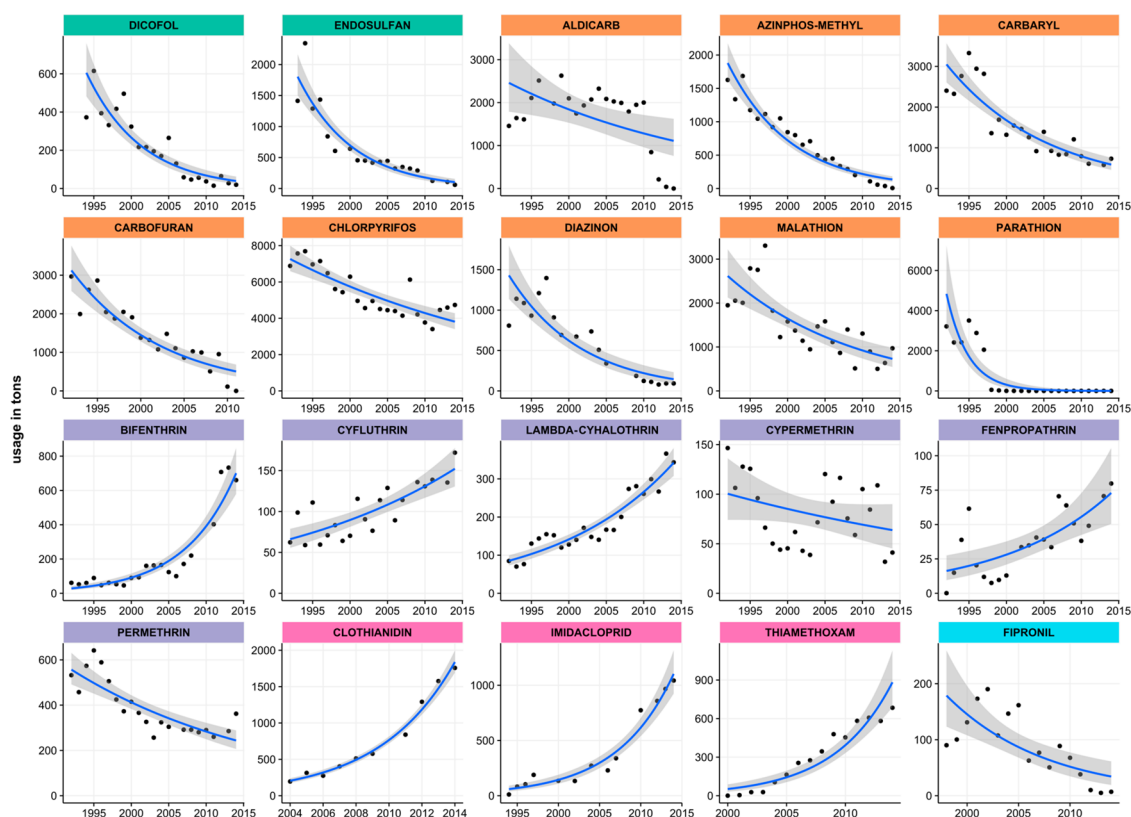


Figure 4. Insecticide use per active ingredient in tons over time.¹³ Temporal use trends are depicted by generalized linear models (blue slope) with their 95% confidence bands (gray). Only insecticides with at least 10 MICs and significant temporal trends (X^2 -test, $\alpha = 0.05$) are depicted. Organophosphates and carbamates (dark orange) are grouped together due to their toxic mode of action.

impact on estuarine waters and impairments to aquatic life in these ecosystems.⁶⁴

Degradates were only found for six different parent compounds across all environmental phases and constituted thus only a minor part of the overall data (6.3%, $n = 367$). Only four degradates (endosulfan sulfate, fipronil sulfone, fipronil sulfide, and desulfinyl fipronil) occurred frequently enough ($n > 10$) to calculate 95% CIs (Figure 2). However, compared to their parent compounds, degradates' MIC to RTL ratios were considerably lower as they are formed only as a fraction of their parent compounds' mass, resulting in lower exposure concentrations while showing comparable toxicity (cf. Table S3). Still, high uncertainty remains regarding the potential impacts of degradates, e.g., substantially higher toxicities of fipronil sulfide and fipronil sulfone suggests that currently used RTLs may considerably underestimate risks.⁴⁶

Regarding acute toxicity, 22.1% of all neonicotinoid MIC_{fw} ($n = 392$) exceeded their RTL_{fw} (Figure 3d), whereas 28.3% of MIC_{fw} surpassed the acute lower 95% confidence limit and 5% hazardous concentrations ($LLHC_5$, Figure 3b, see Morrissey et al.⁴⁴), the latter likely being more protective of aquatic ecosystems as it is based on a broader spectrum of relevant taxa.⁴⁴ However, adequately assessing neonicotinoid toxicity in aquatic systems remains challenging due to its variable toxicity between taxa,⁴⁴ within taxa,⁶⁵ and uncertainties regarding sublethal,³⁰ cumulative⁶⁶ or food-mediated effects.^{67,68} Since many authors argue that neonicotinoids show prolonged and repeated exposure in surface waters, which stresses the importance of chronic toxicity,^{44,69} we also compared MICs to chronic RTL_{fw} and the chronic $LLHC_5$, which revealed considerably higher threshold exceedances of 57.6% (Figure

3a) and 52.3%, respectively (Figure 3c). Imidacloprid (82.7%), thiamethoxam (47.6%), and clothianidin (36.9%) exceeded chronic RTLs most often (Figure 3a), i.e., the neonicotinoids that have been banned most recently for use outside of greenhouses in the EU.¹⁷ Similar to the case of pyrethroids, neonicotinoid risks in U.S. surface waters have not yet been comprehensively evaluated on the national level using available literature, although they are likely of high ecological importance due to their frequent and widespread occurrence.^{70,71}

Use Trends and Future Implications. Annual insecticide use estimates (Figure 4) provide additional information for potential future developments of individual insecticides, reflecting a multitude of macroagronomical factors. Regression analyses suggest that pesticide concentrations (i.e., MIC to RTL ratios) correlate significantly with annual insecticide use estimates on different spatial aggregation scales (see SI Tables S4–6). Therefore, temporal use trends (Figure 4) were used to deduce the potential future implications of the substances. However, prospective risk assessments based on temporal trends are associated with uncertainty regarding their continuity; for instance, they may abruptly change following regulatory action and should therefore only be regarded as risk indications. Acknowledging this uncertainty, significant temporal trends were only used qualitatively, i.e., suggesting increasing or decreasing future risks.

As already known, the use of organochlorine insecticides (endosulfan and dicofol) steadily declined prior their phase-out in 2016, such that their future risk implications are expected to be minimal (Figure 4).^{11,12} The well-known, long presence of organophosphates and carbamates in the

insecticide market⁷² is partly reflected by their large number of available MICs (see Figure 2). However, their use has been in decline (Figure 4), and several substances were subject to regulatory actions (cf. Figure 2), partially in response to their high mammalian toxicity.⁷³ Carbaryl, chlorpyrifos, and malathion represent the remaining substances for which targeted monitoring efforts appear required, despite their receding use trends,^{48,74,75} when assessing substance-specific risks (Figure 2) in combination with recent use estimates.⁷⁶

In response to the reduced use of organophosphates, carbamates, and organochlorines, application of several pyrethroids (cyfluthrin, fenpropathrin, lambda-cyhalothrin, and bifenthrin) increased substantially between 1992 and 2014 (Figure 4). Particularly pyrethroids are associated with most regular and severe RTL exceedances, which highlight their continuing potential for ecologically adverse effects (Figure 2). The application of first-generation pyrethroids (permethrin and cypermethrin) may be declining but likely due to replacements by newer pyrethroids with similar use profiles. Meanwhile, adoption of neonicotinoids has also increased drastically alongside several pyrethroids (Figure 4), a trend that is likely paralleled by more frequent RTL exceedances. From an environmental perspective, several pyrethroids and neonicotinoids may continue to be problematic substances among those currently registered in the U.S., impairing aquatic systems at the national scale.

In general, insecticides responsible for RTL exceedances appear to be changing over time (cf. Figures 1 and 2, Tables S4–S6), yet risk reductions are not observable when past exceedances (48.9%, $n = 2425$, 1960–2001) are compared to more recent data (49.2%, $n = 3280$, 2002–2015), suggesting that risks remain unchanged. In part, potential shortcomings of risk assessment schemes might require a re-evaluation to reduce future risks for nontarget ecosystems resulting from agricultural insecticide use. However, the U.S. EPA's lack of statutory authority to effectively regulate nonpoint source pollution under the Clean Water Act^{77–79} limits the agency's ability to address the shortcomings of prospective risk assessment schemes. In the future, pesticide application is expected to increase in the U.S. due to increased pest pressure resulting from climate change,⁸⁰ a trend even more pronounced for insecticides.¹⁶ As a result, the adverse effects of insecticides on aquatic communities are expected to increase in the U.S.⁸¹ and need to be addressed at both a regulatory and legislative level. Although, the present analysis has focused on insecticides, we suggest conducting similar assessments for herbicides and fungicides to provide a comprehensive evaluation of pesticide impacts on aquatic ecosystems in the U.S. In conclusion, the scenarios presented here are not exclusive to the U.S.; rather, they indicate a global trend of overexposing water resources to insecticides, subsequently causing profound alterations to these ecosystems.²³

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b04651.

Literature search methodology, derivation of threshold levels for sediments, specificity of sampling locations, insecticide use trends, regulatory threshold levels of insecticides, and catchment size distributions (PDF)

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Notes

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■ REFERENCES

- (1) Food and Agriculture Organization of the United Nations Agricultural land (% of land area) - United States. <https://data.worldbank.org/indicator/AG.LND.AGR.LZS?locations=US> (accessed Nov 23, 2017).
- (2) Bigelow, D. P.; Borchers, A. *Major Uses of Land in the United States, 2012*; United States, Department of Agriculture, Economic Research Service: August 2017.
- (3) Oerke, E.-C. Crop losses to pests. *J. Agric. Sci.* **2006**, *144* (1), 31–43.
- (4) Pimentel, D. Environmental and economic costs of the application of pesticides primarily in the United States. *Environ. Dev. Sustainability* **2005**, *7* (2), 229–252.
- (5) Bebbler, D. P.; Holmes, T.; Gurr, S. J. The global spread of crop pests and pathogens. *Global Ecol. Biogeogr.* **2014**, *23* (12), 1398–1407.
- (6) Paini, D. R.; Sheppard, A. W.; Cook, D. C.; De Barro, P. J.; Worner, S. P.; Thomas, M. B. Global threat to agriculture from invasive species. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (27), 7575–7579.
- (7) Pimentel, D.; Zuniga, R.; Morrison, D. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econ.* **2005**, *52* (3), 273–288.
- (8) Popp, J.; Pető, K.; Nagy, J. Pesticide productivity and food security. A review. *Agron. Sustainable Dev.* **2013**, *33* (1), 243–255.
- (9) Strange, R. N.; Scott, P. R. Plant disease: a threat to global food security. *Annu. Rev. Phytopathol.* **2005**, *43* (1), 83–116.
- (10) Fernandez-Cornejo, J.; Nehring, R. F.; Osteen, C.; Wechsler, S.; Martin, A.; Vialou, A. *Pesticide use in US agriculture: 21 selected crops, 1960–2008*; U.S. Government Printing Office: Washington, DC, 2014.
- (11) U.S. EPA. Notice of Receipt of Request to Voluntarily Cancel Certain Pesticide Registrations. *Fed. Regist.* **2011**, *76* (120), 36535–36537.
- (12) U.S. EPA. Notice of Receipt of Request to Voluntarily Cancel Certain Pesticide Registrations. *Fed. Regist.* **2012**, *77* (120), 12299–12301.
- (13) Baker, N. T. *Agricultural Pesticide Use Estimates for the USGS National Water Quality Network, 1992–2014*; U.S. Geological Survey data release, 2016.
- (14) Denholm, I.; Devine, G. J.; Williamson, M. S. Insecticide Resistance on the Move. *Science* **2002**, *297* (5590), 2222–2223.
- (15) Werner, I.; Hitzfeld, B. 50 Years of Ecotoxicology since Sient Spring—A Review. *GAIA-Ecological Perspectives for Science and Society* **2012**, *21* (3), 217–224.
- (16) Koleva, N. G.; Schneider, U. A. The impact of climate change on the external cost of pesticide applications in US agriculture. *International Journal of Agricultural Sustainability* **2009**, *7* (3), 203–216.

- (17) European Environmental Agency. Regulation (EU) No 485/2013. Official Journal of the European Union: https://eur-lex.europa.eu/eli/reg_impl/2013/485/oj, 2013.
- (18) Simon-Delso, N.; Amaral-Rogers, V.; Belzunces, L. P.; Bonmatin, J. M.; Chagnon, M.; Downs, C.; Furlan, L.; Gibbons, D. W.; Giorio, C.; Girolami, V.; Goulson, D.; Kreutzweiser, D. P.; Krupke, C. H.; Liess, M.; Long, E.; McField, M.; Mineau, P.; Mitchell, E. A. D.; Morrissey, C. A.; Noome, D. A.; Pisa, L.; Settele, J.; Stark, J. D.; Tapparo, A.; Van Dyck, H.; Van Praagh, J.; Van der Sluijs, J. P.; Whitehorn, P. R.; Wiemers, M. Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environ. Sci. Pollut. Res.* **2015**, *22* (1), 5–34.
- (19) Health Canada. *Proposed Special Review Decision PSRD2018-01, Special Review of Clothianidin Risk to Aquatic Invertebrates: Proposed Decision for Consultation*. <https://tinyurl.com/y89wrkha>, 2018.
- (20) Federal Insecticide, Fungicide, and Rodenticide Act. Public Law 104-170, 1996; Code of Federal Regulations: Section 136, Title 7, 1996.
- (21) U.S. EPA. Guidelines for Ecological Risk Assessment. *Fed. Regist.* **1998**, *63* (93), 26846–26924.
- (22) U.S. EPA. *Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs, U.S. Environmental Protection Agency - Endangered and Threatened Species Effects Determinations*. U.S. Government Printing Office: Washington, DC, 2004; p 92.
- (23) Stehle, S.; Schulz, R. Agricultural insecticides threaten surface waters at the global scale. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112* (18), 5750–5755.
- (24) Fowle, J. R.; Dearfield, K. L. *Risk characterization handbook*; Science Policy Council, U.S. Environmental Protection Agency: Washington, DC, 2000.
- (25) Gilliom, R. J.; Barbash, J. E.; Crawford, C. G.; Hamilton, P. A.; Martin, J. D.; Nakagaki, N.; Nowell, L. H.; Scott, J. C.; Stackelberg, P. E.; Thelin, G. P.; Wolock, D. M. *Pesticides in the Nation's Streams and Ground Water, 1992–2001*; 1291; Reston, VA, 2006; p 180.
- (26) Stone, W. W.; Gilliom, R. J.; Martin, J. D. *An overview comparing results from two decades of monitoring for pesticides in the Nation's streams and rivers, 1992–2001 and 2002–2011*; 5154; Reston, VA, 2014; p 32.
- (27) Schulz, R. Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution. *J. Environ. Qual.* **2004**, *33* (2), 419–448.
- (28) Stehle, S.; Knäbel, A.; Schulz, R. Probabilistic risk assessment of insecticide concentrations in agricultural surface waters: a critical appraisal. *Environ. Monit. Assess.* **2013**, *185* (8), 6295–6310.
- (29) Deanovic, L. A.; Stillway, M.; Hammock, B. G.; Fong, S.; Werner, I. Tracking pyrethroid toxicity in surface water samples: Exposure dynamics and toxicity identification tools for laboratory tests with *Hyalella azteca* (Amphipoda). *Environ. Toxicol. Chem.* **2018**, *37* (2), 462–472.
- (30) Beketov, M. A.; Liess, M. Potential of 11 Pesticides to Initiate Downstream Drift of Stream Macroinvertebrates. *Arch. Environ. Contam. Toxicol.* **2008**, *55* (2), 247–253.
- (31) Domagalski, J. L.; Weston, D. P.; Zhang, M.; Hladik, M. Pyrethroid insecticide concentrations and toxicity in streambed sediments and loads in surface waters of the San Joaquin Valley, California, USA. *Environ. Toxicol. Chem.* **2010**, *29* (4), 813–823.
- (32) Schäfer, R. B.; von der Ohe, P. C.; Rasmussen, J.; Kefford, B. J.; Beketov, M. A.; Schulz, R.; Liess, M. Thresholds for the effects of pesticides on invertebrate communities and leaf breakdown in stream ecosystems. *Environ. Sci. Technol.* **2012**, *46* (9), 5134–42.
- (33) Walker, C. H.; Sibly, R.; Hopkin, S.; Peakall, D. B. *Principles of ecotoxicology*; CRC Press: 2005.
- (34) Vörösmarty, C. J.; McIntyre, P. B.; Gessner, M. O.; Dudgeon, D.; Prusevich, A.; Green, P.; Glidden, S.; Bunn, S. E.; Sullivan, C. A.; Liermann, C. R.; et al. Global threats to human water security and river biodiversity. *Nature* **2010**, *467* (7315), 555–561.
- (35) Cropper, M. L.; Fraas, A. G.; Morgenstern, R. D. Looking Backward to Move Regulations Forward. *Science* **2017**, *355* (6332), 1375–1376.
- (36) Cooper, H.; Hedges, L. V.; Valentine, J. C. *The handbook of research synthesis and meta-analysis*; Russell Sage Foundation: New York, 2009; p 632.
- (37) U.S. EPA. *Pesticide Chemical Search*. <https://iaspub.epa.gov/apex/pesticides/?p=CHEMICALSEARCH:1> (accessed Jul 4, 2018).
- (38) Lewis, K. A.; Tzilivakis, J.; Warner, D. J.; Green, A. An international database for pesticide risk assessments and management. *Hum. Ecol. Risk Assess.* **2016**, *22* (4), 1050–1064.
- (39) Hladik, M. L.; Kolpin, D. W.; Kuivila, K. M. Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA. *Environ. Pollut.* **2014**, *193*, 189–196.
- (40) Nowell, L. H.; Norman, J. E.; Ingersoll, C. G.; Moran, P. W. Development and application of freshwater sediment-toxicity benchmarks for currently used pesticides. *Sci. Total Environ.* **2016**, *550*, 835–850.
- (41) Crommentuijn, T.; Sijm, D.; de Bruijn, J.; van Leeuwen, K.; van de Plassche, E. Maximum permissible and negligible concentrations for some organic substances and pesticides. *J. Environ. Manage.* **2000**, *58* (4), 297–312.
- (42) White, H. A. Heteroskedasticity-Consistent Covariance Matrix Estimator and a Direct Test for Heteroskedasticity. *Econometrica* **1980**, *48* (4), 817–838.
- (43) Team R Core, R. *A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing, Austria, 2015; <http://www.R-project.org>.
- (44) Morrissey, C. A.; Mineau, P.; Devries, J. H.; Sanchez-Bayo, F.; Liess, M.; Cavallaro, M. C.; Liber, K. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: a review. *Environ. Int.* **2015**, *74*, 291–303.
- (45) Van den Brink, P. J.; Van Smeden, J. M.; Bekele, R. S.; Dierick, W.; De Gelder, D. M.; Noteboom, M.; Roessink, I. Acute and chronic toxicity of neonicotinoids to nymphs of a mayfly species and some notes on seasonal differences. *Environ. Toxicol. Chem.* **2016**, *35* (1), 128–133.
- (46) Weston, D. P.; Lydy, M. J. Toxicity of the Insecticide Fipronil and Its Degradates to Benthic Macroinvertebrates of Urban Streams. *Environ. Sci. Technol.* **2014**, *48* (2), 1290–1297.
- (47) Beketov, M. A.; Kefford, B. J.; Schäfer, R. B.; Liess, M. Pesticides reduce regional biodiversity of stream invertebrates. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (27), 11039–11043.
- (48) Stone, W. W.; Gilliom, R. J.; Ryberg, K. R. Pesticides in U.S. Streams and Rivers: Occurrence and Trends during 1992–2011. *Environ. Sci. Technol.* **2014**, *48* (19), 11025–11030.
- (49) National Research Council. *Preparing for the Third Decade of the National Water-Quality Assessment Program*; National Academies Press: Washington, DC, 2012; p 200.
- (50) Vannote, R. L.; Minshall, G. W.; Cummins, K. W.; Sedell, J. R.; Cushing, C. E. The river continuum concept. *Can. J. Fish. Aquat. Sci.* **1980**, *37* (1), 130–137.
- (51) Wallace, J. B.; Eggert, S. L.; Meyer, J. L.; Webster, J. R. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* **1997**, *277* (5322), 102–104.
- (52) Lyon, J. G.; Trimble, S. W.; Ward, A. D.; Burckhard, S. R. *Environmental Hydrology*; CRC Press: Boca Raton, FL, 2015; p 695.
- (53) Downing, J. A.; Cole, J. J.; Duarte, C.; Middelburg, J. J.; Melack, J. M.; Prairie, Y. T.; Kortelainen, P.; Striegl, R. G.; McDowell, W. H.; Tranvik, L. J. Global abundance and size distribution of streams and rivers. *Inland Waters* **2012**, *2* (4), 229–236.
- (54) Biggs, J.; Von Fumetti, S.; Kelly-Quinn, M. The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers. *Hydrobiologia* **2017**, *793* (1), 3–39.
- (55) Relyea, R. A. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecol. Appl.* **2005**, *15* (2), 618–627.
- (56) Scholz, N. L.; Truelove, N. K.; French, B. L.; Berejikian, B. A.; Quinn, T. P.; Casillas, E.; Collier, T. K. Diazinon disrupts antipredator

- and homing behaviors in chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.* **2000**, *57* (9), 1911–1918.
- (57) Bondarenko, S.; Spurlock, F.; Gan, J. Analysis of pyrethroids in sediment pore water by solid-phase microextraction. *Environ. Toxicol. Chem.* **2007**, *26* (12), 2587–2593.
- (58) Stehle, S.; Bub, S.; Schulz, R. Compilation and analysis of global surface water concentrations for individual insecticide compounds. *Sci. Total Environ.* **2018**, *639*, 516–525.
- (59) U.S. EPA. *Preliminary Comparative Environmental Fate and Ecological Risk Assessment for the Registration Review of Eight Synthetic Pyrethroids and Pyrethrins*; U.S. EPA: Washington, DC, 2016; p 800.
- (60) Ritter, W. F. Pesticide contamination of ground water in the United States - A review. *J. Environ. Sci. Health, Part B* **1990**, *25* (1), 1–29.
- (61) Quinete, N.; Castro, J.; Fernandez, A.; Zamora-Ley, I. M.; Rand, G. M.; Gardinali, P. R. Occurrence and Distribution of Endosulfan in Water, Sediment, and Fish Tissue: An Ecological Assessment of Protected Lands in South Florida. *J. Agric. Food Chem.* **2013**, *61* (49), 11881–11892.
- (62) Scott, G.; Fulton, M.; Wirth, E.; Chandler, G.; Key, P.; Daugomah, J.; Bearden, D.; Chung, K.; Strozier, E.; DeLorenzo, M.; et al. Toxicological studies in tropical ecosystems: an ecotoxicological risk assessment of pesticide runoff in south Florida estuarine ecosystems. *J. Agric. Food Chem.* **2002**, *50* (15), 4400–4408.
- (63) Li, H.; Cheng, F.; Wei, Y.; Lydy, M. J.; You, J. Global occurrence of pyrethroid insecticides in sediment and the associated toxicological effects on benthic invertebrates: An overview. *J. Hazard. Mater.* **2017**, *324*, 258–271.
- (64) U.S. EPA. *Water Quality Assessment and TMDL Information*; https://ofmpub.epa.gov/waters10/attains_index.home (accessed Jun 12, 2018).
- (65) Roessink, I.; Merga, L. B.; Zweers, H. J.; Van den Brink, P. J. The neonicotinoid imidacloprid shows high chronic toxicity to mayfly nymphs. *Environ. Toxicol. Chem.* **2013**, *32* (5), 1096–1100.
- (66) Liess, M.; Foit, K.; Becker, A.; Hassold, E.; Dolciotti, I.; Kattwinkel, M.; Duquesne, S. Culmination of Low-Dose Pesticide Effects. *Environ. Sci. Technol.* **2013**, *47* (15), 8862–8868.
- (67) Englert, D.; Bundschuh, M.; Schulz, R. Thiacloprid affects trophic interaction between gammarids and mayflies. *Environ. Pollut.* **2012**, *167*, 41–46.
- (68) Englert, D.; Zubrod, J. P.; Pietz, S.; Stefani, S.; Krauss, M.; Schulz, R.; Bundschuh, M. Relative importance of dietary uptake and waterborne exposure for a leaf-shredding amphipod exposed to thiacloprid-contaminated leaves. *Sci. Rep.* **2017**, *7* (1), 16182.
- (69) Tennekes, H. A.; Sanchez-Bayo, F. Time-dependent toxicity of neonicotinoids and other toxicants: implications for a new approach to risk assessment. *J. Environ. Anal. Toxicol.* **2011**, No. 4, 1–8.
- (70) Hladik, M. L.; Kolpin, D. W. First national-scale reconnaissance of neonicotinoid insecticides in streams across the USA. *Environ. Chem.* **2016**, *13* (1), 12–20.
- (71) Starner, K.; Goh, K. S. Detections of the Neonicotinoid Insecticide Imidacloprid in Surface Waters of Three Agricultural Regions of California, USA, 2010–2011. *Bull. Environ. Contam. Toxicol.* **2012**, *88* (3), 316–321.
- (72) Jeschke, P.; Nauen, R.; Schindler, M.; Elbert, A. Overview of the Status and Global Strategy for Neonicotinoids. *J. Agric. Food Chem.* **2011**, *59* (7), 2897–2908.
- (73) Costa, L. G. Current issues in organophosphate toxicology. *Clin. Chim. Acta* **2006**, *366* (1–2), 1–13.
- (74) Ryberg, K. R.; Vecchia, A. V.; Martin, J. D.; Gilliom, R. J. *Trends in pesticide concentrations in urban streams in the United States, 1992–2008*; 2010-5139; USGS: Reston VA, 2010; p 114.
- (75) Sullivan, D. J.; Vecchia, A. V.; Lorenz, D. L.; Gilliom, R. J.; Martin, J. D. *Trends in pesticide concentrations in corn-belt streams, 1996–2006*; 2009-5132; USGS: Reston, VA, 2009; p 89.
- (76) USGS. *Pesticide National Synthesis Project - Estimated Annual Agricultural Pesticide Use*. <https://water.usgs.gov/nawqa/pnsp/usage/maps/county-level/> (accessed July 31, 2018).
- (77) Copeland, C. In *Clean Water Act: A Summary of the Law, 1999*; Congressional Research Service, Library of Congress: Washington, DC, 1999.
- (78) Laitos, J. G.; Ruckriegle, H. The clean water act and the challenge of agricultural pollution. *Vt. L. Rev.* **2012**, *37*, 1033.
- (79) Clean Water Act. Public Law 92-500, 1972; Code of Federal Regulations: Section 1251-1387, 1972.
- (80) Chen, C.-C.; McCarl, B. A. An investigation of the relationship between pesticide usage and climate change. *Clim. Change* **2001**, *50* (4), 475–487.
- (81) Koleva, N. G.; Schneider, U. A. The impact of climate change on aquatic risk from agricultural pesticides in the US. *Int. J. Environ. Stud.* **2010**, *67* (5), 677–704.

Supporting Information

for

**Meta-analysis of Insecticides in U.S. Surface Waters: Status and Future
Implications**

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Methods:

Literature search

The literature search was conducted from October 2016 to October 2017 and expanded upon a previous work of Stehle and Schulz¹, which considered 28 compounds and was concluded in June 2012. Search strings can be divided into three groups: substance keywords of parent compounds (e.g., "pyrethroid*" or "aldicarb") or their degradates (see below, n=40), environmental compartment keywords (e.g., "surface water*" or "headwater*"), and detection keywords (e.g., "concentr*" or "measurement*"). *AND*-operators were used to connect all three keyword groups, while keywords within each group were made interchangeable using *OR*-operators. This method was previously applied and independently confirmed, providing accurate coverage of all relevant publications, and more detailed descriptions can be found in Stehle and Schulz¹. Degradates considered here were screened according to methods also applied by the U.S. EPA during ecological risk assessments of pesticides, which indicate that both toxicity (i.e., greater than 10% of parent compound toxicity) and abundance criteria (i.e., greater than 10% of parent compounds' mass) had to be fulfilled.² All degradates that met these criteria and were used as substance keywords are as follows:

endosulfan sulfate, azinphos-methyl oxygen analog, 3,5,6-trichloro-2-pyridinol, chlorpyrifos oxygen analog, chlorpyrifos oxon, diazoxon, diazinon oxon, malaoxon, isomalathion, malathion dicarboxylic acid, malathion monocarboxylic acid, paraoxon, methyl paraoxon, 3-hydroxycarbofuran, 3-ketocarbofuran, carbofuran phenol, alpha-R-deltamethrin, 6-chloronicotinic acid, imidacloprid guanidine, imidacloprid olefin, imidacloprid olefinic-guanidine, imidacloprid urea, o-p-dichlorobenzophenone, p-p-dichlorobenzophenone, 1,1-bis(p-chlorophenyl)-2,2-dichloroethanol, dichlorobenzhydrol, hydroxyl-dichlorobenzophenone, aldicarb sulfoxide, aldoxycarb, aldicarb sulfone, N-(2-chloro-5-thizolylmethyl)-N'-nitroguanidine, 1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl)-guanidine,

1-methylguanidinium chloride, 3-(methylamino)-9-oxa-2-aza-4-azoniabicyclone-3-enehydrogen succinate, 1-methyl-2-nitroguanidine, 1-methyl-3-(tetrahydro-3-furylmethyl)guanidine, 1-methyl-3-(tetrahydro-3-furylmethyl)-guanidinium dihydrogen phosphate, fipronil sulfone, fipronil sulfide, and desulfinyl fipronil.

Evaluating published studies of monitoring results always comes along with some degree of uncertainty regarding the quality of the analytical results reported. We used the following criteria to ensure this quality: i. peer-reviewed studies, ii. concentrations had to exceed their respective LOQs (LOQs and LODs were looked up in the analytical references for each publication) or in some cases 3xLOD were used by authors as thresholds in cases where no LOQ was provided, iii. estimated concentrations were not used in this analysis (i.e., $\text{LOD} < \text{concentration} < \text{LOQ}$), iv. for every publication the analytical part of Materials & Methods was checked in detail and if necessary, referenced analytical publications were visited, and v. it was checked if RSD/RPD values are in acceptable ranges and that the general procedure appeared adequate (i.e., use of field blanks, surrogate recovery tests).

Measured insecticide concentrations (MICs) had to fulfil additional quality criteria in order to be used in this study. Most importantly, several routes or events of entry, which are not related to agricultural nonpoint sources yet may result in comparably high exposure, were not included in this analysis, namely: public health activities (e.g., vector borne disease control), aquaculture, atmospheric deposition, forest application, sheep dipping, golf course applications, spills and accidental releases, intentional water contamination (e.g., fishing, waste dumping) and in-crop use (e.g., rice fields, cranberry bogs)

Regulatory threshold levels for sediments (RTL_{sed})

The most sensitive endpoints (MSE) used to derive regulatory threshold levels for sediments (RTL_{sed}) were taken from (re)registration eligibility decisions (RED) published by the U.S.

EPA. However, in many cases, no bulk sediment ($\mu\text{g}/\text{kg}$) or organic carbon normalized ($\mu\text{g}/\text{kg}_{\text{oc}}$) sediment concentrations were reported, requested by the Agency, submitted by the registrant or used during risk characterization; thus, no RTL_{sed} could be derived from REDs. Prior to the release of the uniform guidance for “Toxicity Testing and Ecological Risk Assessment Guidance for Benthic Invertebrates” in 2007,³ ecological risk for benthic organisms was mainly assessed using the equilibrium-partitioning-methods (EqP) by the U.S. EPA. Briefly, pore-water concentrations are estimated using substance partitioning coefficients (e.g., K_{oc} and K_d) and are compared to chronic MSEs of benthic invertebrates. While this approach is still scientifically valid, it does not consider uptake paths such as skin adsorption or ingestion. Additionally, pore-water concentrations cannot be readily compared to measured insecticide concentrations in sediments without prior conversion, and there is a considerable degree of uncertainty regarding its adequacy in describing risks for benthic organisms, for instance, due to considerable differences between laboratory derived and in field partitioning coefficients remains.⁴ Therefore, insecticides that were last (re)registered prior to the release of uniform testing guidance for benthic invertebrates often were not supplied with bulk sediment toxicity data, and in these cases alternative sources needed to be considered.

Primarily, threshold effect benchmarks (TEBs), derived by Nowell et al.⁵, were used as RTL_{sed} because they are obtained from sediment toxicity assays using standard test species (*Hyalella azteca* and *Chironomus* spp.) and follow standard test protocols. TEBs are provided as organic carbon normalized ($\mu\text{g}/\text{kg}_{\text{oc}}$) sediment concentrations that require conversion to their respective organic carbon content for each sample. However, measurements of organic carbon content per sample are rarely conducted or reported; thus, an average organic carbon content of 4% was assumed for all samples to allow a comparison of MICs to organic carbon normalized MSEs. Based on findings of Suedel and Rodgers⁶ and references therein, 4%

organic carbon can be considered a conservative estimate given that the average organic carbon content of freshwater systems throughout the continental United States ranges from 1.36% to 4.6% organic carbon (weighted average=2.92%). In addition, during REDs, an average organic carbon content of 4% is assumed in the U.S. EPA Exposure Analysis Modeling System (EXAMS) standard pond scenario that is used to derive estimated environmental concentrations, further supporting the assumption.

In case no TEBs were provided for a specific substance, maximum permissible concentrations (MPC) by Crommentuijn et al.⁷ were used as RTL_{sed} because they are based off the EqP-approach used by the U.S. EPA. MPCs include a safety-factor of ten (i.e., MSE/10) and refer to a standard soil or sediment with an organic carbon content of 10%. Therefore, MPCs were adjusted to reflect the decision process of U.S. EPA REDs by excluding safety-factors and converting MPCs to an average organic carbon content of 4%.

To quantify the impact of different estimates for organic carbon content, sensitivity analyses were conducted using multiple organic carbon scenarios (2%, 4%, 8%, 10%, see Fig. S4). Low organic carbon content estimates (i.e., 2%) are considered less conservative as sediment toxicity increases and thus associated risks. Conversely, high organic carbon content estimates potentially underestimate risks for aquatic systems with moderate or low organic carbon contents in sediments. In general, organic carbon content was shown to be more variable within U.S. regions than between them; thus, no spatial bias should result from our aforementioned assumption.⁶ Overall, RTL_{sed} exceedances increased from 48.2% to 55.1% from the high to low organic carbon content scenario (Fig. S4). RTL exceedance frequency did not differ substantially because the RTL_{sed} values of most abundant substances were based on bulk sediment endpoints, which are not subject to change in these different scenarios.

Sampling location specificity

For every MIC, a corresponding sampling location was attributed. The specificity of sampling locations varied between and within publications and could be classified according to three main categories. Sampling locations were deemed "unspecific" (e.g., agricultural canals, Florida Bay, Florida, USA), if corresponding MICs could not be attributed to either a specific location (e.g., Nishnabotna River at Hamburg, Mississippi watershed, Nebraska, USA) or a single surface water (e.g., Beasley Lake, oxbow lakes in Mississippi watershed, Mississippi, USA). Such categorization was performed to quantify the impacts of unspecific sites on the overall conclusions regarding spatial RTL-exceedances. In Table S1, site-specific RTL exceedance frequencies are listed for all locations (i.e., including unspecific sites) and locations excluding unspecific sites. Overall, the impact of unspecific sites on respective analyses appears to be minimal, with a maximum deviance of 2.1% (Table S1).

Insecticide use trends

Insecticide use estimates are provided by Baker⁸ and were summed at the national level. Insecticide use trends are calculated using generalized linear models (GLMs) with national use estimates as the dependent variable and time as the independent variable. As the family function, "*quasipoisson*" (link function=log) was used due to the data showing over dispersion. Analysis of deviance (chi-square-test, $\alpha=0.05$) was used to test for significance of the predictor (time).

Multiple linear regressions (MLRs) were used to test for significant correlation between substance use and MIC to RTL-ratios (\log_{10} -transformed). Three different aggregation levels of substance use were evaluated: insecticide use at the national level and the state level and state-level use intensity. To allow comparability between substances and simplify interpretation of regression coefficients, national and state use estimates were normalized per

substance (i.e., ranging from 0 to 1). Insecticide use intensity was based on state-level insecticide use estimates (kg yr^{-1}) per million acres of crop land of the respective state. Crop land data were retrieved from the USDA Economic Research Service⁹ and averaged per state from 1992–2012. Using the average crop area per state was necessary to prevent large data gaps, because crop data are only reported every five years, which would result in the omission of a majority of the data points.

MLRs were validated following suggestions of Unwin¹⁰ and references therein. Briefly, the normality distribution of the residuals was checked visually using histograms and QQ-Plots, while the independence of errors was tested using the Durbin-Watson-Test with $1 < d < 3$ representing thresholds that would suggest autocorrelation.¹¹ Homoscedasticity was assessed visually by plotting standardized residuals versus their fitted values. Additionally, the significance of all regression coefficients was checked using White's heteroscedasticity corrected covariance matrix¹² and by calculating regression coefficient 95% confidence intervals. Both tests did not reveal any departure from the initial MLR results. Outliers were assessed with studentized residuals and standardized residuals, while potential leverage points were assessed using hat-values, DFFit, Cook's distance and covariance ratios. Multicollinearity was assessed using variance inflation factors with a threshold level of 3. Only substances with more than 20 MICs were used for all MLRs, and no violations of concern became apparent.

Hydrological information

Data-availability, uniform use of descriptors (e.g., "creek", "river", "small lake"), and individual quality of used descriptors represents a major challenge during data-harmonization in meta-analysis. Thus, additional descriptors (e.g., stream order, sampling location within respective water bodies, physico-chemical conditions of water bodies, etc.) were not recorded

or used in analyses due to high uncertainty associated with those criteria or unsatisfactory data availability. However, additional descriptive statistics of catchment sizes for lotic and lentic systems were provided in Table S2 as further hydrologic context.

References

1. Stehle, S.; Schulz, R., Agricultural insecticides threaten surface waters at the global scale. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, (18), 5750-5755.
2. U.S. EPA, *Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs, U.S. Environmental Protection Agency - Endangered and Threatened Species Effects Determinations*. U.S. Government Printing Office: Washington, DC, 2004; p 92.
3. U.S. EPA, *Toxicity Testing and Ecological Risk Assessment Guidance for Benthic Invertebrates*. Office of Pesticide Programs: Washington, DC, 2007; p 31.
4. Stehle, S.; Bub, S.; Schulz, R., Compilation and analysis of global surface water concentrations for individual insecticide compounds. *Sci. Total Environ.* **2018**, *639*, 516-525.
5. Nowell, L. H.; Norman, J. E.; Ingersoll, C. G.; Moran, P. W., Development and application of freshwater sediment-toxicity benchmarks for currently used pesticides. *Sci. Total Environ.* **2016**, *550*, 835-850.
6. Suedel, B. C.; Rodgers, J. H., Variability of bottom sediment characteristics of the continental United States. *J. Am. Water Resour. Assoc.* **1991**, *27*, (1), 101-109.
7. Crommentuijn, T.; Sijm, D.; de Bruijn, J.; van Leeuwen, K.; van de Plassche, E., Maximum permissible and negligible concentrations for some organic substances and pesticides. *J. Environ. Manage.* **2000**, *58*, (4), 297-312.
8. Baker, N. T., Agricultural Pesticide Use Estimates for the USGS National Water Quality Network, 1992-2014. *U.S. Geological Survey data release* **2016**.
9. Bigelow, D. P.; Borchers, A., *Major Uses of Land in the United States, 2012*. United States, Department of Agriculture, Economic Research Service: August 2017.
10. Unwin, A., *Discovering Statistics Using R* by Andy Field, Jeremy Miles, Zoë Field. *International Statistical Review* **2013**, *81*, (1), 169-170.
11. Durbin, J.; Watson, G. S., Testing for serial correlation in least squares regression. II. *Biometrika* **1951**, *38*, (1/2), 159-177.
12. White, H., A Heteroskedasticity-Consistent Covariance Matrix Estimator and a Direct Test for Heteroskedasticity. *Econometrica* **1980**, *48*, (4), 817-838.

List of Tables

Table S1: Differences in site specific RTL-exceedance frequency and the total number of sides considered between all sites (i.e., including unspecific sites) and specific sites (i.e., exact locations or water bodies).

Site exceedance analysis	Exceedance % of all sites (n)	Exceedance % of specific sites (n)	Δ %	Δ n
Single exceedance, all compartments	69.7 (644)	67.8 (580)	-1.9	-64
Single exceedance, surface water phase	68.3 (451)	66.2 (396)	-2.1	-55
Single exceedance, sediment phase	71.1 (308)	71.2 (280)	0.1	-28
Multiple exceedances, all compartments	63.5 (444)	62.6 (393)	-0.9	-51
Multiple exceedances per year, all compartments	47.5 (444)	46.0 (393)	-1.5	-51
Single exceedance (2002-2016), freshwater	67.9 (396)	66.9 (378)	-1.0	-18

Table S2: Additional descriptive statistics of hydrological information.

Water body category	flow conditions	MICs	Catchment size in km ²				
			min	1 st quart.	median	3 rd quart.	max
estuarine	lentic	12	-	-	-	-	-
estuarine	lotic	572	3.5	800	2,500	4,900	150,000
freshwater	lentic	1145	0.16	4.5	9.15	9.15	70,000
freshwater	lotic	3912	1	20	210	2,000	2,900,000
freshwater	NA	176	-	-	-	-	-

Table S3: Regulatory threshold levels (RTL) for freshwater (RTL_{fw}), sediments (RTL_{sed}) and estuaries (RTL_{est}) for all 40 parent compounds and degradates included in this meta-analysis. RTLs are only provided for the respective substance and environmental compartment, if there are reported concentrations. Only insecticide degradates with reported concentrations are listed. A full list of degradates of potential environmental concern can be found in the literature search paragraph. Please note that organophosphate and carbamate insecticides were grouped in this publication due to their similar mode of action.

Insecticide class	Insecticide	RTL _{fw}	RTL _{sed}	RTL _{est}
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		(µg/L)	(µg/kg)	(µg/L)
Organochlorine	Dicofol	26.5	3680	7.55
	Endosulfan	0.05	3.84	0.02
Organophosphate	Azinphos-methyl	0.08	0.89	0.105
	Chlorpyrifos	0.03	16	0.0175
	Diazinon	0.105	76	2.1
	Malathion	0.295	2.56	1.1
	Parathion-ethyl	0.02	0.325	0.0535
	Parathion-methyl	0.485	20.8	0.175
Carbamate	Aldicarb	10		6
	Carbaryl	0.85	4.4	2.85
	Carbofuran	1.115	1.72	2.3
Pyrethroid	Acrinathrin	0.0087		0.0087
	Allethrin	1.05		1.05
	Bifenthrin	0.0002465	0.25	0.001985
	Cyfluthrin	0.0125	0.53	0.0012
	β-cyfluthrin	0.034		0.0011
	Cypermethrin	0.0018	0.308	0.002375
	α-cypermethrin	0.0018	0.308	0.002375
	ζ-cypermethrin	0.0018	0.308	0.002375
	Deltamethrin	0.0001	0.48	0.00185
	Esfenvalerate	0.000424	7.4	0.00233
	Fenpropathrin	0.001525	12.4	0.0105
Fenvalerate	0.016	2.2	0.004	

	Flucythrinate	0.16		0.004
	λ -cyhalothrin	0.00015	0.31	0.00245
	Permethrin	0.0033	7.4	0.009
	Phenothrin	2.2		0.0125
	Resmethrin	0.14		0.115
	τ -fluvalinate	0.155		0.003
	Tefluthrin	0.03	11.6	0.0265
	Tetramethrin	1.85		1.85
	Tralomethrin	0.0195		0.4225
Neonicotinoid	Acetamiprid	10.5		33
	Clothianidin	11		26.5
	Dinotefuran	49550		395
	Imidacloprid	0.385		16.5
	Nithiazine	16925		16925
	Thiacloprid	18.9		15.65
	Thiamethoxam	17.5	10	3450
Phenylpyrazole	Fipronil	0.11	16	0.07
Degradate	Endosulfan sulfate	0.05	73	0.02
	Chlorpyrifos oxon	0.03	16	0.0175
	Aldicarb sulfoxide	21.5		
	Aldicarb sulfone	184.5		
	Fipronil sulfone	0.36	9.1	0.28
	Fipronil sulfide	1.065	29	0.0385
	Desulfinyl fipronil	100	200	0.75

Table S4: Results of multiple linear regressions (adj. $R^2=0.51$) predicting log-transformed MIC to RTL-ratios (n=4321) using normalized insecticide use at the national level for all environmental compartments and substances of interest (n>20). Substance names were dummy coded as they are categorical variables. Significant coefficient estimates of categorical variables (i.e., substance names) represent mean differences from zero. Multiple linear regressions were significantly different from the null model ($F_{20,4242}=210$, $p<0.001$), and significant independent variables ($p<0.05$) are printed in bold.

Independent variable	Estimate	Std. Error	t value	p-value
Normalized use	0.28	0.073	3.821	<0.001
Endosulfan	-0.356	0.061	-5.875	<0.001
Azinphos-methyl	-0.192	0.145	-1.325	0.185
Chlorpyrifos	-0.173	0.063	-2.758	<0.001
Diazinon	-0.468	0.061	-7.735	<0.001
Malathion	-0.709	0.099	-7.145	<0.001
Parathion-methyl	-0.857	0.074	-11.657	<0.001
Carbofuran	-1.292	0.104	-12.458	<0.001
Bifenthrin	1.626	0.051	32.036	<0.001
Cyfluthrin	0.117	0.124	0.941	0.347
Cypermethrin	0.936	0.118	7.96	<0.001
Zeta-cypermethrin	1.035	0.172	6.008	<0.001
Esfenvalerate	0.43	0.101	4.263	<0.001
Fenpropathrin	-0.335	0.123	-2.719	0.007
Lambda-cyhalothrin	1.355	0.069	19.653	<0.001
Permethrin	0.099	0.065	1.534	0.125

Imidacloprid	-1.255	0.137	-9.142	<0.001
Thiamethoxam	-3.051	0.144	-21.118	<0.001
Dicofol	-2.648	0.17	-15.61	<0.001
Carbaryl	-0.813	0.121	-6.696	<0.001
Clothianidin	-2.868	0.124	-23.045	<0.001
Fipronil	-0.894	0.09	-9.969	<0.001

Table S5: Results of multiple linear regressions (adj. $R^2=0.50$) predicting log-transformed MIC to RTL-ratios (n=4454) using normalized insecticide use at the state level for all environmental compartments and substances of interest (n>20). Substance names were dummy coded as they are categorical variables. Significant coefficient estimates of categorical variables (i.e., substance names) represent mean differences from zero. Multiple linear regressions were significantly different from the null model ($F_{20,4432}=204.3$, $p<0.001$), and significant independent variables ($p<0.05$) are printed in bold.

Independent variable	Estimate	Std. Error	t value	p-value
Normalized use	0.642	0.06	10.709	<0.001
Endosuldan	-0.674	0.061	-11.089	<0.001
Azinphos-methyl	-0.507	0.144	-3.519	<0.001
Chlorpyrifos	-0.304	0.044	-6.86	<0.001
Diazinon	-0.626	0.049	-12.875	<0.001
Malathion	-0.947	0.103	-9.155	<0.001
Parathion-methyl	-0.53	0.207	-2.563	0.01
Carbofuran	-1.544	0.1	-15.42	<0.001
Bifenthrin	1.458	0.041	35.524	<0.001
Cyfluthrin	-0.088	0.11	-0.799	0.424

Cypermethrin	0.965	0.114	8.437	<0.001
Zeta-cypermethrin	1.017	0.171	5.932	<0.001
Esfenvalerate	0.08	0.079	1.024	0.306
Fenpropathrin	-0.593	0.121	-4.894	<0.001
Lambda-cyhalothrin	1.217	0.064	19.049	<0.001
Permethrin	-0.085	0.063	-1.36	0.174
Imidacloprid	-1.1	0.106	-10.403	<0.001
Thiamethoxam	-3.339	0.139	-24.074	<0.001
Dicofol	-2.669	0.148	-18.04	<0.001
Carbaryl	-0.901	0.12	-7.493	<0.001
Clothianidin	-3.165	0.117	-26.956	<0.001
Fipronil	-0.431	0.105	-4.127	<0.001

Table S6: Results of multiple linear regressions (adj. $R^2=0.49$) predicting log-transformed MIC to RTL-ratios (n=4452) using state level insecticide use intensity for all environmental compartments and substances of interest (n>20). Substance names were dummy coded as they are categorical variables. Significant coefficient estimates of categorical variables represent mean differences from zero. Multiple linear regressions were significantly different from the null model ($F_{20,4430}=201.6$, $p<0.001$), and significant independent variables ($p<0.05$) are printed in bold.

Independent variable	Estimate	Std. Error	t value	p-value
Use intensity	9.692	0.896	10.817	<0.001
Endosulfan	-0.48	0.053	-8.979	<0.001
Azinphos-methyl	-0.26	0.139	-1.871	0.061
Chlorpyrifos	-0.448	0.054	-8.236	<0.001

Diazinon	-0.531	0.043	-12.363	<0.001
Malathion	-0.206	0.098	-2.093	0.036
Parathion-methyl	-0.429	0.207	-2.072	0.038
Carbofuran	-1.206	0.092	-13.038	<0.001
Bifenthrin	1.602	0.038	42.031	<0.001
Cyfluthrin	0.223	0.106	2.103	0.035
Cypermethrin	1.05	0.114	9.177	<0.001
Zeta-cypermethrin	1.113	0.172	6.486	<0.001
Esfenvalerate	0.328	0.075	4.377	<0.001
Fenpropathrin	-0.17	0.114	-1.49	0.136
Lambda-cyhalothrin	1.481	0.059	25.262	<0.001
Permethrin	0.192	0.055	3.469	0.001
Imidacloprid	-0.663	0.095	-6.986	<0.001
Thiamethoxam	-2.821	0.13	-21.753	<0.001
Dicofol	-2.559	0.147	-17.413	<0.001
Carbaryl	-0.817	0.119	-6.843	<0.001
Clothianidin	-2.684	0.106	-25.299	<0.001
Fipronil	-0.258	0.103	-2.49	0.013

List of Figures

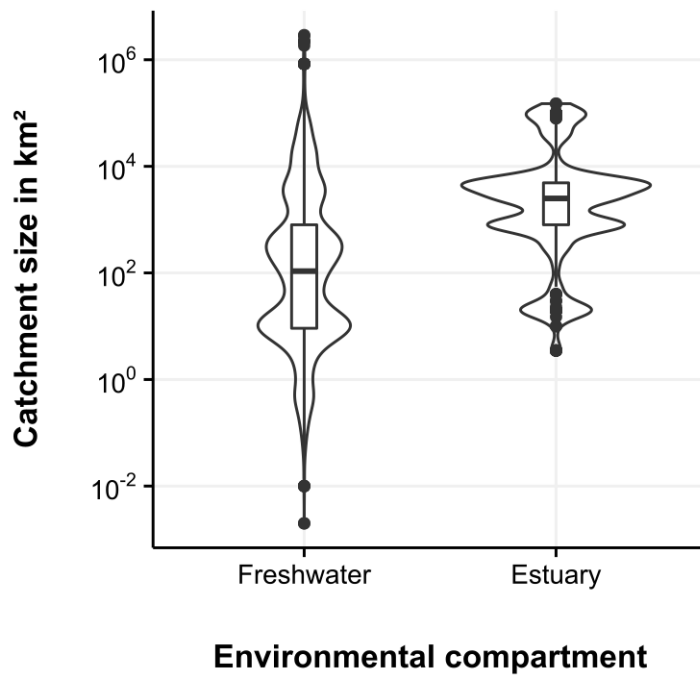


Figure S1: Catchment size distributions in km² of freshwater MICs (n=4182) and estuarine MICs (n=544). Median catchment size of estuaries (2500 km²) differed significantly ($p < 0.001$) from the median catchment size of freshwater (108 km²).

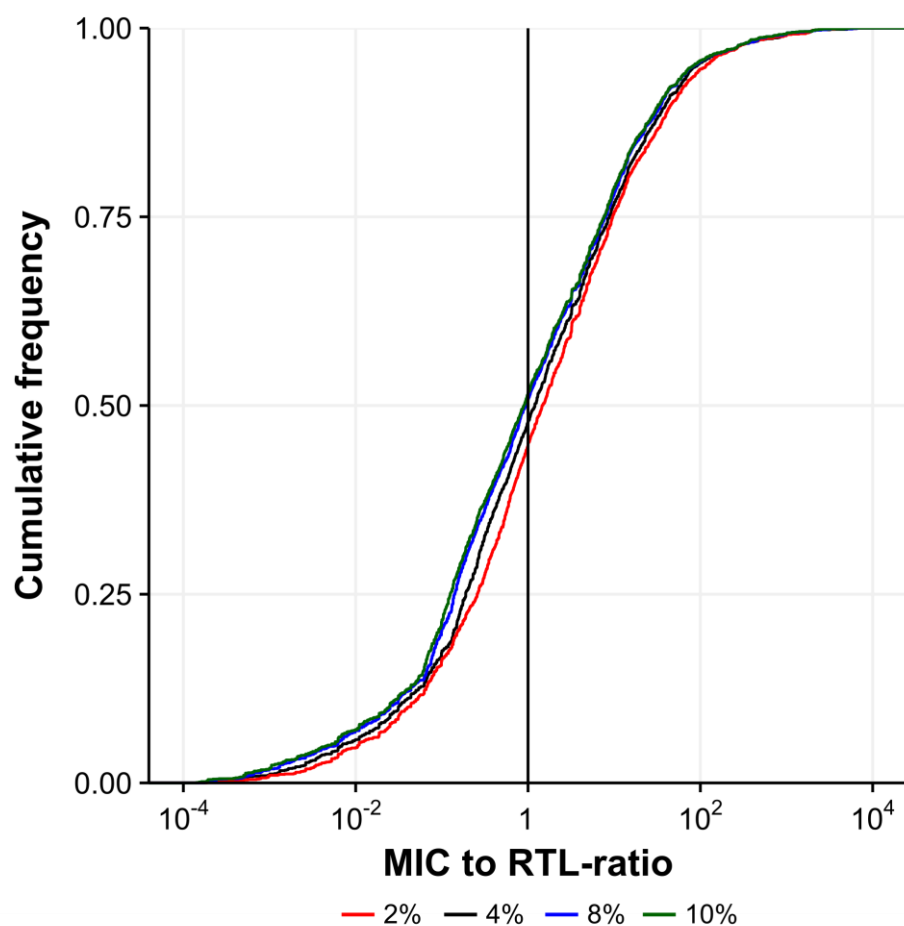


Figure S2: Comparison of RTL exceedance frequencies in sediments using different assumptions regarding the average organic carbon content in percent. The RTL_{sed} is depicted with a black vertical line.

Insecticide Risk in US Surface Waters: Drivers and Spatiotemporal Modeling

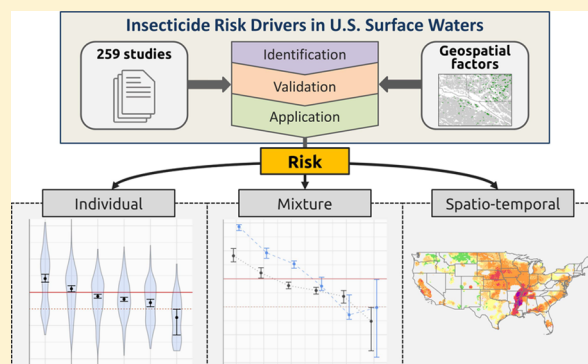
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Supporting Information

ABSTRACT: Although pesticide contamination in agricultural surface waters is a common phenomenon, large-scale studies dealing with the responsible drivers are rare. We used data from 259 publications reporting 5830 individual water or sediment concentrations of 32 insecticides and their metabolites in 644 US surface waters to determine the factors driving insecticide risks, that is, exceedance of regulatory threshold levels (RTLs). Multiple linear regressions (R^2 adj. = 49.6–76.5) revealed that toxicity-normalized agricultural insecticide use (i.e. use divided by toxicity) was the most important driver. Burst rainfall erosivity and irrigation practices also had risk-promoting effects, whereas time, catchment size, and sampling interval had risk-demoting effects. A regression model (R^2 adj. = 62.2, $n = 1833$) for small, medium, and large running waters was validated and used for risk mapping at the national scale, highlighting multiple regions, where the comparison of predicted insecticide concentrations with their RTLs indicate adverse conditions for aquatic organisms. Particularly in smaller streams, risks were most pronounced with an average RTL exceedance frequency of 27.7% in all grid cells ($n = 9968$). Finally, mixture toxicity was mainly (about 76.7%) explained by the most toxic compound in the mixture, causing ~95.7% of RTL exceedances. Identifying the factors, which drive exposure for all relevant insecticide classes, and subsequently mapping these risks for surface waters of various sizes across the U.S., will support future risk management.



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INTRODUCTION

Agricultural intensification plays a central role in global biodiversity decline and ecosystem degradation.^{1,2} In the conterminous United States, an estimated 18.4% of the land surface is currently being used as agricultural cropland,³ supporting a \$400 billion industry. Here, applied pesticides can enter nontarget aquatic ecosystems via various pathways (e.g., runoff, spray drift, and drainage),⁴ often co-occur as mixtures,^{5,6} and adversely affect the ecosystem structure and functions therein.^{7,8}

Insecticides exhibit aquatic toxicities^{9–11} approximately 2 orders of magnitude higher than other types of pesticides (e.g., herbicides and fungicides).¹² Like all pesticides, they have to be registered by the US Environmental Protection Agency (EPA) prior to their use in the United States.¹³ During the mandatory ecological risk assessment accompanying insecticide registration, regulatory threshold levels (RTLs, see Stehle and Schulz¹⁰) are derived from the most sensitive end point of a variety of tested species, that is, algae, invertebrate, and fish, by multiplying the measured toxicity with a so-called “level of concern” (0.05–1).¹⁴ These RTLs denote concentrations above which unacceptable ecological effects in nontarget ecosystems, such as surface waters, may occur.^{10,15} Therefore, RTL exceedances—referred to here as “risks”—indicate adverse conditions for most sensitive aquatic taxa. For many

modern insecticides, that is, pyrethroids or neonicotinoids, because of their mode of action, the most sensitive group in the regulatory risk assessment are invertebrates (also see Stehle and Schulz¹⁰). However, some groups of organisms, such as fish, are known to be most sensitive to organophosphate insecticides.

The potential of individual or co-occurring insecticides adversely affecting nontarget aquatic ecosystems is well characterized,^{5,6,10,11} whereas factors promoting the spatio-temporal variation of RTL exceedances (i.e., “risk drivers”) are less well characterized. One major risk driver is the applied mass of an insecticide, as has been shown both on the local¹⁶ and the national scale.^{11,17} Meteorological, geological, land-use, and hydrological characteristics have also been recognized to affect the complex process of nonpoint source insecticide pollution.¹⁷ In 2013, Stone et al.¹⁷ successfully quantified some of these drivers and developed an elaborate regression model (watershed regressions for pesticides, WARP; $R^2 > 80\%$) that predicts in-stream concentrations for a vast selection ($n = 112$) of herbicides and various insecticides. However, WARP does

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not cover many newer insecticides, such as pyrethroids and neonicotinoids.¹¹ Moreover, lower-order streams (<75 km²), the predominant hydrological features in agricultural landscapes^{18–20} that are most likely exposed to nonpoint source insecticide pollution,^{4,10} were not included in WARP.¹⁷ Thus, we intend to evaluate insecticides including these newer compounds also considering small streams.

Here, we use the data set of US insecticide concentrations (measured insecticide concentrations, MICs) compiled from peer-reviewed studies. These data have been described extensively in an earlier study that focused on substance-specific risks,¹¹ whereas in the present study, the data were used to identify and apply spatiotemporal factors driving insecticide risks in US surface waters in the following way: first, analytic model building was applied to quantify and compare most important risk drivers in water and sediment phases. Then, a model was developed, validated, and applied based on various spatiotemporal descriptors identified during the first step (e.g., land-use, meteorological, and hydrological data), enabling us to characterize and map current and previous insecticide risks in surface waters of various size classes at the national scale. In addition, an evaluation of insecticide mixture toxicity was conducted, detailing the potential impact of co-occurring insecticides and their interaction with risk drivers. Therefore, the present study, for the first time, provides for all relevant agricultural insecticide classes in US surface waters, including small water bodies, an analysis of factors that affect RTL exceedance, as an indicator of aquatic risk, and employs this information for national risk mapping.

MATERIALS AND METHODS

Meta-Analysis Data. For the present study, MICs of 32 insecticides and 6 degradates (for details, see Table S1) in US agricultural surface waters were taken from 259 peer-reviewed studies. Insecticide compounds were selected because of their agricultural application within the last 2 decades (see Baker²¹). Degradates that were considered for this meta-analysis are listed in the Supporting Information (also see Wolfram et al.¹¹). The data were compiled following extensive search protocols,¹⁰ and analytical procedures used to quantify MICs were checked for each publication individually to ensure the integrity of the reported values. More detailed descriptions of search methodologies, quality control measures, validity criteria, and considered substances can be found in Wolfram et al.¹¹ In total, 5830 measured insecticide concentrations quantified in surface waters (MIC_{sw}) or their sediments (MIC_{sed}) were used in this study. The MICs, quantified between 1960 and 2015,¹¹ originate from at least 644 surface water sampling sites in 37 US states with considerable agricultural activity.

Regulatory Threshold Levels. RTLs (see Wolfram et al.¹¹), as threshold levels above which adverse effects in aquatic ecosystems are expected to occur (i.e., MIC to RTL ratio > 1),^{14,22} were used for the assessment of MICs in surface waters (RTL_{sw}) or sediments (RTL_{sed}). RTL_{sw} contain both RTLs for freshwater (RTL_{fw}) and estuarine waters (RTL_{est}) and were applied accordingly (Table S1). When RTL_{sed} was not available from regulatory documents, threshold effect benchmarks taken from Nowell et al.²³ or adjusted maximum permissible concentrations from Crommentuijn et al.²⁴ were used (see Wolfram et al.¹¹ for details).

Insecticide Mixture Toxicity. Two concepts were applied here to deduce the potential toxicity of insecticide mixtures:

concentration addition (CA) and maximum individual component toxicity (MICT).²⁵ CA assumes the additivity of concentrations, described as the sum of individual toxic units (sumTU) per sample, represented here by the sum of MIC to RTL ratios per sample. MICT assumes that risk distributions are heavily skewed because of substantially different toxicities of individual insecticides within a mixture.¹¹ Thus, mixture effects may be sufficiently described by the most toxic component per sample (maxTU), represented here by the maximum MIC to RTL ratio per sample.²⁶ Primarily, aquatic risks of mixtures (i.e., sumTU and maxTU, $n = 913$) were compared (i) between both concepts and (ii) with sole MIC-to-RTL ratios ($n = 2792$), that is, those not occurring in mixtures.

Covariates and Geospatial Factors. MICs were attributed with various covariates and geospatial factors, such as hydrological features, applied analytical methods, co-occurrence of chemicals, location names, and so forth when reported in the peer-reviewed publication (for a full list, see Table S2). Missing catchment sizes were added from secondary sources, such as USGS StreamStats,²⁷ where possible. Sampling intervals were calculated if exact sampling dates were available; otherwise, descriptions (e.g., “weekly sampling”) were expressed in days. In the case of event sampling, that is, sampling during rainfall-induced runoff or spray drift events, a sampling interval of 1 h was assigned (i.e., 0.0417 d).

Spatially explicit MICs ($n = 4391$) were attributed with geospatial information derived from a radial buffer area around the sampling location, named here “regional context area” (RCA). A 50 km RCA was selected for this study as convergence analysis resulted in the highest explained variance for this radius compared to others (15, 30, 75, 100, 250, and 500 km). Radial buffers were used as they provide a simple approach to consider the landscape context of a given site, particularly regarding those parameters analyzed here. With such a generalizing approach, future model application may not be hindered by the lack of detailed catchment data on higher spatial scales or spatial uncertainties associated with literature-derived coordinates.

The following spatial attributes were derived from the RCA: the annual county-level pesticide use estimates for the United States (1992–2014) were taken from Baker²¹ and projected onto crop land estimates (excluding pastures and silviculture, Cropland Data Layer) available from the US Department of Agriculture National Agricultural Statistics Service.⁵ Insecticide use estimates were normalized by respective RTLs (toxicity-normalized use) to reflect potential risks (i.e., MIC-to-RTL ratios). County boundaries for 2017 were obtained from the US Census Bureau.²⁸ Adjusted rainfall erosivity factors (R -factors) for the conterminous United States, such as the modified Fournier or Burst R -factor, were retrieved from Biasutti and Seager.²⁹ Estimates of soil-restrictive layers within the upper 25 cm of agricultural land were obtained from Nakagaki.³⁰ The Moderate Resolution Imaging Spectroradiometer Irrigated Agriculture Data set for the United States was taken from Brown and Pervez³¹ and used to calculate the percentage of agricultural land area that is actively irrigated.

Statistical Analysis. Prior to all statistical analyses, covariate integrity was checked and MIC-to-RTL ratios were log₁₀-transformed. For all statistical tests, an α -level of 0.05 was used. Mean (parametric) or median (nonparametric) 95% confidence intervals (95% CI) of MIC–RTL distributions of

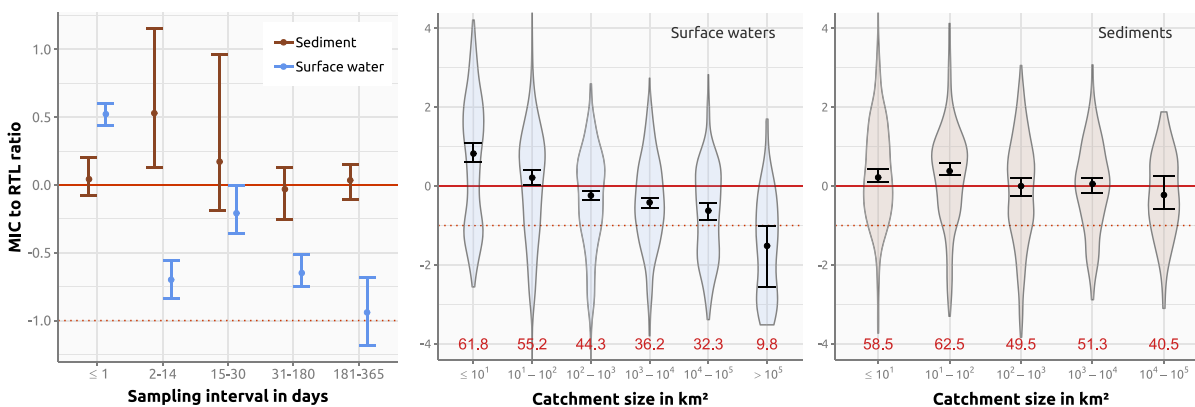


Figure 1. MIC-to-RTL ratios (\log_{10} -transformed) for surface waters (blue) and their sediments (brown) with their median (dot), their corresponding 95% CI (black error bars), and smoothed distributions (violin plots). RTLs are depicted with a solid red line, and threshold concentrations of adverse effects on regional biodiversity are represented by dotted lines.^{7,10} Annotations (red) detail the RTL exceedance frequencies (%) per respective group. Please note that the horizontal axis of the left plot is discrete and data are offset slightly horizontally to circumvent the overlap of medians with CIs.

various groups (e.g., catchment size $\leq 10 \text{ km}^2$) were compared with their RTL. Two sample tests, describing significant differences between MIC–RTL distributions (i.e., insecticide risk) of the different groups, were performed using either Student's *t*-test (parametric) or Mann–Whitney's *U*-test (nonparametric). All calculations and figures were produced using R (R base: Ver. 3.5.1, 64-bit, Windows 10) and QGIS (Ver. 3.6.0 Noosa, 64-bit, Windows 10).^{32,33} All figures can also be accessed online in an interactive format (<https://static.magic.eco/drivers>).

Driver Identification. Multiple linear regressions (MLRs) were used to test for significant relationships between insecticide risks (i.e., MIC-to-RTL ratio as an dependent variable) and covariates (independent variables, see the Supporting Information), here generally referred to as risk drivers. MLRs were built manually to describe individual MIC-to-RTL ratios (freshwater, sediments) or mixture MIC-to-RTL ratios (freshwater) and aimed at providing good explanatory power while remaining as parsimonious as possible. Significant independent variables, providing only marginal gains in explanatory power ($<1\%$), were removed from the model to prevent overfitting. Factorial attributes were dummy-coded and only substances with more than 20 MICs were used for modeling. Automatic model building (e.g., bidirectional stepwise variable selection) was not used here because of the drawbacks associated with this method.^{34,35}

Regression Validation. MLRs were primarily validated using established test protocols detailed in Unwin.³⁶ Briefly, the normality distribution of residuals, homoscedasticity, independence of errors, significance of predictors, presence of outliers or leverage points, and potential collinearity of predictors were individually assessed as detailed in the Supporting Information. Adjusted Pearson correlation coefficients (adj. R^2) were used to describe the correlation of MLRs.

Model variability (i.e., robustness) was additionally assessed following internal validation procedures using a bootstrapping approach ($n = 10\,000$), although the risk of overfitting models can be considered very low because of high sample-to-predictor ratios ($n \gg 20$).^{37,38} On the basis of these simulation results, 95% CIs were calculated for each model's adjusted R^2 to determine its robustness, and 95% CIs of predictor estimates were calculated to test for their statistical

significance. Model predictors were only used in final models if their significance was determined in the main model and based on bootstrapped 95% CIs.

Model Application. National risk estimations for 20 insecticide compounds were calculated for 2017 on an equal-area $20 \times 20 \text{ km}$ raster grid using the previously validated MLR for individual MIC-to-RTL ratios in the freshwater phase (see Table S3 and Figure S1). Predicted insecticide concentration-to-RTL ratios will be referred to as “PIC-to-RTL ratios” and describe acute risks. Most recent and complete insecticide use estimates (2014–2016) were used to define which insecticides may occur in respective RCAs and to attribute their average toxicity-normalized use (2014–2016) per RCA.

For each raster cell ($n = 19\,531$), the average PIC-to-RTL ratio was determined based on simulation runs ($n = 1000$ Monte-Carlo runs), (i) assuming a daily sampling interval, (ii) randomly selecting divergent-adjusted upstream catchment areas³⁹ (i.e., catchment size) in close proximity to each raster cell's centroid ($\leq 10 \text{ km}$), (iii) applying toxicity-normalized insecticide use of the respective RCA, (iv) using the average burst factor per RCA,²⁹ (v) introducing the percentage of irrigated agricultural land per RCA,³¹ and (vi) exemplifying risks for the year of 2017 (for other scenarios, see https://static.magic.eco/drivers_map). Divergent-adjusted upstream areas from the NHDPlus hydrogeographic data set³⁹ provide a sufficient representation of local catchments of all size classes; therefore, small, yet highly vulnerable catchments ($\leq 100 \text{ km}^2$) were also accounted for on the local scale. Finally, risk estimation was limited to areas with at least 5% land-use for crops (excluding pastures and silviculture), resulting in risk estimations for approximately 94.3% of US agricultural areas.

Additionally, the total flow lengths of all available stream features (NHDPlus,³⁹ $n = 2\,691\,339$) were summed across all raster cells to determine the total extent (i.e., flow length in 1000 km) of stretches with potentially adverse in-stream concentrations for small ($\leq 100 \text{ km}^2$), medium ($>100\text{--}1000 \text{ km}^2$), and large ($>1000 \text{ km}^2$) watersheds. For sediments, MLRs were deemed too unspecific with regard to spatial factors affecting regional insecticide risks; therefore, no national risk estimation was conducted.

Table 1. Detailed Statistics Summarizing the MLR Results of Individual MIC-to-RTL Ratios for Freshwaters (R^2 adj. = 62.17, $n = 1,833$, $p < 0.001$)^a

predictor variable	estimate	std. error	t-value	p-value	std. beta	SBR ^b	VIF
intercept	-1.927	0.275	-6.996	<0.001			
catchment size [km ²] ^c	-0.150	0.020	-7.387	<0.001	-0.146	4	1.897
sampling interval [d] ^c	-0.198	0.017	-11.496	<0.001	-0.189	3	1.311
time [y]	-0.057	0.004	-13.200	<0.001	-0.287	2	2.283
toxicity-normalized use [kg × RTL ⁻¹] ^c	0.386	0.020	18.815	<0.001	0.381	1	1.987
burst factor	0.015	0.002	5.990	<0.001	0.131	5	2.310
irrigated agricultural land-use [%]	0.426	0.073	5.832	<0.001	0.101	6	1.448
organoph./carb. [factor]	0.240	0.227	1.055	0.292			1.653
pyrethroids [factor]	1.228	0.231	5.315	<0.001			1.653
neonicotinoids [factor]	0.431	0.237	1.818	0.069			1.653
phenylpyrazole [factor]	1.234	0.254	4.855	<0.001			1.653

^aSignificant predictors ($p < 0.05$) are printed in bold. Standardized betas with their respective rank (SBR) detail numerical predictors' impact on insecticide risks. Variance inflation factors (VIFs) larger than three would indicate collinearity of predictors. Insecticide classes represent the average difference to the reference insecticide class of organochlorines. Organophosphates and carbamates ("Organoph./Carb.") are grouped because of their similar toxic mode of action (Table S5). ^bRanked standardized betas of linear model. ^cPredictor variable was transformed by the decadal logarithm.

RESULTS AND DISCUSSION

Risk Drivers. In surface waters ($n = 3699$), median risks expressed as MIC-to-RTL ratios decreased sharply with increasing sampling intervals (Figure 1). Daily and event sampling (high-frequency sampling, HFS, ≤ 1 d) quantified MICs significantly above the respective RTLs (based on 95% CIs) by a factor of approximately 3.3, suggesting that MICs can be commonly detected above RTLs in agricultural water bodies if temporal sampling resolution is sufficiently high.²¹ In comparison, bimonthly and monthly sampling strategies (low-frequency sampling, LFS) produced median MIC-to-RTL ratios that differed by factors of 16.6 and 5.4, respectively, lower than those from HFS (Figure 1). In line with our results, Spycher et al.⁴⁰ recently showed that pesticide risks in agriculturally dominated watersheds are underestimated by an order of magnitude or more if temporal sampling resolution is low. Similarly, Stehle and Schulz¹⁰ showed at the global scale that risks increase drastically if HFS is applied. In light of monitoring schemes, commonly applying fixed-interval samplings with time intervals >7 days between individual measurements (cf. Stehle et al.⁴¹), the differences between HFS and LFS may indicate a general underestimation of insecticide risk by monitoring. While the results underscore how increasing sampling intervals strongly affected risk characterizations negatively, it is noteworthy that the substantially lower risks of 2–14 d sampling may be disproportionately affected by other covariates, an effect addressed using a modeling approach (see Table 1).

In sediments ($n = 1686$), average MIC-to-RTL ratios did not significantly differ between sampling strategies (based on 95% CIs, Figure 1). Downstream sediment transport is assumed to be slower than in the overlaying water column; thus, particle-bound insecticides can be quantified more reliably. In addition, residence times are also higher because of substances' significantly ($n = 16$, $p < 0.001$) higher stability in sediments (i.e., DT_{50} , Table S4). Therefore, assessing risks in sediment-dwelling communities appear less prone to errors regarding the magnitude of MICs, resulting from different sampling strategies.

Risks also differed significantly between water bodies of varying catchment sizes (Figure 1), in line with assessments on the national⁴⁰ and global scale.¹⁰ In headwater streams (≤ 10

km², $n = 857$, Figure 1), 61.8% of MIC_{sw} significantly exceeded respective RTL_{sw} by an average factor of 6.6, marking a clear deviation from regulatory-acceptable concentrations that are assumed to be protective of aquatic life and their functional integrity.^{14,22} Even when catchment sizes increase ($10 < \text{area} \leq 100$ km², $n = 603$), 55.2% of MIC_{sw} exceeded their RTL by a factor of approximately 1.63 (Figure 1). Moreover, in headwater streams (≤ 10 km²), 76.4% of MIC_{sw} ($n = 857$, Figure 1) exceeded thresholds at which regional aquatic biodiversity is significantly reduced by at least 12% (=“biodiversity thresholds”).^{7,10} Low-order streams, which appear disproportionately exposed to insecticides,⁴ represent pivotal ecosystems of high local biodiversity, providing critical functions such as recolonization refuges, spawning grounds for endangered species, and sources of organic matter breakdown crucial for downstream consumers.^{18,42–44} Our results underscore that these important ecosystems across the United States are exposed to various insecticides at ecologically relevant concentrations for decades, likely contributing to the ongoing biodiversity decline.² In contrast, large water bodies (>1000 km²) exhibited substantially lower risks than small water bodies, a result most likely caused by less direct inputs because of smaller riparian length-to-volume ratios, higher dissipation (e.g., degradation and adsorption), and dilution.

In sediments, risks were distributed more evenly among catchment sizes with only a slight negative trend (Figure 1). Sediment erosion decreases further downstream and sedimentation outweighs erosion at a certain point;⁴³ therefore, a risk profile that is more equally distributed appears reasonable as contaminated sediments are transported from headwaters to larger, slow-flowing parts of the river continuum. As a result, sediment toxicity was found to be more widespread compared to water-phase exposures, yet peak sediment exposures in headwaters are less pronounced. Binding and subsequent distribution of sediment-associated insecticides, however, is a complex environmental process that depends on chemical (i.e., DT_{50} and K_{OC}) and highly variable hydrological properties⁴⁵ (e.g., flow speed, turbidity, f_{OC} , and grain size distribution) determining exposure at the respective sampling sites. This process may also vary locally, given the interaction of aforementioned factors (see Hall et al.⁴⁶). Overall, our data suggest that decades of repeated insecticide applications may

Table 2. Detailed Statistics Summarizing the MLR Results of Individual MIC-to-RTL Ratios for Sediments (R^2 adj. = 49.59, n = 857, $p < 0.001$)^a

predictor variable	estimate	std. error	t-value	p-value	std. beta	SBR ^b	VIF
intercept	-2.390	0.301	-7.948	<0.001			
catchment size [km ²] ^c	-0.101	0.027	-3.742	<0.001	-0.097	6	1.150
sampling interval [d] ^c	-0.052	0.019	-2.775	<0.001	-0.075	7	1.257
time [y]	-0.067	0.009	-7.863	<0.001	-0.215	2	1.274
toxicity-normalized use [kg × RTL ⁻¹] ^c	0.186	0.036	5.201	<0.001	0.170	3	1.808
lentic bodies [factor]	-0.370	0.106	-3.502	<0.001	-0.098	5	1.336
irrigated agricultural land-use [%]	0.483	0.103	4.683	<0.001	0.126	4	1.236
K_{OC} [L/kg] ²	0.770	0.063	12.302	<0.001	0.506	1	2.879
organoph./carb. [factor]	-0.793	0.173	-4.578	<0.001			1.368
pyrethroids [factor]	-0.423	0.168	-2.519	0.012			1.368
phenylpyrazole [factor]	-0.815	0.418	-1.949	0.052			1.368

^aSignificant predictors ($p < 0.05$) are printed in bold. Standardized betas with their respective rank (SBR) detail numerical predictors' impact on insecticide risks. VIFs larger than 3 would indicate collinearity of predictors. Insecticide classes represent the average difference to the reference insecticide class of organochlorines. Organophosphates and carbamates ("Organoph./Carb.") are grouped because of their similar toxic mode of action (Table S5). ^bRanked standardized betas of linear model. ^cPredictor variable was transformed by the decadal logarithm.

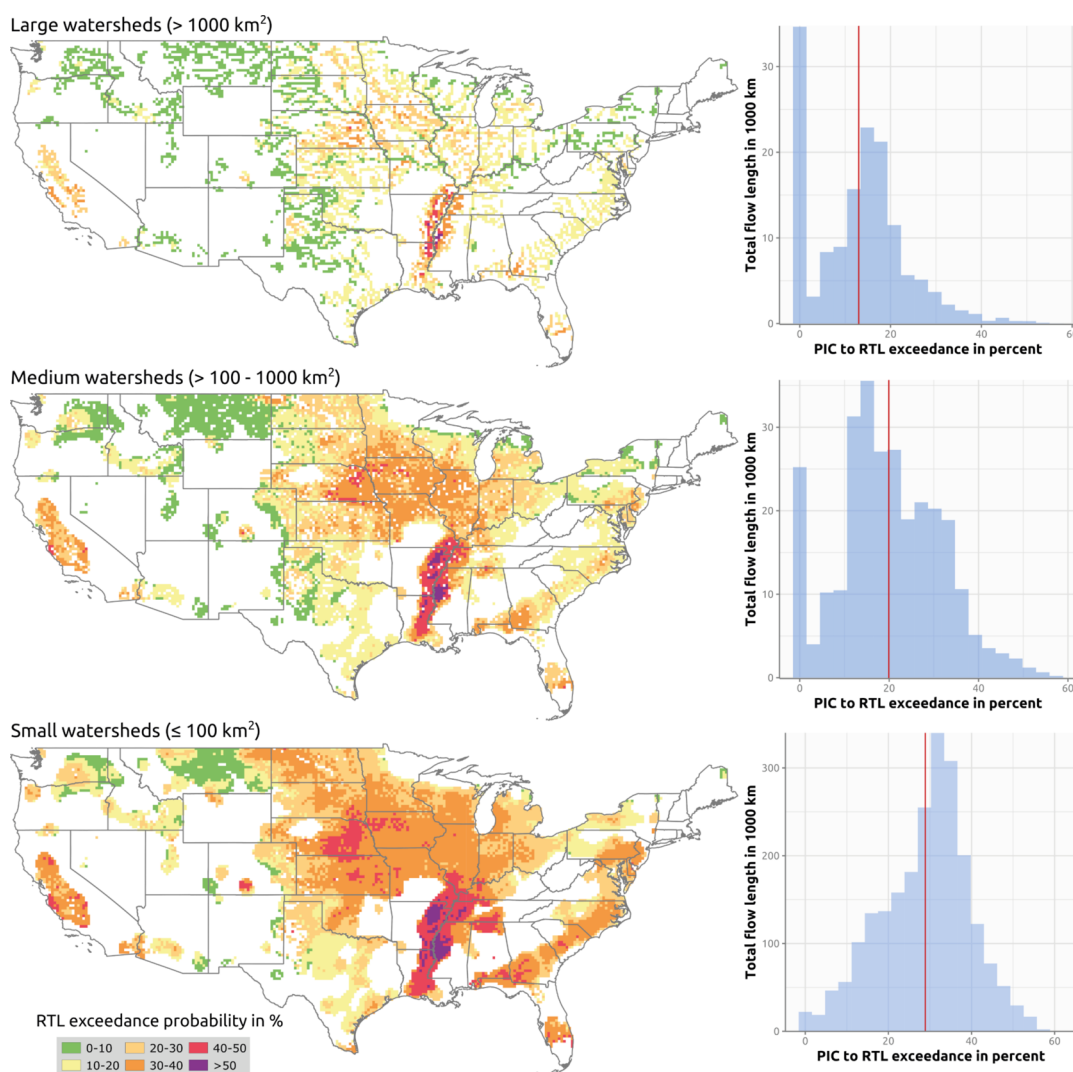


Figure 2. National model application of PIC-to-RTL ratios in freshwaters for small (≤ 100 km², bottom), medium (>100 – 1000 km², center), and large (>1000 km², top) watersheds in the United States. Histograms show total flow lengths of streams or rivers relative to their RTL exceedance probability and the red line depicts average RTL exceedance probability weighted by flow length. Simulation results are based on MICs (i.e., quantifications) and thus refer to respective exceedance probabilities of quantified insecticide concentrations.

have resulted in widespread and long-term contamination of sediments along the entire longitudinal gradient of lentic

systems, also driven by the insecticides' higher persistence in sediments (e.g., bifenthrin, esfenvalerate, and fipronil, see

Table S4), a risk scenario that may be furthermore exacerbated when accounting for the presence of legacy pesticides, such as DDT and lindane.⁴⁷

Identification and weighing of risk drivers against each other were achieved using MLR (R^2 adj. = 57.69, n = 2,031, p < 0.001, Table 1), and additional model validation can be found in the Supporting Information (Table S3, Figure S1), for substance-specific discussions, see Wolfram et al.¹¹ MIC-to-RTL ratios, judged by standardized betas (cf. Table 1), were by far most dependent on insecticide use, with a substantially lower influence of average burst rainfall erosivity. Pronounced use of substances, which may reach hazardous concentrations (e.g., bifenthrin and fipronil), combined with irregular erosive rainfall events (expressed by the burst factor) facilitates a strong runoff potential with subsequent high transient toxicant loads, observed as “first flushes” during winter months, for example, in California.⁴⁸ Risks are furthermore positively related with areas characterized by high degrees of irrigated agricultural land-use (Table 1). Various reasons exist, why local irrigation practices may promote risks, such as higher drainage potentials, an increased water to land interface area because of higher densities of smaller water bodies (e.g., discharge channels), or higher agricultural intensification indicated by irrigation practices, all of which cause higher amounts of insecticides to enter nontarget aquatic ecosystems.^{49–53} Sampling intervals and catchment sizes correlate negatively with average MIC-to-RTL ratios, in line with previously discussed results (see Figure 1). Since 1992, the average insecticide risk has decreased slightly over time (Table 1) while, at the same time, the use of some high-risk insecticides has increased.¹¹ A shift in the focus of recent monitoring toward neonicotinoids, measured at relatively low concentrations in rather large waterbodies,^{54,55} may explain this phenomenon. Finally, insecticide classes showed significantly different inherent risk characteristics (cf. Table 1) and a detailed discussion can be found in Wolfram et al.¹¹

In sediments, a correlation (R^2 adj. = 49.59, n = 857, p < 0.001) between MIC-to-RTL ratios and risk drivers was also found (Table 2), and additional model validation can be found in the Supporting Information (Table S6, Figure S2); for substance-specific discussion, see Wolfram et al.¹¹ The average risks in sediments were most dependent on the substances' tendency to partition onto organic matter (i.e., K_{OC} , Table 2). Highly sorptive substances, such as pyrethroids (e.g., deltamethrin, bifenthrin, and lambda-cyhalothrin) or organochlorines (e.g., endosulfan and dicofol), may pose disproportionate risks to sediment-dwelling organisms in aquatic ecosystems, which may currently be underestimated.⁵⁶ Spatial risk drivers (i.e., toxicity-normalized use and percent-irrigated land-use, Table 2) affected average risks in sediments similarly to surface water risks (cf. Tables 1 and 2), underlining that risk may be most pronounced in highly intensified areas such as the Mississippi Portal, California, and the central United States. However, within RCAs, rainfall erosivity²⁹ and the presence of soil restrictive layers³⁰ did not significantly describe local sediment risks. This is surprising, as both factors were found to play critical roles regarding pesticide fate¹⁷ and sediment loss to runoff.⁵⁷ Sampling intervals and catchment sizes, as previously discussed in detail (see Figure 1), were found to explain average sediment risks only to a lower extent than it has been shown for water. In lotic systems, sediment risks were significantly lower (Table 2), by a factor of approximately 3, when compared to lotic systems. Lentic systems may in

many cases require direct insecticide inputs, for instance, because of adjacency to agricultural fields (e.g., runoff and spray drift) in order to show contamination, whereas in lotic systems, transport of contaminated sediments from upstream areas, which also have substantially higher water–land intersect areas, can result in higher sediment exposures in depositional areas. While sediment risks are also slowly declining over time (Table 2), risk drivers in sediments appear less conclusive, potentially because of lower data availability and more even distributions through time and space when compared to the water phase (cf. Tables 1 and 2).

National Risk Estimations. Model application (Figure 2), yielding PIC-to-RTL ratios in freshwaters (R^2 = 62.17, p < 0.001, see Table 1), highlighted geographical regions, in which current risk drivers suggest acute effects for nontarget aquatic organisms. Please note that the PIC-to-RTL exceedance probabilities are based on quantified insecticide concentrations; thus, they detail estimated probabilities of a respective insecticide exceeding their RTL, when detected, as opposed to each measurement, which often can be a non-detect.⁴¹ Furthermore, interactive simulation results for different years can be accessed at https://static.magic.eco/drivers_map.

Small watersheds (≤ 100 km²) showed the highest insecticide risks, with grid cells on average showing RTL exceedance probabilities of 27.7% (n = 9968). As a result, half of the total stream length is estimated to experience at least 28.9% of RTL exceedances in small watersheds, increasing even to 60.5% in some regions (Figure 2). Risks were most widespread, occurring predominantly in four major crop production regions: the Southern Seaboard including Florida, the Mississippi Portal, coastal and northern parts of California, and the Heartland.⁵⁸ Cropping patterns are highly different in these regions; nonetheless, the results show that differing application patterns can lead to aquatic risks in agro-economically different regions. Further investigation and application of suitable risk reduction techniques on lower spatial scales are required in these regions^{50,59} if environmentally sustainable agricultural practices are to be achieved, protecting freshwater systems that are currently at peril. In contrast, the average risks of all grid cells for medium-sized and large watersheds decreased sharply to 20.1% (n = 9031) and 13.1% (n = 4495), respectively, comparing well with the risk reductions detailed in Figure 1. Risks were substantially lower in these freshwaters as only half of the total stream length of medium and large watersheds is estimated to show RTL exceedances above 19.9 and 13.0% (Figure 2). Moreover, 10.2 and 22.2% of medium-sized and large watersheds, respectively, are estimated to have no RTL exceedances. Most of the stream length with elevated risks is located in the Mississippi Portal, while in other parts of the United States, risks are distributed more sparsely (Figure 2). Thus, insecticide risks may be most prominent in the Mississippi Portal, a hydrologically and biologically diverse river ecosystem that provides habitat for several endangered or threatened species.⁶⁰

Overall, our analysis revealed a widespread risk of insecticides impairing predominantly low-order streams that amount to 5.9 times the total stream length compared to larger catchments (>100 km², cf. Figure 2), suggesting that currently applied insecticides pose risks to surface water integrity at the national scale, and comprehensive reevaluation of their safe-use, as conducted by the US EPA for pyrethroids,⁶¹ appears justified. These small water bodies and current-use modern insecticides are not yet contained in the WARP approach;¹⁷

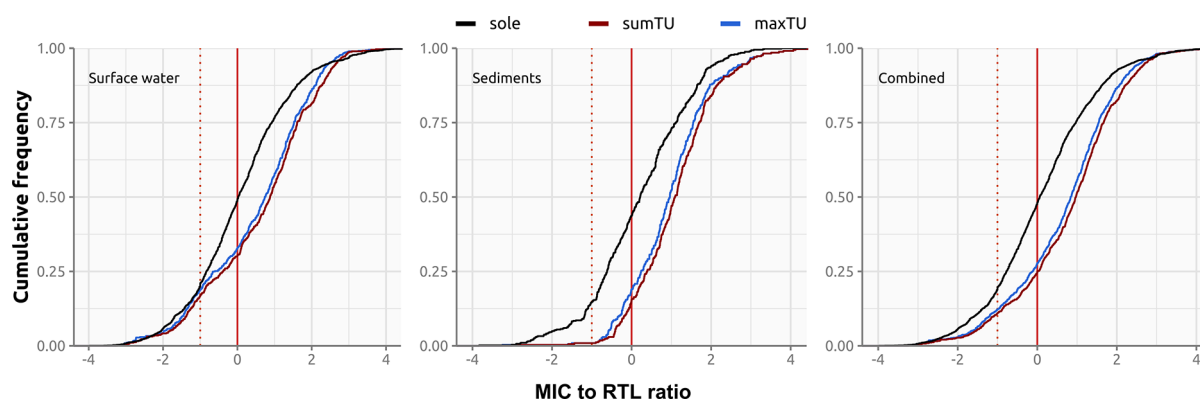


Figure 3. Cumulative frequency distributions of MIC-to-RTL ratios (\log_{10} -transformed) determined for insecticide mixtures for surface waters (left), their sediments (center), and the combination of both compartments (right). Frequency distributions using sole MIC-to-RTL ratios (i.e., those not occurring in mixtures, black) are compared to distributions of mixtures either using only the most toxic component (maxTU) in blue or the sum of all components (sumTU) in red. RTLs are depicted with a solid red line, and threshold concentrations of adverse effects on regional biodiversity^{7,10} are represented by dotted lines.

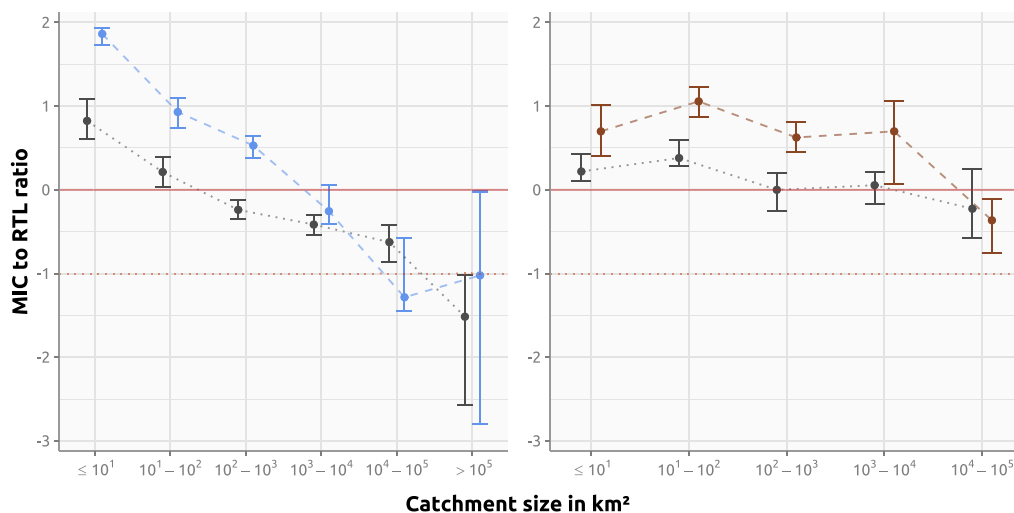


Figure 4. Median MIC-to-RTL ratios (\log_{10} -transformed) of maxTUs (dot) with their 95% CIs (bars) of surface waters (blue, left) and sediments (brown, right) for different catchment-size classes in square kilometers. Individual MIC-to-RTL ratios (black), as depicted in Figure 1, are provided for comparison purposes. RTLs are depicted with a solid red line, and threshold concentrations of adverse effects on regional biodiversity^{7,10} are represented by dotted lines. Please note that the horizontal axis is discrete and data have been offset slightly horizontally to circumvent the overlap of medians with CIs.

thus, the present model provides, for the first time, a comprehensive aquatic risk characterization for a wide range of relevant insecticides at the national level, highlighting most vulnerable regions for watersheds of various sizes.

It is important to note that a modeling approach, using data obtained via comprehensive meta-analysis, is limited by several aspects. First, high-resolution time series data, for instance, obtained via targeted integrative monitoring, are generally not broadly available. Therefore, fine-grained, small-scale (i.e., local) temporal risk profiles cannot be obtained with the present approach and need to be supplemented with different methodologies (see Ippolito and Fait⁶²). Second, with modeling results representing acute exposures, prolonged, repeated, diet-related, or indirect effects are not estimated. Third, nonagricultural insecticide use, which is estimated to account for approximately 40% of US insecticide use, is not considered in the current model because of lacking data availability. Nonagricultural insecticide use is shown to adversely affect nontarget aquatic systems;^{63,64} thus, especially in mixed land-use settings, risks may differ considerably.

On the other hand, the model presented here uses a generalizing approach, contrasting elaborate watershed-scale models,¹⁷ thus enabling the future application on large spatial scales in combination with novel chemical data information tools.⁶⁵ In these larger contexts, small-scale models (e.g., DEM-hydrographic models) are rarely comprehensively available and integrative trans-national data on effects of pesticide use are still very sparse.^{10,66}

Insecticide Mixture Toxicity. The frequency of sole MICs exceeding respective RTLs in surface waters was approximately 50.7% ($n = 2208$), whereas the frequency increased to 67.1 and 69.7% in mixtures ($n = 577$) based on their maxTU and sumTU, respectively (Figure 3). In sediments, sole MICs exceeded respective RTLs in 56.3% of cases ($n = 584$), whereas exceedances amounted to 81.0 and 84.5% in mixtures ($n = 336$), based on maxTU and sumTU, respectively (Figure 3). MICs detected in mixtures yielded a 45.0% RTL exceedance ($n = 3038$, data not shown), when assessed as individual concentrations.

Table 3. Detailed Statistics Summarizing the MLR Results of Maximum Toxicant TUs (maxTU) for Surface Waters (R^2 adj. = 76.42, $n = 421$, $p < 0.001$)^a

predictor variable	estimate	std. error	t-value	p-value	std. beta	SBR ^b	VIF
intercept	-1.736	0.275	-6.302	<0.001			
catchment size [km ²] ^c	-0.314	0.044	-7.218	<0.001	-0.246	3	2.071
sampling interval [d] ^c	-0.130	0.035	-3.694	<0.001	-0.106	7	1.470
burst factor	0.018	0.005	3.955	<0.001	0.138	6	2.180
time [y]	-0.063	0.006	-10.435	<0.001	-0.269	2	1.180
toxicity-normalized use [kg × RTL ⁻¹] ^c	0.447	0.031	14.209	<0.001	0.411	1	1.490
number of detected substances	0.073	0.015	4.955	<0.001	0.151	5	1.665
irrigated agricultural land-use [%]	0.990	0.135	7.307	<0.001	0.209	4	1.453

^aSignificant predictors ($p < 0.05$) are printed in bold. Standardized betas with their respective rank (SBR) detail individual predictors' impact on insecticide risks. VIFs larger than 3 would indicate collinearity of predictors. ^bRanked standardized betas of linear model. ^cPredictor variable was transformed by the decadal logarithm.

The maxTU approach was found to be capable of describing risks similarly well compared to sumTUs (cf. Figure 3), as on average, ~76.7% of mixtures' toxicities and ~95.7% of exceedances were explained by the maxTU. Insecticide exposures are highly variable; thus, MIC-to-RTL ratios generally range over orders of magnitude rather than within magnitudes.⁶⁷ As a result, the sum of individual MIC-to-RTL ratios appears less sensitive to lower-risk exposures, which may be due to a low chance of transient insecticide peaks overlapping, insecticides showing heavily skewed toxicities, and applications being dominated by few compounds. Hence, risks are mainly explained by the highest MIC-to-RTL ratio within the respective samples. In addition, when determining sumTUs in the context of RTLs, one needs to keep in mind that most sensitive end points used to derive RTLs originate from different species or taxa, and the assumption of additivity does not necessarily apply to substances with dissimilar toxic modes of action (see Table S5), which was the case for 55.9% of mixtures here. Thus, the maxTU is associated with fewer limitations while describing risk comparatively well (Figure 4); therefore, it was used for further analyses and discussions. Still, it remains noteworthy that synergistic and antagonistic effects are not accounted for in both concepts, despite synergistic adjuvants being regularly used especially with high risk pyrethroids (e.g., piperonyl butoxide).

Our results underline that insecticide mixtures are important, as RTL exceedances increased by a factor of ~1.32 in surface waters and ~1.44 in sediments when assessing them based on maxTUs in comparison to sole concentrations (Figure 3). Yet, in situations, when comparably toxic compounds had been detected, which are often represented, for example, by pyrethroid insecticides, one single insecticide may explain up to 99% of the observed toxicity. Moreover, when considering aquatic toxicity from a regulatory perspective, herbicides and fungicides are considerably less toxic,^{9,68} and it may be argued that assessing the most toxic insecticide present in a sample sufficiently describes a sample's overall toxicity. This, however, should not be misinterpreted in a way that under specific conditions and for specific species, the risks of other pesticide types are not relevant. Finally, 81.1% of aforementioned MIC mixtures in surface waters ($n = 577$), 99.1% in sediments ($n = 336$), and 87.7% in both compartments combined ($n = 913$), exceeding biodiversity thresholds described by Stehle and Schulz,¹⁰ suggest that single insecticides, such as bifenthrin, cypermethrin, lambda-cyhalothrin, and chlorpyrifos (see also Wolfram et al.¹¹), may be the main culprit in regional biodiversity losses of agriculturally

dominated water bodies (Figure 3); thus, targeted risk reduction strategies for specific insecticides could substantially reduce toxic pressures throughout the United States, underscoring the potential for fiscally efficient water-quality improvements.

Strong correlation was found between previously identified risk drivers and maxTUs of mixtures (R^2 adj. = 75.17, $n = 496$, $p < 0.001$, Table 3), and additional model validation results can be found in the Supporting Information (Table S7, Figure S3). Higher correlation of maxTUs compared to individual MICs (cf. Tables 1 and 3) may be due to the removal of tailing insecticide concentrations that may precede or follow entry events.⁴⁰ The results also show that the total number of detected insecticides correlated positively with average risks. This is most likely a stochastic effect, such that the probability of detecting a high-risk substance increases alongside the number of detected substances. In particular, potential risks in low-order streams appear more pronounced when assessing mixture toxicity based on maxTUs (Figure 4, Table 3). A converging trend was found, resulting in significantly higher risks (based on 95% CIs) in small- to medium-sized watersheds (≤ 1000 km²), whereas in larger water bodies (> 1000 km²), no significant difference was found between individual and mixture risks (Figure 1). On an average, RTLs were exceeded by nearly 2 orders of magnitude in headwaters (≤ 10 km²; Figure 4), which underlines the potential threat that insecticides currently pose to low-order streams, whereas their risk potential is strongly reduced in large watersheds, even when co-occurring. In conclusion, individual MIC assessments tend to considerably underestimate actual risks in the field, where insecticides co-occur (Figure 4). In sediments, mixture toxicities appeared to be distributed more evenly across differently sized surface waters (Figure 4), which again highlights the importance of spatially large scale and temporally long-term contamination of sediments, in contrast to the more variable risk characteristics in the water phase.

Implications. In the future, following our results, some risk drivers may gain further importance in promoting nonpoint source insecticide contamination. Burst rainfall erosivity, in response to climate change,²⁹ is expected to increase across the majority of the United States. Also, patterns of insecticide use are changing as multiple substances have been phased-out or restricted in their use, while other insecticides, which, because of their comparably high toxicity, pose considerable risks to aquatic invertebrates (e.g., pyrethroids), have taken their place instead.¹¹ It is noteworthy that few substances in this analysis can be considered legacy insecticides (e.g., endosulfan, dicofol,

parathion, and azinphos-methyl), whereas the majority is still in active use. Legacy insecticides (e.g., DDT and lindane) often exhibit different physicochemical properties compared to most current-use insecticides, that is, higher persistence, hydrophobicity, vertebrate toxicity, and lower target selectivity, resulting in accumulation in bed sediments, biota, and constant redissolution into the aquatic phase.⁶⁹ As a result, their implications for aquatic organisms, particularly fish and chronic toxicity, should not be overlooked.⁴⁷ In conclusion, the interaction of risk drivers currently suggests that insecticides remain a large-scale and substantial threat to aquatic biodiversity and ecosystems functions and may, in some cases, even increase in the future.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.est.9b04285](https://doi.org/10.1021/acs.est.9b04285).

Covariates, MLR validation, internal validation, MLR simulation results, toxic modes of action, and insecticide stability data (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Campbell, B. M.; Beare, D. J.; Bennett, E. M.; Hall-Spencer, J. M.; Ingram, J. S.; Jaramillo, F.; Ortiz, R.; Ramankutty, N.; Sayer, J. A.; Shindell, D. Agriculture production as a major driver of the Earth system exceeding planetary boundaries. *Ecol. Soc.* **2017**, *22*, 8.
- (2) Vörösmarty, C. J.; McIntyre, P. B.; Gessner, M. O.; Dudgeon, D.; Prusevich, A.; Green, P.; Glidden, S.; Bunn, S. E.; Sullivan, C. A.; Liermann, C. R. Global threats to human water security and river biodiversity. *Nature* **2010**, *467*, 555–561.
- (3) U.S. Department of Agriculture USDA. National Agricultural Statistics Service, 2018 Crop Frequency Layers. <https://tinyurl.com/y47armyz> (accessed March 12, 2019).
- (4) Schulz, R. Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution. *J. Environ. Qual.* **2004**, *33*, 419–448.
- (5) Stone, W. W.; Gilliom, R. J.; Ryberg, K. R. Pesticides in U.S. Streams and Rivers: Occurrence and Trends during 1992–2011. *Environ. Sci. Technol.* **2014**, *48*, 11025–11030.
- (6) Van Metre, P. C.; Alvarez, D. A.; Mahler, B. J.; Nowell, L.; Sandstrom, M.; Moran, P. Complex mixtures of Pesticides in Midwest US streams indicated by POCIS time-integrating samplers. *Environ. Pollut.* **2017**, *220*, 431–440.
- (7) Beketov, M. A.; Kefford, B. J.; Schäfer, R. B.; Liess, M. Pesticides reduce regional biodiversity of stream invertebrates. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 11039–11043.
- (8) Schäfer, R. B.; von der Ohe, P. C.; Rasmussen, J.; Kefford, B. J.; Beketov, M. A.; Schulz, R.; Liess, M. Thresholds for the effects of pesticides on invertebrate communities and leaf breakdown in stream ecosystems. *Environ. Sci. Technol.* **2012**, *46*, 5134–5142.
- (9) Stehle, S.; Elsaesser, D.; Gregoire, C.; Imfeld, G.; Niehaus, E.; Passepport, E.; Payraudeau, S.; Schäfer, R. B.; Tournebize, J.; Schulz, R. Pesticide risk mitigation by vegetated treatment systems: a meta-analysis. *J. Environ. Qual.* **2011**, *40*, 1068–1080.
- (10) Stehle, S.; Schulz, R. Agricultural insecticides threaten surface waters at the global scale. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 5750–5755.
- (11) Wolfram, J.; Stehle, S.; Bub, S.; Petschick, L. L.; Schulz, R. Meta-Analysis of Insecticides in United States Surface Waters: Status and Future Implications. *Environ. Sci. Technol.* **2018**, *52*, 14452–14460.
- (12) Stehle, S.; Schulz, R. Pesticide authorization in the EU-environment unprotected? *Environ. Sci. Pollut. Res.* **2015**, *22*, 19632–19647.
- (13) *Federal Insecticide, Fungicide, and Rodenticide Act. Public Law 104-170, 1996; Code of Federal Regulations: Section 136, Title 7, 1996.*
- (14) U.S. EPA. *Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs*; U.S. Environmental Protection Agency—Endangered and Threatened Species Effects Determinations, U.S. Government Printing Office: Washington, DC, 2004; p 92.
- (15) Fowle, J. R.; Dearfield, K. L. *Risk Characterization Handbook*; Science Policy Council, U.S. Environmental Protection Agency: Washington, DC, 2000.
- (16) Zhang, X.; Luo, Y.; Goh, K. S. Modeling spray drift and runoff-related inputs of pesticides to receiving water. *Environ. Pollut.* **2018**, *234*, 48–58.
- (17) Stone, W. W.; Crawford, C. G.; Gilliom, R. J. Watershed Regressions for Pesticides (WARP) Models for Predicting Stream Concentrations of Multiple Pesticides. *J. Environ. Qual.* **2013**, *42*, 1838–1851.
- (18) Biggs, J.; Von Fumetti, S.; Kelly-Quinn, M. The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers. *Hydrobiologia* **2017**, *793*, 3–39.
- (19) Lyon, J. G.; Trimble, S. W.; Ward, A. D.; Burckhard, S. R. *Environmental Hydrology*; CRC Press: Boca Raton, 2015; p 695.
- (20) Smith, S. V.; Renwick, W. H.; Bartley, J. D.; Buddemeier, R. W. Distribution and significance of small, artificial water bodies across the United States landscape. *Sci. Total Environ.* **2002**, *299*, 21–36.
- (21) Baker, N. T. *Agricultural Pesticide Use Estimates for the USGS National Water Quality Network, 1992–2014*; U.S. Geological Survey data release, 2016.
- (22) U.S. EPA. Guidelines for Ecological Risk Assessment. *Fed. Regist.* **1998**, *63*, 26846–26924.
- (23) Nowell, L. H.; Norman, J. E.; Ingersoll, C. G.; Moran, P. W. Development and application of freshwater sediment-toxicity benchmarks for currently used pesticides. *Sci. Total Environ.* **2016**, *550*, 835–850.
- (24) Crommentuijn, T.; Sijm, D.; de Bruijn, J.; van Leeuwen, K.; van de Plassche, E. Maximum permissible and negligible concentrations for some organic substances and pesticides. *J. Environ. Manage.* **2000**, *58*, 297–312.
- (25) Nowell, L. H.; Norman, J. E.; Moran, P. W.; Martin, J. D.; Stone, W. W. Pesticide Toxicity Index-A tool for assessing potential toxicity of pesticide mixtures to freshwater aquatic organisms. *Sci. Total Environ.* **2014**, *476-477*, 144–157.
- (26) Schäfer, R. B.; Pettigrove, V.; Rose, G.; Allinson, G.; Wightwick, A.; von der Ohe, P. C.; Shimeta, J.; Kühne, R.; Kefford, B. J. Effects of pesticides monitored with three sampling methods in 24 sites on macroinvertebrates and microorganisms. *Environ. Sci. Technol.* **2011**, *45*, 1665–1672.
- (27) USGS StreamStats. <https://streamstats.usgs.gov/ss/> (accessed March 12, 2019).

- (28) U.S. Bureau of Census Cartographic Boundary Shapefiles—Counties. https://www.census.gov/geo/maps-data/data/cbf/cbf_counties.html (accessed March 12, 2019).
- (29) Biasutti, M.; Seager, R. Projected Changes in US Rainfall Erosivity. *Hydrol. Earth Syst. Sci.* **2015**, *19*, 2945.
- (30) Nakagaki, N. S.; Wesley, W.; Wiczorek, M. E. Estimates of the Soil Restrictive Layer in the Upper 25,35,45, and 55 centimeters of agricultural land in the conterminous United States. https://water.usgs.gov/GIS/metadata/usgswrd/XML/ssurgo_srlag.xml (accessed March 12, 2019).
- (31) Brown, J. F.; Pervez, M. S. Merging remote sensing data and national agricultural statistics to model change in irrigated agriculture. *Agric. Syst.* **2014**, *127*, 28–40.
- (32) Team R Core. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Austria, 2015. In ISBN 3-900051-07-0: URL <http://www.R-project.org>, 2018.
- (33) Team, Q. D. QGIS Geographic Information System; Open Source Geospatial Foundation Project, 2016.
- (34) Whittingham, M. J.; Stephens, P. A.; Bradbury, R. B.; Freckleton, R. P. Why do we still use stepwise modelling in ecology and behaviour? *J. Anim. Ecol.* **2006**, *75*, 1182–1189.
- (35) Babyak, M. A. What you see may not be what you get: a brief, nontechnical introduction to overfitting in regression-type models. *Psychosom. Med.* **2004**, *66*, 411–421.
- (36) Unwin, A. Discovering Statistics Using R by Andy Field, Jeremy Miles, Zoë Field. *Int. Stat. Rev.* **2013**, *81*, 169–170.
- (37) Steyerberg, E. W.; Harrell, F. E., Jr.; Borsboom, G. J. J. M.; Eijkemans, M. J. C.; Vergouwe, Y.; Habbema, J. D. F. Internal validation of predictive models. *J. Clin. Epidemiol.* **2001**, *54*, 774–781.
- (38) Harrell, F. E., Jr. *Regression Modeling Strategies: with Applications to Linear Models, Logistic and Ordinal Regression, and Survival Analysis*; Springer, 2015.
- (39) McKay, L.; Bondelid, T.; Dewald, T.; Johnston, J.; Moore, R.; Rea, A. *NHDPlus Version 2: User Guide*; US Environmental Protection Agency, 2012.
- (40) Spycher, S.; Mangold, S.; Doppler, T.; Junghans, M.; Wittmer, I.; Stamm, C.; Singer, H. Pesticide Risks in Small Streams—How to Get as Close as Possible to the Stress Imposed on Aquatic Organisms. *Environ. Sci. Technol.* **2018**, *52*, 4526–4535.
- (41) Stehle, S.; Knäbel, A.; Schulz, R. Probabilistic risk assessment of insecticide concentrations in agricultural surface waters: a critical appraisal. *Environ. Monit. Assess.* **2013**, *185*, 6295–6310.
- (42) Relyea, R. A. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecol. Appl.* **2005**, *15*, 618–627.
- (43) Vannote, R. L.; Minshall, G. W.; Cummins, K. W.; Sedell, J. R.; Cushing, C. E. The river continuum concept. *Can. J. Fish. Aquat. Sci.* **1980**, *37*, 130–137.
- (44) Vander Vorste, R.; McElmurray, P.; Bell, S.; Eliason, K.; Brown, B. Does stream size really explain biodiversity patterns in lotic systems? A call for mechanistic explanations. *Diversity* **2017**, *9*, 26.
- (45) Suedel, B. C.; Rodgers, J. H. Variability of bottom sediment characteristics of the continental United States. *J. Am. Water Resour. Assoc.* **1991**, *27*, 101–109.
- (46) Hall, L. W., Jr.; Anderson, R. D.; Killen, W. D. Mapping the spatial extent of depositional areas in agricultural, urban, and residential California Streams: implications for pyrethroid toxicity. *Hum. Ecol. Risk Assess. Int. J.* **2012**, *18*, 368–392.
- (47) Rasmussen, J. J.; Wiberg-Larsen, P.; Baatrup-Pedersen, A.; Cedergreen, N.; McKnight, U. S.; Kreuger, J.; Jacobsen, D.; Kristensen, E. A.; Friberg, N. The legacy of pesticide pollution: An overlooked factor in current risk assessments of freshwater systems. *Water Res.* **2015**, *84*, 25–32.
- (48) Weston, D. P.; Chen, D.; Lydy, M. J. Stormwater-related transport of the insecticides bifenthrin, fipronil, imidacloprid, and chlorpyrifos into a tidal wetland, San Francisco Bay, California. *Sci. Total Environ.* **2015**, *527*–528, 18–25.
- (49) Howell, T. A. Enhancing water use efficiency in irrigated agriculture. *Agron. J.* **2001**, *93*, 281–289.
- (50) Kröger, R.; Moore, M. T.; Locke, M. A.; Cullum, R. F.; Steinriede, R. W., Jr.; Testa, S.; Bryant, C. T.; Cooper, C. M. Evaluating the influence of wetland vegetation on chemical residence time in Mississippi Delta drainage ditches. *Agric. Water Manag.* **2009**, *96*, 1175–1179.
- (51) Mize, S. V.; Porter, S. D.; Demcheck, D. K. Influence of fipronil compounds and rice-cultivation land-use intensity on macroinvertebrate communities in streams of southwestern Louisiana, USA. *Environ. Pollut.* **2008**, *152*, 491–503.
- (52) Oster, J. D.; Grattan, S. R. Drainage water reuse. *Irrig. Drain. Syst.* **2002**, *16*, 297–310.
- (53) Rhoades, J. *Sustainability of Irrigation: An Overview of Salinity Problems and Control Strategies*; US Salinity Laboratory: Riverside, California, 1997.
- (54) Hladik, M. L.; Kolpin, D. W. First national-scale reconnaissance of neonicotinoid insecticides in streams across the USA. *Environ. Chem.* **2016**, *13*, 12–20.
- (55) Hladik, M. L.; Kolpin, D. W.; Kuivila, K. M. Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA. *Environ. Pollut.* **2014**, *193*, 189–196.
- (56) Rogers, H. A.; Schmidt, T. S.; Dabney, B. L.; Hladik, M. L.; Mahler, B. J.; Van Metre, P. C. Bifenthrin causes trophic cascade and altered insect emergence in mesocosms: implications for small streams. *Environ. Sci. Technol.* **2016**, *50*, 11974–11983.
- (57) Dabney, S. M.; Yoder, D. C.; Vieira, D. A. N.; Bingner, R. L. Enhancing RUSLE to include runoff-driven phenomena. *Hydrol. Process.* **2011**, *25*, 1373–1390.
- (58) Heimlich, R. *Farm Resource Regions*; United States Department of Agriculture - Economic Research Service, 2000; p 2.
- (59) Moore, M. T.; Cooper, C. M.; Smith, S.; Cullum, R. F.; Knight, S. S.; Locke, M. A.; Bennett, E. R. Diazinon mitigation in constructed wetlands: influence of vegetation. *Water, Air, Soil Pollut.* **2007**, *184*, 313–321.
- (60) U.S. Fish & Wildlife Service Listed Species Believed or Known to Occur in Mississippi. <https://tinyurl.com/y25vlqco> (accessed July 10, 2019).
- (61) U.S. EPA. *Preliminary Comparative Environmental Fate and Ecological Risk Assessment for the Registration Review of Eight Synthetic Pyrethroids and Pyrethrins*; U.S. EPA: Washington, DC, 2016; p 800.
- (62) Ippolito, A.; Fait, G. Pesticides in surface waters: from edge-of-field to global modelling. *Curr. Opin. Environ. Sustain.* **2019**, *36*, 78–84.
- (63) Amweg, E. L.; Weston, D. P.; You, J.; Lydy, M. J. Pyrethroid insecticides and sediment toxicity in urban creeks from California and Tennessee. *Environ. Sci. Technol.* **2006**, *40*, 1700–1706.
- (64) Atwood, D.; Paisley-Jones, C. *Pesticides Industry Sales and Usage: 2008–2012 Market Estimates*; United States Environmental Protection Agency: Washington, DC, USA, 2017.
- (65) Bub, S.; Wolfram, J.; Stehle, S.; Petschick, L.; Schulz, R. Graphing Ecotoxicology: The MAGIC Graph for Linking Environmental Data on Chemicals. *Data* **2019**, *4*, 34.
- (66) Malaj, E.; von der Ohe, P. C.; Grote, M.; Kühne, R.; Mondy, C. P.; Usseglio-Polatera, P.; Brack, W.; Schäfer, R. B. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, 9549–9554.
- (67) Stehle, S.; Bub, S.; Schulz, R. Compilation and analysis of global surface water concentrations for individual insecticide compounds. *Sci. Total Environ.* **2018**, *639*, 516–525.
- (68) Zubrod, J. P.; Bundschuh, M.; Arts, G.; Brühl, C. A.; Imfeld, G.; Knäbel, A.; Payraudeau, S.; Rasmussen, J. J.; Rohr, J.; Scharmüller, A.; Smalling, K.; Stehle, S.; Schulz, R.; Schäfer, R. B. Fungicides: An Overlooked Pesticide Class? *Environ. Sci. Technol.* **2019**, *53*, 3347–3365.
- (69) McKnight, U. S.; Rasmussen, J. J.; Kronvang, B.; Binning, P. J.; Bjerg, P. L. Sources, occurrence and predicted aquatic impact of legacy and contemporary pesticides in streams. *Environ. Pollut.* **2015**, *200*, 64–76.

Supporting Information

for

Insecticide Risk in United States Surface Waters: Drivers and Spatiotemporal Modeling

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Methods:

List of degradation products

In the following, a list of all degradation products that were deemed to be potentially relevant to aquatic environments, is provided and more detailed descriptions for their inclusion in this meta-analysis can be found in Wolfram et al.¹:

Endosulfan sulfate, azinphos-methyl oxygen analog, 3,5,6-trichloro-2-pyridinol, chlorpyrifos oxygen analog, chlorpyrifos oxon, diazoxon, diazinon oxon, malaoxon, isomalathion, malathion dicarboxylic acid, malathion monocarboxylic acid, paraoxon, methyl paraoxon, 3-hydroxycarbofuran, 3-ketocarbofuran, carbofuran phenol, alpha-R-deltamethrin, 6-chloronicotinic acid, imidacloprid guanidine, imidacloprid olefin, imidacloprid olefinic-guanidine, imidacloprid urea, o-p-dichlorobenzophenone, p-p-dichlorobenzophenone, 1,1-bis(p-chlorophenyl)-2,2-dichloroethanol, dichlorobenzhydrol, hydroxyl-dichlorobenzophenone, aldicarb sulfoxide, aldoxycarb, aldicarb sulfone, N-(2-chloro-5-thizolylmethyl)-N'-nitroguanidine, 1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl)-guanidine, 1-methylguanidinium chloride, 3-(methylamino)-9-oxa-2-aza-4-azoniabicyclone-3-enehydrogen succinate, 1-methyl-2-nitroguanidine, 1-methyl-3-(tetrahydro-3-furylmethyl)guanidine, 1-methyl-3-(tetrahydro-3-furylmethyl)-guanidinium dihydrogen phosphate, fipronil sulfone, fipronil sulfide, and desulfinyl fipronil.

Multiple linear regression validation

MLRs were validated following suggestions of Unwin² and references therein. Briefly, the normality distribution of the residuals was checked visually using histograms and QQ-Plots, while the independence of errors was tested using the Durbin-Watson-Test with $1 > d > 3$ representing thresholds that would suggest autocorrelation.³ Homoscedasticity was assessed visually by plotting standardized residuals versus their fitted values. Additionally, the

significance of all regression coefficients was checked using White's heteroscedasticity corrected covariance matrix⁴ and by calculating regression coefficient 95% confidence intervals. Both tests did not reveal any departure from the initial MLR results. Outliers were assessed with studentized residuals and standardized residuals, while potential leverage points were assessed using hat-values, DFFit, Cook's distance and covariance ratios. Multicollinearity was assessed using variance inflation factors with a threshold level of 3. For MLRs using factorial attributes with more than one degree of freedom (df), generalized variance inflation factors were calculated (GVIF, see Fox and Monette⁵). GVIFs were then transformed back via squaring, allowing comparison with traditionally used threshold levels (i.e. $VIF < 3$). Only substances with more than 20 MICs were used for all MLRs, and no violations of concern became apparent.

Internal validation via simulation

Following aforementioned standard validation exercises, every model was further tested for robustness and validity applying internal validation techniques,^{6,7} using a simulation approach (i.e. bootstrapping, $n = 10,000$) of randomly drawn data (with replacements).

Surface water risk (individual substances)

Detailed summary statistics about bootstrapping simulation runs ($n = 10,000$) of the linear model for individually measured MICs in freshwaters ($n = 1,833$, R^2 adj. = 62.17, $p < 0.001$) are provided in Table S3. Bootstrapping simulation runs ($n = 10,000$) and their resulting adjusted R^2 are summarized in Figure S3.

Sediment risk (individual substances)

Detailed summary statistics about bootstrapping simulation runs ($n = 10,000$) of the linear model for individually measured MICs in sediments ($n = 478$, R^2 adj. = 48.10, $p < 0.001$) are

provided in Table S6. Bootstrapping simulation runs ($n = 10,000$) and their resulting adjusted R^2 are summarized in Figure S2.

Surface water risk (maxTU)

Detailed summary statistics about bootstrapping simulation runs ($n = 10,000$) of the maximum toxicant pressure (i.e. maxTU) model for surface waters ($n = 496$, R^2 adj. = 75.17, $p < 0.001$) are provided in Table S7. Bootstrapping simulation runs ($n = 10,000$) and their resulting adjusted R^2 are summarized in Figure S3.

References

1. Wolfram, J.; Stehle, S.; Bub, S.; Petschick, L. L.; Schulz, R., Meta-Analysis of Insecticides in United States Surface Waters: Status and Future Implications. *Environ. Sci. Technol.* **2018**, *52*, (24), 14452-14460.
2. Unwin, A., Discovering Statistics Using R by Andy Field, Jeremy Miles, Zoë Field. *International Statistical Review* **2013**, *81*, (1), 169-170.
3. Durbin, J.; Watson, G. S., Testing for serial correlation in least squares regression. II. *Biometrika* **1951**, *38*, (1/2), 159-177.
4. White, H., A Heteroskedasticity-Consistent Covariance Matrix Estimator and a Direct Test for Heteroskedasticity. *Econometrica* **1980**, *48*, (4), 817-838.
5. Fox, J.; Monette, G., Generalized collinearity diagnostics. *Journal of the American Statistical Association* **1992**, *87*, (417), 178-183.
6. Harrell Jr, F. E., *Regression modeling strategies: with applications to linear models, logistic and ordinal regression, and survival analysis*. Springer: 2015.
7. Steyerberg, E. W.; Harrell Jr, F. E.; Borsboom, G. J.; Eijkemans, M.; Vergouwe, Y.; Habbema, J. D. F., Internal validation of predictive models: efficiency of some procedures for logistic regression analysis. *J. Clin. Epidemiol.* **2001**, *54*, (8), 774-781.
8. Lewis, K. A.; Tzilivakis, J.; Warner, D. J.; Green, A., An international database for pesticide risk assessments and management. *Human and Ecological Risk Assessment: An International Journal* **2016**, *22*, (4), 1050-1064.

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Table S1: Regulatory threshold levels (RTL) for freshwater (RTL_{fw}), sediments (RTL_{sed}) and estuaries (RTL_{est}) for all 32 parent compounds. Only insecticide degradates with reported concentrations are listed and provided with a RTL. A full list of degradates of potential environmental concern can be found in the first paragraph of the SI. Please note, this table can also be found in Wolfram et al.¹

Insecticide class	Insecticide	RTL _{fw} (µg/L)	RTL _{sed} (µg/kg)	RTL _{est} (µg/L)
Organochlorine	Dicofol	26.5	3680	7.55
	Endosulfan	0.05	3.84	0.02
Organophosphate	Azinphos-methyl	0.08	0.89	0.105
	Chlorpyrifos	0.03	16	0.0175
	Diazinon	0.105	76	2.1
	Malathion	0.295	2.56	1.1
	Parathion-ethyl	0.02	0.325	0.0535
	Parathion-methyl	0.485	20.8	0.175
Carbamate	Aldicarb	10		6
	Carbaryl	0.85	4.4	2.85
	Carbofuran	1.115	1.72	2.3
Pyrethroid	Bifenthrin	0.0002465	0.25	0.001985
	Cyfluthrin	0.0125	0.53	0.0012
	Cypermethrin	0.0018	0.308	0.002375
	ζ-cypermethrin	0.0018	0.308	0.002375
	Deltamethrin	0.0001	0.48	0.00185
	Esfenvalerate	0.000424	7.4	0.00233
	Fenpropathrin	0.001525	12.4	0.0105
	Fenvalerate	0.016	2.2	0.004
	λ-cyhalothrin	0.00015	0.31	0.00245
	Permethrin	0.0033	7.4	0.009
	Resmethrin	0.14		0.115
	τ-fluvalinate	0.155		0.003
	Tefluthrin	0.03	11.6	0.0265
	Tetramethrin	1.85		1.85
	Tralomethrin	0.0195		0.4225
	Neonicotinoid	Acetamiprid	10.5	
Clothianidin		11		26.5
Dinotefuran		49550		395
Imidacloprid		0.385		16.5
Thiamethoxam		17.5	10	3450
Phenylpyrazole	Fipronil	0.11	16	0.07
Degradate	Endosulfan sulfate	0.05	73	0.02
	Aldicarb sulfoxide	21.5		
	Aldicarb sulfone	184.5		
	Fipronil sulfone	0.36	9.1	0.28

Fipronil sulfide	1.065	29	0.0385
Desulfinyl fipronil	100	200	0.75

Table S2: List of covariates and attributes with their respective data type and availability in percent that were extracted from publications and assigned to individual MICs.

Covariate	Data type	Description	Available %
Substance name	factor	Name of detected insecticide	100
Chemical class	factor	Insecticide class (e.g. Organophosphate)	100
TU identifier	integer	Shared identifier per sample	100
Reference	factor	Name and date of the respective publication	100
Compartment	integer	Integer detailing if MIC was detected in the water phase (1) or sediment phase (2)	100
Sampling interval	numeric	Time in days between individual samples per location	77.4
Catchment size	numeric	Size in km ² of contributing catchment area per sampling location	81.7
Sampling date	numeric	Date of sampling event	98.1
Location name	factor	Name of sampling location	100
Agricultural produce	factor	List of crops grown in respective catchment	71.2
Sampling location (state)	factor	Federal state in which the sampling location is situated	97.8
Sampling location (county)	factor	County in which the sampling location is situated	78.7
Water body type	factor	Water body type (e.g. creek, estuary, river) as detailed in the publication	95.6
Freshwater	integer	Integer detailing if the respective water body contains freshwater (1) or estuarine water (2)	100
Flow type	integer	Integer detailing if respective water body is flowing (1) or standing (2)	97
Number of detects	integer	Number of detections per substance as detailed in the publication	97.7
Number of non-detects	integer	Number of non-detections per substance as detailed in the publication	83.1
Filtration	factor	Description of filtration procedures used for pyrethroid samples	95.7
LOD	numeric	Analytical limit of detection for respective substance	39.9
LOQ	numeric	Analytical limit of quantification for respective substance	51.1
Additional pesticides	factor	Factor indicating if additional pesticides (excluding insecticides) were analyzed	97.7
Additional insecticides	factor	Factor indicating if additional insecticides were analyzed	100
Total pesticides	integer	Number of total detected pesticides (excluding insecticides) per sample	100
Total insecticides	integer	Number of total detected insecticides per sample	65.2
Total Pesticides/Insecticides	factor	Factor detailing how many pesticides/insecticides were detected	99
Detected pesticides	factor	List of substance names detected per sample	61.7
Agricultural origin	factor	Factor describing certainty that MICs originated from agricultural NPS as detailed in the publication	100

Agricultural proximity	factor	Factor describing if any information was provided regarding the proximity of agricultural fields to the sampling location	60.9
Highest spatial accuracy	integer	Integer detailing if MIC was attributable to a location (1), county (2), or the federal state (3)	100
Latitude	numeric	Latitude of sampling location (GPS)	75.3
Longitude	numeric	Longitude of sampling location (GPS)	75.3
RTL	numeric	respective regulatory threshold level	100
RTL class	integer	Integer detailing if MIC was attributable to a freshwaters (1), sediments (2), or estuarine waters (3)	100

Table S3: Detailed summary statistics of model validation simulation runs (bootstrapping, $n = 10,000$) and resulting bootstrapped 95% confidence intervals for the freshwater linear regression of individual MIC to RTL ratios. Standardized betas with their respective rank (SBR) and variance inflation factors (VIF) of the main model are provided for reference.

Predictor variable	Lower 95%-confidence limit	2 nd quartile (median)	Upper 95%-confidence limit	Sign. ¹	Std. beta	SBR ²	VIF
Intercept	-2.302	-1.928	-1.542	*			
Catchment size [km ²] ³	-0.183	-0.151	-0.120	*	-0.146	4	1.897
Sampling interval [d] ³	-0.227	-0.199	-0.170	*	-0.189	3	1.311
Time [y]	-0.064	-0.057	-0.050	*	-0.287	2	2.283
toxicity-normalized use [kg × RTL ⁻¹] ³	0.350	0.385	0.422	*	0.381	1	1.987
Burst factor	0.011	0.015	0.019	*	0.131	5	2.310
Irrigated agricultural land-use [%]	0.289	0.427	0.560	*	0.101	6	1.448
Organophosphates	-0.034	0.242	0.518				1.653
Pyrethroids	0.944	1.231	1.516	*			1.653
Neonicotinoids	0.130	0.436	0.726	*			1.653
Phenylpyrazole	0.807	1.235	1.678	*			1.653

¹ Determined based on confidence level overlap

² Ranked standardized betas of linear model

³ Predictor variable was transformed by the decadal logarithm

Table S4: Comparison of insecticides' stability in days (i.e. DT₅₀) in different compartments and the resulting delta in days. Data was obtained from Lewis et al.⁸.

Substance	DT ₅₀ in days (sediment)	DT ₅₀ in days (water)	Delta
Malathion	0.4	0.4	0
Diazinon	10.4	4.3	6.1
Endosulfan	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>
Chlorpyrifos	36.5	5	31.5
Bifenthrin	161	8	153
λ-cyhalothrin	15.1	0.24	14.86

Esfenvalerate	56	30	26
Fenpropathrin	28	1	27
Carbaryl	5.8	3.1	2.7
Carbofuran	9.7	6.1	3.6
Permethrin	40	23	17
Deltamethrin	65	17	48
Cypermethrin	17	3	14
Fenvalerate	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>
Parathion-methyl	4.3	3.5	0.8
Cyfluthrin	8	1	7
Cypermethrin-zeta	2	0.1	1.9
Fipronil	68	54	14
Desulfinyl-Fipronil	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>
Fipronil sulfide	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>
Fipronil sulfone	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>
Dicofol	29	<i>NA</i>	<i>N/A</i>

Table S5: Toxic modes of action for respective insecticide classes used in the present study.

Insecticide class	Toxic Mode of Action (TMoA)
Organochlorine	GABA-gated chloride channel antagonist
Organophosphate	Acetylcholinesterase inhibitor
Carbamate	Acetylcholinesterase inhibitor
Pyrethroid	Sodium channel modulators
Neonicotinoid	Nicotinic acetylcholine receptor agonists
Phenylpyrazole	GABA _A -gated chloride channel antagonist

Table S6: Detailed summary statistics of model validation simulation runs (bootstrapping, $n = 10,000$) and resulting bootstrapped 95% confidence intervals for the sediment linear regression of individual MIC to RTL ratios. Standardized betas with their respective rank (SBR) and variance inflation factors (VIF) of the main model are provided for reference.

Predictor variable	Lower 95%-confidence limit	2 nd quartile (median)	Upper 95%-confidence limit	Sign. ¹	Std. beta	SBR ²	VIF
Intercept	-3.239	-2.414	-1.602	*			
Catchment size [km ²] ²	-0.142	-0.101	-0.061	*	-0.097	6	1.150
Sampling interval [d] ²	-0.085	-0.053	-0.021	*	-0.075	7	1.257
Time [y]	-0.085	-0.067	-0.049	*	-0.215	2	1.274
Toxicity-normalized use [kg × RTL-1] ²	0.087	0.188	0.281	*	0.170	3	1.808
Lentic bodies [factor]	-0.528	-0.367	-0.210	*	-0.098	5	1.336
Irrigated agricultural land-use [%]	0.312	0.481	0.656	*	0.126	4	1.236
KOC [L/kg] ²	0.617	0.769	0.965	*	0.506	1	2.879
Organophosphates [factor]	-1.326	-0.783	-0.301	*			1.368

Pyrethroids [factor]	-0.946	-0.421	0.063	1.368
Phenylpyrazole [factor]	-2.117	-0.798	0.379	1.368

¹ Determined based on confidence level overlap

² Predictor variable was transformed by the decadal logarithm

³ Ranked standardized betas of linear model

Table S7: Detailed summary statistics of model validation simulation runs (bootstrapping, $n = 10,000$) and resulting bootstrapped 95% confidence intervals for the mixture toxicity (maxTU, freshwaters) linear regression of individual MIC to RTL ratios. Standardized betas with their respective rank (SBR) and variance inflation factors (VIF) of the main model are provided for reference.

Predictor variable	Lower 95%- confidence limit	2 nd quartile (median)	Upper 95%- confidence limit	Sign. ¹	Std. beta	SBR ²	VIF
Intercept	-2.219	-1.736	-1.243	*			
Catchment size [km ²] ³	-0.393	-0.314	-0.238	*	-0.246	3	2.071
Sampling interval [d] ³	-0.194	-0.130	-0.063	*	-0.106	7	1.470
Burst factor	0.010	0.018	0.025	*	0.138	6	2.180
Time [y]	-0.074	-0.063	-0.053	*	-0.269	2	1.180
Toxicity-normalized use [kg × RTL ⁻¹] ³	0.394	0.448	0.503	*	0.411	1	1.490
Number of detected substances	0.050	0.073	0.097	*	0.151	5	1.665
Irrigated agricultural land-use [%]	0.726	0.989	1.254	*	0.209	4	1.453

¹ Determined based on confidence level overlap

² Ranked standardized betas of linear model

³ Predictor variable was transformed by the decadal logarithm

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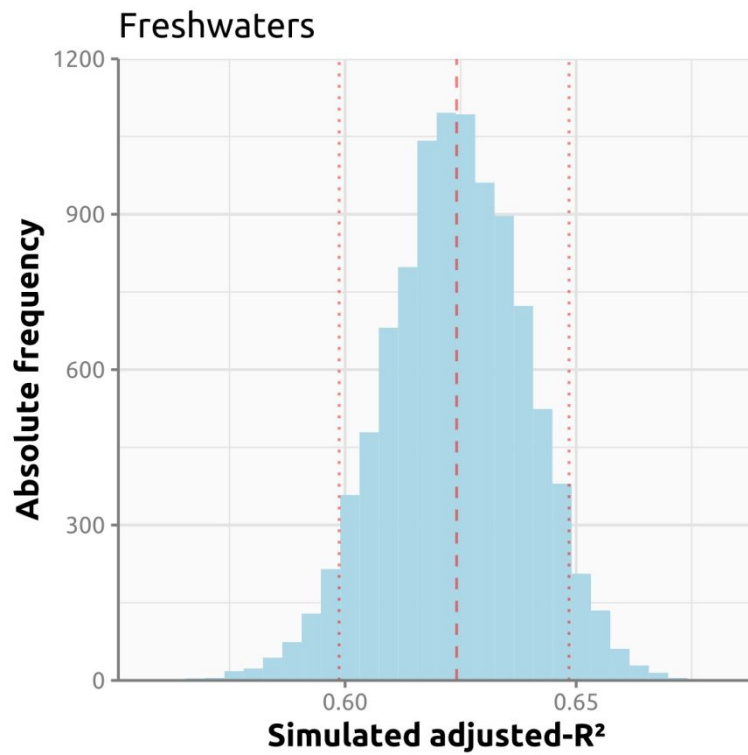


Figure S1: Frequency plot detailing the distribution of bootstrap simulated ($n = 10,000$) adj. R^2 of the multiple linear regression for insecticide risks in freshwaters. Bootstrapped upper and lower 95% confidence intervals are depicted by dotted (orange) lines and the median is depicted by a red dashed line.

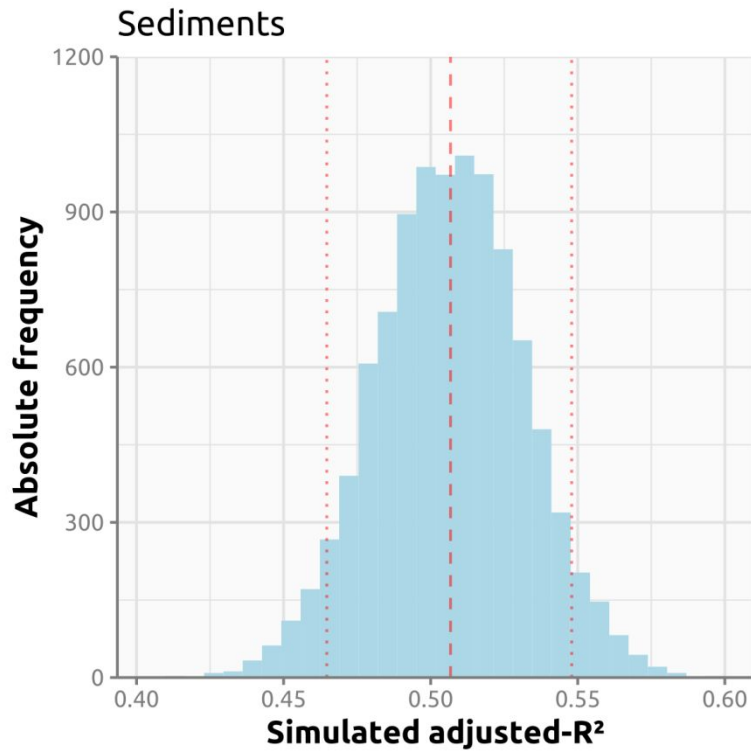


Figure S2: Frequency plot detailing the distribution of bootstrap simulated ($n = 10,000$) adj. R^2 of the multiple linear regression for insecticide risks in sediments. Bootstrapped upper and lower 95% confidence intervals are depicted by dotted (orange) lines and the median is depicted by a red dashed line.

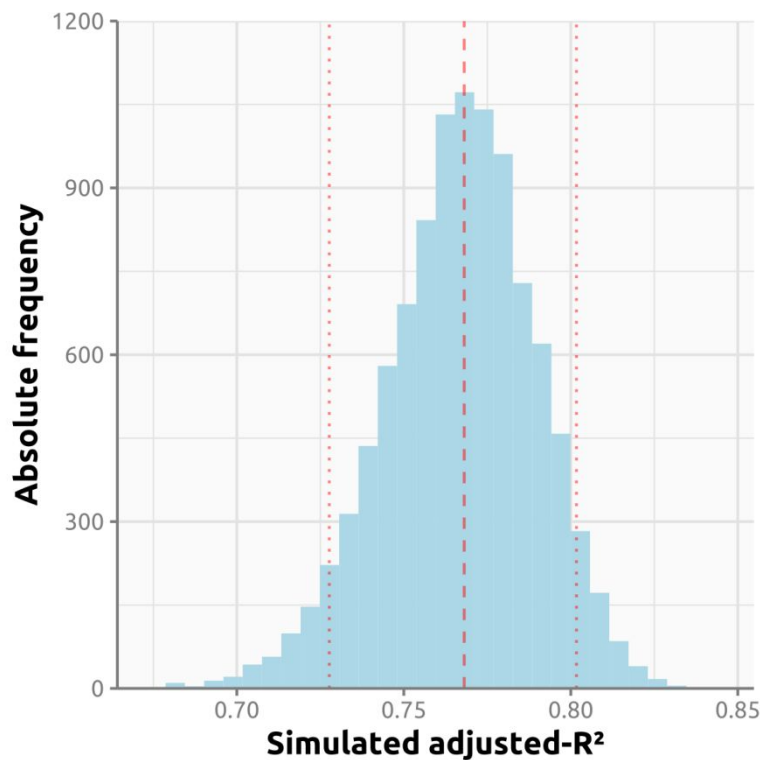


Figure S3: Frequency plot detailing the distribution of bootstrap simulated ($n = 10,000$) adj. R^2 of the maxTU multiple linear regression. Bootstrapped upper and lower 95% confidence intervals are depicted by dotted (orange) lines and the median is depicted by a red dashed line.



Water quality and ecological risks in European surface waters – Monitoring improves while water quality decreases

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ABSTRACT

Aquatic ecosystems are at risk of being impaired by various organic chemicals, however comprehensive large-scale evaluations of waterbodies' status and trends are rare. Here, surface water monitoring data, gathered as part of the EU Water Framework Directive and comprising the occurrence of 352 organic contaminants (>8.3 mil. measurements; 2001–2015; 8213 sites) in 31 European countries, was used to evaluate past and current environmental risks for three aquatic species groups: fish, invertebrates, plants. Monitoring quality indices were defined per country and found to improve over time. Relationships became apparent between countries' monitoring quality index and their success in detecting contaminants. Across the EU, contaminants were more frequently found in recent years. Overall, 35.7% ($n = 17,484$) of sites exceeded at least one acute regulatory threshold level (RTL) each year, and average risks significantly increased over time for fish ($\tau = 0.498$, $p = 0.01$) and aquatic invertebrates ($\tau = 0.429$, $p = 0.03$). This indicates an increased chemical pressure to Europe's waterbodies and overall large-scale threshold exceedances. Pesticides were identified as the main risk drivers (>85% of RTL exceedances) with aquatic invertebrates being most acutely at risk in Europe. Agricultural land-use was clearly identified as the primary spatial driver of the observed aquatic risks throughout European surface waters. Issues in monitoring data heterogeneity were highlighted and also followed by subsequent improvement recommendations, strengthening future environmental quality assessments. Overall, aquatic ecosystem integrity remains acutely at risk across Europe, signaling the demand for continued improvements.

1. Introduction

A plethora of chemicals is being currently used, distributed, or sold in Europe. More than 20,000 industrial chemicals are registered under REACH (EFSA, 2020), a regulation that outlines the registration, (eco)-toxicological hazard evaluation, and authorization of industrial chemicals in the European Union. In addition, at least 450 pesticides (European Commission, 2020) in more than 1700 product formulations are applied in agriculture (BVL, 2020), and more than 3000 pharmaceuticals are currently in use (Owens, 2015). As a result, surface waters, as receiving ecosystems, are at risk of being exposed to many of these chemicals, their byproducts and transformation products via point sources (e.g. waste water outlets) or diffuse sources (e.g. farmland runoff and drainage) (Stone et al., 2014; Stone et al., 2014; Stehle et al., 2018). Aquatic biota can thus be exposed to a vast array of chemical contaminants that may adversely affect local populations, the ecosystem structure and its functioning, or the provision of vital ecological services

(Beketov et al., 2013; Beketov et al., 2008; Schäfer et al., 2007; Schäfer et al., 2012). In response to this potential large-scale threat to ecosystem integrity, resulting from the increased chemization of environments (Vörösmarty et al., 2010), the Water Framework Directive (WFD, Directive 2000/60/EC) entered into force in December 2000, requiring the EU member states – among other objectives - to assess the condition of and achieve a “good ecological status” of their surface waters (European Council, 2000). This effort has produced more than 33 million records of water quality related parameters (e.g. nutrients, organic chemicals, temperature, pH, etc.), consolidated into the Water Information System for Europe – Water Quality database version 6 (=WISE6) (European Environment Agency, 2020).

Recently, the WFD has entered its third and last decadal cycle, providing an ample opportunity to comprehensively analyze the status, temporal trends, and ecological implications of pan-European water quality. In this context, Malaj et al. (2014) provided a critical overview concerning the status of European waterbodies for 2006–2010 using

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WISE4 data, demonstrating continental impairments of aquatic systems. However, it remains unclear how water quality has evolved over time and if improvements have been reached. Further studies have provided fundamental information mostly aiming at improving the efforts of the Directive: von der Ohe et al. (2011) constructed extensive prioritization recommendations on more than 500 chemicals to improve WFD monitoring; Geissen et al. (2015) identified conceptual challenges in current monitoring protocols, especially with regards to emerging pollutants; similarly, Brack et al. (2017) extensively reviewed the current state of WFD chemical monitoring and developed solution-oriented recommendations for improvement of ecological impact assessments supporting targeted risk reduction strategies; Carvalho et al. (2019) provided a holistic analysis of novel approaches advancing current WFD monitoring in support of water management policies and highlighted a multitude of issues, organizational hindrances or lack of public stakeholder awareness. Other conceptual recommendations have also been proposed by various studies: for instance; the prioritization and assessment of chemical mixtures (Faust et al., 2019); the advancement of the linkage between analytical and ecological response interactions (Altenburger et al., 2019); the derivation of ecological status estimates from species trait-indices in comparison to exposure measurements (von der Ohe et al., 2007); and the derivation of robust environmental quality standards from *in vitro* and *in vivo* tests for WFD water quality evaluation (Escher et al., 2018). However, to this date, no comprehensive evaluation has been conducted using the entirety of available occurrence data for organic chemicals (i.e. since 2001). Here, we fill this gap with a comprehensive risk evaluation with respect to all four principal ecotoxicological dimensions (and their interactions; i.e. space, time, biotic effects, and chemicals) in order to improve our understanding of the trajectory of aquatic ecosystem health in Europe. We hypothesize that data quality, hence monitoring quality, improved over time (H1, Fig. 1). Furthermore, we hypothesize that the occurrence frequency of organic contaminants decreased over time (H2a), leading to reduced aquatic risks over time (H2b, Fig. 1), partially in response to WFD efforts.

To precede any risk evaluation and answer H1, monitoring network characteristics (i.e. data availability per country over time) were derived to identify potential sources of data heterogeneity and uncertainties, highlighting areas where improvements are needed and data-related limitations that apply to the study itself. These monitoring network characteristics were assessed qualitatively by derivation of a monitoring-quality-index (MQI) per country to deduce differences in monitoring strategies and the impact of varying strategies in capturing organic contaminants. Second, the occurrence frequency of chemicals was detailed in addition to chemical occurrence frequency trends over time to answer H2a. Third, regarding H2b, an ecotoxicological risk evaluation was conducted and based on regulatory threshold levels (RTL) (Petschick et al., 2019; EFSA, 2013), which were applied to three main aquatic organism groups: aquatic invertebrates, fish, and aquatic plants. Additionally, more general risk evaluations were conducted on the most sensitive out of the three aforementioned species groups. With this approach, 286 organic contaminants could be assessed, covering about 91.2% of samples (n = 8,383,640) from 8209 monitoring sites in 7227 waterbodies across 31 European countries between 2001 and 2015. Therefore, the current study provides extensive ecotoxicological insights into the complete WISE6 dataset, that has been gathered since the initiation of the WFD in December 2000. Environmental risk evaluations were conducted based on RTL with respect to the ecotoxicological dimensions space, time and chemicals (H2b, Fig. 1). Furthermore, environmental risk evaluations were contextualized via comparison with existing Environmental Quality Standards for priority substances as given in Directive 2008/105/EC and its amendment (Directive 2013/39/EU). Also, land-use characteristics and hydrogeographic information were deduced for 88.3% (n = 7249) of the upstream catchments of monitoring sites to provide further explanations for the representativeness of sampled sites and land-use specific impacts on water quality in Europe. Finally, data-related challenges were identified and recommendations elaborated to solve issues, aiding to improve future regulatory actions.

2. Materials and methods

2.1. Monitoring data

European monitoring data were obtained from the WISE Water Quality database (WISE6, published July 3rd 2020) (European Environment Agency, 2020) and procured as part of the WFD (European Council, 2000) to characterize, among other factors, the chemical status of European waterbodies. Variables not referring to individual organic compounds, like “oxygen content”, “mixture of pesticides”, “sum of DDT metabolites”, or “nitrogen”, were omitted from further analysis (for details, see SI Data Processing Protocols). In total, 352 organic contaminants, measured at 8209 locations in 7227 waterbodies for 31 countries between 2001 and 2015, were identified and listed along with their chemical class and other attributes in Table S1. Organic contaminants were assigned to eleven major chemical classes: anti-fouling agents; flame retardants; fungicides; herbicides; industrial chemicals; insecticides; plasticizers; solvents; pharmaceuticals; hormones; and miscellaneous chemicals. Data prior to 2001 (i.e. the initiation of the WFD) and after 2015 were found to be too sparse or heterogeneous and were omitted from the analysis. Hence, the dataset contains at least 8,383,640 samples, which were aggregated per chemical and location to average and maximum “annual aggregate concentrations” (=AACs; n = 971,381). The highest risk (i.e. concentration / toxicity endpoint), hence presumably most ecotoxicologically relevant (Wolfram et al., 2019) AAC per site, year, and organism group (=MRAA), was determined and used for most risk analyses. For a detailed description of the terms sample, AAC, and MRAA, see SI Measurement Definitions. All data were checked for reliability, relevance, and completeness, with data being removed if quality control criteria were not met (for details, see SI Data Processing Protocols). Any reported concentration below its limit of quantification

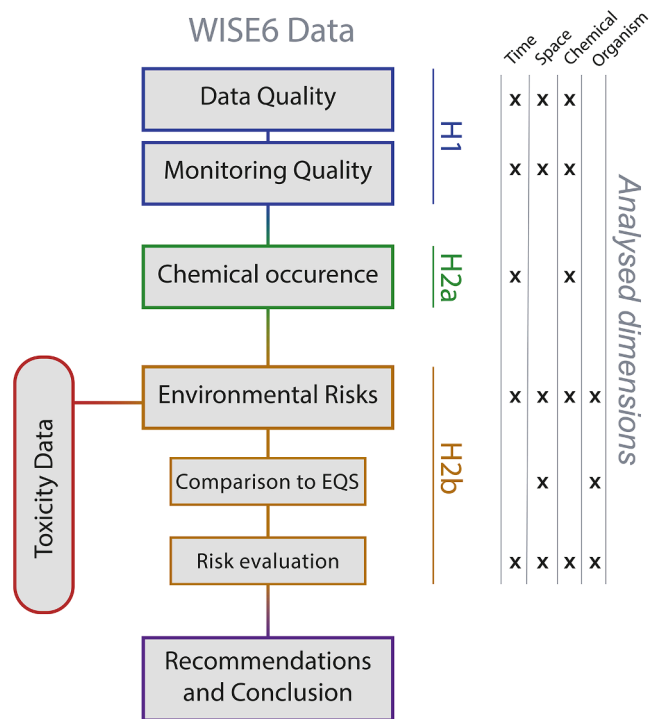


Fig. 1. Overview of the study’s outline, detailing the main analysis blocks and the corresponding main hypotheses (H1, H2a, H2b). The principal ecotoxicological dimensions, which were analyzed and discussed per chapter, are depicted as well.

was regarded as a non-quantification and not used in risk evaluations. This was done to ensure that risk characterizations were not inflated by non-detects and non-quantifications, avoiding a source of uncertainty (James et al., 2009). A minor part of all data refers to non-EU member states (e.g. Norway), therefore terms such as “Europe” rather than “EU” will be used.

2.2. Ecotoxicological endpoints and regulatory threshold levels

Acute ecotoxicological effect data (i.e. endpoints) for standard test species (for details see EFSA, 2013 and Petschick et al., 2019) were obtained from three sources to derive the most relevant and reliable acute endpoint for aquatic invertebrates, fish, and aquatic plants for all chemical classes. Additionally, a most sensitive endpoint representing all three organism groups was derived, representing the level at which one of those groups is acutely affected. First, the EFSA OpenFoodTox (Dorne et al., 2017) database that lists curated ecotoxicological test data for various chemical classes, which passed rigorous review and are used during ecological risk assessments in the EU (e.g. Regulation (EC) No 1107/2009, EFSA, 2013), was used as primary source for acute endpoints. Second, regulatory endpoint estimates, derived according to Petschick et al. (European Council, 2000), were used to complement the EFSA OpenFoodTox data. These estimates mirror the manual validation process applied for environmental risk assessments under Regulation (EC) No 1107/2009 and FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act, 1996) in deriving valid endpoints from standard ecotoxicological test data, achieving a high accordance with endpoints derived during environmental risk assessments (European Council, 2000). Third, the EnviroTox database (Connors et al., 2019) curates data from multiple ecotoxicological databases and was used to fill data gaps where possible. However, preliminary analyses (see SI Deduction of Robust EnviroTox Endpoints) indicated that most sensitive standard toxicity endpoints from the EnviroTox provided too sensitive endpoints compared to those reliable estimates from Petschick et al. (2019). Thus, to prevent overestimations of aquatic risks, further aggregation and validation was conducted for EnviroTox data prior to their use (see SI Deduction of Robust EnviroTox Endpoints). The three aforementioned databases were used hierarchically as listed above, i.e. an endpoint derived from the EnviroTox for aquatic invertebrates was only used, if endpoints from the OpenFoodTox (Dorne et al., 2017) or Petschick et al. (2019) were unavailable. An overview of all chemicals, endpoints and their respective database source can be found in Table S1.

The ecological risk assessment scheme for pesticides, Regulation (EC) No 1107/2009, is based on elaborate and strict data quality criteria, thus yielding robust and comparable thresholds (EFSA, 2013). Following this methodology, all acute endpoints were applied with assessment factors as used during pesticide registration under Regulation (EC) No 1107/2009 (see EFSA, 2013). Assessment factors serve the purpose of capturing different sources of uncertainty that are inherent to standardized laboratory ecotoxicological data, such as intra- and inter-species variation, inter-laboratorial variation, and sources of variability, such as developmental, physiological, behavioral, or other deleterious biological effects (Beketov et al., 2013; Schäfer et al., 2007; Schäfer et al., 2012; Beketov and Liess, 2005; Berenzen et al., 2005; Brock and Van Wijngaarden, 2012; Lepper, 2005; Liess, 2002; Liess and von der Ohe, 2005; van Wijngaarden et al., 2005; van Wijngaarden et al., 2015; EFSA Scientific Committee, 2016a, 2016b). A detailed discussion regarding the use of assessment factors based on a literature review can be found in the Supporting Information (SI Use of Assessment Factors). A uniform methodology for threshold derivation was applied to all organic chemicals ($n = 352$) to provide comparable risk estimates across various chemical classes. Therefore, acute endpoints for aquatic invertebrates and fish were divided by an assessment factor of 100, and acute endpoints for aquatic plants (mostly algae) were divided by an assessment factor of 10, yielding so called regulatory threshold levels (Stehle and Schulz, 2015), i.e. $RTL_{\text{aquatic-inverts}}$ ($n = 254$), RTL_{fish} ($n =$

251), and $RTL_{\text{aquatic-plants}}$ ($n = 218$). Surface water RTLs (RTL_{sw} , $n = 286$) were derived as well, based on the most sensitive organism group per substance. RTLs delineate unacceptable risks for aquatic species groups and are indicative of ecosystem impairments if they are exceeded, e.g. resulting in biodiversity losses in freshwater systems (Stehle and Schulz, 2015). RTLs are comparable to regulatory acceptable concentrations, often referred to as RACs, used in the European context. Their use may be further expanded to other geographical regions (Wolfram et al., 2019; Stehle and Schulz, 2015; Wolfram et al., 2018) as they are based on the majority of publicly curated ecotoxicological effect data following strict quality criteria protocols. For a complete list of RTLs used in this study, see Table S1.

Priority substances that may adversely impact European waterbodies were identified as part of the WFD derivation (Directive 2000/60/EC) and listed in its Annex X. With Directive 2008/105/EC and its amendment (Directive 2013/39/EU), Environmental Quality Standards (i.e. acute effect threshold concentrations, EQS) were assigned to 45 priority substances in order to evaluate their potential environmental risks. In this study, of these 45 substances, 36 could be classified as organic contaminants with an acute effect threshold (EQS). Risk evaluations using both EQS or RTLs were subsequently compared.

2.3. Spatial data

Spatial data were used to deduce watershed features upstream of monitoring sites and national or watershed-specific land-use characteristics. For land-use characteristics, the Corine Land Cover layer 2012 was used with an areal resolution of 500 m, i.e. the highest resolution available (European Environment Agency, 2020). Corine Land Cover data assigns land-use characteristics to 44 categories, which were furthermore condensed in this study to 5 main land-use categories used for statistical analysis: urban, industrial, agricultural, silvicultural (i.e. forestry), and natural (see Table S2).

For the derivation of upstream watershed features (i.e. catchment polygons), the “European Catchment and Rivers Network System” (ECRINS, version 1.2) was used, which provides extensive hydrological geoinformation for Europe (European Environment Agency, 2012). Upstream catchment polygons were derived for 7250 monitoring sites based on functional elementary catchments using string-pattern matching (see SI Spatial Data Appendix). As such, for most monitoring sites (88.3%, $n = 7249$), complete upstream catchment polygons could be derived directly or indirectly after processing workflows were optimized (see SI Spatial Data Appendix). Zonal statistics were applied to derive land-use characteristics within all respective catchments using the Corine Land Cover data.

2.4. Monitoring data evaluation

Environmental monitoring of surface waters is understood here as an analytical effort of multiple dimensions, primarily in space, time, and chemicals. Nationally organized monitoring schemes may differ substantially in quantity and quality depending on how thoroughly these dimensions are probed. To provide a basis for comparison among countries, we derived an indicative index, named here monitoring-quality-index (MQI), which incorporates the most important dimensions that could be derived from the present data (for details see SI Monitoring Data Evaluation):

- i. The network density (ND) per anthropogenically-used land surface area. ND is a measure of the spatial network resolution that details the number of monitoring stations per country in relation to its surface area that is classified as urban, industrial, or agricultural in 1000 square kilometers (European Environment Agency, 2020).
- ii. The average sampling frequency (SF) per year and site, averaged per country, which details the intra-year temporal resolution.

iii. The average temporal continuity (TC) of monitoring sites. TC represents a measure of how temporally complete data are for each monitoring station. TC is scaled from 0 to 1, with 1 equaling data being available for all years from 2001 to 2015 for a specific

site. TC is averaged per country and represents the inter-year temporal resolution.

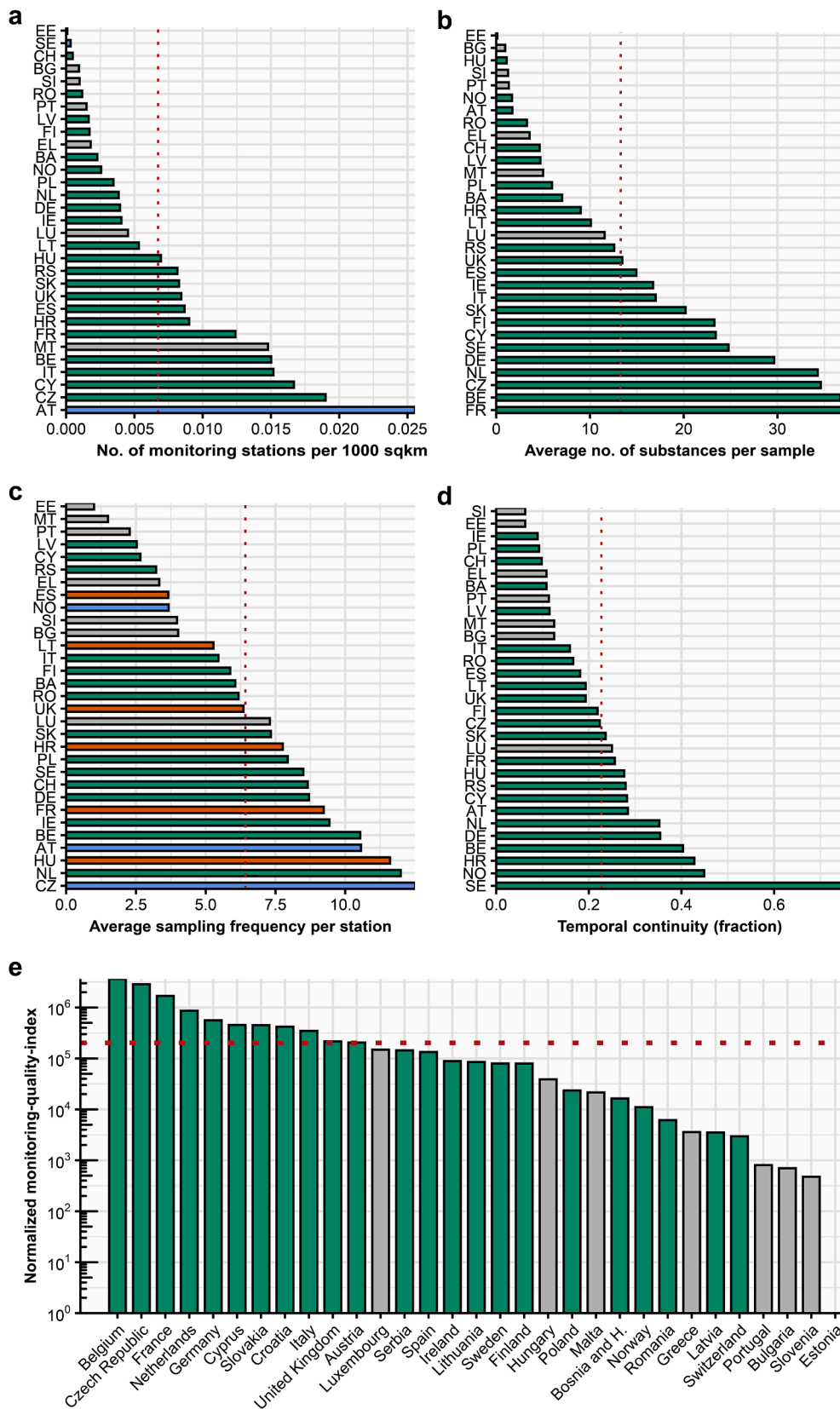


Fig. 2. Overview of different monitoring program parameters per country that were used to calculate the monitoring-quality-index (MQI). (a) Monitoring network density (ND) in No. of stations per 1000 km² of anthropogenically-used land. (b) Average number of chemicals analyzed per sample taken (CS). (c) Average sampling frequency of monitoring stations per year (SF). (d) Average temporal continuity (TC), (e) MQI per country normalized by the lowest observed value (Estonia = 1). Average rates of change for the respective indicator (a-e) are indicated as follows. Negative change is given in orange, positive change is given in blue, and insufficient data (<5 years) is given in grey. Average scores in a-e are depicted with orange dotted lines. Full country names and their corresponding abbreviation (i.e., ISO 3166-1 country codes used in a-d) are given in Table S3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

- iv. The chemical spectrum (CS) per site and year, averaged per country. CS represents how many chemicals are monitored on average per country.

The MQI per country i is calculated as

$$MQI_i = ND_i \times SF_i \times TC_i \times CS_i$$

A more detailed description of the derivation of the individual factors can be found in the SI Monitoring Data Evaluation.

2.5. Statistical analysis

For all statistical tests, an α -level of 0.05 was used, and resulting p -value thresholds were Bonferroni-adjusted in case of multiple comparisons to account for alpha-error inflation. Two-sample tests, for significant differences between distributions of different groups, were performed as either Student's T -test (parametric) or Mann-Whitney's U test (non-parametric). The non-parametric Mann-Kendall test was used for monotonic trend analysis, as it provides robust trend estimates for time-series. Correlations were assessed using Pearson product-moment correlation, univariate linear regressions, general additive or linear models (GAM, GLM), and LOESS regressions depending on the underlying data structure. For detection frequency analyses, all eleven chemical classes were used; however, to improve the readability of figures in all subsequent risk analyses, only the six most impactful chemical classes (fungicides, herbicides, industrial chemicals, insecticides, plasticizers, and solvents) are shown, with the chemical class "others" containing all the remaining data. All calculations and figures were produced using R (R base: Ver. 3.6.1, 64-bit, Windows 10) and QGIS (Ver. 3.12.0 Bucuresti, 64-bit, Windows 10) (QGIS Development Team, 2016; Team R Core, 2015). Additional, interactive graphics depicting chemical class specific quantification profiles (Sankey-diagrams), AAC and MRAA exceedance profiles (Sankey-diagrams), and chemical association networks per country (Chord-diagrams) can be found at <https://magic.eco/WebApp/WFD>.

3. Results and discussion

3.1. Quality of monitoring

Monitoring quality was found to be highly variable among European countries, based on sampling frequencies (SF), chemical substance spectra (CS), temporal continuities (TC), and network densities (ND; see Fig. 2a-d). Resulting monitoring-quality-indices differed by large degrees, however they only serve as indicators how monitoring data quality may differ among countries. Three countries have achieved what may be classified as exemplary monitoring efforts, judged by their above-average performance and MQI scores compared to other countries: namely Belgium, the Czech Republic, and France. Hence, in the remaining cases, MQI scores were lower, often by rather wide margins (Fig. 2e). For instance, twenty or ten countries scored lower by a factor of 10 or 100, respectively, if compared to the MQI of France. Especially in countries where current MQIs are far below the European average, reinvigorated efforts should be primed to narrow the gap. However, average annual rates of SF, CS, TC, and ND changes were largely positive (93.5%, $n = 124$), suggesting that countries generally improved those central monitoring metrics over time (Table S3). Resulting MQIs uniformly increased over time so that Hypothesis 1 was accepted, demonstrating that monitoring strategies improved over time. While the MQI covers the years 2001–2015, it is noteworthy that Italy and the UK have established spatial dense, high frequency monitoring networks in recent years, although they score low due to poor temporal continuities. A noteworthy drawback of such heterogeneous data, as evidenced by the differing MQIs, is that the occurrence of chemicals will inevitably be underestimated spatiotemporally, and, in consequence, also the resulting risks for aquatic species in general (Wolfram et al., 2019; Stehle and

Schulz, 2015; Crawford, 2004). Therefore, our analyses harbor a possibility that transient exposure peaks are missed. In light of the quality discrepancies, further adjustments of minimal monitoring obligations (Directive 2000/60/EC Annex V Table 1.3ff) should be discussed to further harmonize data quality among contributing European countries. The data reporting pace per country (Fig. 2) is also highly variable, with some countries (e.g. Czech Republic, Germany) being nearly up-to-date (i.e. 2018), and many others lying far behind with their latest data reports (2008–2014).

3.2. Measurements and detection frequencies

The WISE6 database contains more than 8.3 million measurements (2001–2015) for the 352 organic contaminants considered here. A strong increase in monitoring activity took place since 2005 and peaked in 2012 with more than 1.5 million samples taken annually (Fig. 3). Detection frequencies have increased since 2007 with a continuous upward trend identified based on general additive modeling (GAM, Fig. 3). Also, of 238 contaminants within more than 5 years of data, 58 showed a significantly increasing trend ($\tau > 0$, $p < 0.05$), 7 significantly decreasing trend ($\tau < 0$, $p < 0.05$), and 173 insignificant detection trends ($p > 0.05$) over time. Improvement of analytical techniques was, however, only marginally able to explain this observation. Only 6.5% ($n = 26$) of substances showed significantly decreasing limits of quantifications (LOQ) over time ($\tau < 0$, $p < 0.05$), whereas for 3.8% ($n = 15$) and 77.1% ($n = 179$) of substances LOQs significantly increased or remained unchanged. More so, the overlap between substances with decreasing LOQs (i.e. improved detection capabilities) and increased detection frequencies was minimal ($n = 6$), accounting only for 9.5% of increasingly detected chemicals. Furthermore, no indication was found of correlations between the number of samples taken and detection frequencies, which may suggest stochastic effects influencing the observed increase in detection frequencies. Briefly, of 14,297 correlation tests per chemical and site (≥ 5 years of data), only 3.9% ($n = 551$, $p < 0.05$) showed significantly positive relationships suggesting that detection frequencies increased alongside the number of samples taken, whereas

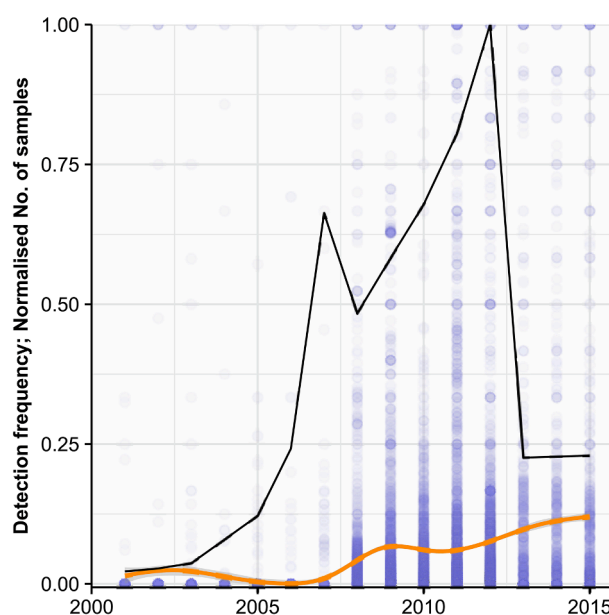


Fig. 3. Average detection frequencies at each monitoring station ($n = 8209$) and year (blue dots) with GAM regression (orange) depicting their temporal trend. The black line represents the normalized number of samples taken per year. Please note that for 2013–2015 data are only available for BE, CZ, DE, and ES. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

also 3.9% ($n = 552$, $p < 0.05$) showed significantly negative relationships. As such, it is probable that some organic contaminants are transported more frequently into non-target aquatic systems, where they are detected, although other factors could potentially improve detection frequencies as well. For instance, prioritization of sampling in periods during which substances are likely to occur (i.e. periods of pesticide runoff) and/or a prior screening of samples in order to improve targeting of relevant analytes (Geissen et al., 2015). Inversely, regular sampling during low or base flow conditions can improve detection of some organic contaminant classes (e.g. pharmaceuticals) originating from point sources (e.g. waste water effluents) (Guillon et al., 2015; Shala and Foster, 2010).

Increasing overall detection frequencies (Fig. 3) reflect increased detections of nearly all chemical classes (Fig. 4). Average detection frequencies (i.e. across all sites, 5.2%) of most chemical classes ranged between 2 and 9%, with herbicides (5.4%), plasticizers (9.2%), and industrial chemicals (9.3%) being detected more frequently, whereas insecticides (1.7%), fungicides (2.3%), and flame retardants (2.2%) representing more elusive substances. Their differing detection frequencies were likely the product of varying application or use patterns both regarding their mass and spatial extent (Fernandez-Cornejo et al., 2014; Baker, 2016), in addition to differing physico-chemical properties and entry pathways (Vörösmarty et al., 2010; Hollender et al., 2008; Schwarzenbach et al., 2006). For instance, insecticides, are generally applied less frequently and at lower masses, often as a reactionary pest

management measure, contrasting the high volume and partially preventive use of herbicides (Fernandez-Cornejo et al., 2014). Higher hydrophobicity of insecticides (i.e. K_{OC} , K_{OW}) also fosters faster dissipation rates from the aquatic phase when compared to herbicides. Similarly, a plethora of industrial chemicals are currently used (EFSA, 2020), and plasticizers are found throughout many consumer and professional products (Białecka-Florjańczyk et al., 2007; Li and Suh, 2019). Thus, introduction via waste water disposal or indirect leaching would explain the frequent detection of some of these chemicals in aquatic ecosystems. It is important to note that low substance- or chemical class specific detection frequencies are not indicative of low environmental risks (see also the chapter Environmental Risks below). On the whole, several lines of evidence point towards increasing detection of organic chemicals throughout Europe, therefore, Hypothesis 2a was rejected.

Overall, 32% ($n = 26,808$) of monitoring stations did not detect any chemical in a given year. Further analysis of these potentially “unexposed” sites revealed significantly different monitoring characteristics compared to those sites where chemicals were found regularly. At the sites in question, analyzed substance spectra were significantly lower by 53.9% ($p < 0.001$) and sampling frequencies were significantly lower by 41.8% ($p < 0.001$), both influencing detection frequencies negatively, thereby questioning if the sites were truly devoid of organic contamination. However, significantly lower anthropogenic land-use ($p < 0.001$) in the contributing catchment (44.5% vs. 61.6%) partially explains the observations. Nonetheless, a relationship became evident

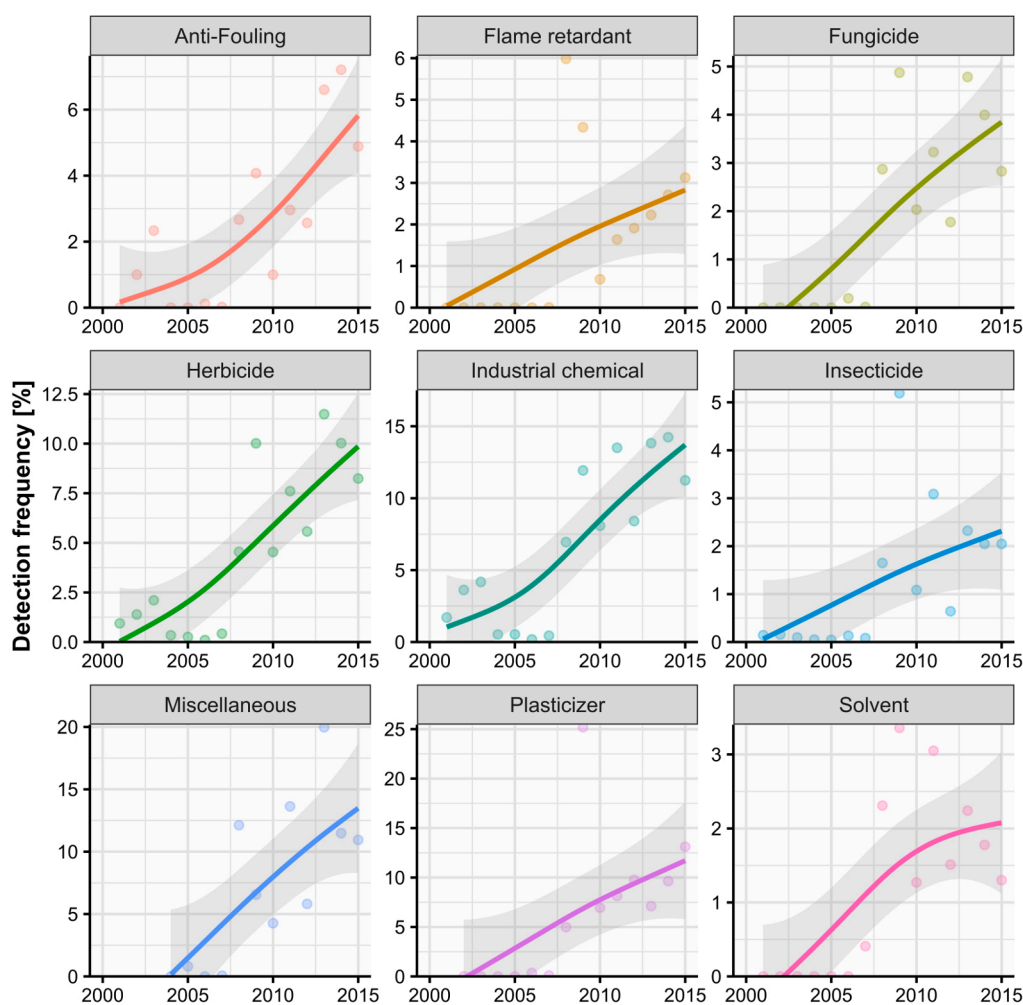


Fig. 4. Average annual detection frequencies in percent for each of the 10 chemical classes across Europe with LOESS regressions to indicate temporal trends. At least 8 years of consecutive data was required for the depiction of temporal trends, thus pharmaceutical and hormone data were omitted. Please note that y-axes are scaled individually. Trends were similar, although less robust, for those four countries that provide data for 2013–2015 (data not shown).

between countries' MQI and the fraction of monitoring stations that did quantify any organic contaminants (Fig. 5a). A similar relationship can also be observed on the level of chemical classes (Fig. 5b). These considerations underline the importance of proper monitoring strategies for finding contaminants in the aquatic environment and of the MQI's suitability to be used as a crude monitoring quality score. Therefore, if MQI metrics were improved, then some of those currently "unexposed" sites would likely show actual organic contamination with proper monitoring protocols in place.

Pharmaceuticals were only monitored in recent years (i.e. since 2010, $n = 35,341$) and showed detection frequencies of 58.3% on average, much higher than those of any other chemical class currently monitored. In this context, von der Ohe et al. (2011) found, for example, that regularly used pharmaceuticals (diclofenac, ibuprofen) were detectable in > 90% of measurements in river basins in Northern Germany, suggesting that high-use pharmaceuticals can be regularly present in anthropogenically influenced watersheds, as they mainly enter surface waters via the permanent outflow of waste water treatment plants (WWTPs). Especially during baseflow pharmaceuticals are found frequently at higher concentrations following their introduction via WWTPs (Shala and Foster, 2010), however, exposure profiles become more variable in watersheds with diverse urban and agricultural sources (Guillon et al., 2015). Furthermore, a recent study found 69 pharmaceuticals (mainly anti-inflammatory drugs) to be regularly present in Spanish rivers, with multiple sites experiencing exposures expected to adversely impact aquatic biota (Fonseca et al., 2020). In the present study, non-steroidal anti-inflammatory drugs were detected most often ($n = 9809$), followed by the anti-convulsant drug carbamazepin ($n = 4475$), and several antibiotics ($n = 2952$). Non-steroidal anti-inflammatory drugs and antibiotics have been linked to detrimental effects in aquatic ecosystems (Geissen et al., 2015; Godoy et al., 2015; Jonsson et al., 2015; Katipoglu-Yazan et al., 2015; Roose-Amsaleg and Laverman, 2016), suggesting that continued monitoring of these substances is critical. The large number of pharmaceuticals currently in circulation (Owens, 2015) and the potential of some of them to bio-accumulate or bio-magnify through foodwebs and to cross the aquatic-terrestrial interface (Richmond et al., 2018) gives further reason to monitor this group of contaminants.

The hormonal compounds 17-beta-estradiol (E2), Estrone (E1), and 17-alpha-ethinylestradiol (EE2) were detected in 8.3% of all samples. These substances, being main agents in oral contraception medication, are also expected to be mainly transported into surface waters via WWTPs. While their occurrence is not expected to evoke acute toxic effects at current exposure levels, their regular presence in aquatic systems poses risks of endocrine activity, such as increased vitellogenin

production in fish, shifting sex ratios within vertebrate communities or producing intersexuality (Brack et al., 2017). The presence of hormonal substances is also poorly documented in the present data as only few monitoring stations started probing for them recently resulting in only 157 measurements between 2012 and 2015. Analysis of all the data, i.e. data from 2015 onwards, shows even higher detection frequencies of 11.9% ($n = 1064$). The indication of high detection frequencies, which are comparable to those of plasticizers or industrial chemicals, underscores that further investigation of the long-term presence of hormonal compounds in European surface waters is required.

3.3. Environmental risks

Contrasting the comparatively low detection frequencies of most chemical classes (Fig. 3; Fig. 4), single organic contaminants still posed substantial risks to non-target aquatic species. We found that 23.9% ($n = 17,162$), 18.9% ($n = 16,588$), and 17.8% ($n = 17,053$) of MRAAs exceeded their respective $RTL_{\text{aqua-inverts}}$, RTL_{fish} , and $RTL_{\text{aqua-plants}}$ (Fig. 6a). RTL exceedances of AACs were expectedly lower, as this approach – in contrast to the MRAA – did not account for the co-occurrence of substances in space and time, which may be considered an "environmental risk dilution" (for details see SI AAC Risk Analyses). Noteworthy, risk metrics relate to individual substances, thus not accounting for additive or potentiating effects of contaminant mixtures, which may reveal further environmental issues, if temporally more granular data become available. Overall, more than one third (35.4%, $n = 17,358$) of MRAAs exceeded their ecological threshold (RTL_{sw}), putting at least one of the organism groups at significant risk once per year (Fig. 6b). As such, we found that, throughout their operation, 36.7% of the 8209 monitoring sites have recorded concentrations that are adversely affecting aquatic ecosystems representing 38.2% of all waterbodies ($n = 7227$).

Acute ecological risks (i.e. MRAA to RTL_{sw} ratios > 1, $n = 6143$, Fig. 6a) were mainly posed by insecticides (56.5%), herbicides (27.4%), and industrial chemicals (10.9%), whereas all other chemical classes (5.2%) contributed substantially less (Fig. 7c). Finding that pesticides were responsible for 85.0% of all RTL_{sw} exceedances (Fig. 7c) in European surface waters further highlights that agricultural non-point source pollution, and to some lesser degree point sources pollution (Kreuger and Nilsson, 2001), remain a widespread problem throughout Europe. As such, continued risk reduction and management strategies are urgently needed to avoid pesticides' subsequent transport into non-target aquatic systems. However, more ambitious or even paradigm-shifting policy changes may be necessary to significantly curb aquatic pesticide risks, given their dominant impact on water quality on the

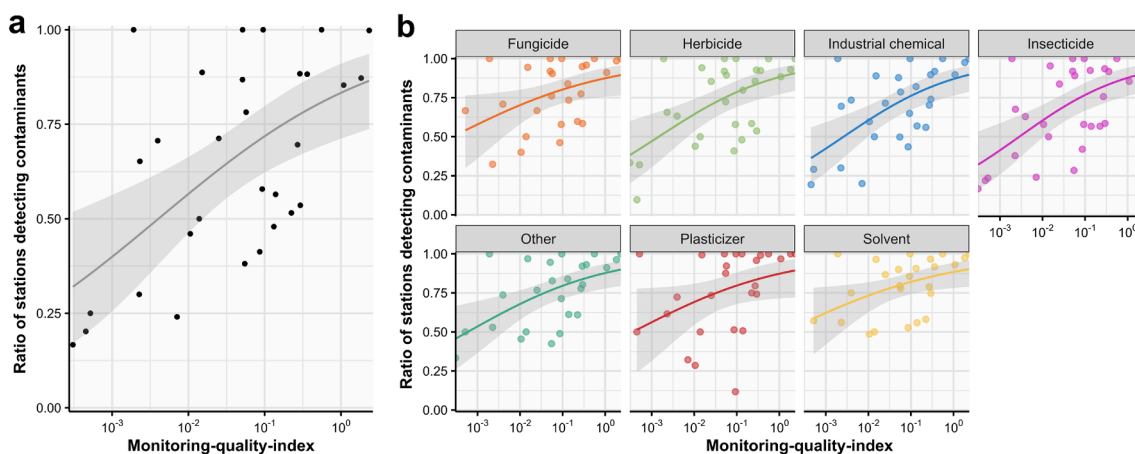


Fig. 5. (a) GLM regressions (quasibinomial, link = logit) between countries' MQI scores and the fraction of countries' monitoring stations that detected organic contaminants. (b) GLM regressions (quasibinomial, link = logit) between countries' MQI scores and the fraction of countries' monitoring stations that were able to detect the 7 main chemical classes. Please note that pharmaceuticals and anti-fouling agents are now grouped into "other", see also Methods.

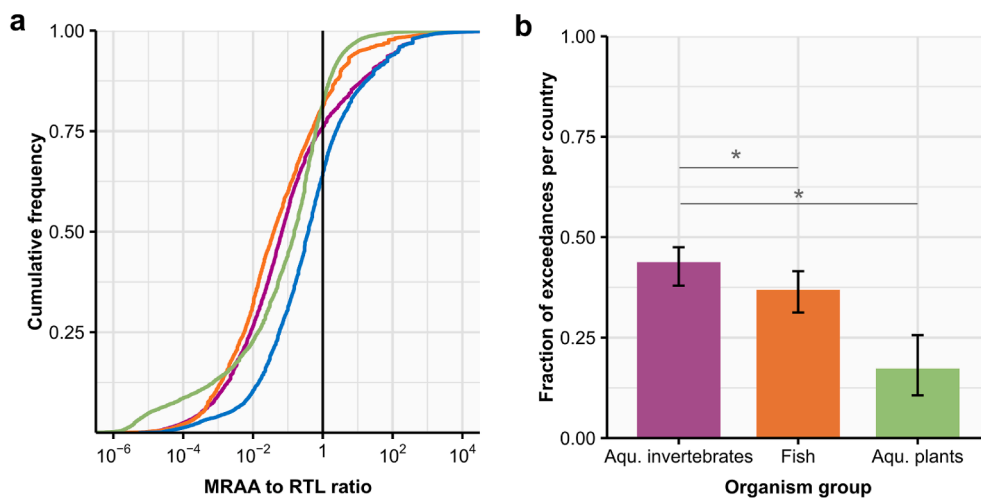


Fig. 6. (a) Cumulative frequency distributions of \log_{10} -transformed MRAA to RTL ratios with the black solid line depicting respective RTLs. Aquatic invertebrates (magenta), fish (orange), aquatic plants (green), and ecologically most relevant species (blue). (b) Barplots and their 95%-CIs depicting the contribution of each species group (fraction) to the total number of ecological threshold exceedances for countries ($n = 20$) with at least 30 spatiotemporally distinct entities. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

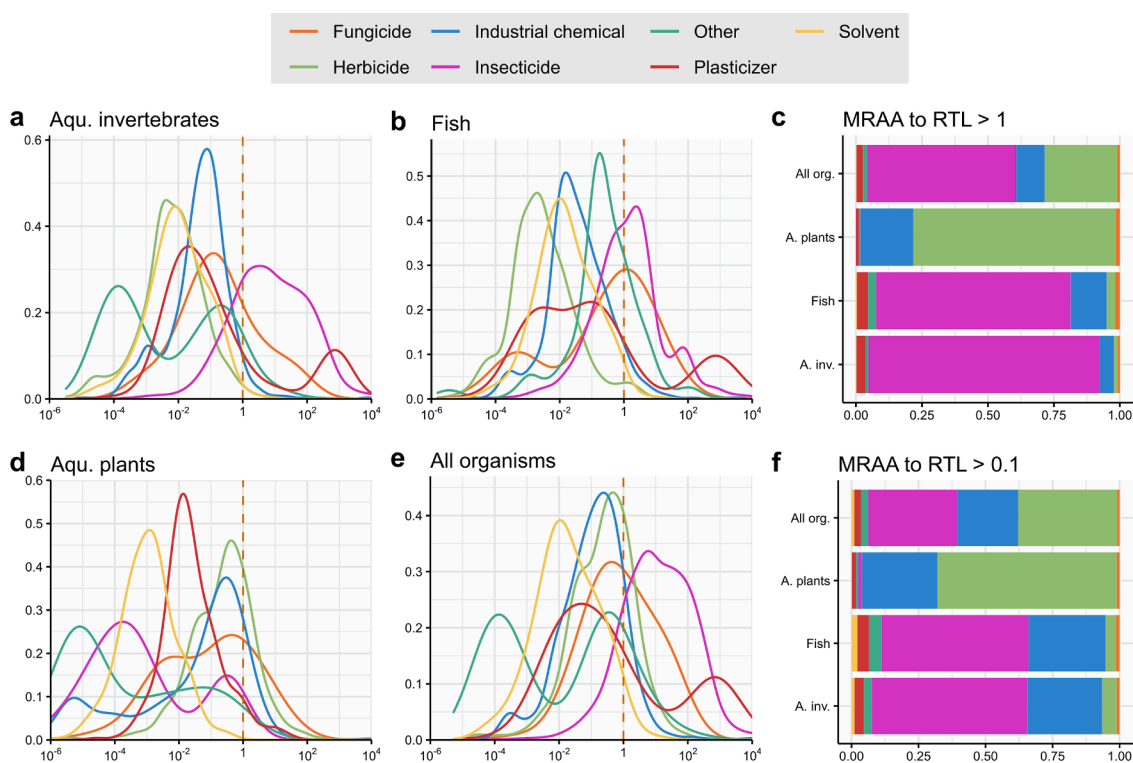


Fig. 7. Density distributions of MRAA to RTL ratios per chemical class and organism group (a, b, d, e). Density distributions depict how MRAAs of certain chemical classes compare to their respective RTLs (dashed line, equality of MRAA and RTL) but do not provide any information about the absolute number of concentrations that occur in these ranges. In (c), the fraction of all MRAA to RTL exceedances is provided per chemical class to deduce their relative impact on water quality. In (f), the fraction of MRAA to $(0.1 \times \text{RTL})$ exceedances is depicted.

continental scale. Current debates held by some member states with the aim to substantially reduce pesticide use and their associated environmental impact by 2030 (European Commission, 2020) should be seen as a necessary endeavor. Although, whether such ambitions match the gravity of pesticides' impacts, remains yet to be seen. Insecticides' large contribution to the overall risk profile (Fig. 7c) also requires special attention, because these substances are generally difficult to capture during monitoring efforts due to their fast dissipation rates. Especially in larger waterbodies, which are over-proportionally represented by the WFD data (SI Spatial Data Appendix), insecticide risks are expectedly lower compared to lower order streams (i.e. up to a factor of 100, see Stehle and Schulz, 2015 or Wolfram et al., 2019), where nonetheless

they are frequently found at adverse concentrations. Especially pyrethroid insecticides are extremely elusive yet biologically active in or below nanograms per liter ranges that may still be unperceived in the current dataset (Rösch et al., 2019). As such, it is evident that detection frequencies and actual aquatic risks are not directly related, rather the contrary appears to be the case here, as insecticides, which are one of the most elusive chemical classes, define aquatic risks on a European scale.

The chemical classes driving acute ecological risk in surface waters differ considerably among organism groups (Fig. 7). Threshold exceedances in invertebrates (Fig. 7a) and vertebrates (Fig. 7b) are mainly driven by insecticides and to a lesser degree by industrial chemicals. In algae and plants (Fig. 7d), herbicides and industrial chemicals represent

95% of exceedances. There are large differences within the group of industrial chemicals regarding their impact on aquatic plants and invertebrates, as no significant correlation was found between each substance's contribution and either group's exceedance rate (Pearson = 0.08, $p = 0.60$). This shows that different organism groups are susceptible to different high-risk chemicals within the group of industrial chemicals.

From an ecosystem perspective, insecticides and herbicides are the primary risk drivers, followed by industrial chemicals (Fig. 7e). We found that industrial chemicals contributed substantially more when considering concentrations that reach at least 10% of their threshold level (Fig. 7f). This finding shows that industrial chemicals occur frequently in concentrations that are close to their RTL and potentially affect organisms, even if they are not indicative for unacceptable risk as currently defined (see Methods). Additionally, it cannot be ruled out with certainty that acute ecological risks arising from industrial chemicals may be more pronounced than documented here, because ecotoxicological testing of these chemicals is less expansive compared to, for instance, pesticides (c.f. Regulation (EC) No 1907/2007, Regulation (EC) No 1107/2009).

With the present methodology, no acute risk could be identified for pharmaceuticals, however, this may only be the result of missing toxicity data, requiring extrapolative approaches to fill these gaps (Owens, 2015; Fonseca et al., 2020). In this context, recorded peak concentrations of 3.8 $\mu\text{g/L}$ (ibuprofen), 4.4 $\mu\text{g/L}$ (diclofenac), and 0.25 $\mu\text{g/L}$ (azitromycin) are within ranges for which Fonseca et al. (Fonseca et al., 2020) derived aquatic risks; specifically, diclofenac and azitromycin were among the most ecologically detrimental substances identified in their study. Pharmaceuticals' regular occurrence (i.e. 58.3% of samples) also suggests that chronic exposure pathways are ecologically relevant with first indications that their long-term presence can negatively affect aquatic ecosystems (Fonseca et al., 2020). Hence, pharmaceuticals should receive more attention in ecotoxicological

research and monitoring.

3.4. Evaluation of WFD environmental quality standards

Ecotoxicological evaluation of AACs ($n = 971,381$) was possible for 30.9% ($n = 300,603$) when using EQS and for 91.6% ($n = 889,734$) when using RTL_{sw} . Usage of both threshold types resulted in exceedance frequencies with similar magnitude for AACs: 15.7% (EQS, $n = 63,723$) and 9.6% (RTL_{sw} , $n = 127,999$, Fig. 8a). Assessment of MRAAs showed only minor differences in exceedance frequencies with 33.1% ($n = 4714$, $N = 14,247$) using EQS and 35.7% ($n = 6233$, $N = 17,484$) using RTL_{sw} (Fig. 8b). While exceedance frequencies are mostly similar, some key differences between the usage of EQSs and RTLs remain. First, 22.9% more AAC exceedances can be found when using RTL instead of EQS. In return, the majority of AAC exceedances, found using RTLs, were also indicated using EQS. Second, spatiotemporal coverage of assessable sites decreased by 18.5% (14,146 instead of 17,358 sites \times year combinations) when using EQSs instead of RTLs, and nearly one quarter of ecological impairments (24.0%, $\text{MRAA to RTL}_{\text{sw}} > 1$) would have been overlooked. Third, all MRAAs comprise of significantly ($p < 0.01$) fewer chemicals when using EQS, i.e. on average only 36.0% ($\text{SD} = \pm 23.6\%$) of samples, constituting an MRAA, are assessable with EQS, compared to the average chemical coverage of 88.9% ($\text{SD} = \pm 18.8\%$) provided by the RTL_{sw} .

Overall, monitoring was mostly successful in probing both for priority substances and for additional contaminants of concern. Our results show that the RTL approach provides very good spatial coverage of Europe (Fig. 8c), with the exception of Hungary. EQS characterize a smaller fraction of exposure compared to RTL in most countries, except for Austria, Portugal and Malta, where sampled substance spectra are mostly limited to the priority substances covered by EQS (Fig. 8c). WFD priority substances and their EQS appear to be an adequate initial selection of organic contaminants that are ecotoxicologically relevant in

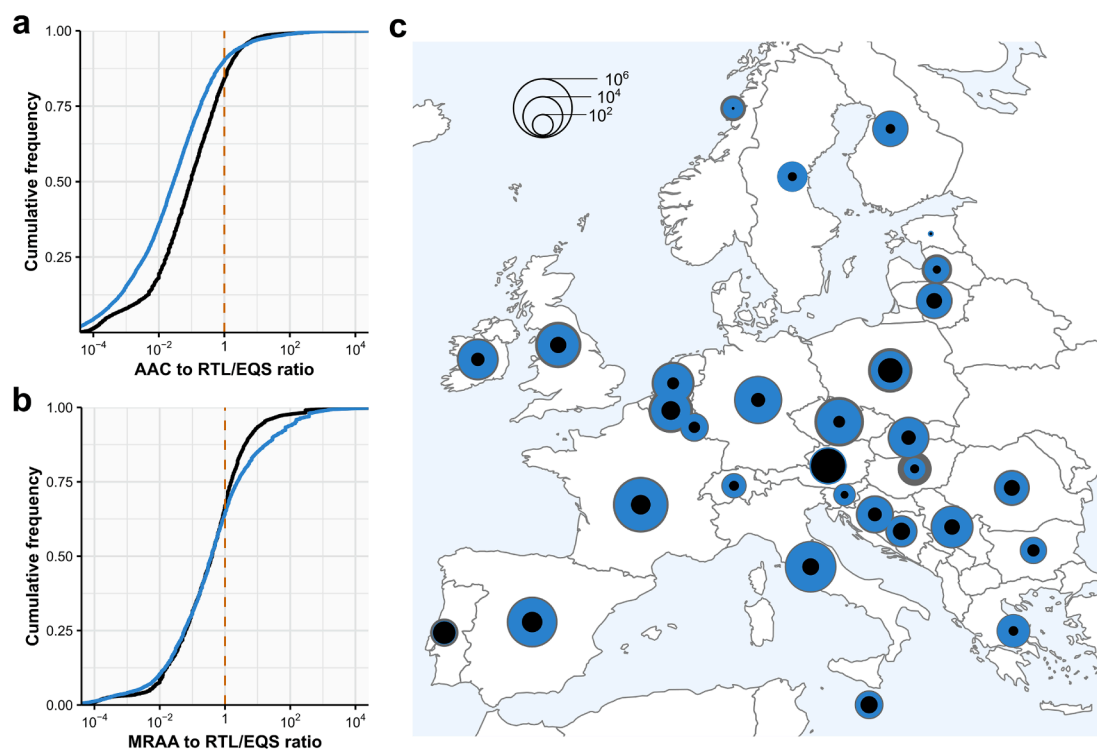


Fig. 8. Cumulative frequency distributions of (a) AAC to RTL_{sw} ratio (blue) and AAC to EQS ratio (black), (b) MRAA to RTL_{sw} ratio (blue) and MRAA to EQS ratio (black). (c) Map of measurements per country (grey), those assessable with RTL_{sw} (blue) and those assessable with EQS (black). The size of each circle corresponds to each country's number of AACs taken (grey), assessable with the RTL_{sw} approach (blue and black), or assessable with the EQS approach (black). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Europe. Consideration of non-priority substances that reflect local land-use practices, however, remains critically important for a complete acute risk characterization of surface waters but is not uniformly practiced yet (c.f. Fig. 2b). Especially, newer, yet highly toxic insecticides (e.g. pyrethroids, neonicotinoids), are relevant in this context, as they were shown to particularly affect aquatic ecosystem integrity in both agricultural and urbanized landscapes (Stehle et al., 2018; Wolfram et al., 2018; Stehle et al., 2019).

3.5. Risk trends

Risk trend analyses, i.e. aquatic risks being understood as the development of MRAA to RTL ratios over time, show that further changes to current management strategies are needed to improve the chemical quality of European waterbodies. Monotonic trend analysis suggested that median risks remained statistically unchanged ($p > 0.05$) for aquatic plants and all organism groups combined (Fig. 9). Median risks significantly increased since 2001 for aquatic vertebrates ($\tau = 0.498$, $p = 0.01$; Fig. 9a) and invertebrates ($\tau = 0.429$, $p = 0.03$; Fig. 9b) between 0.5 and 1 order of magnitude. This can be either interpreted as a deterioration of the chemical quality of European streams and lakes for fish and invertebrate assemblages, or, alternatively, as a continuation of chemical risk, which is becoming more apparent as monitoring efforts were found to improve over time (c.f. Table S3). Which of those aforementioned cases applies, or if it is a combination of both, is however beyond the scope of this study. In either case, the results still underline that a substantial fraction of European waterbodies frequently and recently experienced chemical exposures that adversely impact biota, posing unacceptable risks to the environment such that Hypothesis 2b is rejected.

Of 1917 monitoring stations, for which at least 5 years of monitoring data were available, the largest share ($n = 1826$, 95.8%) showed no

significant improvements ($p > 0.05$; RTL_{sw}) over time. Only 24 sites (1.3%) showed significantly decreasing risks and at 55 sites (2.9%), risks significantly increased over time. Risk trends may be dissimilar at the national level, as a result of varying national management strategies aiming at reducing certain types of surface water pollution. Results on the national scale, based on data from countries with at least 4 years of consecutive data and at least 10 distinct sampling sites annually ($n = 21$ countries), were comparable: 85.3% ($n = 58$) of national temporal risk trends for all organism groups did not change significantly. Only Belgium was able to significantly reduce average aquatic risk nationally for all four organism groups from 2001 to 2015, indicating that applied management strategies (Belgian Federal Government, 2014) have a positive effect on national water quality (see Figure S8). In the remaining cases of significantly decreasing trends, average risks were only reduced for either fish (France, United Kingdom) or aquatic plants (France, Netherlands, United Kingdom). It is furthermore noteworthy that no country, except for Belgium, succeeded in significantly reducing the average risk towards aquatic invertebrates. Most importantly, currently applied management strategies appear insufficient in meaningfully decreasing risks in most European waterbodies. Hence, achieving the goals set out by the WFD remains a challenge.

Recent studies' conclusions that the health of aquatic invertebrate communities is improving globally and in Europe as well, would initially appear to be in opposition to our results (Outhwaite et al., 2020; van Klink et al., 2020). As we found that aquatic invertebrates were the organism group most acutely at risk, based on three principal aspects assessed here: Probabilistically, MRAAs most frequently exceeded their $RTL_{aqua-inverts}$ compared to other organism groups (Fig. 6a); Temporally, trend analysis showed significantly increasing risk (Fig. 9), which indicates continuation or deterioration of surface water quality for invertebrates; Spatially, threshold exceedances per country were significantly more widespread for aquatic invertebrates than fish ($p =$

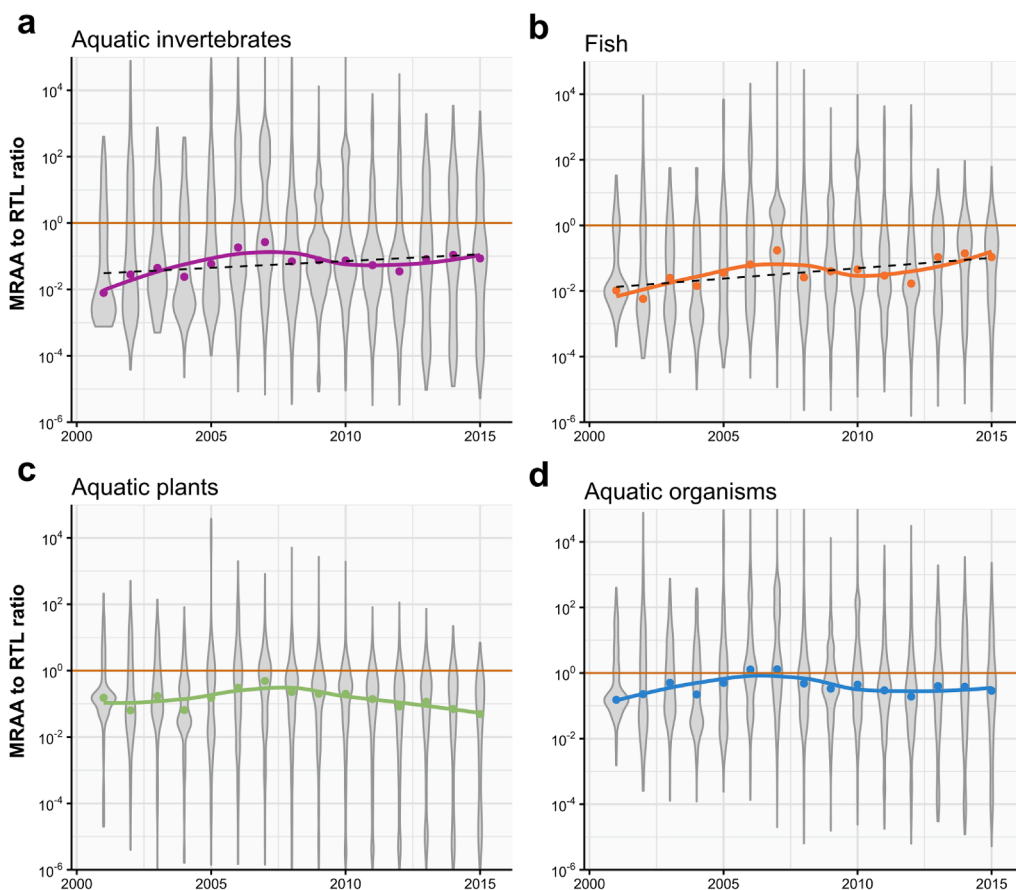


Fig. 9. Median MRAA (dots) to (a) $RTL_{aqua-inverts}$, (b) RTL_{fish} , (c) $RTL_{aqua-plants}$, and (d) RTL_{sw} ratios over time with the underlying data distribution per year depicted with violin plots (grey). Temporal trends are indicated by LOESS regression. Significant monotonic temporal trends (Mann-Kendall, $p < 0.05$) are depicted with dashed lines (black). Specific temporal trend analyses of the most impactful chemical classes (insecticides, herbicides, and industrial chemicals) can be found in SI Chemical class specific risk trends.

0.03) or aquatic plants ($p < 0.001$, Fig. 6b), indicating a pan-European phenomenon that most severely puts aquatic invertebrates at risk. However, we found that pesticides are the main driver of observed aquatic risks but Outhwaite et al. (2020) as well as van Klink et al. (2020) could not derive results that are directly attributable to agricultural practices, which furthermore highlights the present ecotoxicological knowledge-gap with respect to large-scale and comprehensive links between agriculture and aquatic invertebrate health. Our results should therefore motivate further investigations into this complex but central task of deciphering invertebrate community integrity on a trans-national scale, particularly with respect to pesticide contamination.

3.6. Spatial analysis

Unacceptable aquatic risks were found throughout Europe, only a few spatial clusters did not show at least sporadic average RTL exceedances for MRAA (Fig. 10a-d). This indicates regular occurrences of organic chemicals at concentrations likely impairing aquatic biota. Chemical risks showed noticeable spatial differences between organism groups. For instance, risks for aquatic plants compared to those for aquatic invertebrates revealed spatial clusters where aquatic plants are exposed to substantially higher toxic pressures (i.e. the difference between both risk estimates) than invertebrates (see Figure S9), namely in parts of northern Italy, Austria, and western France. Spatial differences in toxic pressures towards certain organism groups likely result from

differing regional land-use practices. For instance, coastal regions in Spain (e.g. Catalonia, Andalusia, Figure S9, Fig. 11), where higher toxic pressures towards invertebrates were found, are intensified agricultural regions dominated by orchards, citrus farms, vineyards and olive groves. These land-uses rely on considerable insecticide use to combat fruit flies and other pests (Baker, 2016). Conversely, some northern parts of Italy (e.g. Piedmont, Lombardy) are diverse agricultural regions with higher risk for aquatic plants (Piedmont) or aquatic invertebrates (Lombardy, c. f. Figure S9). Both regions are characterized by intensive irrigation practices (eurostat, 2018), which are known to promote transport of pesticides into adjacent surface waters by runoff (Wolfram et al., 2019). Varying land-use of both regions might at least partly explain the different risks for aquatic plants and invertebrates (eurostat, 2018). Identifying the chemical class most commonly defining MRAs per site further show that insecticides are the dominant risk driver in the aforementioned regions in Spain (Fig. 11), and herbicides in Austria and Northern Italy (Piedmont; Fig. 11). However, the higher toxic pressure towards aquatic plants in Austria should be interpreted with care, because monitoring strategies were also shown to be restricted to WFD priority substances (Fig. 8c), so that the risk of many other relevant organic contaminants (including insecticides) remains opaque. Risk reduction strategies need to be tailored towards local or regional land-use characteristics, as latter vary regionally, producing discernible differences in aquatic risks for organism groups.

Importantly, agricultural land-use in the upstream-catchment plays

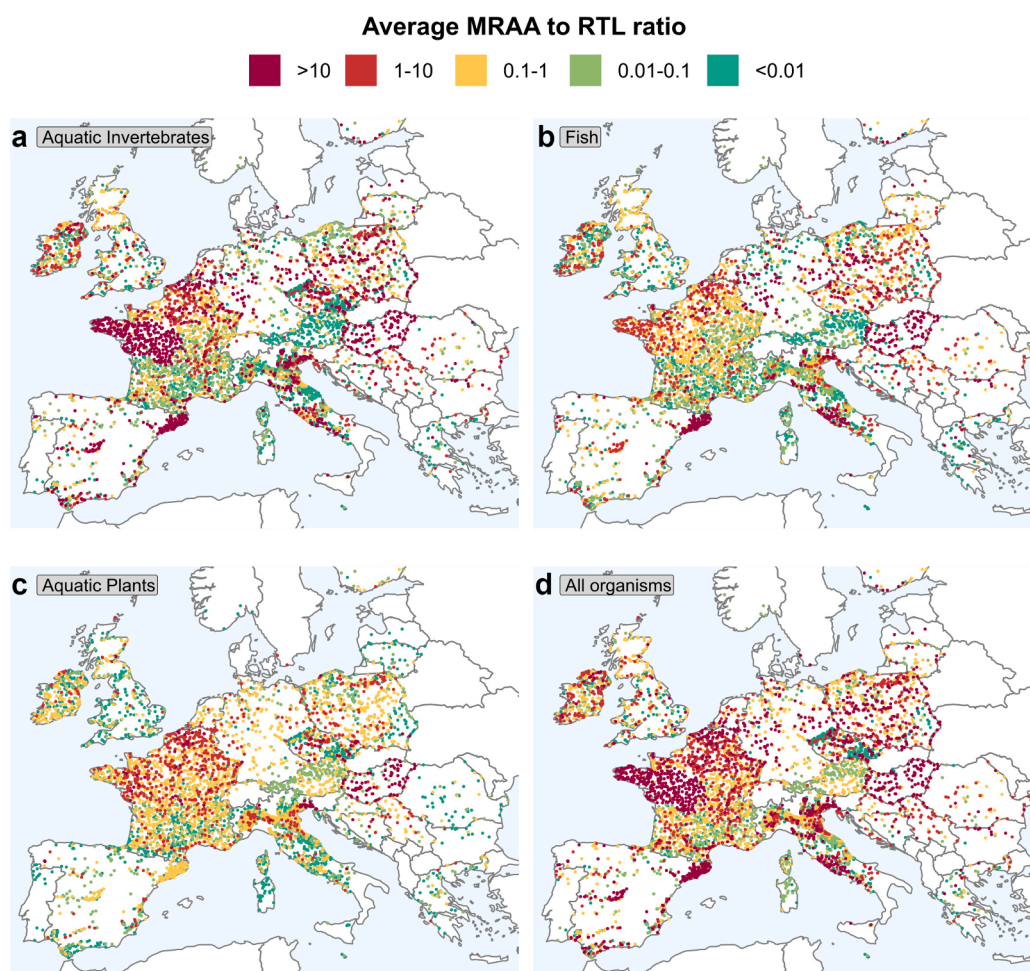


Fig. 10. Average MRAA to RTL ratios per monitoring site for (a) aquatic invertebrates, (b) fish, (c) aquatic plants, and (d) all organisms. Data were not aggregated per waterbody or catchment to give an exact representation of the spatially attributable sampling locations (88.3%, $n = 8209$). However, this necessitates care when interpreting this figure, as spatially denser countries (e.g. France) may appear to have higher aquatic risks compared to less densely represented countries; a conclusion that should not be drawn from this figure.

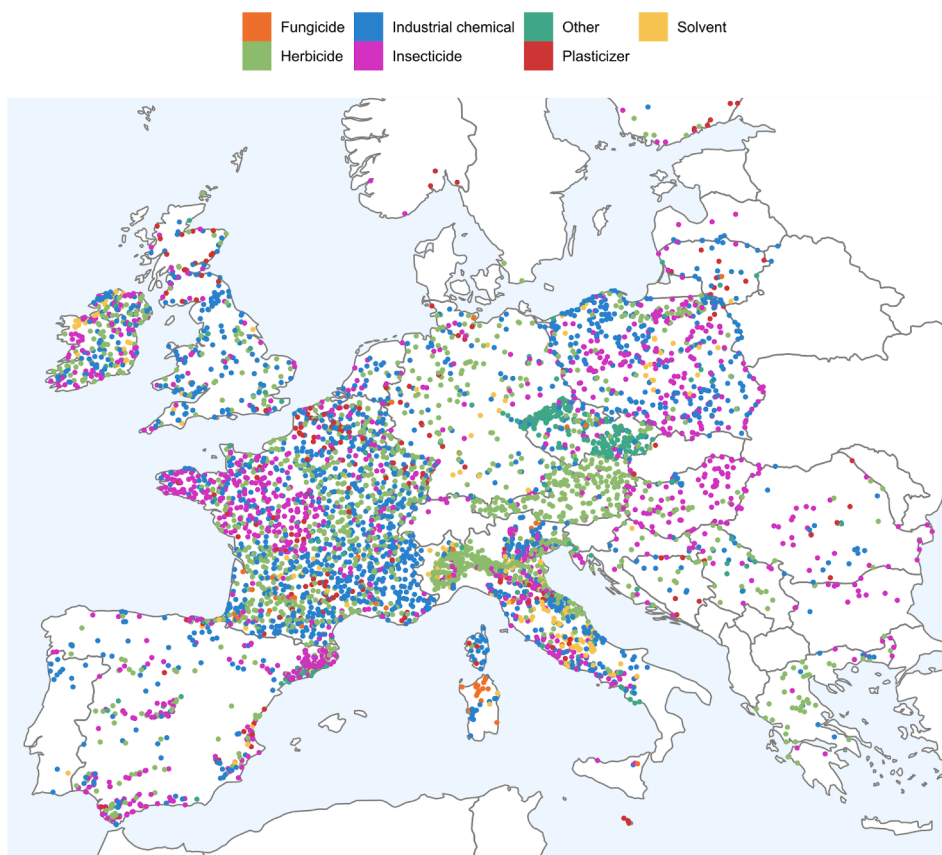


Fig. 11. Chemical class per monitoring site that most commonly defines the MRAA to RTL_{sw} ratio, i.e. the chemical class most commonly responsible for the highest risk in all organism groups combined per site.

the central role in deriving aquatic risks for invertebrates, aquatic plants, and aquatic organisms in general (Fig. 12). MRAA to RTL ratios (i.e. risks) differed by approximately an order of magnitude on average between sites with low (<15%) versus high (>80%) agricultural land-use, demonstrating a clear link between agricultural intensification and average risks (Fig. 12). Pesticides are, however, also defining MRAAs in surface waters with minimal agricultural land-use (Fig. 12). Here, herbicides most commonly defined MRAAs, which is in accord with their diverse commercial and private application patterns, such as silvicultural, roadside, railway, industrial, and private infrastructure weed control (Heap and Duke, 2018). Conversely, average risks in highly intensified regions are not solely defined by pesticides (see

Fig. 12d), but to a lesser degree by other chemical classes (industrial chemicals, solvents, and plasticizers). Thus, spatially small land-use features (e.g. residential WWTPs and waste-water outlets) can still pose local aquatic risks for all organism groups. In comparison, other land-use characteristics (e.g. industrial, urban) did not show clear relationships between areal fractions in the upstream-catchment and MRAA to RTL ratios. Thus, landscape-level management schemes aiming to reduce the use of pesticides or prevent their subsequent transport into non-target aquatic systems are an immediate opportunity to curb environmental risks in highly intensified regions.

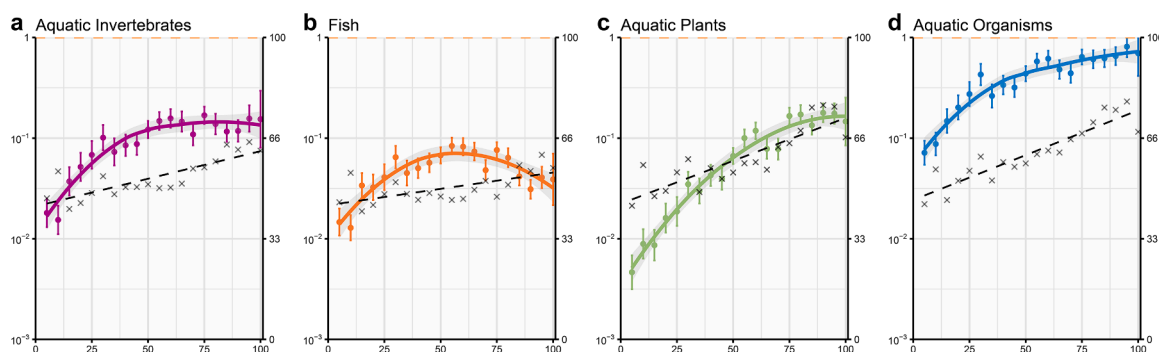


Fig. 12. Average aquatic risks ($\pm 95\%$ CI, MRAA to RTL ratios, primary y-axis) as a function of agricultural land-use (aggregated into evenly-spaced 5% intervals) for (a) aquatic invertebrates, (b) fish, (c) aquatic plants, and (d) all organism groups combined (RTL_{sw}). LOESS regressions depict the approximate relationship between the degree of agricultural intensification within respective catchments and observed average risks for each organism group (a-d). Pesticides (i.e. herbicides, insecticides, fungicides) percentage contribution to the MRAA is depicted with black crosses, and corresponding linear trends are depicted with black dashed lines (secondary y-axis).

3.7. Recommendations

Whilst providing a comprehensive risk analysis for European waterbodies, data-related issues became evident that arise from heterogeneous network characteristics (Fig. 2, Table S3), which currently hinder additional analyses. We therefore want to encourage improvement of the existing WFD networks – or subsequent monitoring schemes – to further support the environmental protection goals that were formulated nearly two decades ago (Directive 2000/60/EC). Acknowledgment of the multidimensionality of aquatic risks (i.e. chemical, organismic, temporal, spatial risks) requires continuous improvements to existing monitoring systems in order to both advance our understanding of emerging ecological challenges and to provide a factual basis that is able to support sound management and regulatory actions. Advancements remain needed, considering that environmental conditions have either remained unchanged or deteriorated (Fig. 9), as the last phase of the WFD has begun. Thus, we formulate six recommendations aimed at improving aforementioned data-related shortcomings, which are ordered from an “ease-of-implementation” point of view to aid in prioritizing easy-to-achieve data improvements. These recommendations may also find broader application, for instance outside Europe, when monitoring networks are organized by multiple stakeholders, potentially resulting in considerable data heterogeneity that requires continuous harmonization efforts.

1. *Acceleration of data reporting, processing (i.e. QA/QC) and publication.* Most recent data for the majority of contributing countries is dating back to 2012, which is unsuitable when it comes to assessing efficacy of management practices in a timely manner. Only five countries provided data for 2016 onwards (Czech Republic, Belgium, Germany, Spain, and Romania), with the Czech Republic spearheading the reporting pace by providing results of approx. 791,000 samples since 2016. Developing process workflows based on Czech protocols may provide member states a “tried-and-true” blueprint to improve their internal workflows.
2. *Complete provision of disaggregated data for improved spatiotemporal analysis.* Currently, raw (i.e. disaggregated) data are incomplete for some countries (e.g. Germany, Spain) compared to the aggregated data, which hinders further temporal co-occurrence analyses that are required for mixture toxicity evaluations, the development of chemical association networks, and chronic toxicity analyses. In view of frequent detections of chemical classes (Fig. 4) such as herbicides, industrial chemicals, and pharmaceuticals, the need for temporally more granular data is highlighted to assess potential long-term ecological risks and that of additive or interactive effects of contaminant mixtures, which currently remain unknown.
3. *Expansion of monitoring for hormonal and pharmaceutical chemicals.* As monitoring protocols are already in place for most countries, expanding analytical protocols to include common pharmaceuticals and hormones should not critically increase operational costs and so offers a cost-efficient solution to a potentially unperceived environmental issue. In addition, current gaps in ecotoxicological effect data for most pharmaceuticals, especially with respect to chronic toxicity data, need to be closed to enable comprehensive risk evaluations (see EC COM (2019), 128/F1), for instance via the use of QSAR-derived or extrapolative toxicity estimates (Fonseca et al., 2020).
4. *Pesticide application information in support of spatial ecotoxicological assessment.* In light of the land-use dependent risk profiles of pesticides, harmonized and publicly available pesticide use data for Europe is needed so that monitoring efforts can improve substance targeting and more source-oriented spatial analyses are enabled.
5. *Country specific MQIs differ substantially.* A comparable data basis between countries (i.e. similarity in MQIs) is fundamental for more reliable and *trans*-national risk mitigation or reduction strategies, if a “good ecological status” is to be achieved by 2027. However,

formulation and implementation of more uniform monitoring requirements will demand long preparation and legislative support.

6. *Intensifying monitoring programs in upstream regions to guide risk reduction strategies.* National monitoring efforts that focus on smaller waterbodies (e.g. upstream reaches) provide vital information about the true sources and annual loads of contaminants (Neale et al., 2020; Spycher et al., 2018). Existing monitoring networks with this focus may be used as blueprints for *trans*-national implementation strategies. Such efforts would also improve the representativeness of WFD data with respect to the actual distribution of waterbodies in Europe (see SI Spatial Data Appendix).

4. Conclusions

While we provide a comprehensive risk analysis for European waterbodies, it should be remembered that such analyses are dependent on the underlying data and its quality. Therefore, the shown spatio-temporal heterogeneity (see Fig. 2, Fig. 3) warrants care when interpreting our results, but it may also obscure actual risk in the waterbodies. Quantifying these relevant aspects of data heterogeneity by means of a comparable index (i.e. MQI) should generally find further application in monitoring or related research so that sources of uncertainty can readily be contextualized and, importantly, further reduced in the future. To minimize influences of data heterogeneity, chained aggregation was generally applied when possible to construct robust estimates, detailing overarching trends. In addition, multiple lines of evidence were employed by analyzing a single phenomenon (e.g. increasing detection frequencies) with multiple tools (e.g. GAM, LOESS) or from differing points of view in order to evaluate if conclusions are supported by multiple analyses.

However, further challenges with the present monitoring data remain, and should form the basis for future conceptual improvements. For instance, due to highly variable physico-chemical characteristics of currently used chemicals, water phase sampling of pollutants represents only one piece of the puzzle for understanding actual pollutant dynamics in aquatic systems and the resulting environmental risks. System-based approaches that expand on this assessment method by incorporating further environmental compartments, such as sediments (Smalling et al., 2007; Smalling et al., 2013; Wang et al., 2012; Weston et al., 2015), biota (Richmond et al., 2018), and biofilms (Mahler et al., 2020), would greatly improve our understanding of pollutant dynamics as well as form a basis that may be better suited for deducing actual shifts in ecosystem structure and functioning. The environmental magnitude of effects, i.e. the severity of impairments, is also difficult to assess. Whilst direct acute effects can be deduced with the current approach, the transduction of these effects through the connected ecological matrix (e.g. via indirect or food-chain effects) can – at best – be only estimated. Also, chronic ecological risks, due to the lack of temporally more granular data, remain currently unknown but require attention in light of high detection frequencies for several chemical classes. Our analysis should therefore prime new vigor for expanding, improving, and integrating the WFD monitoring schemes, or any scheme that may follow in its footsteps, based on expansive recommendations (Altenburger et al., 2019; Brack et al., 2017; Carvalho et al., 2019; Faust et al., 2019; Geissen et al., 2015), because the WFD remains one of the most ambitious environmental policies that – despite its shortcomings – can achieve continental improvements to surface water quality.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2021.106479>.

References

- Altenburger, R., Brack, W., Burgess, R.M., Busch, W., Escher, B.I., Focks, A., Hewitt, L.M., Jacobsen, B.N., de Alda, M.L., Ait-Aissa, S., 2019. Future water quality monitoring: improving the balance between exposure and toxicity assessments of real-world pollutant mixtures. *Environ. Sci. Eur.* 31 (1), 1–17.
- Baker, N.T., 2016. Agricultural Pesticide Use Estimates for the USGS National Water Quality Network, 1992–2014. U.S. Geological Survey Data Release.
- Beketov, M.A., Schäfer, R.B., Marwitz, A., Paschke, A., Liess, M., 2008. Long-term stream invertebrate community alterations induced by the insecticide thiacloprid: effect concentrations and recovery dynamics. *Sci. Total Environ.* 405 (1–3), 96–108.
- Beketov, M.A., Kefford, B.J., Schäfer, R.B., Liess, M., 2013. Pesticides reduce regional biodiversity of stream invertebrates. *Proc. Natl. Acad. Sci. USA* 110 (27), 11039–11043.
- Beketov, M.A., Liess, M., 2005. Acute contamination with esfenvalerate and food limitation: chronic effects on the mayfly, *Cloeon dipterum*. *Environ. Toxicol. Chem.* 24 (5), 1281–1286.
- Belgian Federal Government, Belgian action plan to reduce the risks and impacts linked to pesticides. In Force, N. T., Ed. 2014.
- Berenzen, N., Kumke, T., Schulz, H.K., Schulz, R., 2005. Macroinvertebrate community structure in agricultural streams: impact of runoff-related pesticide contamination. *Ecotoxicol. Environ. Saf.* 60 (1), 37–46.
- Bialecka-Florjańczyk, E., Florjańczyk, Z., 2007. Chapter 22 - Solubility of Plasticizers, Polymers and Environmental Pollution. In: Letcher, T.M. (Ed.), *Thermodynamics, Solubility and Environmental Issues*. Elsevier, Amsterdam, pp. 397–408.
- Brack, W., Dulio, V., Ågerstrand, M., Allan, I., Altenburger, R., Brinkmann, M., Bunke, D., Burgess, R.M., Cousins, I., Escher, B.I., 2017. Towards the review of the European Union Water Framework management of chemical contamination in European surface water resources. *Sci. Total Environ.* 576, 720–737.
- Brock, T.C., Van Wijngaarden, R.P., 2012. Acute toxicity tests with *Daphnia magna*, *Americamysis bahia*, *Chironomus riparius* and *Gammarus pulex* and implications of new EU requirements for the aquatic effect assessment of insecticides. *Environ. Sci. Pollut. Res.* 19 (8), 3610–3618.
- Carvalho, L., Mackay, E.B., Cardoso, A.C., Baattrup-Pedersen, A., Birk, S., Blackstock, K. L., Borics, G., Borja, A., Feld, C.K., Ferreira, M.T., 2019. Protecting and restoring Europe's waters: An analysis of the future development needs of the Water Framework Directive. *Sci. Total Environ.* 658, 1228–1238.
- Connors, K.A., Beasley, A., Barron, M.G., Belanger, S.E., Bonnell, M., Brill, J.L., de Zwart, D., Kienzler, A., Krailler, J., Otter, R., 2019. Creation of a curated aquatic toxicology database: EnviroTox. *Environ. Toxicol. Chem.* 38 (5), 1062–1073.
- Crawford, C.G., 2004. Sampling strategies for estimating acute and chronic exposures of pesticides in streams. *JAWRA J. Am. Water Resources Assoc.* 40 (2), 485–502.
- Dorne, J.L., Richardson, J., Kass, G., Georgiadis, N., Monguidi, M., Pasinato, L., Cappe, S., Verhagen, H., Robinson, T., 2017. OpenFoodTox: EFSA's open source toxicological database on chemical hazards in food and feed. *EFSA J.* 15 (1), e15011.
- EFSA, 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. *EFSA J.* 11 (7), 3290.
- BVL, 2020. Verzeichnis zugelassener Pflanzenschutzmittel. <https://bit.ly/2iZzV5a> (accessed November 9th, 2020).
- EFSA Scientific Committee, 2016. Guidance to develop specific protection goals options for environmental risk assessment at EFSA, in relation to biodiversity and ecosystem services. *EFSA J.* 14(6).
- EFSA Scientific Committee, 2016. Coverage of endangered species in environmental risk assessments at EFSA. *EFSA J.* 14(2), 4312.
- EFSA Database of registered chemicals under REACH. <https://bit.ly/3mwYqDS> (accessed November 9th, 2020).
- Escher, B.I., Ait-Aissa, S., Behnisch, P.A., Brack, W., Brion, F., Brouwer, A., Buchinger, S., Crawford, S.E., Du Pasquier, D., Hamers, T., 2018. Effect-based trigger values for in vitro and in vivo bioassays performed on surface water extracts supporting the environmental quality standards (EQS) of the European Water Framework Directive. *Sci. Total Environ.* 628, 748–765.
- European Commission, 2020. EU pesticides database. <https://bit.ly/3h1bZum> (accessed November 9th, 2020).
- European Commission, 2020. Farm to fork strategy: for a fair, healthy and environmentally-friendly food system. DG SANTE/Unit 'Food information and composition, food waste'.
- European Council, 2000. Water Framework Directive - A Framework for Community Action in the Field of Water Policy. <https://bit.ly/3asirt1> (accessed November 9th, 2020).
- [dataset] European Environment Agency, 2020. Corine Land Cover - 100 2020. <https://bit.ly/2KhEat1> (accessed June 6th, 2020).
- [dataset] European Environment Agency, 2012. European Catchments and Rivers Network System (Ecrins, version 1.2). <https://bit.ly/3nCTIeY> (accessed June 6th, 2020).
- [dataset] European Environment Agency, 2020. Waterbase - Water Quality ICM (WISE6, version 6). <https://bit.ly/3r8lqvA> (accessed November 9th, 2020).
- Faust, M., Backhaus, T., Altenburger, R., Dulio, V., van Gils, J., Ginebreda, A., Kortenkamp, A., Munthe, J., Posthuma, L., Slobodnik, J., 2019. Prioritisation of water pollutants: the EU Project SOLUTIONS proposes a methodological framework for the integration of mixture risk assessments into prioritisation procedures under the European Water Framework Directive. *Environ. Sci. Eur.* 31 (1), 1–4.
- eurostat, 2018. Agricultural census in Italy. <https://bit.ly/3nypnZg> (accessed September 12th, 2020).
- Federal Insecticide, Fungicide, and Rodenticide Act. Public Law 104-170, 1996; Code of Federal Regulations: Section 136, Title 7, 1996.
- Fernandez-Correo, J., Nehring, R.F., Osteen, C., Wechsler, S., Martin, A., Vialou, A., 2014. Pesticide use in US agriculture: 21 selected crops, 1960–2008. U.S. Government Printing Office, Washington, DC.
- Fonseca, E., Hernández, F., Ibáñez, M., Rico, A., Pitarch, E., Bijlsma, L., 2020. Occurrence and ecological risks of pharmaceuticals in a Mediterranean river in Eastern Spain. *Environ. Int.* 144, 106004.
- Geissen, V., Mol, H., Klumpp, E., Umlauf, G., Nadal, M., van der Ploeg, M., van de Zee, S. E., Ritsema, C.J., 2015. Emerging pollutants in the environment: a challenge for water resource management. *Int. Soil Water Conserv. Res.* 3 (1), 57–65.
- Godoy, A.A., Kummrow, F., Pamplin, P.A.Z., 2015. Occurrence, ecotoxicological effects and risk assessment of antihypertensive pharmaceutical residues in the aquatic environment-A review. *Chemosphere* 138, 281–291.
- Guillon, A., Noyon, N., Gogot, C., Robert, S., Bruchet, A., Esperanza, M., 2015. Study on veterinary and human antibiotics in raw and treated water from a French basin. *Water Sci. Technol. Water Supply* 15 (6), 1275–1284.
- Heap, I., Duke, S.O., 2018. Overview of glyphosate-resistant weeds worldwide. *Pest Manag. Sci.* 74 (5), 1040–1049.
- Hollender, J., Singer, H., McArdell, C. S., 2008. Polar organic micropollutants in the water cycle. In *Dangerous pollutants (xenobiotics) in urban water cycle*, Springer, pp 103–116.
- James, A., Bonnomet, V., Morin, A., Fribourg-Blanc, B., 2009. Implementation of requirements on priority substances within the context of the Water Framework Directive. Prioritization Process: Monitoring-Based Ranking 58.
- Jonsson, M., Ershammar, E., Fick, J., Brodin, T., Klaminder, J., 2015. Effects of an antihistamine on carbon and nutrient recycling in streams. *Sci. Total Environ.* 538, 240–245.
- Katipoglu-Yazan, T., Merlin, C., Pons, M.-N., Ubay-Cokgor, E., Orhon, D., 2015. Chronic impact of tetracycline on nitrification kinetics and the activity of enriched nitrifying microbial culture. *Water Res.* 72, 227–238.
- Kreuger, J., Nilsson, E., 2001. In *Catchment scale risk-mitigation experiences - key issues for reducing pesticide transport to surface waters*. British Crop Protection Council Symposium Proceedings 2001, 319–324.
- Lepper, P., 2005. Manual on the methodological framework to derive environmental quality standards for priority substances in accordance with Article 16 of the Water Framework Directive (2000/60/EC). Schmallenberg, Germany: Fraunhofer-Institute Mol. Biol. Appl. Ecol. 51.
- Li, D., Suh, S., 2019. Health risks of chemicals in consumer products: A review. *Environ. Int.* 123, 580–587.
- Liess, M., 2002. Population response to toxicants is altered by intraspecific interaction. *Environ. Toxicol. Chem.* 21 (1), 138–142.
- Liess, M., von der Ohe, P.C., 2005. Analyzing effects of pesticides on invertebrate communities in streams. *Environ. Toxicol. Chem.* 24 (4), 954–965.
- Mahler, B.J., Schmidt, T.S., Nowell, L.H., Qi, S.L., Van Metre, P.C., Hladik, M.L., Carlisle, D.M., Munn, M.D., May, J., 2020. Biofilms Provide New Insight into Pesticide Occurrence in Streams and Links to Aquatic Ecological Communities. *Environ. Sci. Technol.* 54 (9), 5509–5519.
- Malaj, E., Peter, C., Grote, M., Kühne, R., Mondy, C.P., Usseglio-Polatera, P., Brack, W., Schäfer, R.B., 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proc. Natl. Acad. Sci. USA* 111 (26), 9549–9554.
- Neale, P.A., Braun, G., Brack, W., Carmona, E., Gunold, R., König, M., Krauss, M., Liebmann, L., Liess, M., Link, M., Schäfer, R.B., Schlichting, R., Schreiner, V.C., Schulze, T., Vormeier, P., Weisner, O., Escher, B.I., 2020. Assessing the Mixture Effects in In Vitro Bioassays of Chemicals Occurring in Small Agricultural Streams during Rain Events. *Environ. Sci. Technol.* 54 (13), 8280–8290.
- Outhwaite, C.L., Gregory, R.D., Chandler, R.E., Collen, B., Isaac, N.J.B., 2020. Complex long-term biodiversity change among invertebrates, bryophytes and lichens. *Nat. Ecol. Evol.* 4 (3), 384–392.
- Owens, B., 2015. Pharmaceuticals in the environment: a growing problem. *Pharm. J. Petschick, L.L., Bub, S., Wolfram, J., Stehle, S., Schulz, R., 2019. Modeling Regulatory Threshold Levels for Pesticides in Surface Waters from Effect Databases. Data 4 (4), 150.*
- QGIS Development Team, QGIS geographic information system. Open Source Geospatial Foundation Project 2016.
- Richmond, E.K., Rosi, E.J., Walters, D.M., Fick, J., Hamilton, S.K., Brodin, T., Sundelin, A., Grace, M.R., 2018. A diverse suite of pharmaceuticals contaminates stream and riparian food webs. *Nat. Commun.* 9 (1), 1–9.
- Roose-Amsaleg, C., Laverman, A.M., 2016. Do antibiotics have environmental side-effects? Impact of synthetic antibiotics on biogeochemical processes. *Environ. Sci. Pollut. Res.* 23 (5), 4000–4012.
- Rösch, A., Beck, B., Hollender, J., Singer, H., 2019. Picogram per liter quantification of pyrethroid and organophosphate insecticides in surface waters: a result of large enrichment with liquid–liquid extraction and gas chromatography coupled to mass

- spectrometry using atmospheric pressure chemical ionization. *Anal. Bioanal. Chem.* 411 (14), 3151–3164.
- Schäfer, R.B., Caquet, T., Siimes, K., Mueller, R., Lagadic, L., Liess, M., 2007. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. *Sci. Total Environ.* 382 (2–3), 272–285.
- Schäfer, R.B., von der Ohe, P.C., Rasmussen, J., Kefford, B.J., Beketov, M.A., Schulz, R., Liess, M., 2012. Thresholds for the effects of pesticides on invertebrate communities and leaf breakdown in stream ecosystems. *Environ. Sci. Technol.* 46 (9), 5134–5142.
- Schwarzenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson, C.A., Von Gunten, U., Wehrli, B., 2006. The challenge of micropollutants in aquatic systems. *Science* 313 (5790), 1072–1077.
- Shala, L., Foster, G.D., 2010. Surface water concentrations and loading budgets of pharmaceuticals and other domestic-use chemicals in an urban watershed (Washington, DC, USA). *Arch. Environ. Contam. Toxicol.* 58 (3), 551–561.
- Smalling, K.L., Orlando, J.L., Kuivila, K.M., 2007. Occurrence of pesticides in water, sediment, and soil from the Yolo Bypass, California. *San Francisco Estuary Watershed Sci.* 5 (1).
- Smalling, K.L., Reilly, T.J., Sandstrom, M.W., Kuivila, K.M., 2013. Occurrence and persistence of fungicides in bed sediments and suspended solids from three targeted use areas in the United States. *Sci. Total Environ.* 447, 179–185.
- 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 (8), 4526–4535.
- Stehle, S., Schulz, R., 2015. Agricultural insecticides threaten surface waters at the global scale. *Proc. Natl. Acad. Sci. USA* 112 (18), 5750–5755.
- Stehle, S., Bub, S., Schulz, R., 2018. Compilation and analysis of global surface water concentrations for individual insecticide compounds. *Sci. Total Environ.* 639, 516–525.
- Stehle, S., Blin, A., Bub, S., Petschick, L.L., Wolfram, J., Schulz, R., 2019. Aquatic pesticide exposure in the US as a result of non-agricultural uses. *Environ. Int.* 133, 105234.
- Stone, W. W., Gilliom, R. J., Martin, J. D., 2014. An overview comparing results from two decades of monitoring for pesticides in the Nation's streams and rivers, 1992-2001 and 2002-2011; 5154; Reston, VA, p. 32.
- Stone, W.W., Gilliom, R.J., Ryberg, K.R., 2014. Pesticides in U.S. Streams and Rivers: Occurrence and Trends during 1992-2011. *Environ. Sci. Technol.* 48 (19), 11025–11030.
- Team R Core, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Austria, 2015. In ISBN 3-900051-07-0: URL <http://www.R-project.org>: 2018.
- van Klink, R., Bowler, D.E., Gongalsky, K.B., Swengel, A.B., Gentile, A., Chase, J.M., 2020. Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances. *Science* 368 (6489), 417–420.
- van Wijngaarden, R.P., Brock, T.C., Van Den Brink, P.J., 2005. Threshold levels for effects of insecticides in freshwater ecosystems: a review. *Ecotoxicology* 14 (3), 355.
- van Wijngaarden, R.P., Maltby, L., Brock, T.C., 2015. Acute tier-1 and tier-2 effect assessment approaches in the EFSA Aquatic Guidance Document: are they sufficiently protective for insecticides? *Pest Manage. Sci.* 71 (8), 1059–1067.
- von der Ohe, P.C., Prüb, A., Schäfer, R.B., Liess, M., de Deckere, E., Brack, W., 2007. Water quality indices across Europe—a comparison of the good ecological status of five river basins. *J. Environ. Monit.* 9 (9), 970–978.
- von der Ohe, P.C., Dulio, V., Slobodnik, J., De Deckere, E., Kühne, R., Ebert, R.-U., Ginebreda, A., De Cooman, W., Schüürmann, G., Brack, W., 2011. A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European Water Framework Directive. *Sci. Total Environ.* 409 (11), 2064–2077.
- Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R., 2010. Global threats to human water security and river biodiversity. *Nature* 467 (7315), 555–561.
- Wang, W., Delgado-Moreno, L., Conkle, J.L., Anderson, M., Amrhein, C., Ye, Q., Gan, J., 2012. Characterization of sediment contamination patterns by hydrophobic pesticides to preserve ecosystem functions of drainage lakes. *J. Soils Sed. Im.* 12 (9), 1407–1418.
- Weston, D.P., Chen, D., Lydy, M.J., 2015. Stormwater-related transport of the insecticides bifenthrin, fipronil, imidacloprid, and chlorpyrifos into a tidal wetland, San Francisco Bay, California. *Sci. Total Environ.* 527, 18–25.
- Wolfram, J., Stehle, S., Bub, S., Petschick, L.L., Schulz, R., 2018. Meta-Analysis of Insecticides in United States Surface Waters: Status and Future Implications. *Environ. Sci. Technol.* 52 (24), 14452–14460.
- Wolfram, J., Stehle, S., Bub, S., Petschick, L.L., Schulz, R., 2019. Insecticide Risk in US Surface Waters: Drivers and Spatiotemporal Modeling. *Environ. Sci. Technol.* 53 (20), 12071–12080.

Supplementary Materials

for

**Water Quality and Ecological Risks in European Surface Waters
– Monitoring Improves While Water Quality Declines**

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1 Supplementary Materials and Methods

1.1 Data Processing Protocols

Original aggregated WFD data (WISE6)¹ was processed as follows to ensure its integrity, reliability, and relevance with resulting data exclusions shown in Figure S1: First, data from Iceland and the former Yugoslav Republic of Makedonia were removed, as the former does not relate to continental Europe, and the latter national state does not exist anymore. Then, all measurements relating to groundwater were removed. Few measurements relate to concentrations of particle bound contaminants (i.e. suspended sediments), which were removed as well. A large fraction of measurements details the occurrence of non-organic contaminants (e.g. nitrogen, metals), mixtures (e.g. “sum of DDT metabolites”), or other abiotic water parameters (e.g. temperature, salinity, pH), all of which were removed. Other entries that relate to (i) sediment concentrations, (ii) water concentrations in non-conform formats (i.e. not given as concentrations), (iii) those identified as duplicates in space \times time \times chemical dimensions, and finally, (iv) those being deemed as unreliable, because they failed a simple logical rule (i.e. minimum concentration $<$ maximum concentration), were all removed as well. Finally, data were reduced to the relevant time frame of the analysis, i.e. 2001 – 2015. After data processing was completed, 971,381 annual aggregate concentrations (AAC), which are based on at least 8,383,640 individual samples, remained, detailing the occurrence of organic contaminants throughout European surface waterbodies.

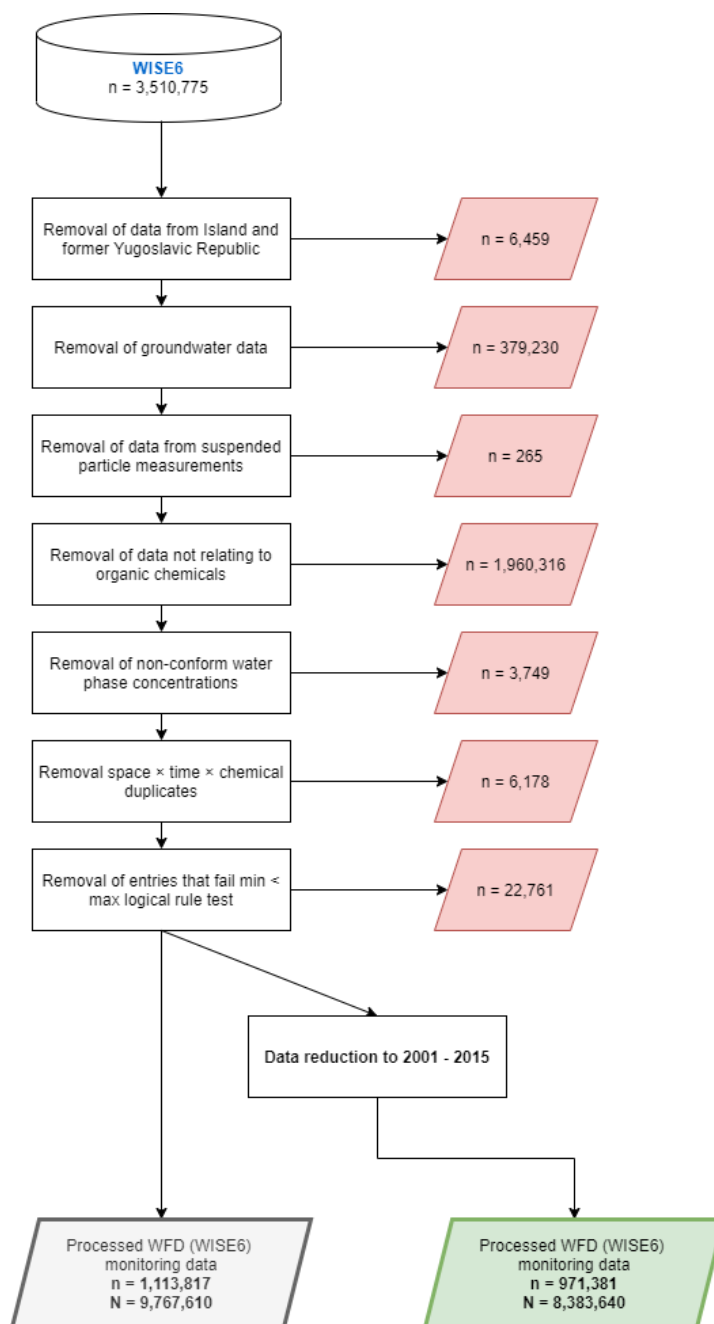


Figure S1: Flow chart of data processing steps taken to ensure the integrity, reliability, and relevance of WISE6 data to derive monitoring results of organic contaminants ($n = 352$) measured (i.e. “samples”, $N, N = 8,383,640$) and aggregated in AACs ($n; n = 971,381$) throughout Europe. The final dataset used in this study, i.e. relating to measurements between 2001 – 2015, is depicted in green.

1.2 Measurement Definitions

Three types of organic contaminant measurements were used in the present study that were either directly available or derivable from the WISE6 dataset. “Samples” refer in this study to individual measurements of contaminants that are distinct in space \times time (date) \times chemical (Figure S2). Hence, they are the most detailed spatiotemporal information about chemicals’ occurrences, however, they can only be derived from the pre-aggregated WISE6 data

indirectly. “Annual aggregate concentrations” (AACs) are provided by the WISE6 datasets directly as minimum, average, and maximum concentrations, hence minima and maxima AACs are distinct in space × time (year) × chemical (Figure S2). In this study, AAC maxima were used for all acute risk evaluations. The “most relevant annual aggregates” (MRAAs) can be deduced from AACs and represent the highest risk contamination per organism group (Figure S2), i.e. MRAAs are distinct in space × time (year) × organism. Risk is evaluated by comparing the measured AAC with the receptive ecotoxicological threshold per organism group, i.e. the most toxic concentration has the highest concentration to threshold ratio. As such, MRAAs are specific to the organism group in question, representing the highest risk peak exposure a monitoring site has experienced in a given year with respect to the organism group. Thus, MRAAs can differ despite being based on the same samples or AACs. For instance, the MRAA provided in Figure S2 may refer to aquatic plants, as the glyphosate concentration has the highest toxicity, however, for aquatic invertebrates or fish 1.1 µg/L PCB-231 may be more toxic, hence constituting their MRAA.

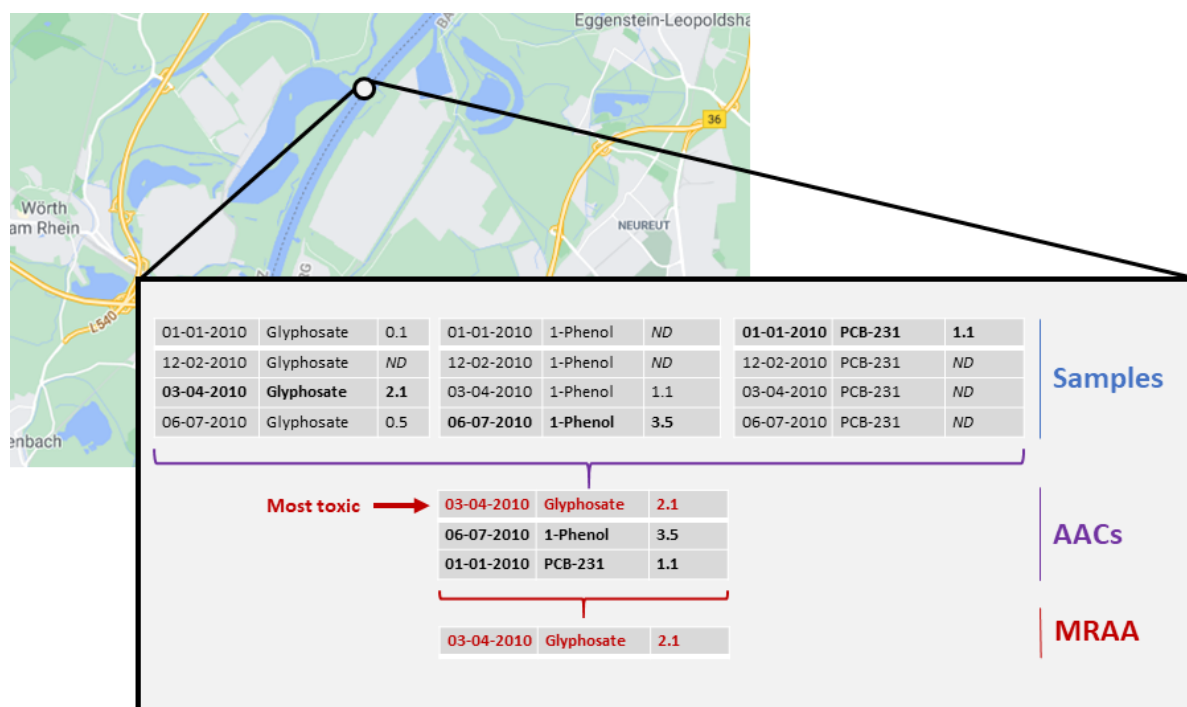


Figure S2: Depiction of theoretical monitoring results and the relation of samples to AACs to MRAAs, as used in the present study. ND stands for non-detect. The most toxic concentration for aquatic plants in space and time is highlighted in red.

1.3 Deduction of Robust EnviroTox Endpoints

Most sensitive endpoints used during the (i) EFSA ERA and (ii) those derived by Petschick et al.², had to pass rigorous quality control procedures in order to be judged as reliable. For data

obtained from the curated EnviroTox,³ some of these quality criteria could not be applied, because such attributes are currently not coded.³ Thus, identification of the most sensitive endpoint per organism group in the EnviroTox resulted in selection of endpoints that may be more sensitive than those identified during risk assessment procedures or those derived by Petschick et al.² even after available quality criteria were evaluated (Figure S3a,d,g). For instance, for the substance benzo-a-pyrene the most sensitive invertebrate toxicity data listed in the EnviroTox were based on UV-induced toxicity trials.^{4,5} Although UV-enhanced toxicity is a relevant aspect of many organic contaminants,^{4,5} it would not be realistic to assume that measured exposures also refer to full UV-sunlight penetration in all scenarios. As such, permanently basing risk estimation on these rather specific cases would overestimate risks in general. Therefore, relevant EnviroTox endpoints were aggregated to derive a robust and conservative ecotoxicological estimate per substance and organism group. Then, aggregated results were compared to endpoints from Petschick et al.² for validation and accuracy. Median aggregation per substance of relevant EnviroTox endpoints showed very good estimation for each substance and organism group, resulting in conservative estimates, i.e. rare deduction of endpoints that are more sensitive than those derived by Petschick et al.² (Figure S3). Also, such an approach preserves the actuality of endpoints compared to other measures (e.g. mean).

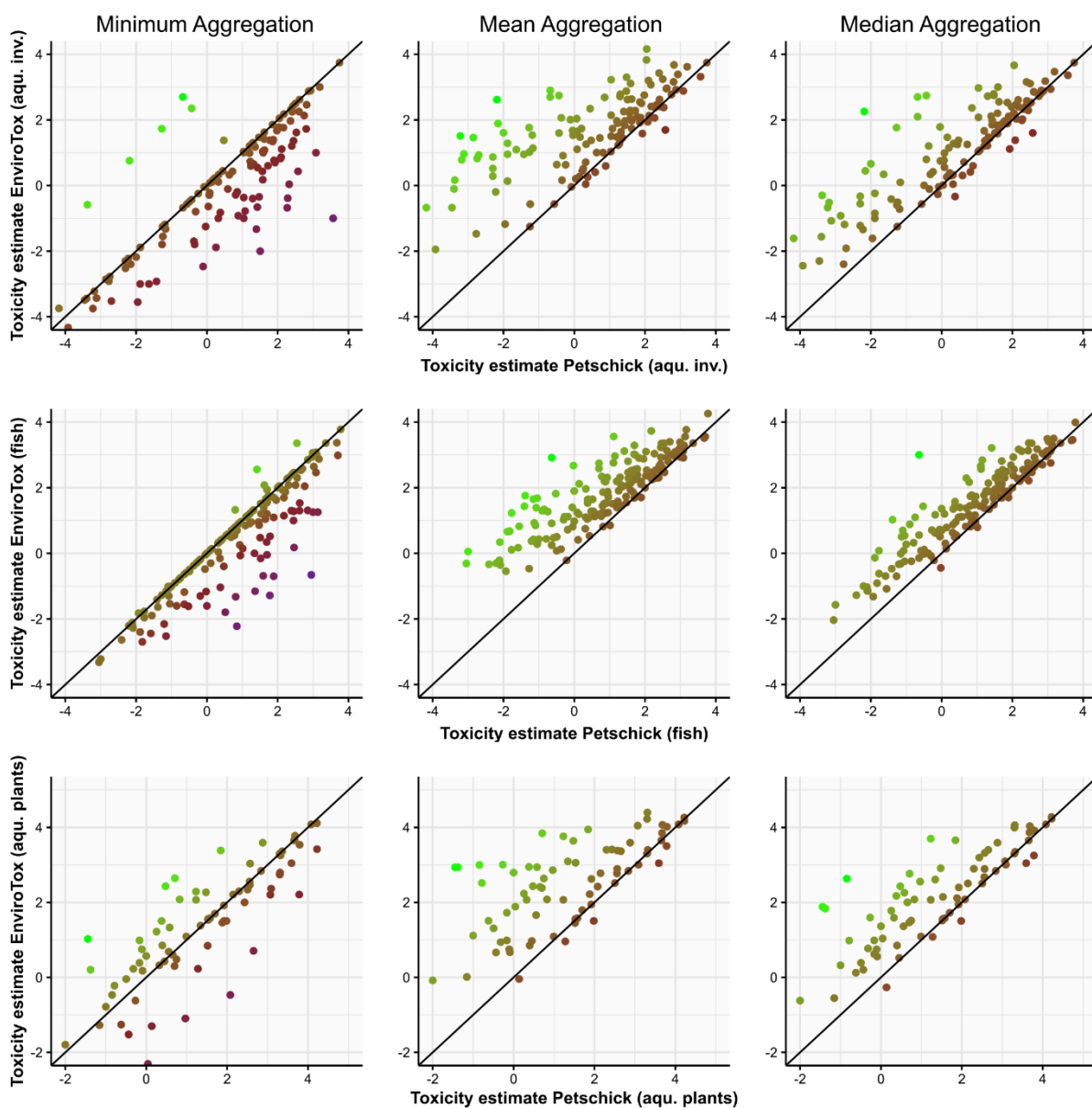


Figure S3: Accuracy estimates when comparing EnviroTox data with endpoints derived by Petschick et al.² using minima, means, or medians for aquatic invertebrates (a-c), fish (d-f), and aquatic plants (g-i). The diagonal line represents exact matches. Points laying below the diagonal line (orange to red gradient) indicate too sensitive (i.e. too low) toxicity endpoints, resulting in risk overestimations.

1.4 Use of Assessment Factors

Previous work by Malaj et al.⁶ used data from 2006 - 2010 without any further temporal resolution for an assessment of WFD monitoring data. In their analysis, acute risk was assessed based on actual and estimated (i.e. QSAR, read-across) ecotoxicological data from three standard test species (*D. magna*, *P. promelas*, *P. subcapitata*), each representing one central organism group in aquatic environments (i.e. fish, invertebrates, and aquatic plants). An uncertainty factor, here “assessment factor” (AF), of 10 was applied to all endpoints to derive thresholds at which acute ecological effects are expected. Such an approach can be regarded as

highly conservative, i.e. resulting in comparably low risk estimations, yet producing threshold levels at which profound alterations in community structure and functioning are certain.⁷ However, we reviewed existing literature concerning this topic and found that multiple lines of evidence strongly suggest that acute environmental effects occur at concentrations of at least one order of magnitude lower, thus requiring the application of higher safety factors (e.g. 100 – 1000) to reflect varying susceptibilities of aquatic organisms towards differing chemical stressors in addition to multiple other factors contributing to effects in the field:

Berenzen et al.⁸ found that invertebrate community compositions changed significantly in streams exposed to pesticide runoff equaling the use of an AF = 100. van Wijngaarden et al.⁹ evaluated the protectiveness of higher tier studies (i.e. micro and mesocosms) in comparison to single species tests, concluding that applying an AF of 100 to single species tests should provide a sufficiently protective threshold for insecticides, whereas the application of an AF of 10 would already result in regular effects for most sensitive species.¹⁰ In addition, while mesocosm studies are currently the most ecologically complex toxicity tests conducted, they remain simplified test systems when compared to natural environments. Using micro-/mesocosms as baselines to delineate ecological effects bears uncertainties, hence potentially underestimating actual effects in aquatic environments, due to differing community compositions,¹¹ mixture and joint toxicity of chemicals, and delayed or sublethal effects,¹²⁻¹⁴ which further supports the notion of using an AF = 100. Brock and Van Wijngaarden¹⁵ also provided an extensive comparison between single organism tests and endpoints derived from mesocosm studies. Their review showed that the inclusion of additional standard test species (*A. bahia*, *G. pulex*, *Chironomus* spp.) was necessary to improve protectiveness when using an AF = 100, as chemical stressors can exert highly variable toxicity towards different aquatic species. For instance, crustaceans are generally known to be orders of magnitude less sensitive towards pyrethroid and neonicotinoid insecticides than aquatic insects. Despite their use of a wider organism spectrum, using an AF = 100 still resulted in sporadic underestimations of risks, especially for newer substances such as neonicotinoids and insect growth regulators.

Schäfer et al.¹⁶ assessed invertebrate community structures and leaf litter breakdown rates in 29 streams in France and Finland, which showed that community structure already changed at concentrations of 100th and 1000th of those derived from 48h-LC₅₀ for *D. magna*. Schäfer et al.⁷ furthermore demonstrated that community composition already changed at concentrations equaling the use of an AF of 1,000 to 10,000 based on *D. magna* acute tests and, strikingly, reducing relative abundances of sensitive species by 27 to 61% when using an AF = 100.

Substantial losses of γ -diversity in affected streams would directly violate efforts of curbing the ongoing biodiversity loss in European waterbodies as outlined by recent EFSA communications and guidance.¹²⁻¹⁴ Liess and von der Ohe¹⁷ delineated clear effects in community structures at levels equaling or below an AF of 100 to 1,000 based on acute *D. magna* toxicity tests. Beketov and Liess¹² demonstrated that even highly transient exposures (1h) of the insecticide esfenvalerate were able to reduce fecundity of *C. dipterum* at concentrations 1000 times lower than those evoking lethality in food-scarce environments. As such, von der Ohe et al.¹⁸ have recommended and applied even stricter criteria (i.e. AF = 1,000) in order to derive thresholds from standard toxicity data that complies with the protection goals outlined under the Water Framework Directive for 500 organic contaminants.¹⁹ Their reasoning was based on preceding analyses showing that using AFs = 1,000 should provide thresholds that still delineate a “good ecological status”, whereas the use of AF = 100 would provide thresholds where altered (i.e. “disturbed”) communities were found.²⁰ Most importantly, their expansive analysis applies to a large array of organic contaminants, i.e. not only pesticides, which are commonly focused on, thus underlining the suitability of an AF = 100 for organic contaminants in general.

From a regulatory perspective, the application of an AF = 100 for most relevant fish and invertebrate standard test species and an AF = 10 for aquatic plants, is oftentimes conducted to derive regulatory acceptable concentrations (RACs) that indicate risks for specific organism groups, if they are exceeded.²¹ Such an approach, as conducted in the present study, is following recent efforts to derive “specific protection goals” (=SPGs), by which (i) aquatic biodiversity,^{22,23} (ii) endangered species,²⁴ and (iii) vital ecological services may be protected throughout waterbodies in Europe.^{22,25} This first tier assessment procedure follows the assumption of hierarchical protection, i.e. the idea that the protection of individual organisms groups also safeguards higher organizational structures, such as meta-populations, ecosystems or habitats. Here, the use of an AF = 100 was estimated to protect the majority of fish (97%), insects (96.2%), and crustaceans (94.4%), based on extensive randomized sensitivity analysis (for details see EFSA Scientific Committee²⁴). Highlighted by their results, if only an AF = 10 would be used then substantial fractions of each organism group (12.1% fish, 16.9% of insects, and 19.5% of crustaceans) would be put at risk, due to the differing sensitivities within these organism groups.²⁴ A similar analysis, using only standard test species for comparisons, showed that 21.4% of insects, 16.0% of crustaceans and 5.0% of fish would be at risk when applying an AF = 10.²⁴ Thus, according to the EFSA, the use of an AF = 100 could currently

cover between 99.3% - 82.2% of known within-species-groups sensitivities derived from laboratory experiments. It is however important to note that this analysis only covered sensitivities within organism groups, as such uncertainties regarding long-term, delayed effects, sublethal, and behavioral effects, in addition to uncertainties relating to non-chemical stressors, joint toxicities, conceptual, etc. can also negatively impact organisms in the field.^{7,8,11-14,16-20,26}

In consideration of these multiple lines of evidence outlined above, the application of an AF = 100 for fish and invertebrate species on standardized ecotoxicological test data should account for several sources of uncertainty, although it could be argued that even higher AFs may be warranted for some bioactive substances with fewer available test data (e.g. pharmaceuticals) or to account for further sources of uncertainty (see above). Thus, the approach provides a robust threshold estimation at which relevant acute ecological effects are expected in aquatic environments across Europe. This is also in accordance with EFSA based protection goals and mimicking currently applied regulatory assessment procedures.²¹⁻²⁴

1.5 Monitoring Data Evaluation

For each country in the WISE6 dataset, 4 principal (quantitative) indicators were deduced that allow for comparisons between each country's qualitative effort in probing a certain dimension that is relevant to chemical surface water monitoring. These four non-weighted indicators were summed up in order to deduce the monitoring-quality-index (MQI) per country *i*.

Network density of anthropogenically-used land surface area (ND) represents the spatial dimension by producing a comparable estimate between countries regarding their spatial effort of probing for contaminants. Anthropogenically used surface area per country (i.e. urban, industrial, agricultural) was derived with zonal histograms using the Corine Land Cover for 2012 (resolution = 500 m, see Table S2 for land-use information) in combination with polygons representing national territorial areas provided by ECRINS v1.2. It was deemed necessary to base the network density on anthropogenically-used surface areas rather than the national surface area, because anthropogenic land-use differs greatly between some Nordic countries, such as Sweden (ca 11%) and Norway (ca. 6%) when compared to more densely populated countries such as the Netherlands (ca. 85%) or Germany (ca. 66%). ND was calculated as the average number of monitoring stations over time (n = 16, i.e. 2001 – 2015) per anthropogenically-used land surface area, which was then again averaged over time per country. It is important to note that the frequency or temporal coverage of each monitoring

sites are explicitly not included in this metric, because they will be incorporated separately in following metrics (see below).

Sampling frequency (SF) represents one of two principal temporal dimensions, because it details the average intra-year temporal resolution of each countries' monitoring effort. Because most organic contaminants occur transiently or periodically, sufficient intra-year temporal resolutions are fundamental to generate reliable concentration estimates.²⁷⁻²⁹ Thus, the SF was derived from the sampling frequency per monitoring station and year, which was then again averaged over time.

Temporal continuity (TC) represents the second of the two principal temporal dimensions, because it details how complete the operating of each monitoring station was over time, i.e. the inter-year temporal resolution. Thus, TC can serve as a penalizing measure for countries were, for instance, in recent years substantial numbers of monitoring stations have been established throughout space, potentially skewing ND estimates, despite the fact that for the majority of time no data were generated from these stations. Hence, TC is an average presence/absence estimate over time for each monitoring station, which is then again averaged over time for each country.

Chemical spectra (CS) describe the principal chemical dimension, because it represents the spectrum of chemicals that can be detected during monitoring efforts. Without proper or expansive substance spectra, true environmental risks cannot be deduced, given the vast suite of chemicals that can occur in surface waters. CS was calculated as the average substance spectrum per country and year, which was averaged again over time to derive a single estimate per country.

1.6 Spatial Data Appendix

For each monitoring station with spatial coordinates, the contributing upstream watershed was derived using the ECRINS v1.2 dataset,³⁰ which provides comprehensive hydrological geoinformation for Europe. First, all monitoring sites were attributed to its nearest flow feature (i.e. the drainage line representing e.g. rivers or creeks) so that upstream catchments could be derived. Flow features are already attributed with vital information, for instance (i) the size of the contributing upstream catchment, which was later used for methods validation, (ii) the flow feature's Strahler order, and (iii) the so-called "Code Arbo", which is a recursive algorithm-derived string that can be used to readily deduce all upstream catchment polygons via string

pattern matching. However, selecting all upstream catchment areas based solely on the “Code Arbo” resulted in nonsensical results for very small streams, mainly those of Strahler order 1–2. Here, large discrepancies were found between the pre-calculated and polygon-derived upstream catchment areas, i.e. average discrepancy factors of 300 and 50 for Strahler order 1 and 2 streams, respectively, thus vastly overestimating the actual catchment size. This phenomenon is due to the limited spatial resolution of subcatchments available in ECRINS v1.2. For many lower order flow features (<50 km²), no individual catchments are derived, as they are prone to errors and substantially increase computational load (see [dataset] European Environment Agency³⁰). Thus, most Strahler order 1–2 streams lay within a subcatchment that belongs to a higher order stream (≥ 50 km²). Although, this subcatchment is correctly linked to other upstream catchments via the “Code Arbo”, these upstream catchments do, however, not always have a hydrological connection to the lower order streams in question. This issue was addressed by only considering the immediate (i.e. directly surrounding) subcatchment for Strahler order 1-2 streams. Size discrepancies were reduced to an average factor of approx. 4 (formerly 300) and 1.5 (formerly 50) for Strahler order 1 and 2 streams, respectively. For all other stream features of Strahler order >2, the “Code Arbo” was used to derive upstream catchment polygons, yielding results with an average size accuracy between 87.5 – 100% (see Figure S4). Lower spatial inaccuracies could not be achieved, as the current methodology already used the highest spatial resolution that is available in ECRINS v1.2. Overall, polygon-derived catchment areas compared well to precalculated values, achieving a correlation coefficient of 89.5% and 100% for log₁₀-transformed and untransformed data respectively (df = 7525, see Figure S4).

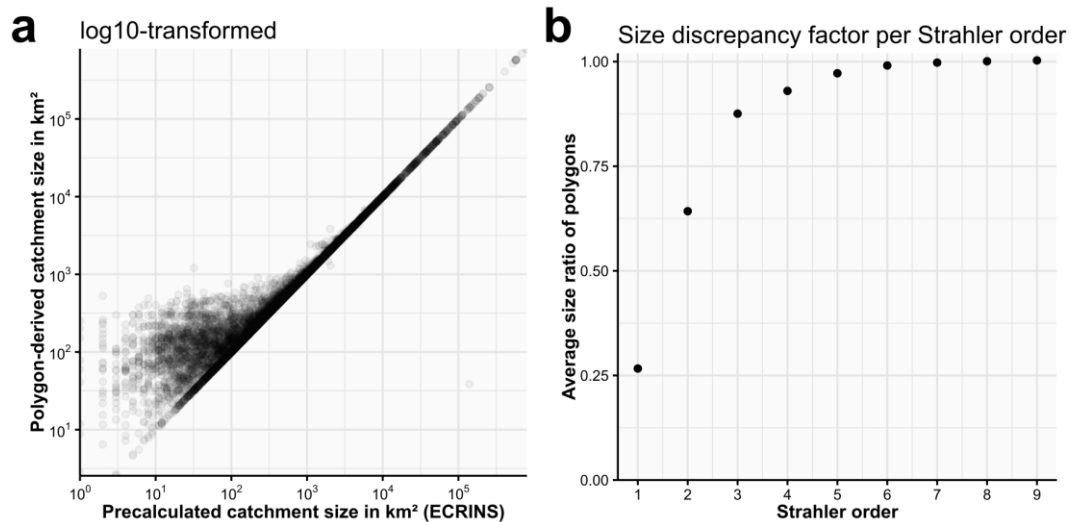


Figure S4: Results depicting the accuracy of polygon-derived catchments in km² when compared to precalculated estimates given per flow-feature in ECRINS v1.2. (a) Comparison of size estimates is given for based on log₁₀-transformed data, (b) the average size discrepancy is given when comparing precalculated catchment sizes with polygon derived sizes. Please note that the site DERW_DEHH_VIP_SEEMANNSH had to be removed from spatial analyses as the derived catchment was faulty.

Risk characteristics in waterbodies are known to be dependent of various physico-chemical features, such as the contributing catchment size and land-use therein.^{28,29,31} Thus, deriving the size and characteristics of upstream catchments for all monitoring sites in the WISE6 data provides central information about its representativeness for European waterbodies. For instance, a focus on small waterbodies would suggest above average risks throughout Europe, which would, however, not be representative of the entirety of European waterbodies.

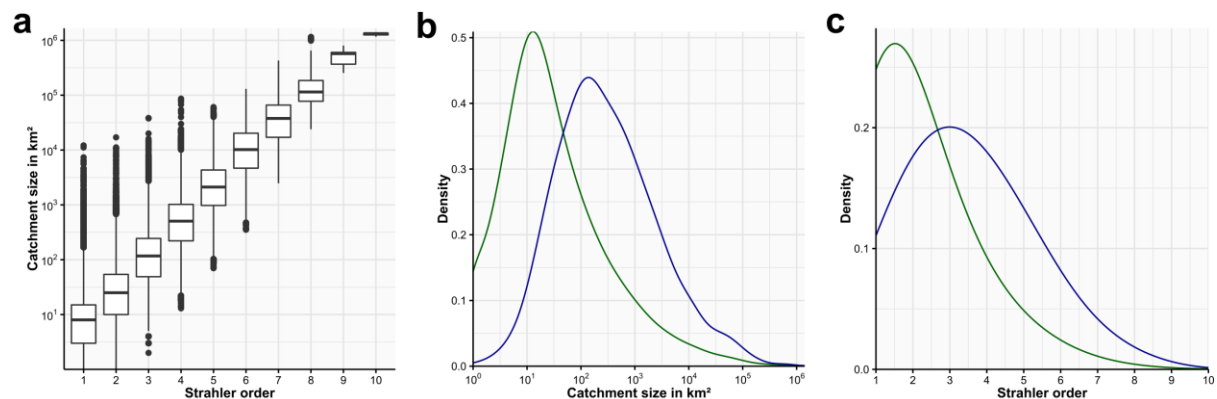


Figure S5: Comparison of catchment sizes and Strahler orders for those derived from the WFD data and those naturally distributed in Europe (ECRINS). (a) Watershed size as a function of the Strahler order in Europe. (b) Size distributions of all upstream catchments listed in ECRINS (green) and those probed in the WFD (blue), (c) Strahler order distributions of all flow features listed in ECRINS (green) versus those probed in the WFD (blue)

The median catchment size of the WISE6 data is 238 km², thus approx. one order of magnitude larger than those naturally occurring (i.e. indicated by ECRINS) with a median size of 20 km² (see Figure S5a). Most likely, the natural size distribution can be expected to show an even heavier left tail, i.e. having a median watershed size <20 km², as DEM derived catchments in

ECRINS reach conceptual limitations at $<50 \text{ km}^2$. This is illustrated by Strahler order density distribution in Figure S5b, which is closer to the true natural distribution, i.e. forming an exponential decay function (see also Smith et al.³²). In addition to the right-leaning WISE6 distribution, the distribution itself is also dampened when compared to the natural distribution, such that again an over-proportional fraction of very large waterbodies represented in the dataset in comparison to the natural distribution is present. The fact that the WISE6 data do not represent the natural size distribution in the environment, is however neither surprising nor reason for substantial critique, because such an effort would pose immense fiscal and logistical challenges. It may also be more cost-efficient to probe larger watersheds, serving as natural reservoirs for a larger population, than focusing on smaller streams.^{33,34} Nonetheless it is important to be aware of this discrepancy, due to the fact that chemical risks are often a function of upstream watershed size, resulting from processes such as dilution, increased dissipation, and degradation of contaminants.²⁹ Thus, in reality, the true risk in European waterbodies is likely higher than shown here, as those smaller and more vulnerable streams are not proportionally represented by the WFD monitoring schemes.

2 Supplementary Results and Discussion

2.1 AAC Risk Analyses

When comparing all annual peak concentrations (AACs) with their respective risk thresholds (Figure S6a), we found that 6.7% ($n = 114,210$), 4.9% ($n = 103,064$), and 3.5% ($n = 109,224$) of those concentrations exceed the $RTL_{\text{aqua-invert}}$, RTL_{fish} , and $RTL_{\text{aqua-plants}}$, respectively. Consequently, only a small fraction of AACs pose acute risks to aquatic organisms. In return, such an approach does not reflect spatio-ecological relevance, because it does not account for the fact that in most cases (79.1%, $n = 18,072$) multiple substances occur at a specific site each year. To expand on this, an average of 8.0 substances ($SD = \pm 9.5$) are detected at each site per year. Thus, aquatic organisms are in most cases exposed to a multitude of organic contaminants, yet exposures from single substances already suffice in impairing biota per site. Therefore, the MRAA approach identifies the contamination posing the highest risk for each organisms group per site and year, in order to deduce if adverse effects can be expected there, whereas the AAC approach gives a complete overview of all quantifications and yet may “dilute” actual risk estimates, due to the consideration of many comparatively unimportant co-occurring contaminants that may be co-occurring alongside more detrimental chemicals.

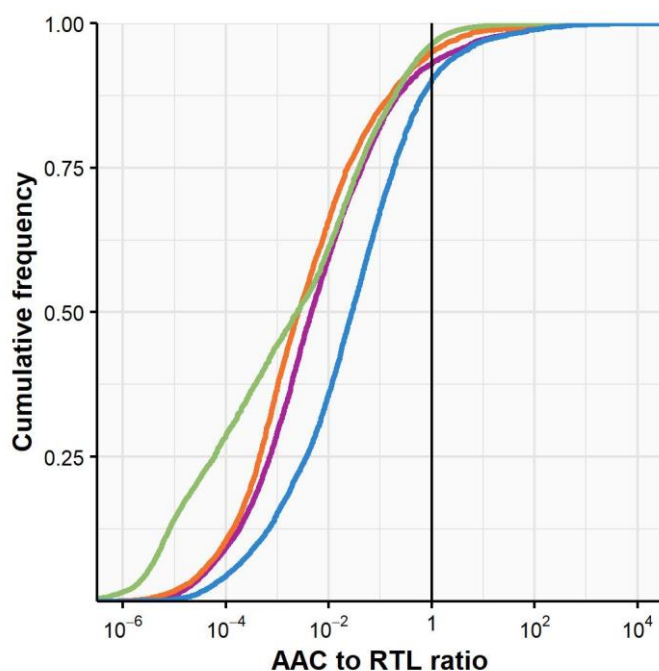


Figure S6: Cumulative frequency distributions of AAC to RTL ratios. Aquatic invertebrates (magenta), aquatic plants (green), fish (orange), and all organisms combined (blue). RTLs are depicted with a black solid line.

2.2 Chemical class specific risk trends

In Figure S7, risk trends are depicted for the three most impactful chemical classes, i.e. insecticides, herbicides, and industrial chemicals. Smoothed trend analyses (LOESS) primarily depict two principal risk characteristics per organism group and chemical class, i.e. the average risk (MRAA to RTL ratio) for each chemical class and its development over time. However, it is important to note that comparisons between differing chemical classes should be interpreted with care, because the underlying sample sizes differs considerably (e.g. 5,516 vs. 11,886, Figure S7a). This is mainly the result of varying detection frequencies and analyzed substance spectra for these chemical classes. In spite of this limitation, it is evident that the average risk per chemical class over time corresponds well to these chemical classes' exceedance frequencies, as depicted in Figure 7. Furthermore, the comparison within a chemical class (e.g. herbicides) but between organism groups, should not suffer from such drawbacks, as the spatio-temporal overlap is almost complete, i.e. deviances ranging from 1.2% (industrial chemicals) to 5.6% (insecticides).

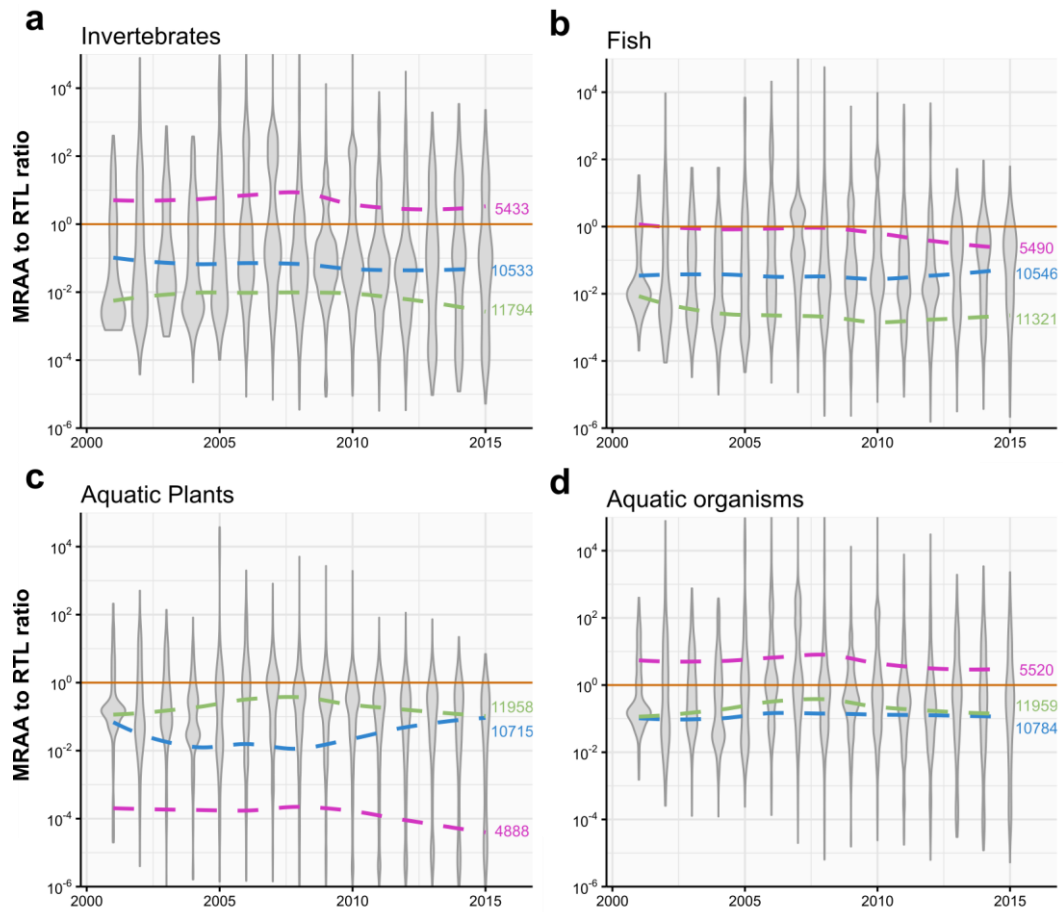


Figure S7: Median MRAA to (a) $RTL_{\text{aqua-invert}}$, (b) RTL_{fish} , (c) $RTL_{\text{aqua-plants}}$, and (d) RTL_{sw} ratios over time depicted by LOESS regressions for specific chemical classes, i.e. insecticides (pink), herbicides (green), and industrial chemicals (blue). The complete underlying data distribution per year is depicted with violin plots (grey). Please note that LOESS regressions depicted here are specific to the respective chemical class, which results in differing sample sizes (noted on the right-hand side per graph), depending on the number of monitoring stations that probed and subsequently quantified chemicals of a specific chemical class.

3 SI Figures

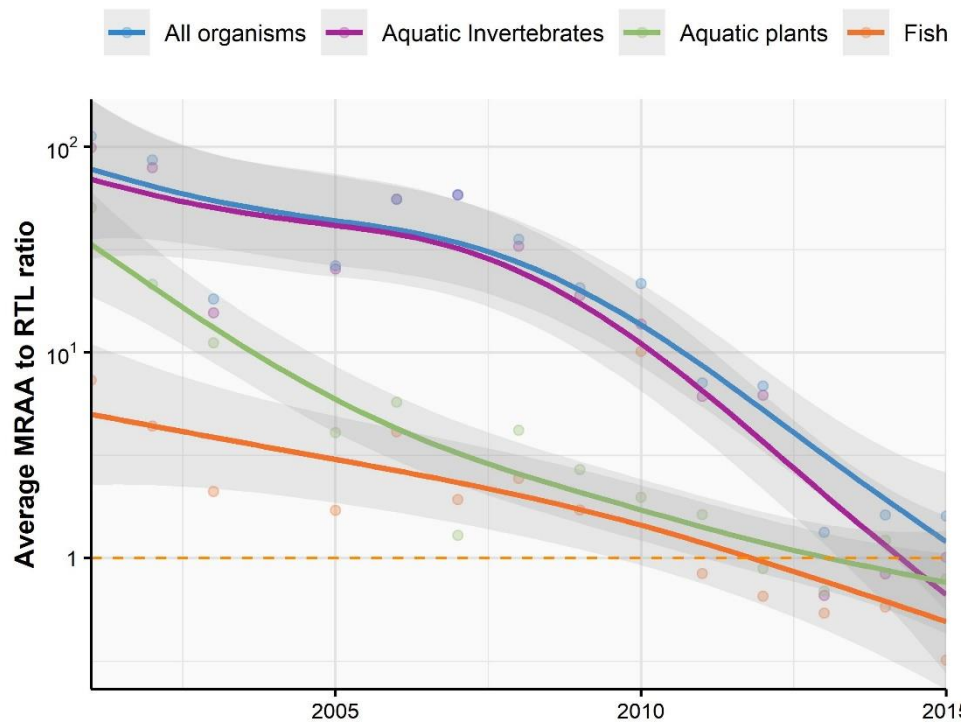


Figure S8: Average risk expressed as MRAA to RTL ratios in Belgian surface waters over time (points) and per organism group (see legend). Temporal trends are depicted with LOESS regressions and their 95%-confidence bands (grey).

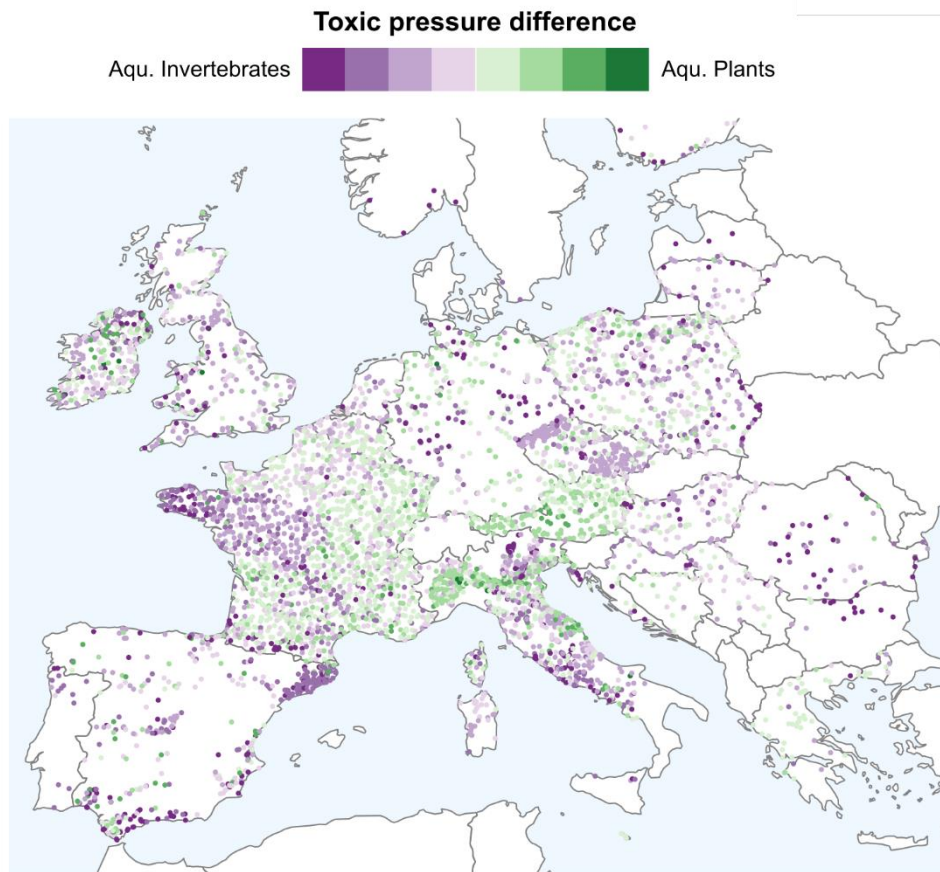


Figure S9: Difference of average risk between aquatic plants and aquatic invertebrates (i.e. toxic pressures) with darker shades of purple depicting higher local risks for aquatic invertebrates than aquatic plants. Darker shades of green depict higher toxic pressures to aquatic plants than aquatic invertebrates.

4 SI Tables

Table S1: Chemical data overview of all 352 organic contaminants assessed here with their CAS-RN, associated use type, RTLs, sources of RTLs, and their acute Environmental Quality Standard. Use types are abbreviated: A = Anti-Fouling, FR = Flame Retardant, F = Fungicide, H = Herbicide, Ho = Hormone, Ind = Industrial Chemical, Ins = Insecticide, M = Miscellaneous, Ph = Pharmaceutical, PI = Plasticizer, S = Solvent.

Substance	CAS RN	Use type	RTL _{aqua-invert}	Source RTL _{aqua-invert}	RTL _{fish}	Source RTL _{fish}	RTL _{aqua-plant}	Source RTL _{aqua-plant}	RTL _{sw}	EQS
Pentachlorophenol	87-86-5	A	0.92	2	0.1	2	5	2	0.1	1
Tributyltin-cation	36643-28-4	A	-	-	-	-	-	-	-	0.0015
Pentachlorobenzene	608-93-5	FR	11	2	2.875	3	663	3	2.875	-
Carbon tetrachloride	56-23-5	FR	970.5	3	414	2	35.3	3	35.3	-
BDE 47	5436-43-1	FR	0.92	3	128.35	3	-	-	0.92	-
BDE 154	207122-15-4	FR	-	-	-	-	-	-	-	-
BDE 153	68631-49-2	FR	-	-	-	-	-	-	-	-
BDE 100	189084-64-8	FR	-	-	-	-	-	-	-	-
BDE 99	60348-60-9	FR	0.54	3	-	-	-	-	0.54	-
Pentabromodiphenylether	32534-81-9	FR	-	-	-	-	-	-	-	0.14
Octabromodiphenylether	32536-52-0	FR	-	-	-	-	-	-	-	-
Bis(pentabromophenyl)ether	1163-19-5	FR	-	-	-	-	-	-	-	-
Bromodichloromethane	75-27-4	FR	-	-	195	3	920	3	195	-
PCB 209	2051-24-3	FR	-	-	-	-	-	-	-	-
BDE 183	68928-80-3	FR	-	-	-	-	-	-	-	-
BDE 85	182346-21-0	FR	-	-	-	-	-	-	-	-
BDE 138	182677-30-1	FR	-	-	-	-	-	-	-	-
Pentabromoethylbenzene	85-22-3	FR	-	-	-	-	-	-	-	-
Hexachlorobenzene	118-74-1	F	0.3	3	23	2	2.5	3	0.3	0.05
Epoxiconazole	133855-98-8	F	86.9	1	31.4	1	0.098	1	0.098	-
Propiconazole	60207-90-1	F	5.1	1	26	1	900	1	5.1	-
Quinoxifen	124495-18-7	F	0.83	2	2.7	2	14.9	1	0.83	2.7
Metalaxyl	57837-19-1	F	125	2	184	2	2030	2	125	-
Formaldehyde	50-00-0	F	146	2	14.1	2	489	3	14.1	-
Cybutryne	28159-98-0	F	53	2	7.5	2	0.01	2	0.01	0.016
Captan	133-06-2	F	34	1	0.98	1	160	1	0.98	-
Carbendazim	10605-21-7	F	1.5	1	0.19	1	770	1	0.19	-
Fenpropimorph	67564-91-4	F	0.24	1	23	1	0.5	1	0.24	-
Thiram	137-26-8	F	2.1	3	0.07	2	4.5	2	0.07	-
Quintozene	82-68-8	F	7.7	2	1	2	69	3	1	-
Ethylenethiourea (ETU)	96-45-7	F	269	2	75000	3	2300	2	269	-
Fluquinconazole	136426-54-5	F	-	-	13.4	1	1.4	1	1.4	-
Ziram	137-30-4	F	0.48	2	0.08	2	6.7	2	0.08	-
Metalaxyl-M	70630-17-0	F	97	1	350	2	1995	1	97	-
Alachlor	15972-60-8	H	25	2	2.4	2	0.164	2	0.164	0.7
Deisopropyldeethylatrazine	3397-62-4	H	-	-	-	-	-	-	-	-
Simazine	122-34-9	H	11	2	50	2	3.6	2	3.6	4
Atrazine	1912-24-9	H	7.2	2	49	2	1.1	2	1.1	2

Acetochlor OA	194992-44-4	H	-	-	-	-	-	-	-	-	-
Alachlor OA	171262-17-2	H	-	-	-	-	-	-	-	-	-
Metolachlor ESA	171118-09-5	H	-	-	-	-	-	-	-	-	-
Bifenox	42576-02-3	H	6.6	1	1275	3	0.0175	1	0.0175	0.04	-
Hydroxyterbuthylazine	66753-07-9	H	-	-	-	-	-	-	-	-	-
Dimethachlor	50563-36-5	H	85.4	1	39	1	0.217	1	0.217	-	-
MCPB	94-81-5	H	-	-	33	2	-	-	33	-	-
Tri-allate	2303-17-5	H	0.91	1	9.5	1	1.3	1	0.91	-	-
Dichlobenil	1194-65-6	H	62	1	72	1	2.79	1	2.79	-	-
Aminomethylphosphonic acid (AMPA)	1066-51-9	H	-	-	-	-	-	-	-	-	-
Acetochlor ESA	187022-11-3	H	-	-	-	-	8130	3	8130	-	-
Alachlor ESA	142363-53-9	H	-	-	-	-	-	-	-	-	-
Metolachlor OA	152019-73-3	H	-	-	-	-	-	-	-	-	-
Hydroxyatrazine	2163-68-0	H	-	-	-	-	-	-	-	-	-
Sebuthylazine	7286-69-3	H	-	-	-	-	-	-	-	-	-
Desethylterbuthylazine	30125-63-4	H	-	-	-	-	-	-	-	-	-
Chloridazon methyl desphenyl	17254-80-7	H	-	-	-	-	1500	2	1500	-	-
Lenacil	96639	H	-	-	-	-	0.77	1	0.77	-	-
Fenoprop	93-72-1	H	660	3	6	2	-	-	6	-	-
Pyridate	55512-33-9	H	4.9	1	11.9	1	82	1	4.9	-	-
Dinoseb	88-85-7	H	2.4	3	0.32	2	121.5	3	0.32	-	-
Prometon	1610-18-0	H	257	2	42	2	9.8	2	9.8	-	-
2,4-DB	94-82-6	H	150	2	19.7	2	-	-	19.7	-	-
Clopyralid	1702-17-6	H	7502	3	530	1	3000	1	530	-	-
Diketo-metribuzin	56507-37-0	H	-	-	-	-	-	-	-	-	-
Desamino-diketo-metribuzin	52236-30-3	H	-	-	-	-	-	-	-	-	-
Dichlorprop-P	15165-67-0	H	5580	2	-	-	7.6	1	7.6	-	-
Mecoprop-P (MCP-P)	16484-77-8	H	-	-	-	-	2.69	1	2.69	-	-
2,4-dichlorophenol	120-83-2	H	28	1	77.5	2	150	1	28	-	-
Chloridazon desphenyl	6339-19-1	H	-	-	-	-	-	-	-	-	-
Chlorsulfuron	64902-72-3	H	3700	2	500	2	0.035	1	0.035	-	-
Desmedipham	13684-56-5	H	3.5	1	14.1	1	6.4	1	3.5	-	-
Sulfosulfuron	141776-32-1	H	-	-	-	-	66.9	1	66.9	-	-
Metsulfuronmethyl	74223-64-6	H	431	1	1500	3	0.0365	1	0.0365	-	-
2,6-dichlorobenzamide	2008-58-4	H	8560	3	4690	2	10000	3	4690	-	-
Dinitro-o-cresol (DNOC)	534-52-1	H	1.45	2	0.66	2	3100	3	0.66	-	-
Carbetamide	16118-49-3	H	810	1	1650	3	12800	1	810	-	-
Trietazine	1912-26-1	H	60	3	10	3	-	-	10	-	-
Dalapon	75-99-0	H	110	2	1050	2	-	-	110	-	-
Chlorthiamid	1918-13-4	H	-	-	330	3	-	-	330	-	-
Bromoxynil octanoate	1689-99-2	H	0.26	1	0.41	1	5.1	2	0.26	-	-
Nitrophen	1836-75-5	H	2.168	2	16	3	-	-	2.168	-	-
Oxadiazon	19666-30-9	H	5.3	2	12	1	0.318	1	0.318	-	-
17beta-estradiol (E2)	50-28-2	Ho	29.7	3	19.85	3	-	-	19.85	-	-
Estrone (E1)	53-16-7	Ho	-	-	-	-	-	-	-	-	-
17alpha-ethinylestradiol (EE2)	57-63-6	Ho	41.9	2	-	-	44.7	3	41.9	-	-

1,2,4-trichlorobenzene	120-82-1	Ind	16.8	2	12.17	2	421.5	3	12.17	-
Anthracene	120-12-7	Ind	0.95	3	0.0278	2	29.405	3	0.0278	0.1
Benzo(b)fluoranthene	205-99-2	Ind	10.24	3	-	-	-	-	10.24	0.017
Benzo(k)fluoranthene	207-08-9	Ind	-	-	-	-	-	-	-	0.017
Trichloromethane	67-66-3	Ind	657	2	707	2	50000	3	657	-
Benzo(a)pyrene	50-32-8	Ind	0.1715	3	12.9	3	1	3	0.1715	0.27
Fluoranthene	206-44-0	Ind	0.45	2	0.95	2	3495	3	0.45	0.12
Benzene	71-43-2	Ind	978	3	53	2	52500	3	53	50
Benzo(g,h,i)perylene	191-24-2	Ind	-	-	-	-	-	-	-	0.0082
1,2-dichloroethane	107-06-2	Ind	1550	2	1360	2	16600	2	1360	-
Naphthalene	91-20-3	Ind	16	2	2.13	2	3300	3	2.13	130
Indeno(1,2,3-cd)pyrene	193-39-5	Ind	-	-	-	-	0.0395	3	0.0395	-
1,2-dichloropropane	78-87-5	Ind	559	2	1270	2	6200	2	559	-
1,4-dichlorobenzene	106-46-7	Ind	130	2	8.8	2	2800	3	8.8	-
1,1-dichloroethane	75-34-3	Ind	-	-	-	-	-	-	-	-
1,2-dibromoethane	106-93-4	Ind	500	3	250	3	-	-	250	-
Ethylbenzene	100-41-4	Ind	26.9	3	90.9	2	360	2	26.9	-
O-xylene	95-47-6	Ind	60	3	164	2	470	3	60	-
Chlorobenzene	108-90-7	Ind	79	2	77	2	11410	3	77	-
1,3-dichlorobenzene	541-73-1	Ind	42.3	2	78	2	3000	3	42.3	-
Toluene	108-88-3	Ind	336.5	3	67.8	2	12500	3	67.8	-
Chloroethene (vinylchloride)	75-01-4	Ind	-	-	-	-	-	-	-	-
1,1-dichloroethene	75-35-4	Ind	509	3	1080	3	912	3	509	-
2-chlorotoluene	95-49-8	Ind	447.2	3	87	3	920	3	87	-
4-chlorotoluene	106-43-4	Ind	35.7	3	59.2	3	550	3	35.7	-
1,2,3-trichlorobenzene	87-61-6	Ind	14.5	3	23.4	3	100	3	14.5	-
1,3,5-trichlorobenzene	108-70-3	Ind	-	-	33	3	179.5	3	33	-
Octylphenol	140-66-9	Ind	0.9	3	4.74	3	11.5	3	0.9	-
Chrysene	218-01-9	Ind	10	3	-	-	-	-	10	-
Acenaphthylene	208-96-8	Ind	-	-	-	-	-	-	-	-
Pyrene	129-00-0	Ind	0.36	3	20	3	122.425	3	0.36	-
Benzo(a)anthracene	56-55-3	Ind	0.1	3	-	-	1.37	3	0.1	-
4-nonylphenol	104-40-5	Ind	1.4	2	1.28	2	50	2	1.28	-
Phenanthrene	85-01-8	Ind	1.17	2	2.34	2	49.9	3	1.17	-
Fluorene	86-73-7	Ind	4.3	2	7.6	2	1550	3	4.3	-
Polychlorinated biphenyls	1336-36-3	Ind	-	-	3.9	3	5.705	3	3.9	-
Naphthalene, chloro derivatives	70776-03-3	Ind	-	-	-	-	-	-	-	-
Acenaphthene	83-32-9	Ind	1.2	2	5.8	2	52	3	1.2	-
Phenols	64743-03-9	Ind	-	-	-	-	-	-	-	-
Chloroalkanes C10-13	85535-84-8	Ind	-	-	-	-	-	-	-	1.4
Trichlorobenzenes (all isomers)	12002-48-1	Ind	72.95	3	18.2	2	-	-	18.2	-
Isopropylbenzene	98-82-8	Ind	80	3	57.1	3	260	3	57.1	-
Dibutyltin	1002-53-5	Ind	-	-	-	-	-	-	-	-
Octylphenol	1806-26-4	Ind	-	-	5.74	3	16.5	3	5.74	-
Methylbenzotriazol	136-85-6	Ind	-	-	-	-	-	-	-	-
PFOS	1763-23-1	Ind	380	3	680	3	-	-	380	36

Nitrophenol	100-02-7	Ind	60	2	45	2	489	3	45	-
Dichlorophenol	25167-81-1	Ind	63	3	-	-	-	-	63	-
2,4,6-tri-tert-butylphenol	732-26-3	Ind	-	-	0.609	2	-	-	0.609	-
Acrylonitrile	107-13-1	Ind	106	3	150	2	-	-	106	-
Gamma-HCH (Lindane)	58-89-9	Ins	0.01	2	0.017	2	141	2	0.01	-
Chlorpyrifos	2921-88-2	Ins	0.00035	2	0.013	2	91.9	3	0.00035	0.1
Dichlorvos	62-73-7	Ins	0.00066	2	5.5	1	6060	2	0.00066	7.00E-04
Endrin	72-20-8	Ins	0.00076	2	0.00089	2	30	3	0.00076	-
Isodrin	465-73-6	Ins	-	-	0.12	3	-	-	0.12	-
Aldrin	309-00-2	Ins	0.013	2	0.026	2	6	3	0.013	-
Dieldrin	60-57-1	Ins	0.005	2	0.0062	2	10	3	0.005	-
Pirimicarb	23103-98-2	Ins	0.17	1	790	1	14000	1	0.17	-
DDT, p,p'	50-29-3	Ins	0.0018	2	0.015	2	-	-	0.0018	-
DDT, o,p'	789-02-6	Ins	-	-	-	-	-	-	-	-
Beta-Endosulfan	33213-65-9	Ins	2.05	3	0.066	3	-	-	0.066	-
Alpha-Endosulfan	959-98-8	Ins	2.49	3	0.0055	3	80	3	0.0055	-
Heptachlor epoxide	1024-57-3	Ins	1.2	3	0.053	2	-	-	0.053	3.00E-04
Heptachlor	76-44-8	Ins	0.005	2	0.062	2	2.82	2	0.005	3.00E-04
Omethoate	1113-02-6	Ins	0.21	2	50	2	2.00E+05	3	0.21	-
Methamidophos	10265-92-6	Ins	0.00042	2	12.8	2	365	2	0.00042	-
Chlordane	57-74-9	Ins	2.4	2	0.09	2	33.8	2	0.09	-
Dimethoate	60-51-5	Ins	20	1	302	1	9040	1	20	-
Diazinon	333-41-5	Ins	0.0041	1	2.7	1	640	1	0.0041	-
Chlorfenvinphos	470-90-6	Ins	0.007	2	0.23	2	-	-	0.007	0.3
p,p'-DDD	72-54-8	Ins	0.006	2	0.14	2	-	-	0.006	-
p,p'-DDE	72-55-9	Ins	0.3	3	0.32	2	-	-	0.3	-
Alpha-HCH	319-84-6	Ins	9	3	13	3	1000	3	9	-
Delta-HCH	319-86-8	Ins	68	3	1.2	2	-	-	1.2	-
Endosulfan	115-29-7	Ins	0.023	2	0.001	2	42.8	3	0.001	0.01
Beta-HCH	319-85-7	Ins	-	-	16.3	3	-	-	16.3	-
Epsilon-HCH	1537222	Ins	-	-	-	-	-	-	-	-
Demeton-S-methyl	919-86-8	Ins	300	3	-	-	-	-	300	-
Hexachlorocyclohexane	608-73-1	Ins	0.78	2	0.09	2	-	-	0.09	0.04
Carbofuran	1563-66-2	Ins	0.094	1	1.8	1	650	1	0.094	-
cis-1,3-dichloropropene	10061-01-5	Ins	-	-	-	-	-	-	-	-
Dicofol	115-32-2	Ins	6.5	2	0.53	2	1900	3	0.53	-
Malathion	121-75-5	Ins	0.0072	1	0.22	1	410	1	0.0072	-
Fenitrothion	122-14-5	Ins	0.086	1	13	1	130	1	0.086	-
Parathion-methyl	298-00-0	Ins	0.0014	2	6.5	2	30	2	0.0014	-
Phosalone	2310-17-0	Ins	0.00396	1	6.3	1	41	1	0.00396	-
Parathion	56-38-2	Ins	4.00E-04	2	0.178	2	-	-	4.00E-04	-
1,1-dichloropropene	563-58-6	Ins	-	-	-	-	-	-	-	-
trans-1,3-dichloropropene	10061-02-6	Ins	-	-	-	-	-	-	-	-
1,2-dibromo-3-chloropropane	96-12-8	Ins	515	3	355	2	4100	3	355	-
Cypermethrin	52315-07-8	Ins	5.30E-05	1	0.0283	1	11600	3	5.30E-05	6.00E-04
Diethyltoluamide (DEET)	134-62-3	Ins	750	2	712.5	2	7200	3	712.5	-

o,p'-DDE	3424-82-6	Ins	-	-	-	-	-	-	-	-	-
o,p'-DDD	53-19-0	Ins	-	-	-	-	-	-	-	-	-
Methoxychlor	72-43-5	Ins	0.005	2	0.017	2	-	-	0.005	-	-
Chlorpyrifos-methyl	5598-13-0	Ins	0.0111	2	0.126	2	-	-	0.0111	-	-
Demeton-S-methylsulfon	17040-19-6	Ins	-	-	-	-	-	-	-	-	-
Thiamethoxam	153719-23-4	Ins	0.35	2	-	-	-	-	0.35	-	-
Mirex	2385-85-5	Ins	19.7	2	0.23	2	-	-	0.23	-	-
Ethanimidamide	135410-20-7	Ins	0.209	2	1000	1	-	-	0.209	-	-
3-hydroxycarbofuran	16655-82-6	Ins	-	-	-	-	-	-	-	-	-
Imidacloprid	138261-41-3	Ins	0.341	1	2110	1	1000	1	0.341	-	-
Thiacloprid	111988-49-9	Ins	0.37	2	197	1	9670	1	0.37	-	-
1,3-dichloropropene	542-75-6	Ins	6.4	1	8.7	1	235	1	6.4	-	-
Isobenzane	297-78-9	Ins	320	3	1.7515	3	8	3	1.7515	-	-
Chlordecone (Kepone)	143-50-0	Ins	1.8	2	0.23	2	35	2	0.23	-	-
trans-Nonachlor	39765-80-5	Ins	0.223	3	-	-	-	-	0.223	-	-
Permethrin-cis+trans	52645-53-1	Ins	6.60E-05	2	0.0079	2	9.2	3	6.60E-05	-	-
Methomyl	16752-77-5	Ins	0.17	1	6.2	1	9400	1	0.17	-	-
Tebufozide	112410-23-8	Ins	3	1	30	1	4.6	1	3	-	-
Iodofenphos	18181-70-9	Ins	-	-	0.162	2	-	-	0.162	-	-
Terbufos	13071-79-9	Ins	0.0017	2	0.0077	2	2459.5	3	0.0017	-	-
Fenazaquin	120928-09-8	Ins	0.041	1	0.032	1	760	2	0.032	-	-
Fenbutatin oxide	13356-08-6	Ins	0.476	1	0.0114	1	1.8	2	0.0114	-	-
Methiocarb	2032-65-7	Ins	0.077	1	6.5	1	82	1	0.077	-	-
Toxaphene	8001-35-2	Ins	0.013	2	0.0082	2	38	2	0.0082	-	-
Clothianidin	210880-92-5	Ins	0.22	2	-	-	1000	2	0.22	-	-
Dibenzo(a,h)anthracene	53-70-3	M	2.4878	3	-	-	-	-	2.4878	-	-
Caffeine	58-08-2	M	5470	3	4915	3	-	-	4915	-	-
Tolyltriazole	13351-73-0	M	-	-	-	-	-	-	-	-	-
Musk xylene	81-15-2	M	-	-	-	-	-	-	-	-	-
Triclosan	3380-34-5	M	3.9	2	2.5	2	0.07	2	0.07	-	-
Dichlorodifluoromethane	75-71-8	M	-	-	-	-	-	-	-	-	-
4-chloro-2-methylphenol	1570-64-5	M	2.9	3	23	3	-	-	2.9	-	-
2,6-dichlorobenzoic acid	50-30-6	M	-	-	1200	2	-	-	1200	-	-
Pentachloroanisole	1825-21-4	M	0.272	2	6.5	2	-	-	0.272	-	-
Bronopol	52-51-7	M	16	2	111.7	2	2	2	2	-	-
1-methylnaphthalene	90-12-0	M	19	3	90	3	230	3	19	-	-
2,4,6-trinitrotoluene	118-96-7	M	117	3	16	2	76	3	16	-	-
2,5-dimethyl-phenol	95-87-4	M	74.5	3	108	3	-	-	74.5	-	-
2,3-dimethyl-phenol	526-75-0	M	118	3	-	-	-	-	118	-	-
Atenolol	29122-68-7	Ph	1732	3	-	-	62000	3	1732	-	-
Metoprolol	37350-58-6	Ph	363.5	3	-	-	730	3	363.5	-	-
Clarithromycin	81103-11-9	Ph	336	3	-	-	2.4	3	2.4	-	-
Trimethoprim	738-70-5	Ph	1450	3	-	-	7265	3	1450	-	-
Bezafibrate	41859-67-0	Ph	707	3	-	-	-	-	707	-	-
Carbamazepin	298-46-4	Ph	877.5	3	199	2	10005	3	199	-	-
Naproxen	22204-53-1	Ph	600	3	-	-	4100	3	600	-	-

Ibuprofen	15687-27-1	Ph	514	3	-	-	-	-	514	-
Diclofenac	15307-86-5	Ph	801	3	-	-	12540	3	801	-
Sulfamethoxazol	723-46-6	Ph	908	3	5630	3	153.5	3	153.5	-
Azitromycin	83905-01-5	Ph	-	-	-	-	-	-	-	-
Erythromycin	114-07-8	Ph	240	3	3490	3	13	3	13	-
Di(2-ethylhexyl)phthalate (DEHP)	117-81-7	Pl	110	3	702.5	3	96	2	96	-
PCB 153	35065-27-1	Pl	0.013	3	0.013	3	-	-	0.013	-
PCB 28	7012-37-5	Pl	1.6	3	1.6	3	-	-	1.6	-
PCB 52	35693-99-3	Pl	0.3	3	0.3	3	-	-	0.3	-
PCB 180	35065-29-3	Pl	-	-	-	-	-	-	-	-
PCB 138	35065-28-2	Pl	-	-	-	-	-	-	-	-
PCB 101	37680-73-2	Pl	2.1	2	0.1	3	-	-	0.1	-
BDE 28	41318-75-6	Pl	-	-	-	-	-	-	-	-
Dibutylphthalate	84-74-2	Pl	21	2	3.5	2	120	2	3.5	-
Butyl benzyl phthalate (BBP)	85-68-7	Pl	21.5	3	19	3	60	3	19	-
PCB 194	35694-08-7	Pl	0.002	3	0.002	3	-	-	0.002	-
Di-ethyl phthalate	84-66-2	Pl	740	3	318	2	2300	3	318	-
Dipropyl phthalate	131-16-8	Pl	-	-	-	-	90	3	90	-
Di-iso-butyl phthalate	84-69-5	Pl	30	3	9	2	122	3	9	-
PCB 157	69782-90-7	Pl	-	-	-	-	-	-	-	-
Dipentyl phthalate	131-18-0	Pl	-	-	-	-	-	-	-	-
PCB 189	39635-31-9	Pl	-	-	-	-	-	-	-	-
PCB 123	65510-44-3	Pl	-	-	-	-	-	-	-	-
PCB 170	35065-30-6	Pl	-	-	-	-	-	-	-	-
Dichloromethane	75-09-2	S	14650	3	5020	2	24200	3	5020	-
Hexachlorobutadiene	87-68-3	S	2.1	2	0.9	2	200	3	0.9	0.6
1,1,1-trichloroethane	71-55-6	S	112	2	580	2	53.6	3	53.6	-
Trichloroethylene	79-01-6	S	490	3	282.8	2	3650	3	282.8	-
Tetrachloroethylene	127-18-4	S	74.9	2	49.9	2	364	3	49.9	-
1,1,2-trichloroethane	79-00-5	S	400	2	451.17	2	16700	2	400	-
1,2-dichlorobenzene	95-50-1	S	120	2	15.8	2	1400	3	15.8	-
1,1,2,2-tetrachloroethane	79-34-5	S	230	2	203	2	4700	2	203	-
PFOA	335-67-1	S	4130	3	2620	3	36900	3	2620	-
Bromoform	75-25-2	S	450	3	290	3	3860	3	290	-
1,1,1,2-tetrachloroethane	630-20-6	S	240	3	200	3	-	-	200	-
Bromochloromethane	74-97-5	S	-	-	670	3	-	-	670	-
Trichlorofluoromethane	75-69-4	S	-	-	-	-	-	-	-	-
1,2-dichloroethene	540-59-0	S	-	-	1400	3	-	-	1400	-

¹ EFSA OpenFoodTox database

² see Petschick et al.

³ EnviroTox Database

Table S2: Corine Land Cover 2012 (CLC) land-use categories originally found in the data with their respective CLC IDs. Simplified categories denote a condensed form of the original 44 CLC land-use categories into 5 categories.

ID	CLC category name	Simplified category
1	Continuous urban fabric	urban
2	Discontinuous urban fabric	urban
3	Industrial or commercial units	industrial
4	Road and rail networks and associated land	industrial
5	Port areas	industrial
6	Airports	industrial
7	Mineral extraction sites	industrial
8	Dump sites	industrial
9	Construction sites	industrial
10	Green urban areas	urban
11	Sport and leisure facilities	urban
12	Non-irrigated arable land	agriculture
13	Permanently irrigated land	agriculture
14	Rice fields	agriculture
15	Vineyards	agriculture
16	Fruit trees and berry plantations	agriculture
17	Olive groves	agriculture
18	Pastures	agriculture
19	Annual crops associated with permanent crops	agriculture
20	Complex cultivation patterns	agriculture
21	Land principally occupied by agriculture ...	agriculture
22	Agro-forestry areas	forest
23	Broad-leaved forest	forest
24	Coniferous forest	forest
25	Mixed forest	forest
26	Natural grasslands	natural
27	Moors and heathland	natural
28	Sclerophyllous vegetation	natural
29	Transitional woodland-shrub	natural
30	Beaches dunes sands	natural
31	Bare rocks	natural
32	Sparsely vegetated areas	natural
33	Burnt areas	natural
34	Glaciers and perpetual snow	natural
35	Inland marshes	natural
36	Peat bogs	natural
37	Salt marshes	natural
38	Salines	natural
39	Intertidal flats	natural
40	Water courses	natural
41	Water bodies	natural
42	Coastal lagoons	natural

43	Estuaries	natural
44	Sea and ocean	natural
45	NODATA	no_data

Table S3: Average rate-of-change values for the annually taken No. of samples and monitoring-quality-index parameters, i.e. network densities (ND), analysed chemical spectra (CS), sampling frequencies (SF), and temporal continuities (TC). Negative average rates of change, indicating a deterioration of a specific monitoring metric, are printed in bold.

Country	Country code	No. of samples	ND	CS	SF	TC
Austria	AT	4	0	0.38	0	0.16
Bosnia and Herz.	BA	55	2	0.83	1.7	0.06
Belgium	BE	2980	5	6.92	0.3	0.06
Bulgaria	BG	190	10	1.25	0.2	0.22
Switzerland	CH	1592	1	7.37	7.6	0.17
Cyprus	CY	662	2	7.57	0.3	0.05
Czech Republic	CZ	12927	20	4.67	0	0.05
Germany	DE	4478	9	3.36	0.9	0.03
Estonia	EE	0	0	0.5	0	0.25
Greece	EL	518	11	9.12	-0.9	0.12
Spain	ES	14438	92	6.74	-0.1	0.08
Finland	FI	681	1	5.08	0.4	0.05
France	FR	42220	94	3.91	-0.1	0.06
Croatia	HR	485	4	2.59	-0.4	0.07
Hungary	HU	621	14	1	-1.4	0.09
Ireland	IE	3499	6	8.4	1.1	0.03
Italy	IT	24048	61	4.01	0.3	0.04
Lithuania	LT	10	2	0.27	-0.1	0.01
Luxembourg	LU	753	1	22.3	1.8	0.25
Latvia	LV	17	1	1.67	0.2	0.02
Malta	MT	280	2	20	1	0.5
Netherlands	NL	6490	5	15.9	0.1	0.16
Norway	NO	36	2	1	0	0.23
Poland	PL	23003	101	4.1	1	0.13
Portugal	PT	14	2	1.32	-0.4	0.04
Romania	RO	1882	12	4.56	0.2	0.12
Serbia	RS	2790	9	5.66	0.9	0.07
Sweden	SE	94	0	5.25	0.3	0.08
Slovenia	SI	216	3	9	0.1	0.25
Slovakia	SK	3153	6	4.99	0.9	0.06
United Kingdom	UK	6116	45	1.56	-0.2	0.07

5 References

1. [dataset] European Environment Agency Waterbase - Water Quality ICM (WISE6, version 6). <https://bit.ly/3r8IqvA> (accessed November 9th, 2020).
2. Petschick, L. L.; Bub, S.; Wolfram, J.; Stehle, S.; Schulz, R., Modeling Regulatory Threshold Levels for Pesticides in Surface Waters from Effect Databases. *Data* **2019**, *4*, (4), 150.
3. Connors, K. A.; Beasley, A.; Barron, M. G.; Belanger, S. E.; Bonnell, M.; Brill, J. L.; de Zwart, D.; Kienzler, A.; Krailler, J.; Otter, R., Creation of a curated aquatic toxicology database: EnviroTox. *Environ. Toxicol. Chem.* **2019**, *38*, (5), 1062-1073.
4. Lampi, M. A.; Gurska, J.; McDonald, K. I.; Xie, F.; Huang, X. D.; Dixon, D. G.; Greenberg, B. M., Photoinduced toxicity of polycyclic aromatic hydrocarbons to *Daphnia magna*: Ultraviolet-mediated effects and the toxicity of polycyclic aromatic hydrocarbon photoproducts. *Environ. Toxicol. Chem.* **2006**, *25*, (4), 1079-1087.
5. Pelletier, M. C.; Burgess, R. M.; Ho, K. T.; Kuhn, A.; McKinney, R. A.; Ryba, S. A., Phototoxicity of individual polycyclic aromatic hydrocarbons and petroleum to marine invertebrate larvae and juveniles. *Environ. Toxicol. Chem.* **1997**, *16*, (10), 2190-2199.
6. Malaj, E.; Peter, C.; Grote, M.; Kühne, R.; Mondy, C. P.; Usseglio-Polatera, P.; Brack, W.; Schäfer, R. B., Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, (26), 9549-9554.
7. Schäfer, R. B.; von der Ohe, P. C.; Rasmussen, J.; Kefford, B. J.; Beketov, M. A.; Schulz, R.; Liess, M., Thresholds for the effects of pesticides on invertebrate communities and leaf breakdown in stream ecosystems. *Environ. Sci. Technol.* **2012**, *46*, (9), 5134-5142.
8. Berenzen, N.; Kumke, T.; Schulz, H. K.; Schulz, R., Macroinvertebrate community structure in agricultural streams: impact of runoff-related pesticide contamination. *Ecotoxicol. Environ. Saf.* **2005**, *60*, (1), 37-46.
9. van Wijngaarden, R. P.; Maltby, L.; Brock, T. C., Acute tier-1 and tier-2 effect assessment approaches in the EFSA Aquatic Guidance Document: are they sufficiently protective for insecticides? *Pest Manage. Sci.* **2015**, *71*, (8), 1059-1067.
10. van Wijngaarden, R. P.; Brock, T. C.; Van Den Brink, P. J., Threshold levels for effects of insecticides in freshwater ecosystems: a review. *Ecotoxicology* **2005**, *14*, (3), 355.
11. Beketov, M. A.; Schäfer, R. B.; Marwitz, A.; Paschke, A.; Liess, M., Long-term stream invertebrate community alterations induced by the insecticide thiacloprid: effect concentrations and recovery dynamics. *Sci. Total Environ.* **2008**, *405*, (1-3), 96-108.
12. Beketov, M. A.; Liess, M., Acute contamination with esfenvalerate and food limitation: chronic effects on the mayfly, *Cloeon dipterum*. *Environ. Toxicol. Chem.* **2005**, *24*, (5), 1281-1286.
13. Liess, M., Population response to toxicants is altered by intraspecific interaction. *Environ. Toxicol. Chem.* **2002**, *21*, (1), 138-142.
14. Liess, M.; Schulz, R., Chronic effects of short-term contamination with the pyrethroid insecticide fenvalerate on the caddisfly *Limnephilus lunatus*. *Hydrobiologia* **1996**, *324*, (2), 99-106.
15. Brock, T. C.; Van Wijngaarden, R. P., Acute toxicity tests with *Daphnia magna*, *Americamysis bahia*, *Chironomus riparius* and *Gammarus pulex* and implications of new EU requirements for the aquatic effect assessment of insecticides. *Environmental Science and Pollution Research International* **2012**, *19*, (8), 3610-3618.
16. Schäfer, R. B.; Caquet, T.; Siimes, K.; Mueller, R.; Lagadic, L.; Liess, M., Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. *Sci. Total Environ.* **2007**, *382*, (2-3), 272-285.

17. Liess, M.; von der Ohe, P. C., Analyzing effects of pesticides on invertebrate communities in streams. *Environ. Toxicol. Chem.* **2005**, *24*, (4), 954-965.
18. von der Ohe, P. C.; Dulio, V.; Slobodnik, J.; De Deckere, E.; Kühne, R.; Ebert, R.-U.; Ginebreda, A.; De Cooman, W.; Schüürmann, G.; Brack, W., A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European Water Framework Directive. *Sci. Total Environ.* **2011**, *409*, (11), 2064-2077.
19. Lepper, P., Manual on the methodological framework to derive environmental quality standards for priority substances in accordance with Article 16 of the Water Framework Directive (2000/60/EC). *Schmallenberg, Germany: Fraunhofer-Institute Molecular Biology and Applied Ecology* **2005**, 51.
20. von der Ohe, P. C.; Prüß, A.; Schäfer, R. B.; Liess, M.; de Deckere, E.; Brack, W., Water quality indices across Europe—a comparison of the good ecological status of five river basins. *J. Environ. Monit.* **2007**, *9*, (9), 970-978.
21. EFSA, Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. *EFSA Journal* **2013**, *11*, (7), 3290.
22. EFSA Scientific Committee, Guidance to develop specific protection goals options for environmental risk assessment at EFSA, in relation to biodiversity and ecosystem services. *EFSA Journal* **2016**, *14*, (6).
23. EFSA, EFSA Scientific Colloquium Summary Report: Biodiversity as Protection Goal in Environmental Risk Assessment for EU agro-ecosystems. *EFSA Journal* **2013**.
24. EFSA Scientific Committee, Coverage of endangered species in environmental risk assessments at EFSA. *EFSA Journal* **2016**, *14*, (2), 4312.
25. Norwegian Scientific Committee for Food Environment, Summary Report of Joint VKM and EFSA Symposium on risk assessment and risk management cooperation on environmental protection goals. *EFSA Supporting Publications* **2018**, *15*, (4), 1405E.
26. Beketov, M. A.; Kefford, B. J.; Schäfer, R. B.; Liess, M., Pesticides reduce regional biodiversity of stream invertebrates. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, (27), 11039-11043.
27. Crawford, C. G., Sampling strategies for estimating acute and chronic exposures of pesticides in streams. *JAWRA Journal of the American Water Resources Association* **2004**, *40*, (2), 485-502.
28. Stehle, S.; Schulz, R., Agricultural insecticides threaten surface waters at the global scale. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, (18), 5750-5755.
29. Wolfram, J.; Stehle, S.; Bub, S.; Petschick, L. L.; Schulz, R., Insecticide Risk in US Surface Waters: Drivers and Spatiotemporal Modeling. *Environ. Sci. Technol.* **2019**, *53*, (20), 12071-12080.
30. [dataset] European Environment Agency European Catchments and Rivers Network System (Ecrins, version 1.2). <https://bit.ly/3nCTleY> (accessed June 6th, 2020).
31. Spycher, S.; Mangold, S.; Doppler, T.; Junghans, M.; Wittmer, I.; Stamm, C.; Singer, H., Pesticide Risks in Small Streams—How to Get as Close as Possible to the Stress Imposed on Aquatic Organisms. *Environ. Sci. Technol.* **2018**, *52*, (8), 4526-4535.
32. Smith, S.; Renwick, W.; Bartley, J.; Buddemeier, R., Distribution and significance of small, artificial water bodies across the United States landscape. *Sci. Total Environ.* **2002**, *299*, (1-3), 21-36.
33. Rowe, G. L.; Belitz, K.; Demas, C. R.; Essaid, H. I.; Gilliom, R. J.; Hamilton, P. A.; Hoos, A. B.; Lee, C. J.; Munn, M. D.; Wolock, D. W. *Design of Cycle 3 of the National Water-Quality Assessment Program, 2013-23: Part 2: Science plan for improved water-quality information and management*; 2331-1258; US Geological Survey: 2013.
34. National Research Council, *Preparing for the Third Decade of the National Water-Quality Assessment Program*. National Academies Press: Washington, DC, 2012; p 200.



Pesticide occurrence in protected surface waters in nature conservation areas of Germany



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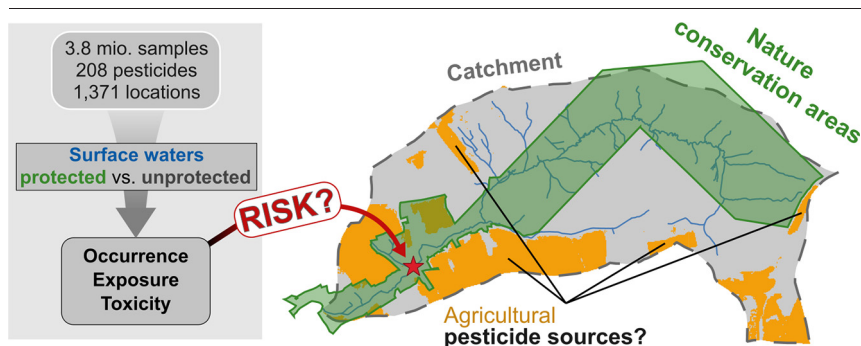
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HIGHLIGHTS

- Large-scale monitoring data of 208 pesticides in protected surface waters
- Pesticides are regularly detected in nature conservation areas.
- Multiple pesticides or metabolites show high detection frequencies.
- Noteworthy environmental risks in protected areas from pesticide exposure
- Unprotected agricultural areas serve as contaminant sources via fluvial inflows.

GRAPHICAL ABSTRACT



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ABSTRACT

A complete ban on pesticide use in sensitive areas, such as nature conservation areas (NCA), is currently being debated in the EU as part of the Sustainable Use Regulation. NCA are strictly protected landscapes in Germany with minimal agricultural activity (<4.5 %) that serve as vital ecological refuges. However, it is largely unknown whether surface waters in German NCA are contaminated by pesticides. The current study uses extensive monitoring data ($n = 3,822,553$ measurements, 1998–2020, 208 pesticides) from the federal state of Saxony (18,416 km²), Germany, to characterize pesticide occurrence, contamination levels and risks (defined as exceedance of regulatory threshold levels) for surface waters in NCA ($n = 68,277$ measurements, mean size = 2.5 km²) in comparison to unprotected areas ($n = 3,754,276$). Pesticide detection frequencies show strong correlation between NCA and unprotected areas (UPA, $R^2 = 0.70$ – 0.92), but concentrations to be ~40 % lower in protected areas. Ecological risk distributions for three major species groups are similar between NCA and UPA (fish = 79.8 % overlap in density distribution, invertebrates = 78.6 %, plants = 81.9 %). Threshold exceedances differ greatly among groups (fish = 0.9 %, aquatic invertebrates = 14.7 %, plants = 20.4 %). Based on principal component analysis, ecological risks for aquatic plants and aquatic invertebrates are strongly correlated with upstream agricultural land use (80.5–82.4 %) and are negatively correlated with upstream (semi)-natural land cover (90.1–97.3 %). Fluvial transport appears to be the most important pathway for contaminants into NCA surface waters, likely due to small conservation area size. Overall, surface waters in NCA are exposed to numerous organic contaminants causing ecological risks, highlighting the need to improve protection of these valuable water resources.

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1. Introduction

The European Commission currently considers a full ban of pesticide use in sensitive areas to reach the risk reduction goals outlined in the Regulation on the Sustainable Use of Plant Protection Products (European Commission Regulation on the Sustainable Use of Plant Protection Products - EC, 2022). Nature conservation areas (“Naturschutzgebiete”, = NCA) are classified as such sensitive areas and belong to one of six nationally designated types of protection areas in Germany. German NCA have an average size of 2.94 km² and are clearly defined areas in which a special protection of nature is necessary to safeguard conservation, development or restoration efforts (Bundesamt für Naturschutz Naturschutzgebiete, 2022). Their goal is to sustainably protect the biological diversity, ecosystem performance and functionality, as well as other intrinsic properties (e.g. beauty, peculiarity) of these areas (Bundestag, 2009). Up to now, pesticide use is usually not restricted in NCA (Mühlenberg et al., 2021), though agricultural land use in NCA is substantially lower (4.5 % of area) compared to the total area of Germany (33.0 % of area). Still, surface waters and other media (e.g. soil, biota) in German NCA may be exposed to pollutants (e.g. pesticides, pharmaceuticals) as evidenced for various protected surface waters outside Germany, indicating a risk of jeopardizing protection goals (Ackerman et al., 2008; Barakat et al., 2013; Bradley et al., 2020; Bradley et al., 2021; Buah-Kwofie and Humphries, 2017; Daly et al., 2007; Elliott and VanderMeulen, 2017; Fellers et al., 2004; Ferrario et al., 2017; Forrester et al., 2017; Gerber et al., 2016; Hageman et al., 2006; LeNoir et al., 1999; Mast et al., 2012; Ribeiro et al., 2005; Roche et al., 2009; Shunthirasingham et al., 2011). The focus of previous studies was, however, predominantly on the occurrence of legacy-use pesticides, such as organochlorine pesticides and other persistent organic pollutants, that were transported into the protected areas via pathways such as atmospheric deposition (Ackerman et al., 2008; Daly et al., 2007; Fellers et al., 2004; Ferrario et al., 2017; Hageman et al., 2006; Hageman et al., 2010; Kaiser, 2011; Klemens et al., 2003; LeNoir et al., 1999; Mast et al., 2002; Mast et al., 2012; McConnell et al., 1998; Shunthirasingham et al., 2011; Smalling et al., 2013; Thurman and Cromwell, 2000) fluvial inflow, (Barakat et al., 2013; Bradley et al., 2020; Bradley et al., 2021; Buah-Kwofie and Humphries, 2017; Camacho-Munoz et al., 2010; Elliott and VanderMeulen, 2017; Gerber et al., 2016; Ribeiro et al., 2005; Roche et al., 2009), or park visitors (e.g. defecation, bathing) (Bradley et al., 2021; Forrester et al., 2017). The fluvial inflow from unprotected upstream areas has yet received less attention, although its impact could be particularly high for small protection areas, which are predominantly found in Germany (Bundesamt für Naturschutz Naturschutzgebiete, 2022). Studies focusing on pesticide contamination in protected areas in Germany are sparse, despite Hallmann et al. (2017) showing a long-term 76 %-decline of flying insects' biomass, which initiated a lasting debate regarding the underlying reasons (Hallmann et al., 2017). Only recently, Brühl et al. (2021) measured an average of 16.7 pesticides in ethanol used for storing flying insects from traps placed in NCA. However, to the best of our knowledge, no comprehensive information about pesticide contamination of surface waters in NCA is available, which is remarkable given the well-known role water bodies play as a sink for pesticides (Malaj et al., 2014; Stehle and Schulz, 2015; Wolfram et al., 2021).

We analyze here extensive monitoring data from the federal state of Saxony, Germany ($n = 3,822,553$ measurements, 1998–2020), comprising occurrences of a total of 208 pesticides at 1371 monitoring sites. The goal of this study is to compare the occurrence and risks of pesticides in surface waters in NCA to unprotected areas (UPA), providing a comprehensive assessment of pesticide contamination and related pathways in NCA. The main hypothesis is that NCA are substantially less exposed to pesticides due to their high protection standards and the considerably limited agricultural land use therein. Pesticide exposure characteristics (detection frequencies, average concentrations) were analyzed in NCA and UPA. We assessed acute aquatic risks using regulatory threshold levels (RTL) (Stehle and Schulz, 2015) for three major species groups (aquatic invertebrates, fish and aquatic plants). Lastly, we explored the influence of upstream land

use on environmental risks in NCA. Quantifying environmental risks and identifying contamination pathways for NCA will be critical for effective management and future debates about their successful protection.

2. Materials and methods

2.1. Monitoring data

Chemical monitoring data from regular, long-term surface water monitoring were obtained from the Federal Agency for the Environment, Agriculture and Geology of Saxony (=LfULG, Landesamt für Umwelt, Landwirtschaft und Geologie). Monitoring data was gathered at 1371 locations (Fig. S1) between 1998 and 2020 with a median catchment size of 25.02 km² ($n = 38$) and 21.28 km² ($n = 1333$) for NCA and UPA, respectively, thus, 2.7 % of sites represent NCAs. On average, 21.9 % (lower quartile = 4.4 %; upper quartile = 33.9 %) of NCA watersheds were covered by the protection area itself. For a spatial distribution of NCA monitoring sites, see Fig. S1 and for spatial NCA statistics see Table S3. NCA sites were furthermore classified as mainstem-sites ($n = 33$) and tributary-sites ($n = 5$) for additional analyses regarding the influence of fluvial inflows as pesticide pathways (see SI discussion).

Measurements not referring to individual pesticides in the water phase (e.g. suspended solids, sediments, biota) were omitted from further analysis (for details see SI Data Processing). In total, 208 organic pesticides ($n = 3,822,553$ measurements) were identified and attributed with their pesticide class (e.g. “herbicides”), year-specific detection or quantification limits and other attributes. Mainly those classifications (e.g. pesticide classes) established by the LfULG were used in the present work, which were found to be comprehensive. Minor errors (e.g. outdated or missing CAS-RN) were corrected applying data quality assurance protocols (see SI Data Processing).

Overall, 141 pesticides in the dataset can be regarded as current-use pesticides, 105 and 92 of which were still actively sold in Germany in 2000 and 2010, respectively, whereas 28 are legacy use pesticides (e.g. aldrin, DDTs) and 39 are classified as metabolites. Over time, the total number of measurements per year increased considerably (see Fig. S2) from ~50 thousand before 2005 to ~230 thousand after 2015. However, pesticide classes were not uniformly represented in the monitoring data (see SI Discussion). Monitoring data for herbicides appear to reflect current-use substances well, whereas for fungicides and insecticides representation was less comprehensive, e.g. lacking detailed data on pyrethroid insecticides (see SI Discussion).

2.2. Spatial data

Spatial data were used to derive catchment features upstream of monitoring sites, catchment-specific land use characteristics and land use characteristics within NCA or within surrounding UPA. For general land use or land cover characteristics (e.g. urban, natural), the Corine Land Cover layer 2012 was used with a resolution of 100 m (European Environment Agency, 2020). Corine Land Cover data assigns land use and land cover characteristics to 44 categories, which were furthermore condensed to five main land use categories used for statistical analysis: urban, industrial, agricultural, forested and natural (Table S1). Detailed crop-distribution data (10 m resolution) for 24 crops (e.g. “winter rye”, “other spring cereal”, “grassland”) in Germany (2017–2019) were used for land use analyses within and surrounding nature conservation areas (Blickensdorfer et al., 2017, 2018 and 2019). Pesticide use data for Saxony was obtained from GfK Kynetec (Kleffmann Group) for the years 2004–2020 detailing the application of 152 active ingredients in 31 crops and crop classes on the governmental district level ($n = 3$, “Regierungsbezirk”) (Kynetec, 2021). Geometries describing the NCA ($n = 226$) were obtained from the LfULG (Landesamt für Umwelt, Landwirtschaft und Geologie, 2021a). NCA cover 3.1 % (570.3 km²) of Saxony's total area (18,401.6 km²) and 1.8 % ($n = 68,277$) of all measurements were taken in NCA, which indicates a good representation given the much lower prevalence of agriculture in NCA.

A geodata-service of the LfULG Saxony which provides detailed hydrological geoinformation about surface waters was used to derive upstream catchment features (Landesamt für Umwelt, Landwirtschaft und Geologie, 2021b). Catchment polygons were derived for all 1371 monitoring sites based on sub-catchments ($n = 6606$, median area = 1.24 km²) via their upstream hydrological connection. Zonal statistics were calculated to describe land use characteristics within all catchments using the Corine Land Cover data. Zonal statistics were also used to derive crop composition estimates for Saxony, NCA and areas surrounding NCA (e.g. 500 m buffers).

2.3. Regulatory threshold levels

RTLs were derived for three major species groups (algae, aquatic invertebrates, fish) from acute Tier-1 ecotoxicity data divided by the respective assessment factor, and represent thresholds above which unacceptable effects on aquatic biota are expected (EFSA, 2013; Stehle and Schulz, 2015). RTL were based on toxicity data which are officially used during the European ecological risk assessment of pesticides (EFSA, 2013). Toxicity data were primarily obtained from the OpenFoodTox database (Dorne et al., 2017) which summarizes the majority of expert-curated effect data that met strict quality and validity criteria and is thus used under guideline EC-1107/2009 (EFSA, 2013). For detailed description see Wolfram et al. (2021). The most sensitive and valid effect endpoint per substance and species group is divided by an assessment factor (algae = 10, fish and invertebrates = 100), yielding the RTL (Stehle and Schulz, 2015). In case of data gaps in the OpenFoodTox database, secondary sources were consulted: effect data from the Pesticide Properties Database was used if it was assigned with high data quality indicators, i.e. A4–A5, indicating their use in regulatory risk assessments (Lewis et al., 2016); else effect data from the Office of Pesticide Program - Pesticide Ecotoxicity Database (Environmental Protection Agency Office of Pesticide Programs, 2022), which procures standardized ecotoxicity effect data with assigned validity criteria used during the U.S. EPA ecological risk assessment, were used. Irrespective of the data source, the same group-specific assessment factors were applied consistently. A complete list of RTL is provided in Table S2.

Measured environmental concentrations were divided by their respective RTL per species group to quantify environmental risks, yielding M/R. M/R are similar to commonly used toxic units (TU) (Backhaus et al., 2004), but use RTL as ecotoxicological effect thresholds, which include a safety factor. M/R were summed up per sample for each species group, i.e. assuming concentration addition (Backhaus et al., 2004; Loewe and Muischnek, 1926), yielding sum(M/R). In addition, sum(M/R)_{max} were derived, which represent the highest sum(M/R) per site and year, a measure that represents the most adverse observed event each year per monitoring site. Sum(M/R) or sum(M/R)_{max} exceeding a threshold of 1 indicate acute environmental risks (Stehle and Schulz, 2015).

2.4. Statistical analysis

Two-sample tests, assessing significant differences between distributions of different groups, were performed as either Student's *t*-tests (parametric) or Mann-Whitney's *U* tests (non-parametric). Correlations were assessed using Pearson product-moment correlation (parametric) or Spearman's rho (non-parametric). Similarities of detection frequencies per pesticide class over time were expressed with correlations. However, data for the first four years of monitoring of each pesticide class were very sparse and hence omitted due to their high leverage on correlation estimates, i.e. inflating correlation estimates. Similarity of risk profiles for aquatic species groups (i.e. distributions of sum(M/R)) were assessed based on the overlap of areas-under-the-curve of density distributions (Pastore and Calcagni, 2019). Principal component analysis (PCA) in combination with k-means clustering was applied to catchment-specific land use characteristics. For all PCAs, the sum of explained variances from individual principal components was aimed to be >80 %. Thus, the number (dimensionality) of principal components used in k-means clustering was dependent of the sum of explained variances. A consensus approach using a combination of various

indices ($n = 30$) was applied to derive the optimal number of clusters when describing the dissimilarity matrix via Euclidean distances (Charrad et al., 2014). Pesticides were also classified as “regular detects” in case of detection frequencies ≥ 50 % in at least four independent measurements per monitoring site, following the classification of “pseudo-persistence” outlined by Weissinger et al. (2018). However, the term “pseudo-persistence” was thus far primarily used in the context of pharmaceuticals occurring regularly or continuously in non-target surface waters due to specific extrinsic properties, e.g. continuous WWTP inflow (Bradley et al., 2016; Bu et al., 2016; Daughton, 2002; Daughton and Brooks, 2011; O'Flynn et al., 2021; Pereira et al., 2020). The term “regular detects” was used instead of “pseudo-persistence” in the current study to prevent any misconceptions regarding pesticides' primary pathways (e.g. continuous WWTP inflows) into surface waters. For all statistical tests, an α -level of 0.05 was used. All calculations and figures were produced using R (R base: Ver. 4.0.3, 64-bit, Windows 10) and QGIS (Ver. 3.16.2 Hannover, 64-bit, Windows 10) (QGIS Development Team, 2016; Team R Core, 2015).

3. Results and discussion

3.1. Description of monitoring data

Pesticide measurements were available for 1371 monitoring sites comprising 3,822,553 individual samples. Over time, the total number of measurements per year increased considerably from ~50 thousand before 2005 to ~230 thousand after 2015 (see Fig. S2), whereas the number of operated sites remained stable since 2005 with 364.6 (± 44.6) sites being operated each year. Overall, 1.8 % of measurements were taken in NCA ($n = 68,277$) whereas the remainder was attributed to UPA (98.2 %, $n = 3,754,276$). The number of analyzed pesticides increased considerably over time from ca. 50 substances (1998–2002) to >200 since 2015. The average number of analyzed pesticides per sample also increased considerably from 30 (1998–2002) to 133 (2016–2020). The 208 analyzed pesticides comprised of 70 herbicides, 56 insecticides, 41 fungicides, 31 metabolites, one algicide and one molluscicide. On average, 6.2 samples are taken each year per monitoring site with no clear change over time. Overall, the Saxon monitoring data provides comprehensive spatiotemporal information about the occurrence and distribution of pesticides in surface waters of UPA and NCA.

3.2. Exposure of NCA to pesticides

Pesticides were detected less frequently in NCA than in UPA, however, temporal trends are similar to those of UPA. Detection frequencies of pesticides were strongly correlated over time for all four pesticide classes when compared between NCA and UPA (Fig. 1, $R^2 = 0.70$ – 0.92). Nonetheless, detection frequencies in NCA were on average considerably lower by 43.2 %, 36.4 %, 42.7 % and 47.7 % for fungicides, herbicides, insecticides and metabolites, respectively (Fig. 1). No significant difference was found between the median number of analyzed substances between NCA (75 analytes) and UPA (81 analytes, $p = 0.05$), as such, lower detection frequencies in NCA were not driven by differences in analyte spectra. Agricultural land use is substantially lower or virtually absent in NCA (Table S1), hence, sources of pesticides are likely located outside the actual protection area. Pesticide sources in the immediate vicinity of the sampling location (i.e. the protection area) are thus rare, which may explain the overall lower detection frequencies and suggests that protection efforts are partially successful in reducing the loads of organic contaminants in protected surface waters. Yet, fluvial inflows or atmospheric transport from unprotected areas likely produce temporal detection frequencies patterns that are closely related to those of UPA as shown here (Fig. 1). Hence, the similarity of detection frequencies over time between NCA and UPA (Fig. 1) suggests that pesticide transport into surface waters is also governed at larger scales, i.e. the catchment scale. In this context, only an average area of 21.9 % of NCA upstream catchments lies within the NCA itself. Smaller scale protection, i.e. the NCA itself, may thus only reduce exposure

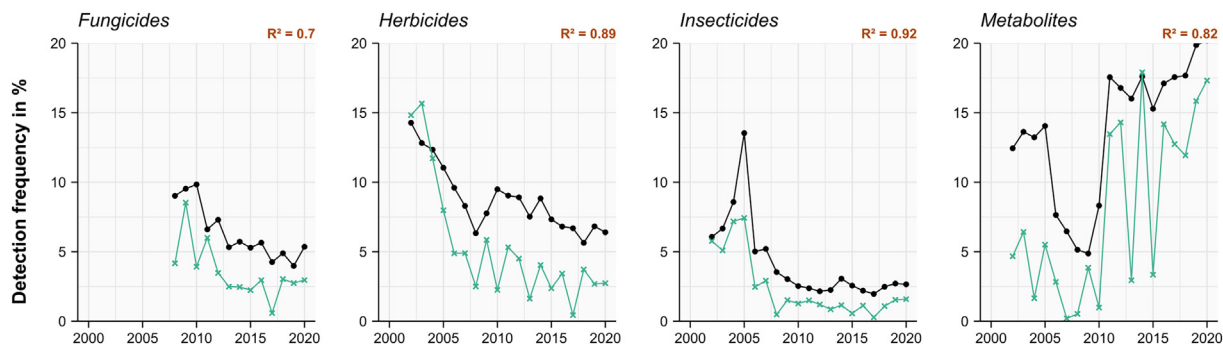


Fig. 1. Overall detection frequencies of the four main pesticides classes over time for surface waters in nature conservation areas (NCA, green) and unprotected areas (UPA, black). Please note that the first four years of monitoring data per pesticide class were omitted here and in correlation estimates (R^2) due to small samples sizes exerting high leverage on correlation estimates and inflating correlation estimates.

frequencies by $\sim 40\%$ (Fig. 1) as upstream catchments still function as significant pesticide sources. Atmospheric deposition can be an additional pathway; however, it primarily applies to certain sub-groups of pesticides (e.g. organochlorines, organophosphates, triazines) with specific physico-chemical characteristics (e.g. volatility, persistence, photolytic stability) that facilitate long-range atmospheric transport, hence the pathway's contribution is likely of lesser importance (Daly et al., 2007; Fellers et al., 2004; Ferrario et al., 2017; Kaiser, 2011; Mast et al., 2012). Still, shorter-range atmospheric transport of 35 current-use pesticides has recently been shown in Austria, potentially contributing to the detection trends in NCA (Zaller et al., 2022). Taken together, detection frequencies in NCA appear to be driven at two scales: the local-scale (i.e. the protection area itself) reducing detection frequency due to its predominantly natural land cover, and the catchment-scale, being largely unprotected and contributing to the transport of pesticides into NCA.

Comparing the average \log_{10} -concentrations of detections per substance between NCA and UPA ($n = 276,362$ detections, Fig. 2) showed medium or strong correlation for metabolites ($R^2 = 0.87$), fungicides ($R^2 = 0.94$) and insecticides ($R^2 = 0.6$), whereas for herbicides ($R^2 = 0.09$) there was no correlation, i.e. average concentrations of herbicides were mostly not proportional (Fig. 2). For instance, atrazine ($n = 10,136$; herbicide) was abundantly measured and generally found in higher concentrations in NCA, whereas glyphosate ($n = 851$; herbicide) was found in substantially higher concentrations in UPA. Glyphosate has been the herbicide with the highest annually applied mass in Saxony since 2008 (Kynetec, 2021). Particularly high applied tonnages in UPA and short dissipation times ($DT_{50\text{-soil}} \sim 6.5$ d, $DT_{50\text{-water}} \sim 9.9$ d), may explain the higher glyphosate concentrations in UPA. Only twelve substances occurred in substantially higher average concentrations in NCA (factor > 2), most of which are legacy-use or phased-out chemicals such as p,p-DDT and its metabolites, atrazine,

malathion and linuron. In addition, 49 substances showed similar average concentrations in NCA and UPA, and 34 substances showed substantially higher concentrations in UPA (factor > 2). The weighted average ratio (weighted by the number of detections) between the concentrations of NCA and UPA were higher by a factor of only 1.70, 1.72, 1.29 and 1.77 in UPA for fungicides, herbicides, insecticides and metabolites, respectively. As such, particularly substances that were frequently detected also showed similar average exposure concentrations in NCA and UPA. Even though average concentrations of most substances are on average lower in NCA, factors lower than two are likely too minor for a noteworthy difference in actual ecological risks, considering that ecotoxicity differences between compounds range many orders of magnitude (Schulz et al., 2021). Thus, pesticide exposure in NCA showed two differing characteristics: On the one hand, detection frequencies were lower. On the other hand, average concentrations did not show large differences, which means that in case of contamination events in NCA, $\text{sum}(M/R)$ could be similar to those of UPA (see below).

3.3. Presence of regularly detected pesticides

Few substances, primarily metabolites, were classified frequently as “regular detects” in surface waters, both in NCA and UPA. Some pesticides occur repeatedly or near-continuously at specific sites, thus being detected frequently ($\geq 50\%$ detections in $n \geq 4$ independent measurements per location), hereafter referred to as “regular detects”. In NCA, 209 instances (i.e. pesticide \times site, 4.6%, $n = 4575$) of “regular detects” of 50 pesticides were found (Fig. S4). In UPA, 11,921 instances of “regular detects” (6.8%, $n = 174,198$) of 135 pesticides were found (Fig. S5). For both NCA and UPA, the majority of instances (NCA = 60.3%, UPA = 59.5%) was attributable to metabolites, such as metazachlor sulfonic acid ($n_{\text{NCA}} = 19$, n_{UPA}

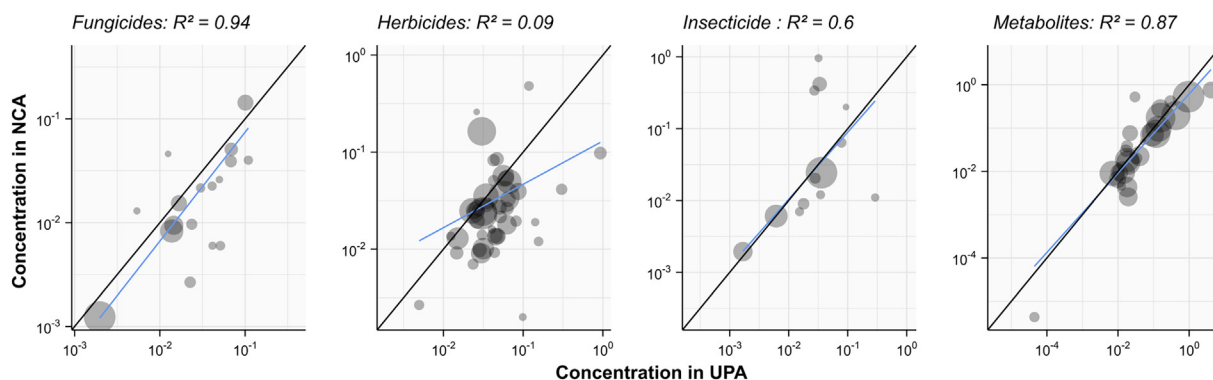


Fig. 2. Comparison of average concentrations per substance (bubble-dot) between UPA (x-axis) and NCA (y-axis) for fungicides, herbicides, insecticides and metabolites. Average concentrations are displayed on a \log_{10} -scale. Bubble size indicates the underlying number of detections used to calculate average concentrations and used as regression weights. Pearson's R^2 (weighted by number of detections) was used as an indicator for the commonality between exposures profiles and the black lines indicate 1:1 relation.

= 658), metolachlor ESA ($n_{NCA} = 17$, $n_{UPA} = 582$) and metazachlor OA ($n_{NCA} = 14$, $n_{UPA} = 594$). Aforementioned metabolites were classified as “regular detects” in 90.5 %, 81.0 % and 66.7 % of all sites in NCA (see Fig. S4) and in 92.9 %, 82.2 % and 84.1 % in UPA, respectively (Fig. S5). Metazachlor and S-metolachlor degrade quickly in agricultural soils (compared to their metabolites), with $DT_{50-soil}$ ranging from one to six weeks depending on soil type. (Lewis et al., 2016) Their metabolites show higher stability in most agricultural soils, with $DT_{50-soil}$ of up to several hundred days. Leachate analysis shows that metabolite concentrations can be orders of magnitude higher than their parent compounds. (EFSA, 2022a; EFSA, 2022b) Large-scale monitoring of small agricultural streams in Germany also showed generally higher concentrations and frequent detections of aforementioned metabolites. (Halbach et al., 2021) Thus, long-term release of metabolites in adjacent surface waters through drainage or additional run-off events could produce continuous exposure profiles that explain their regular detection. This phenomenon again underscores that unprotected upstream areas could serve as pesticide sources and explain the high degree of similarity in “regular detect” behavior within NCA and UPA. Overall, the commonality in “regular detect” behavior between NCA and UPA was high for both the 20 most influential ($\rho = 83.5\%$) and all substances ($\rho = 90.4\%$). However, the frequency in NCA was on average lower by 15.3 percentage points for the most commonly found substances, in line with their aforementioned lower detection frequencies in NCA. Metabolite-parent ratios showed no significant differences between NCA and UPA except for desisopropylatrazine ($p < 0.001$, Table S4). Whilst this may suggest that pesticide sources are close to NCA, i.e. producing no discernible shift in metabolite to parent concentrations, it may also be a result of insufficient sensitivity of the proposed approach (see SI Discussion). Overall, these results show that several substances continuously or repeatedly occur in NCA.

3.4. Ecotoxicological risks

Ecotoxicological risk profiles (i.e. $\text{sum}(M/R)$ distributions) were highly similar between NCA and UPA for all pesticide classes, with aquatic plants and invertebrates being most affected. For aquatic invertebrates, 9.5 % and 14.7 % of $\text{sum}(M/R)_{\max}$ exceeded the threshold of 1 in NCA and UPA, respectively, equaling a $\sim 35.4\%$ lower exceedance of thresholds in NCA than in UPA. The results are thus lower than found in European surface waters, where 23.9 % ($n = 17,162$) of $\text{sum}(M/R)_{\max}$ exceeded invertebrate thresholds (Wolfram et al., 2021). The results are probably not distorted by monitoring characteristics, because the Saxon monitoring programs generally performed better, i.e. having achieved substantially larger average analyte spectra per sample (~ 86 pesticides vs. ~ 13 in EU), higher monitoring network densities (~ 74.4 per thousand km^2 vs. ~ 1.83 per thousand km^2 in EU) and comparable average sampling frequencies ($\sim 57\text{d}$ vs. $\sim 58\text{d}$ in EU), all of which affect the accuracy of deriving robust environmental risk estimates. (Wolfram et al., 2021) Although in Wolfram et al. (2021) organic chemicals, such as industrial chemicals and plasticizers, were also used in risk estimations, their influence on exceedance rates was very low ($<5\%$ of exceedances), hence they do not explain the difference in exceedance frequencies reported here.

In Saxony, insecticides primarily defined risk for aquatic invertebrates (see Fig. S7). Chlorpyrifos-ethyl (organophosphate) and cypermethrin (pyrethroid) were contributing most frequently to acute invertebrate risks.

Pyrethroids have been shown to be the main driver for aquatic risks in the United States (Wolfram et al., 2018) and to increase the total applied toxicities in agriculture (Schulz et al., 2021), in addition to causing trophic shifts in aquatic mesocosms (Rogers et al., 2016), bio-concentrating via biofilm-accumulation (Mahler et al., 2020) and exhibiting particularly high ecotoxicities compared to other insecticides (Schulz et al., 2021). However, in the present monitoring data, high-use pyrethroids are severely underrepresented (e.g. lambda-cyhalothrin, deltamethrin, esfenvalerate), with 10–40 times less samples compared to other insecticides, or missing completely (beta-cyfluthrin). As such, risks for aquatic invertebrates, as reported here, currently do not fully reflect the potential effects in aquatic ecosystems.

For aquatic plants, 15.0 % and 20.4 % of $\text{sum}(M/R)_{\max}$ exceeded their threshold of 1 in NCA and UPA, respectively (see Table 1), a $\sim 26.5\%$ lower exceedance frequency of thresholds in NCA than in UPA. Overall, aquatic plants represent the species group most often adversely affected. Exceedance frequencies are similar to those found in European surface waters (17.8 %, $n = 17,053$) (Wolfram et al., 2021). Herbicide compounds primarily define risks for aquatic plants (Fig. S7) and were most comprehensively represented in the data (Kynetec, 2021), e.g. based on the comparison of monitored substances with application data for Saxony (see SI discussion, Fig. S3). As a result, $\text{sum}(M/R)$ comprise more contaminants, which likely affected the higher exceedances frequencies for aquatic plants. Diflufenican, metazachlor and diuron were the major risk contributors with the former two being intensively used in Germany, with ca. 300 t yr^{-1} and ca. 450 t yr^{-1} , respectively. These substances also show the highest applied toxicities (for details see Schulz et al. (2021)) toward aquatic plants in Germany, underscoring the benefit of ecotoxicity-normalized risk indicators for assessing pesticide application data and their potential link to adverse effects in non-target aquatic ecosystems.

Acute ecotoxicological risks were particularly low for fish (see Table 1), with only 0.9 % and 1.8 % of $\text{sum}(M/R)_{\max}$ exceeding the threshold of 1 in NCA and UPA, respectively. As such, acute ecotoxicological risks for fish appear to be negligible in both systems. The substantially lower threshold exceedances are likely the result of minor quantities of organophosphate and carbamate insecticides being used in Germany during the investigated time period along with the above-mentioned lack of pyrethroid measurements (Schulz et al., 2021). Overall, no relationship was found between detection frequencies and RTL exceedance frequencies (data not shown). Therefore, the frequency with which substances occurred in surface waters was not indicative of their potential to adversely affect the three species groups assessed here.

Risk profiles (i.e. $\text{sum}(M/R)$ and $\text{sum}(M/R)_{\max}$ distributions) between NCA and UPA were overall highly similar based on the overlap of respective density functions, i.e. the commonly shared area-under-the-curve (Pastore and Calcagni, 2019). For $\text{sum}(M/R)$, overlaps ranged between 84.38 and 88.27 %, and for $\text{sum}(M/R)_{\max}$, overlaps were slightly smaller, ranging between 78.64 and 81.86 % (see Fig. 3). Therefore, annual peak environmental risks ($\text{sum}(M/R)_{\max}$) are less pronounced in NCA, although not substantially (e.g. c.f. Fig. 3a,b). In this context, the size of NCA in Saxony (2.52 km^2 average size, 2.53 km median length of streams in NCA) – and in Germany generally – is comparably small. Also, agricultural land use directly surrounding NCA (1000 m buffer, see Fig. S8) is highly similar to the general agricultural land use in Saxony ($R^2 = 0.92$, buffer 1000 m), hence surrounding areas show substantially higher agricultural intensity ($>30\%$) compared to the NCA ($<2.5\%$). Therefore, potential agricultural sources of

Table 1

Overview of exceedance frequencies for $\text{sum}(M/R)$ and $\text{sum}(M/R)_{\max}$ for aquatic invertebrates, fish and aquatic plants for NCA and UPA.

Group	Type	Exceedance frequency $\text{sum}(M/R)$ (%)	$\text{sum}(M/R)$ (N)	Exceedance frequency $\text{sum}(M/R)_{\max}$ (%)	$\text{sum}(M/R)_{\max}$ (N)
Aqu. Invertebrates	UPA	6.94	37,041	14.65	6484
Aqu. Invertebrates	NCA	3.97	504	9.52	126
Fish	UPA	0.46	33,955	1.82	6148
Fish	NCA	0.24	420	0.91	110
Aqu. Plants	UPA	5.58	37,570	20.38	6503
Aqu. Plants	NCA	5.08	512	14.96	127

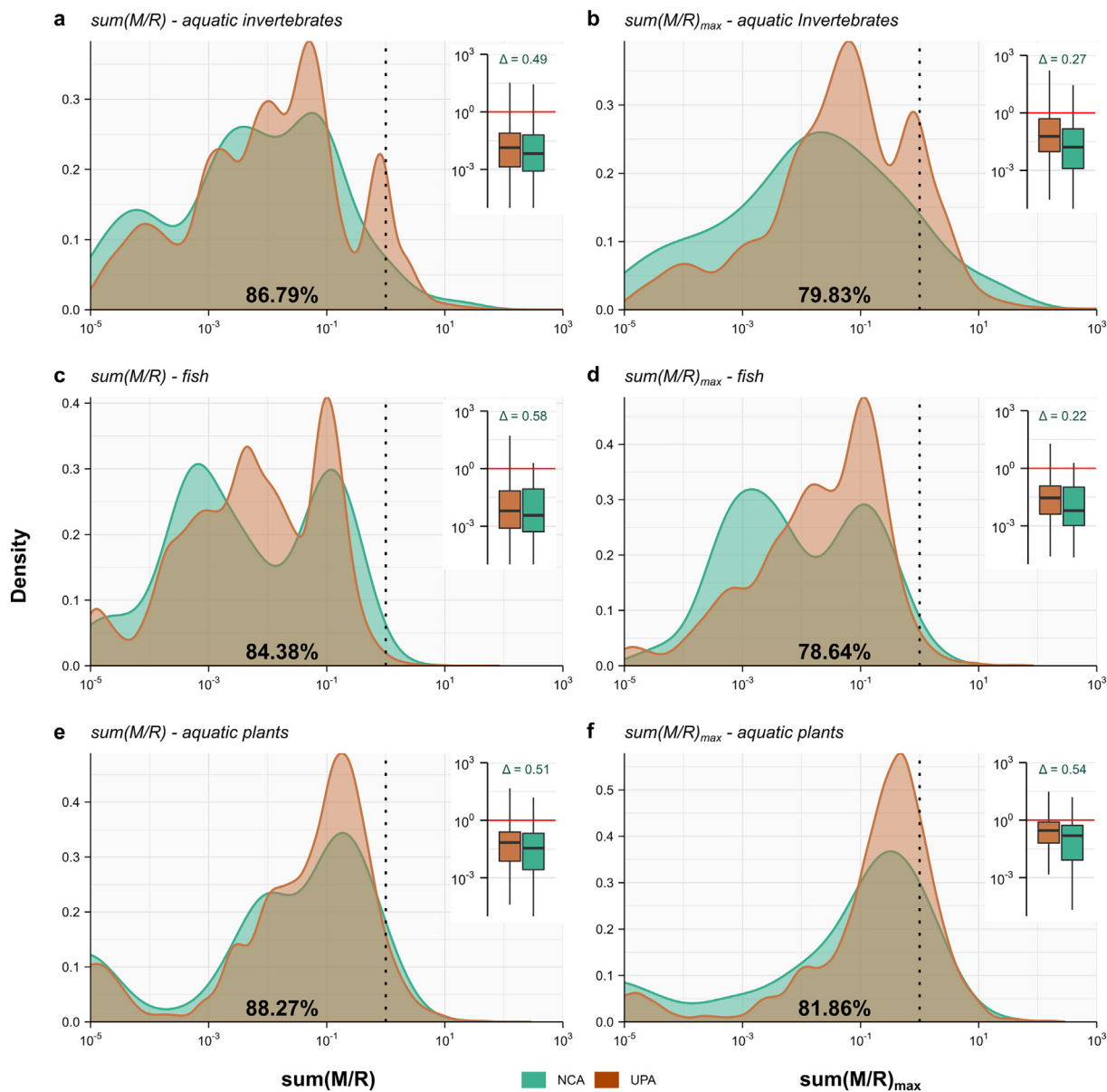


Fig. 3. Density distributions of $sum(M/R)$ are provided for (a) aquatic invertebrates, (c) fish and (e) aquatic plants. Density distributions of $sum(M/R)_{max}$ per site are provided for (b) aquatic invertebrates, (d) fish and (f) aquatic plants. Similarities of risk profiles can be deduced from the overlap (brown) of density distributions for NCA (green) and UPA (orange), annotated in bold (black). Instances of acute risk can be assumed in case of $sum(M/R)$ (left) or $sum(M/R)_{max}$ (right) being larger than 1 (black, dotted vertical line). $sum(M/R)$ and $sum(M/R)_{max}$ are also depicted as boxplots in the upper right corner of the respective panel. The median $sum(M/R)$ or $sum(M/R)_{max}$ of NCA compared to UPA (quotient, untransformed) is annotated with Δ .

pesticides are spatially close to NCA, resulting in possible direct inflows or even drift-ins. Comparisons between mainstem and tributary sites added to this assumption, showing substantially lower detection frequencies at tributary sites, which are primarily surrounded by the protection area, thus lacking major inflow sources (see SI discussion). As previously discussed, average concentrations of pesticides were higher by a factor of 1.29–1.77 in UPA than in NCA. Concentrations of commonly detected substances differed less between NCA and UPA, which would seem to explain the similarities in risk profiles. However, the frequency with which substances were detected, which define aforementioned weighted average concentrations, had no relationship to the observed risks (data not shown). On the contrary, infrequently occurring substances often defined risks (e.g. chlorpyrifos-ethyl, cypermethrin). The discrepancy of regular occurrences and environmental relevance remains a central challenge for precise risk characterizations in ecotoxicology. Furthermore, it highlights the importance of appropriate designs of monitoring schemes (Wolfram et al., 2021), e.g.

achieving sufficient detection limits and obtaining temporally highly resolved results (Spycher et al., 2018). Highly-resolved occurrence and physico-chemical properties data could then form the basis for more detailed analyses regarding pesticides' persistence and resulting chronic risks, which could be particularly important for all regularly detected pesticides (see above). Also, ecotoxicity-based analyses of applied pesticide masses can further help identifying substances that may pose particularly high risks for aquatic ecosystems (Schulz et al., 2021). Importantly, risk profiles were based here in this study on detections, thus it needs to be noted that pesticides were generally detected less frequently in NCA (see above). Nonetheless, it remains evident that, in case of exposure, the actual environmental risks differed only to a small degree overall. Particularly impairments to aquatic invertebrate and plant assemblages (Lozano et al., 2020; Schäfer et al., 2011) can adversely affect stream productivity (e.g. oxygen and biomass production, detritus turnover) or the provision of ecological services (Rumschlag et al., 2020). In addition, pesticides can transfer

from the aquatic phase into the terrestrial phase of NCA, for instance via emerging insects which have been shown to bioaccumulate current-use pesticides (Roodt et al., 2022), or via terrestrial organisms preying aquatic systems (Graf et al., 2020; Wickramasinghe et al., 2004). Particularly bottom-up impairments of aquatic insect assemblages could adversely affect higher trophic groups on large spatial scales, as shown for insectivorous birds in the Netherlands (Hallmann et al., 2014). Finally, pesticides can be registered under the assumption that recolonization or recovery takes place within several weeks at adversely affected sites (EFSA, 2013). Whilst NCA should function as such key recovery refuges and recolonization sources, it appears questionable if they can serve this role in light of the present aquatic risks.

No RTL were available for the three substances most frequently classified as “regular detects” (metazachlor ESA, metolachlor ESA, metazachlor OA) because they were regarded as non-toxic during the registration process. However, Velisek et al. (2020) have shown metazachlor OA to induce significant mortality, reduced ontogeny and oxidative stress in marbled crayfish at 3.2 µg/L over a 40-d exposure period. Here, we observed an upper quartile of concentrations ranging from 0.39 to 15 µg/L. A near-continuous occurrence of metazachlor OA may thus affect crayfish populations adversely, some of which are currently threatened by extinction in Germany (Theissinger et al., 2021). The metabolite metolachlor ESA is currently assumed to be of low toxicity to aquatic plants with the most sensitive endpoint being 21.7 mg/L (14-d EC₅₀, *Lemna gibba*). Still, the long-term effects of aforementioned substances on aquatic primary producers or non-standard test species should receive further attention in light of their omnipresence contrasted by the scarcity of ecotoxicological effect data. Continuous toxic pressure on primary production processes could critically alter productivity fluxes in aquatic ecosystems (Lozano et al., 2020) and indirectly affect respective consumer groups, e.g. macroinvertebrate assemblages, which is currently not reflected in acute risk characterizations (Rumschlag et al., 2020). Finally, ecotoxicological effects of metabolites, particularly from chronic exposures, are less understood compared to those of their parent compounds. Toxicological properties of metabolites are sometimes discovered only decades later, suggesting that their long-term presence in surface waters requires further attention (Fenner et al., 2013).

3.5. Spatial risk factors in NCA

Environmental risks in NCA surface waters were primarily driven by upstream agricultural activity since natural land cover prevailed within NCA. Within NCA with available monitoring data, agricultural land use amounted only to 2.1 % of the protected area on average, which is comparable to the agricultural land use in all NCA in Saxony (2.3 %) and far below

the overall percentage of agriculturally used area in Saxony (36.1 %) (Blickensdörfer et al., 2017, 2018 and 2019). In addition, the majority of latest changes to NCA's individual legal directives were enacted prior to 2010, suggesting that land use therein is unlikely to have changed recently (see Table S1). Thus, the probability of pesticides being emitted directly into surface waters from within NCA appears unlikely and seems in line with generally lower detection frequencies, as outlined above. The degree by which upstream catchments lie within nature conservation areas also differed considerably between sites, ranging from <1 % to 100 % of the catchment area (mean = 21.9 %).

Further analysis underscored that land use in the upstream catchments of NCA showed clear links to instream environmental risks at NCA sites (mean sum(M/R)_{max}) using principal component analysis (Fig. 4). For all three species groups, the first (PC1) and second principal component (PC2) explained 44.4–46.7 % and 24.7–24.9 % of the variability, respectively. PC1 can be interpreted as the anthropogenic land use and environmental risk gradient, whereas PC2 as the agricultural land use gradient (opposing all other land uses). Environmental risks per site were strongly correlated with agricultural land use in their catchments for aquatic plants (82.4 %) and aquatic invertebrates (80.5 %), and were strongly negatively correlated with natural or forested land cover (90.1–96.4 % for invertebrates, 91.2–97.3 % for aquatic plants). Therefore, areal intensity of agriculture in each catchment contributed to the extent to which aquatic plants and invertebrates were at risk. Hence, a major pathway of pesticides into NCA appear to be fluvial inflows, underscoring that contamination of NCA is driven on the catchment-scale, which has been observed in other protected areas as well (Bradley et al., 2021; Gerber et al., 2016; Roche et al., 2009). Conversely, protection or expansion of natural land cover in upstream watersheds can reduce the ecotoxicological risk, as shown here (Bradley et al., 2020). In this context, four NCA monitoring stations with very low agricultural land use in their catchment (≤ 7 % of area) showed that aquatic risks were on average lower by an order of magnitude compared to the remainder of NCA. In addition, detection frequencies were also lower by a factor of 3. Additional analyses of tributary versus mainstem sites furthermore showed that detection frequencies, average exposures and resulting risks were substantially lower at tributary sites, supporting this conclusion (see SI discussion). Other pathways, such as atmospheric deposition or recreational use, thus seem to act as less relevant pesticide sources in these cases, which is in line with previous findings (Bradley et al., 2021). The average risks in NCA for fish were not clearly related to agricultural land use, yet correlated with urban land use (85.0 %), and to a lesser degree, with industrial land use (73.1 %). The reason for this phenomenon is unclear, because the substances contributing most to the observed risks were not associated with particular urban or industrial use-patterns. Some agricultural fungicides (carbendazim, azoxystrobin)

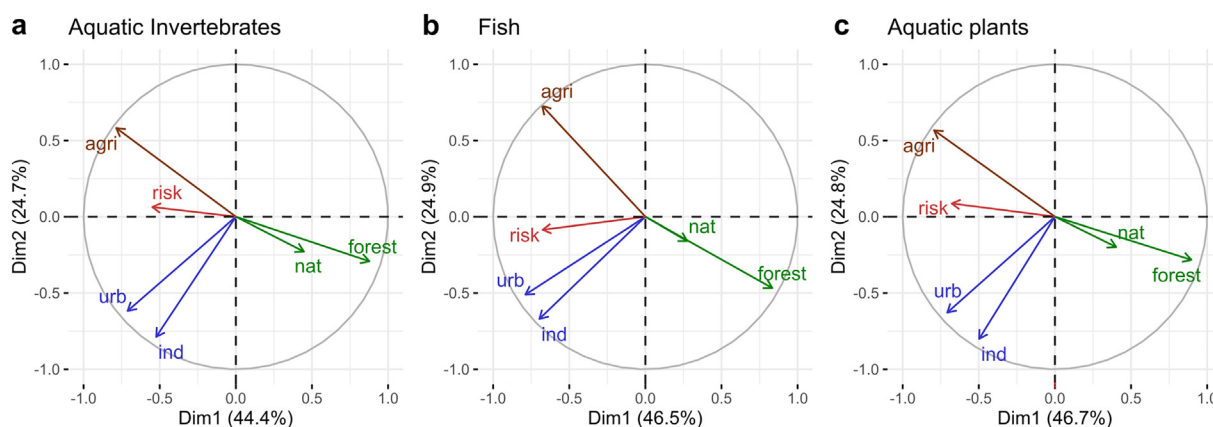


Fig. 4. Principal component analysis of land use compositions within upstream catchments of NCA with their respective influence on average risks over time (= risk, red, mean sum(M/R)_{max}) for (a) aquatic invertebrates, (b) fish and (c) aquatic plants. Variable plots use type-II scaling of principal components, as such, angles between vectors are indicative of variable correlation and orthogonal length of variable-vectors denote their relative influence in building respective principal components. Land use or land cover was aggregated into five groups (see Table S1): urban and industrial (blue), natural and forested (green), and agricultural (brown).

contributed particularly to risks in fish, yet they unlikely stem from urban sources. In the future, adding to present results by analyzing substance-specific persistence characteristics will improve the understanding of pesticides' large-scale distribution, occurrence and long-term detrimental effects.

4. Conclusion

The contamination of aquatic ecosystems by pesticides was similar between NCA and UPA with regards to average concentrations and risk distributions, whereas detection frequencies and the presence of “regular detect” compounds were lower in NCA. As such, the main hypothesis that NCA show substantially different contamination characteristics, was not met. NCA are among the most strictly protected areas in Germany and represent some of the few remaining natural refuges in otherwise intensively used landscapes, yet aquatic systems in these protected areas are subject to similar contamination levels than UPA. The results of the present study support the current goals of the EU Commission to reduce pesticide risks (e.g. by prohibiting application) in sensitive areas, such as NCA. However, the benefit for aquatic ecosystems in NCA may not be overly pronounced, because the primary contamination pathways appears to be fluvial inflow from UPA. In several US national parks, watershed management plans established ~500 m riparian buffer zones to curb diffuse entries of pesticides and achieved convincing reductions in contaminant loads (Bradley et al., 2020). We found considerable environmental risks in NCA, mainly for primary producers and invertebrates, which is likely influenced small sizes of NCA and the lack of protection of upstream areas. Our results also indicate that the loss of biodiversity or insect biomass in NCA could be influenced by the risks observable in their aquatic compartments. In the future, the influence of physico-chemical properties on substances' persistence and resulting long-term adverse effects will require further exploration. Subsequent analyses should also investigate the effect of upstream land use in more detail, particularly, the influence of individual crops, their associated pesticide application patterns and resulting applied toxicities.

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CRediT authorship contribution statement

Jakob Wolfram: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Sascha Bub:** Conceptualization, Methodology, Visualization, Writing – review & editing. **Lara L. Petschick:** Conceptualization, Writing – review & editing. **Anna Schemmer:** Formal analysis, Visualization, Writing – review & editing. **Sebastian Stehle:** Conceptualization, Investigation, Writing – review & editing. **Ralf Schulz:** Conceptualization, Project administration, Supervision, Funding acquisition, Writing – review & editing.

Data availability

All data used in the present work are publicly available or can be obtained upon request from the respective federal agencies (see references). Ecotoxicological thresholds are provided in the SI.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. S.B. is an employee of the University Koblenz-Landau and also works as a part-time freelance consultant in the field of ecotoxicology and environmental risk assessment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.160074>.

References

- Ackerman, L.K., Schwindt, A.R., Massey Simonich, S.L., Koch, D.C., Blett, T.F., Schreck, C.B., Kent, M.L., Landers, D.H., 2008. Atmospherically deposited PBDEs, pesticides, PCBs, and PAHs in western US National Park fish: concentrations and consumption guidelines. *Environ. Sci. Technol.* 42 (7), 2334–2341.
- Backhaus, T., Arrhenius, Å., Blanck, H., 2004. Toxicity of a mixture of dissimilarly acting substances to natural algal communities: predictive power and limitations of independent action and concentration addition. *Environ. Sci. Technol.* 38 (23), 6363–6370.
- Barakat, A.O., Khairy, M., Aukaily, I., 2013. Persistent organochlorine pesticide and PCB residues in surface sediments of Lake qarun, a protected area of Egypt. *Chemosphere* 90 (9), 2467–2476.
- Blickensdörfer, L., Schwieder, Marcel, Pflugmacher, Dirk, Nendel, Claas, Erasmi, Stefan, Hostert, Patrick, 2017, 2018 and 2019. National-scale crop type maps for Germany from combined time series of Sentinel-1, Sentinel-2 and Landsat 8 data accessed August 20th, 2021.
- Bradley, P.M., Journey, C.A., Clark, J., 2016. Effect of wastewater treatment facility closure on endocrine disrupting chemicals in a coastal plain stream. *Remediation* 26 (2), 9–24.
- Bradley, P.M., Romanok, K.M., Duncan, J.R., Battaglin, W.A., Clark, J.M., Hladik, M.L., Huffman, B.J., Iwanowicz, L.R., Journey, C.A., Smalling, K.L., 2020. Exposure and potential effects of pesticides and pharmaceuticals in protected streams of the US National Park Service southeast region. *Sci. Total Environ.* 704, 135431.
- Bradley, P.M., Kulp, M.A., Huffman, B.J., Romanok, K.M., Smalling, K.L., Breitmeyer, S.E., Clark, J.M., Journey, C.A., 2021. Reconnaissance of cumulative risk of pesticides and pharmaceuticals in Great Smoky Mountains National Park streams. *Sci. Total Environ.* 781, 146711.
- Brühl, C.A., Bakanov, N., Köthe, S., Eichler, L., Sorg, M., Hörrn, T., Mühlethaler, R., Meinel, G., Lehmann, G.U., 2021. Direct pesticide exposure of insects in nature conservation areas in Germany. *Sci. Rep.* 11 (1), 1–10.
- Bu, Q., Shi, X., Yu, G., Huang, J., Wang, B., 2016. Assessing the persistence of pharmaceuticals in the aquatic environment: challenges and needs. *Emerg. Contam.* 2 (3), 145–147.
- Buah-Kwofie, A., Humphries, M.S., 2017. The distribution of organochlorine pesticides in sediments from iSimangaliso Wetland Park: ecological risks and implications for conservation in a biodiversity hotspot. *Environ. Pollut.* 229, 715–723.
- Bundesamt für Naturschutz Naturschutzgebiete, 2022. Bundesamt für Naturschutz Naturschutzgebiete. accessed 19.8 <https://www.bfn.de/naturschutzgebiete>.
- Bundestag, 2009. Gesetz über Naturschutz und Landschaftspflege (Bundesnaturschutzgesetz - BNatSchG) vom 29. Juli 2009 (BGBl. I S. 2542), das zuletzt durch Artikel 1 des Gesetzes vom 20. Juli 2022 (BGBl. I S. 1362) geändert worden ist. BGBl. I S. 2542.
- Camacho-Munoz, M., Santos, J., Aparicio, I., Alonso, E., 2010. Presence of pharmaceutically active compounds in Donana Park (Spain) main watersheds. *J. Hazard. Mater.* 177 (1–3), 1159–1162.
- Charrad, M., Ghazzali, N., Boiteau, V., Niknafs, A., 2014. NbClust: an R package for determining the relevant number of clusters in a data set. *J. Stat. Softw.* 61, 1–36.
- Daly, G.L., Lei, Y.D., Teixeira, C., Muir, D.C., Castillo, L.E., Wania, F., 2007. Accumulation of current-use pesticides in neotropical montane forests. *Environ. Sci. Technol.* 41 (4), 1118–1123.
- Daughton, C.G., 2002. Environmental stewardship and drugs as pollutants. *Lancet* 360 (9339), 1035–1036.
- Daughton, C.G., Brooks, B.W., 2011. Active pharmaceutical ingredients and aquatic organisms. *Environmental Contaminants in Biota: Interpreting Tissue Concentrations.* 2.
- Dorne, J.L., Richardson, J., Kass, G., Georgiadis, N., Monguidi, M., Pasinato, L., Cappe, S., Verhagen, H., Robinson, T., 2017. OpenFoodTox: EFSA's open source toxicological database on chemical hazards in food and feed. *EFSA J.* 15 (1), e15011.
- EFSA, 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. *EFSA Journal* 11 (7), 3290.
- EFSA, 2022. Public consultation on the active substance metazachlor. ISBN 3-900051-07-0-Assessment of the Concentrations of the Metazachlor Metabolites in Ground Water under Consideration of Monitoring Results <http://www.R-project.org> <https://www.efsa.europa.eu/en/consultations/call/public-consultation-active-substance-metazachlor-0>.
- EFSA, 2022. Public consultation on the active substance S-metolachlor. Renewal Assessment Report accessed 26 July <https://www.efsa.europa.eu/en/consultations/call/181129-1>.
- Elliott, S.M., VanderMeulen, D.D., 2017. A regional assessment of chemicals of concern in surface waters of four midwestern United States national parks. *Sci. Total Environ.* 579, 1726–1735.
- Environmental Protection Agency Office of Pesticide Programs, 2022. Pesticide Ecotoxicity Database. accessed 19.8 <https://ecotox.ipmcenters.org/>.
- European Commission Regulation on the Sustainable Use of Plant Protection Products - EC 2021/2115 - COM/2022/305 Final. accessed 30.8 <https://bit.ly/3RpGk7q>.

- European Environment Agency, 2020. Corine Land Cover - 100. <https://bit.ly/2KhEat1>. (Accessed 6 June 2020).
- Fellers, G.M., McConnell, L.L., Pratt, D., Datta, S., 2004. Pesticides in mountain yellow-legged frogs (*Rana muscosa*) from the Sierra Nevada Mountains of California, USA. *Environ. Toxicol. Chem.* 23 (9), 2170–2177.
- Fenner, K., Canonica, S., Wackett, L.P., Elsner, M., 2013. Evaluating pesticide degradation in the environment: blind spots and emerging opportunities. *Science* 341 (6147), 752–758.
- Ferrario, C., Finizio, A., Villa, S., 2017. Legacy and emerging contaminants in meltwater of three alpine glaciers. *Sci. Total Environ.* 574, 350–357.
- Forrester, H., Clow, D., Roche, J., Heyvaert, A., Battaglin, W., 2017. Effects of backpacker use, pack stock trail use, and pack stock grazing on water-quality indicators, including nutrients, *E. coli*, hormones, and pharmaceuticals, in Yosemite National Park, USA. *Environ. Manage.* 60 (3), 526–543.
- Gerber, R., Smit, N.J., Van Vuren, J.H., Nakayama, S.M., Yohannes, Y.B., Ikenaka, Y., Ishizuka, M., Wepener, V., 2016. Bioaccumulation and human health risk assessment of DDT and other organochlorine pesticides in an apex aquatic predator from a premier conservation area. *Sci. Total Environ.* 550, 522–533.
- Graf, N., Battes, K.P., Cimpean, M., Entling, M.H., Frisch, K., Link, M., Scharmüller, A., Schreiner, V.C., Szöcs, E., Zubrod, J.P., Schäfer, R.B., 2020. Relationship between agricultural pesticides and the diet of riparian spiders in the field. *Environmental Sciences Europe* 32 (1), 1.
- Hageman, K.J., Simonich, S.L., Campbell, D.H., Wilson, G.R., Landers, D.H., 2006. Atmospheric deposition of current-use and historic-use pesticides in snow at national parks in the western United States. *Environ. Sci. Technol.* 40 (10), 3174–3180.
- Hageman, K.J., Hafner, W.D., Campbell, D.H., Jaffe, D.A., Landers, D.H., Simonich, S.L.M., 2010. Variability in pesticide deposition and source contributions to snowpack in western US National Parks. *Environ. Sci. Technol.* 44 (12), 4452–4458.
- Halbach, K., Möder, M., Schrader, S., Liebmann, L., Schäfer, R.B., Schneeweiss, A., Schreiner, V.C., Vormeier, P., Weisner, O., Liess, M., Reemtsma, T., 2021. Small streams—large concentrations? Pesticide monitoring in small agricultural streams in Germany during dry weather and rainfall. *Water Res.* 203, 117535.
- Hallmann, C.A., Poppen, R.P., van Turnhout, C.A., de Kroon, H., Jongejans, E., 2014. Declines in insectivorous birds are associated with high neonicotinoid concentrations. *Nature* 511 (7509), 341–343.
- Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PloS one* 12 (10), e0185809.
- Kaiser, K., 2011. Preliminary study of pesticide drift into the Maya Mountain protected areas of Belize. *Environ. Contam. Toxicol.* 86 (1), 56–59.
- Klemens, J.A., Wieland, M., Flanagan, V., Frick, J., Harper, R., 2003. A cross-taxa survey of organochlorine pesticide contamination in a Costa Rican wildland. *Environ. Pollut.* 122 (2), 245–251.
- Kynetec, 2021. FarmTrak - Pesticide active ingredient tonnages applied in Saxon agriculture from 2004 - 2020.
- Landesamt für Umwelt, 2021a. Landwirtschaft und Geologie. Schutzgebiete in Sachsen accessed June 18th <https://bit.ly/359bVqJ>.
- Landesamt für Umwelt, 2021b. Landwirtschaft und Geologie. Geodatendownload des Fachbereichs Wasser accessed 30.8 <https://bit.ly/3FHcNz9>.
- LeNoir, J.S., McConnell, L.L., Fellers, G.M., Cahill, T.M., Seiber, J.N., 1999. Summertime transport of current-use pesticides from California's Central Valley to the Sierra Nevada Mountain range, USA. *Environ. Toxicol. Chem.* 18 (12), 2715–2722.
- Lewis, K.A., Tzilivakis, J., Warner, D.J., Green, A., 2016. An international database for pesticide risk assessments and management. *Hum. Ecol. Risk Assess.* 22 (4), 1050–1064.
- Loewe, S., Muischnek, H., 1926. Über Kombinationswirkungen. *Naunyn-Schmiedeberg's Archiv für experimentelle Pathologie und Pharmakologie* 114 (5), 313–326.
- Lozano, V.L., Allen Dohle, S., Vera, M.S., Torremorell, A., Pizarro, H.N., 2020. Primary production of freshwater microbial communities is affected by a cocktail of herbicides in an outdoor experiment. *Ecotoxicol. Environ. Saf.* 201, 110821.
- Mahler, B.J., Schmidt, T.S., Nowell, L.H., Qi, S.L., Van Metre, P.C., Hladik, M.L., Carlisle, D.M., Munn, M.D., May, J., 2020. Biofilms provide new insight into pesticide occurrence in streams and links to aquatic ecological communities. *Environ. Sci. Technol.* 54 (9), 5509–5519.
- Malaj, E., Peter, C., Grote, M., Kühne, R., Mondy, C.P., Usseglio-Polatera, P., Brack, W., Schäfer, R.B., 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proc. Natl. Acad. Sci. U. S. A.* 111 (26), 9549–9554.
- Mast, M.A., Campbell, D.H., Ingersoll, G.P., Foreman, W.T., Krabbenhoft, D.P., 2002. Atmospheric deposition of nutrients, pesticides, and mercury in Rocky Mountain National Park, Colorado. *Water Resour. Invest. Rep. U.S. Geol. Surv.* 2003, 03–4241.
- Mast, M.A., Alvarez, D.A., Zaugg, S.D., 2012. Deposition and accumulation of airborne organic contaminants in Yosemite National Park California. *Environ. Toxicol. Chem.* 31 (3), 524–533.
- McConnell, L.L., LeNoir, J.S., Datta, S., Seiber, J.N., 1998. Wet deposition of current-use pesticides in the Sierra Nevada mountain range, California, USA. *Environ. Toxicol. Chem.* 17 (10), 1908–1916.
- Mühlberg, H.M., Stefan, Sattler, Cornelia, Settele, Josef, 2021. Regelungen zur Anwendung von Pestiziden in Schutzgebieten. Umweltbundesamt, Dessau-Roßlau.
- O'Flynn, D., Lawler, J., Yusuf, A., Parle-McDermott, A., Harold, D., Mc Cloughlin, T., Holland, L., Regan, F., White, B., 2021. A review of pharmaceutical occurrence and pathways in the aquatic environment in the context of a changing climate and the COVID-19 pandemic. *Anal. Methods* 13 (5), 575–594.
- Pastore, M., Calcagni, A., 2019. Measuring distribution similarities between samples: a distribution-free overlapping index. *Front. Psychol.* 10, 1089.
- Pereira, A., Silva, L., Laranjeiro, C., Lino, C., Pena, A., 2020. Selected pharmaceuticals in different aquatic compartments: part I—source, fate and occurrence. *Molecules* 25 (5), 1026.
- QGIS Development Team, 2016. QGIS geographic information system. Open Source Geospatial Foundation Project accessed 30.8.
- Ribeiro, C.O., Vollaire, Y., Sanchez-Chardi, A., Roche, H., 2005. Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the eel (*Anguilla anguilla*) at the camargue nature reserve France. *Aquat. Toxicol.* 74 (1), 53–69.
- Roche, H., Vollaire, Y., Martin, E., Rouer, C., Coulet, E., Grillas, P., Banas, D., 2009. Rice fields regulate organochlorine pesticides and PCBs in lagoons of the Nature Reserve of Camargue. *Chemosphere* 75 (4), 526–533.
- Rogers, H.A., Schmidt, T.S., Dabney, B.L., Hladik, M.L., Mahler, B.J., Van Metre, P.C., 2016. Bifenthrin causes trophic cascade and altered insect emergence in mesocosms: implications for small streams. *Environ. Sci. Technol.* 50 (21), 11974–11983.
- Roodt, A.P., Röder, N., Pietz, S., Kolbenschlag, S., Manfrin, A., Schwenk, K., Bundschuh, M., Schulz, R., 2022. Emerging midges transport pesticides from aquatic to terrestrial ecosystems: importance of compound-and organism-specific parameters. *Environ. Sci. Technol.* 56 (9), 5478–5488.
- Rumschlag, S.L., Mahon, M.B., Hoverman, J.T., Raffel, T.R., Carrick, H.J., Hudson, P.J., Rohr, J.R., 2020. Consistent effects of pesticides on community structure and ecosystem function in freshwater systems. *Nature Communications* 11 (1), 6333.
- Schäfer, R.B., van den Brink, P.J., Liess, M., 2011. Impacts of pesticides on freshwater ecosystems. *Ecological impacts of toxic chemicals.* 2011, pp. 111–137.
- Schulz, R., Bub, S., Petschick, L.L., Stehle, S., Wolfram, J., 2021. Applied pesticide toxicity shifts toward plants and invertebrates, even in GM crops. *Science* 372 (6537), 81–84.
- Shunthirasingham, C., Gouin, T., Lei, Y.D., Ruepert, C., Castillo, L.E., Wania, F., 2011. Current-use pesticide transport to Costa Rica's high-altitude tropical cloud forest. *Environ. Toxicol. Chem.* 30 (12), 2709–2717.
- Smalling, K.L., Fellers, G.M., Kleeman, P.M., Kuivila, K.M., 2013. Accumulation of pesticides in Pacific chorus frogs (*Pseudacris regilla*) from California's Sierra Nevada Mountains, USA. *Environ. Toxicol. Chem.* 32 (9), 2026–2034.
- 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 (8), 4526–4535.
- Stehle, S., Schulz, R., 2015. Agricultural insecticides threaten surface waters at the global scale. *Proc. Natl. Acad. Sci. U. S. A.* 112 (18), 5750–5755.
- Team R Core, 2015. R: A Language and Environment for Statistical Computing. ISBN 3-900051-07-02018. R Foundation for Statistical Computing, Austria <http://www.R-project.org>.
- Theisinger, K., Edsman, L., Maguire, I., Diéguez-Urbeondo, J., Jussila, J., 2021. Editorial: conservation of European freshwater crayfish. *Front. Ecol. Evol.* 9.
- Thurman, E., Cromwell, A.E., 2000. Atmospheric transport, deposition, and fate of triazine herbicides and their metabolites in pristine areas at Isle Royale National Park. *Environ. Sci. Technol.* 34 (15), 3079–3085.
- Velisek, J., Stara, A., Kubec, J., Zuskova, E., Buric, M., Kouba, A., 2020. Effects of metazachlor and its major metabolite metazachlor OA on early life stages of marbled crayfish. *Sci. Rep.* 10 (1), 1–9.
- Weissinger, R.H., Blackwell, B.R., Keteles, K., Battaglin, W.A., Bradley, P.M., 2018. Bioactive contaminants of emerging concern in National Park waters of the northern Colorado plateau, USA. *Sci. Total Environ.* 636, 910–918.
- Wickramasinghe, L.P., Harris, S., Jones, G., Vaughan Jennings, N., 2004. Abundance and species richness of nocturnal insects on organic and conventional farms: effects of agricultural intensification on bat foraging. *Conserv. Biol.* 18 (5), 1283–1292.
- Wolfram, J., Stehle, S., Bub, S., Petschick, L.L., Schulz, R., 2018. Meta-analysis of insecticides in United States surface waters: status and future implications. *Environ. Sci. Technol.* 52 (24), 14452–14460.
- Wolfram, J., Stehle, S., Bub, S., Petschick, L.L., Schulz, R., 2021. Water quality and ecological risks in European surface waters—monitoring improves while water quality decreases. *Environ. Int.* 152, 106479.
- Zaller, J.G., Kruse-Plaf, M., Schlechtriemen, U., Gruber, E., Peer, M., Nadeem, I., Formayer, H., Hutter, H.-P., Landler, L., 2022. Pesticides in ambient air, influenced by surrounding land use and weather, pose a potential threat to biodiversity and humans. *Sci. Total Environ.* 156012.

Supplementary Materials for

**Pesticide occurrence in protected surface waters in nature
conservation areas of Germany**

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1 Supporting Information

1.1 Data processing

The monitoring data offered by the LfULG has a high quality, especially when compared to that other national or trans-national sources (e.g. Wolfram et al.¹). Preestablished classification schemes for all analytes eased the pace of data processing and aggregative steps. Also, year-specific tabulation of the detection or quantification limits per analyte provided vital information about the observable spectrum of analytes. Only minor issues or sporadic errors were found within these data, thus no detailed flowcharts for QA processing were deemed necessary. Briefly, faulty CAS-RN (e.g. outdated) were found in few instances and three metabolites were not attributed with respective CAS-RN, which was subsequently corrected. Space × time duplicate measurements were removed to prevent data inflation. Chemical classes (e.g. “pharmaceutical”, “pesticide”) were reduced to their primary use purpose, as some substances can be classified in multiple groups, e.g. biphenyl can be both an industrial chemical (synthesis-byproduct) and fungicide active ingredient, although its primary use purpose was as a fungicide.

Monitoring data by the LfULG is divided in two databases, one covering the years 1996–2016 followed by a new geoportal for the years 2017–2020. Both databases were harmonized without any noteworthy issues.² Since 2017, i.e. data from the new geoportal, analyte measurements can differentiate between non-detects (<LOD), non-quantifications (LOD < measurement < LOQ) and quantifications (>LOQ). However, in the predecessor database such a distinction was not possible, i.e. measurements could only differentiate between <LOD and >LOD. To provide a uniform basis for comparison, the present study only differentiated between “non-detects” (<LOD) and “detects” (quantifications).

1.2 Representation of pesticide classes

Pesticide classes were heterogeneously represented by monitoring data, when assessing the mean number of measurements per pesticide and number of monitored pesticides (Figure S3). Also, the coverage of currently used pesticides (see Figure S3c) was overall highest for herbicides, followed by fungicides and lowest for insecticides, particularly until 2016. Overall, herbicides appear most comprehensively represented, followed by fungicides and lastly insecticides. Furthermore, pesticides' representation was contextualized with the total annually applied amount for Saxony.³ For each substance and year, the applied tonnage was classified as either “represented”, i.e. in case monitoring data for the respective substance was available, or as “not represented”, i.e. if no monitoring data was available for the substance. Herbicides were well-represented in the dataset, as an average 91.7 % of the applied herbicide tonnage between 2004–2020 was also represented by measurements each year.³ For insecticides and fungicides, the representation was less comprehensive with only 74.8 and 61.7 % of the applied tonnages being represented by measurements. For insecticides, primarily pyrethroids were not fully covered by the monitoring data (e.g. alpha-cypermethrin, beta-cyfluthrin, tau-fluvalinate) which provides a reason for concern, since pyrethroids are particularly toxic to aquatic invertebrates⁴ and can occur in ecologically adverse concentrations.⁵ For fungicides, the coverage was even lower, primarily driven by the omission of high tonnage substances such as chlorothalonil, captan, prothioconazole, and mancozeb from the monitoring programs. The former two still being actively used whereas the latter two having been phased-out recently. Importantly, applied tonnages do not necessarily reflect actual applied ecotoxicities,⁴ hence they should be interpreted with care.

1.3 Mainstem and tributary NCA sites

NCA monitoring sites were classified as either mainstem or tributary sites. A tributary site classification was assigned if (i) the monitored stream flows into a higher order stream and (ii)

was primarily (>90%) situated within the NCA boundaries. The distinction between mainstem and tributary sites was made to further explore the assumption that fluvial inflow from unprotected upstream areas explains the occurrence and risk characteristics discussed in the main text. However, only five monitoring sites could be classified as tributary sites, whereas 33 were classified as mainstem sites. As such, any inference from the following results should be interpreted with care.

In line with the assumption that fluvial inflow is primarily responsible for the transport of pesticides into NCA, tributary sites showed substantially lower detection frequencies, average concentrations and resulting environmental risks: Detection frequencies at mainstem sites were with 5.13 % (n = 62,616) substantially higher than the 0.94 % (n = 5,661) observed at tributary sites. Measured concentrations were also substantially lower in tributary sites overall (mean = 38.8 ng/L) compared to mainstem sites (mean = 84.0 ng/L), more so when comparing average daily concentrations (tributary: mean = 22.2 ng/L, mainstem: mean = 65.1 ng/L). Finally, environmental risks, expressed as sum(M/R), also showed noteworthy difference between tributary and mainstem sites. For aquatic plants, median sum(M/R) were lower by more than three orders of magnitude, whereas the difference was small for invertebrates and fish by ca. 1.5 and 1.3 orders of magnitude, respectively. Overall, tributary sites showed substantially lower detection frequencies of pesticides in addition to lower average concentrations and lower sum(M/R) for all three organism groups. The results add an additional line of evidence that fluvial inflow from upstream areas is the primary input pathway of pesticides into NCA.

1.4 Metabolite-parent ratios

Metabolite-parent ratios for 23,157 cases were available for both NCA and UPA, a case representing if both the parent and metabolite (20 metabolite-parent pairs) were measured at the same location and day. For a complete list of metabolite-parent summary statistics see Table

S4. No significant differences were found for metabolite-parent ratios between NCA and UPA with the exception of desisopropylatrazine ($p < 0.001$, Table S4). Furthermore, no clear trend in effect sizes was observable between NCA and UPA as twelve metabolite-parent ratios were larger in NCA whereas eight were smaller in NCA and generally ranged only within a factor of 2 (see Table S4, see Figure S6). We hypothesized that metabolite-parent ratios could be higher in NCA, if sources are substantially further upstream in unprotected areas, granting more time for parent compounds to degrade. Thus, the lack of significant differences in metabolite-parent ratios (Table S4) could suggest that NCA are too small for differences in contaminant compositions to develop. However, parent compounds for which these metabolite-parent pairs were available have median $DT_{50_{soil}}$ and $DT_{50_{water}}$ of 21.4 d and 48.75 d, respectively.⁶ Furthermore, several parent compounds are classified as photolytically or hydrolytically stable in the water phase.⁶ Compounds' residence times in the water phase may simply be too short (i.e. only amounting to several days) compared to the time required for them to degrade or dissipate in general. As such, it may be unlikely to find significant differences irrespective of the size of the protected area given the scale of the analyzed catchments. Conversely, metabolite-parent ratios could be defined primarily in agricultural soils prior to substances off-target transport into surface waters, hence our proposed method of using metabolite-parent ratios as indicators for the distance of primary non-point sources could be too insensitive. This issue may be further exacerbated by the variability inherent to monitoring data, increasing uncertainty, hence the resulting confidence intervals. Still, the method appears most suitable when investigating historic versus recent applications of highly persistent substances particularly in sediments, as conducted by Buah-Kwofie and Humphries⁷. In their work, longer residence times sediments and a generally longer reference period improve the ability of detecting significant differences. In the present work, the use of metabolite-parent ratios as an

indicator for the distance of pesticide source should only be interpreted with care and may have noteworthy insensitivities.

1.5 SI Figures

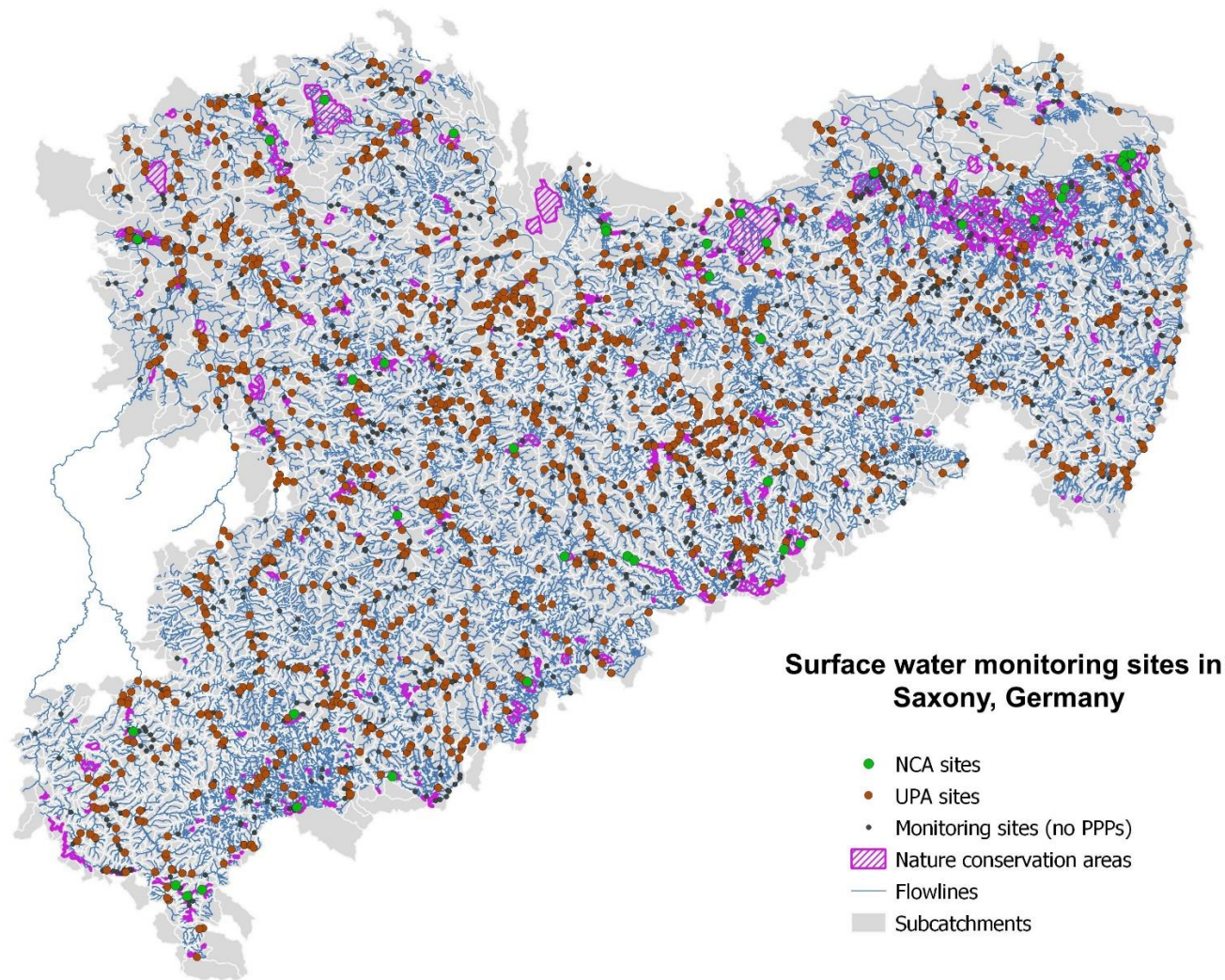


Figure S1: Surface water monitoring sites in Saxony from 1998–2020 and nature conservation areas (NCA, 2020). Monitoring sites in black symbolize sites where no pesticides were analyzed.

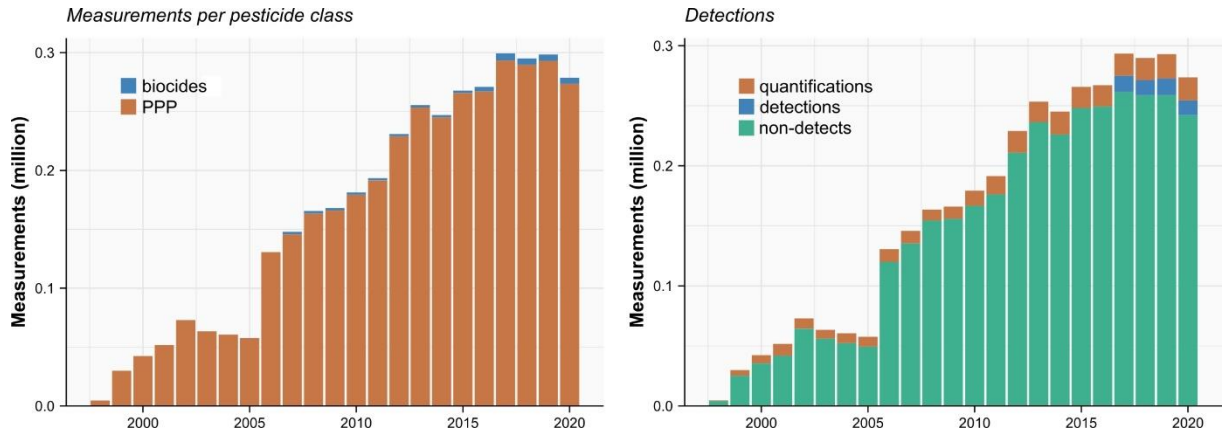


Figure S2: (a) Overview over the total number of measurements per year for pesticides and biocides, (b) the total number of non-detections, non-quantifications and quantifications per year for Saxony.

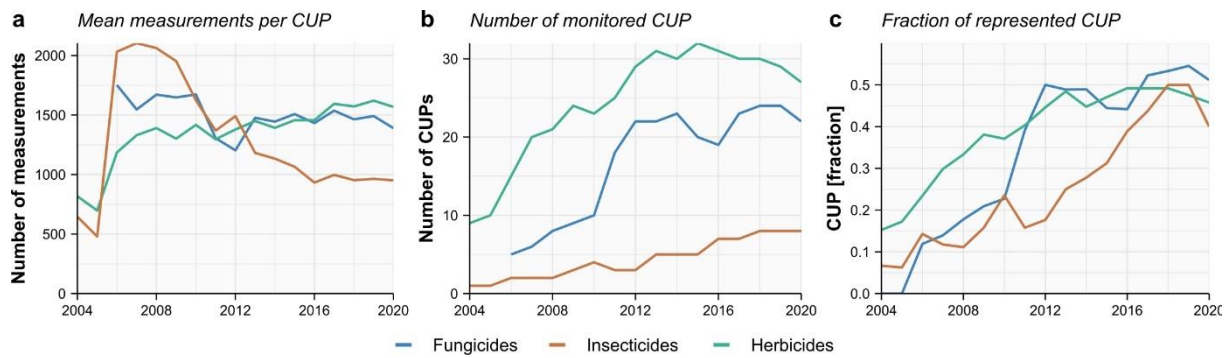


Figure S3: Current-use pesticides (CUPs, $n = 152$) are based on respondent-level application data for Saxony (2004–2020) obtained from GfK Kynetec. (a) depicts the average number of measurements per CUP each year. (b) depicts the number of CUPs per year for which monitoring data was available. (c) depicts the fraction of CUPs represented by monitoring data per year.

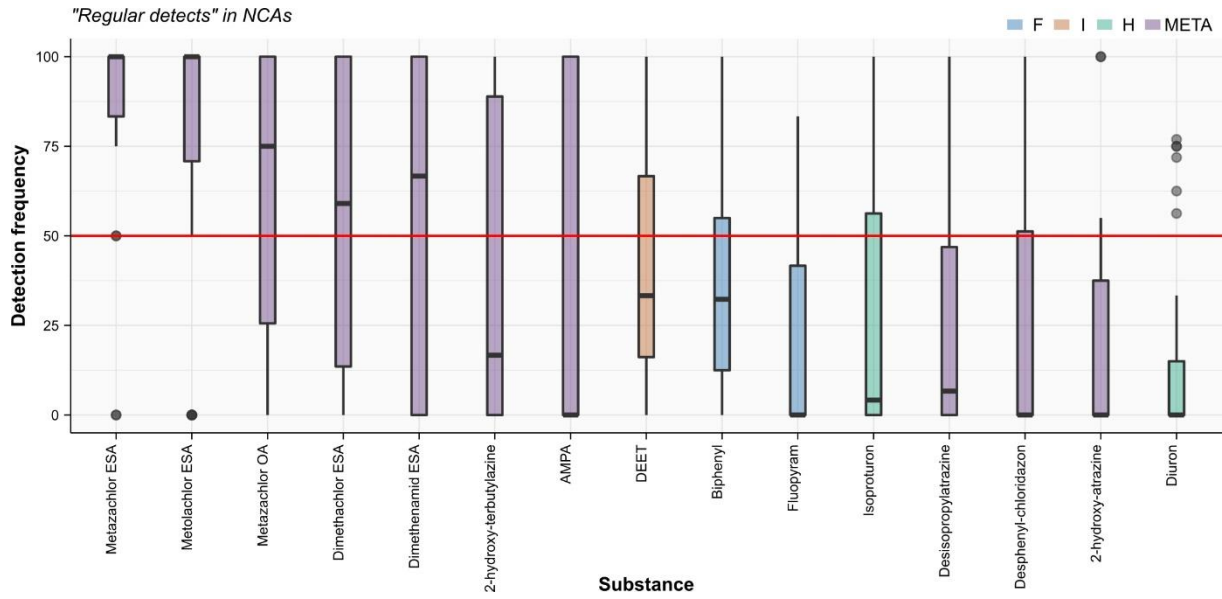


Figure S4: “Regular detect” distributions of the 15 most commonly detected substances in NCA ordered by their frequency of “regular detect” classification. A single point represents a substance’s detection frequency per monitoring site (≥ 4 distinct measurements) and a detection frequency $\geq 50\%$ assumes regular detection. For instance, metazachlor ethane sulfonic acid (metabolite) showed only a single instance in which it was not “regularly detected” (detection frequency = 0 %). ESA = ethane sulfonic acid; OA = oxanilic acid. Fungicide = F, insecticide = I, herbicide = H, metabolite = META.

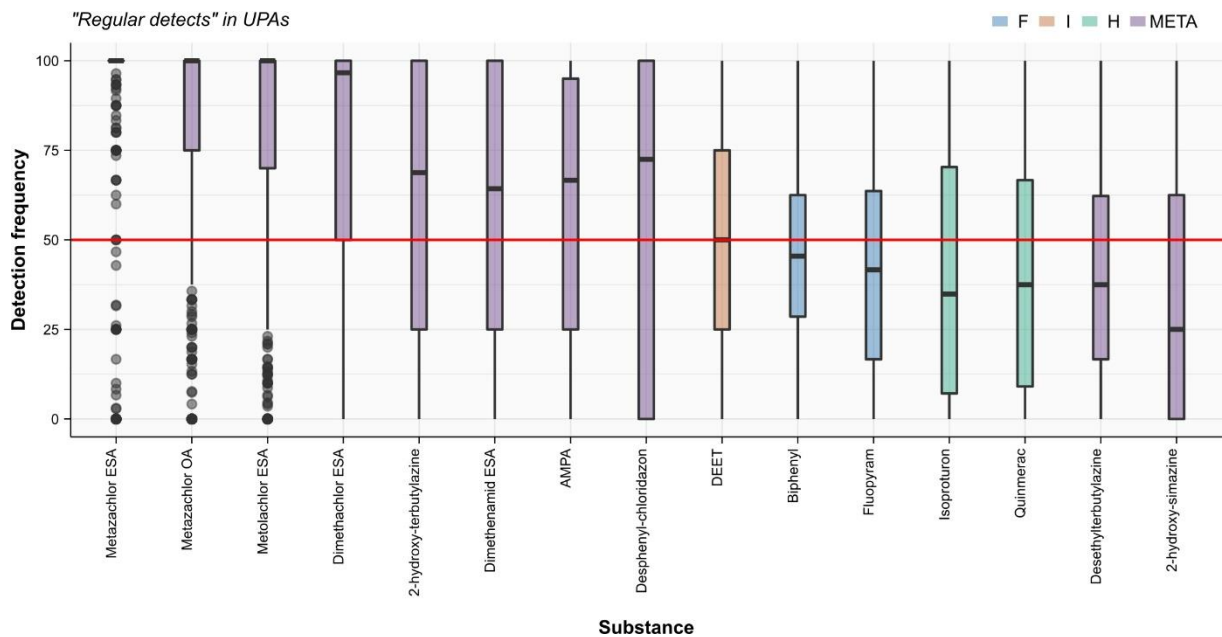


Figure S5: “Regular detect” distributions of the 15 most commonly detected substances in UPA ordered by their frequency of “regular detect” classification. A single point represents a substance’s detection frequency per monitoring site (≥ 4 distinct measurements) and a detection frequency $\geq 50\%$ assumes regular detection. ESA = ethane sulfonic acid; OA = oxanilic acid. Fungicide = F, insecticide = I, herbicide = H, metabolite = META.

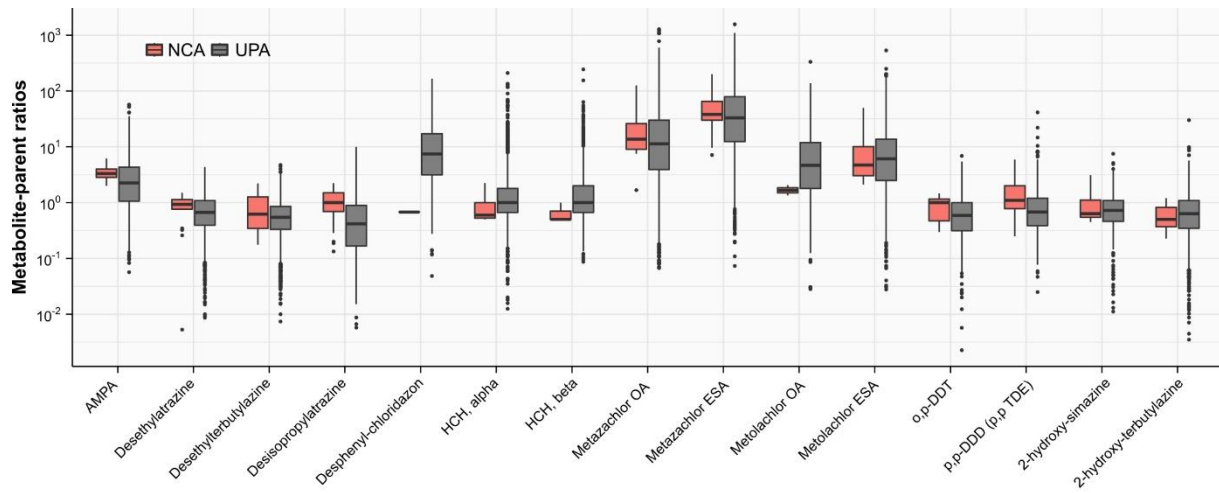


Figure S6: Comparison of metabolite-parent ratios between NCA and UPA for the 15 most data-abundant pairs. ESA = ethane sulfonic acid; OA = oxanilic acid.

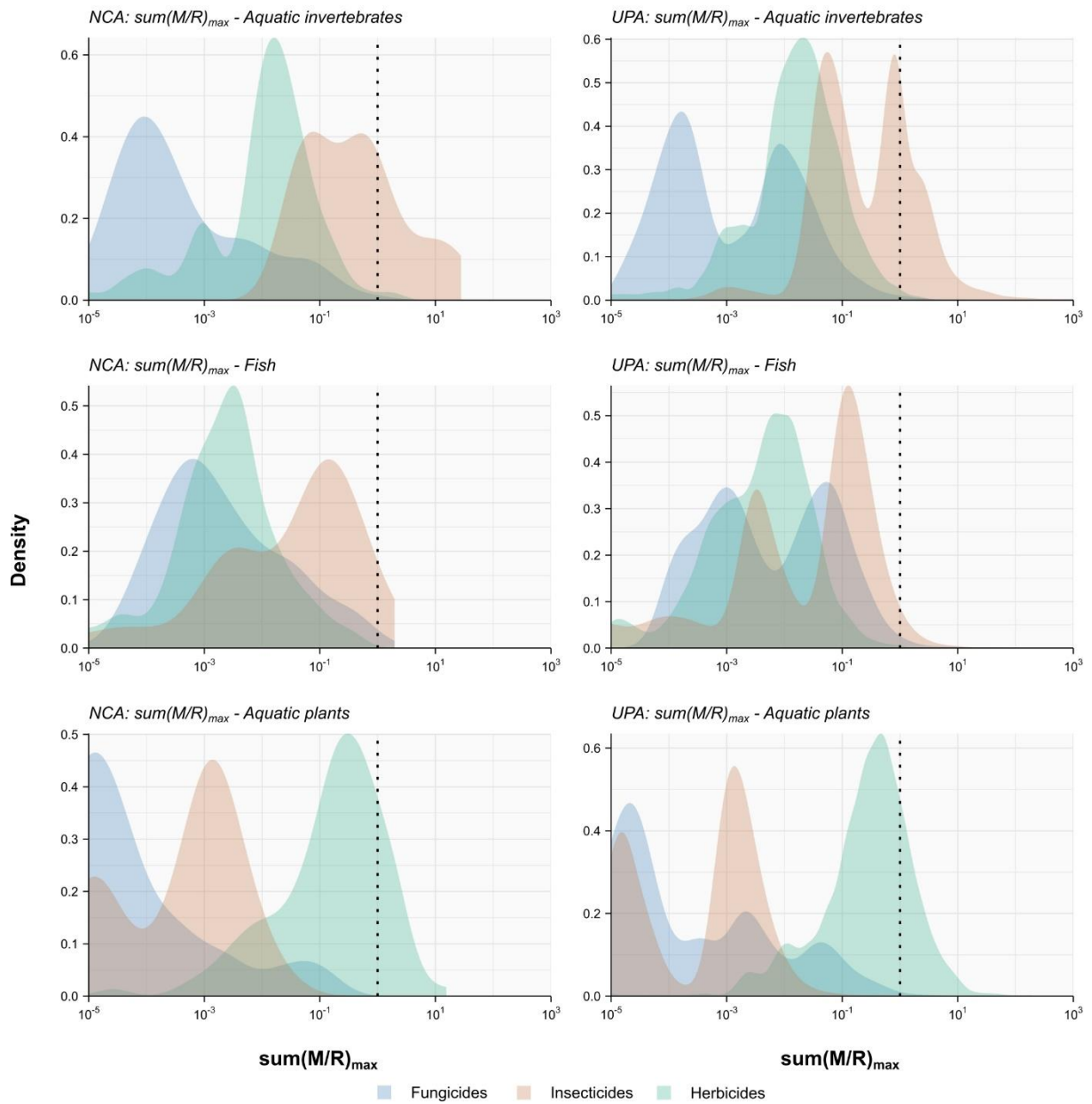


Figure S7: Density distributions of $\text{sum}(M/R)$ and $\text{sum}(M/R)_{\max}$ for the three primary pesticides classes (fungicides, insecticides, herbicides) and with respect to the three species groups (aquatic invertebrates, fish, aquatic plants) for NCA (left) and UPA (right). The $\text{sum}(M/R)$ or $\text{sum}(M/R)_{\max}$ threshold of 1 is depicted with a vertical, dotted line.

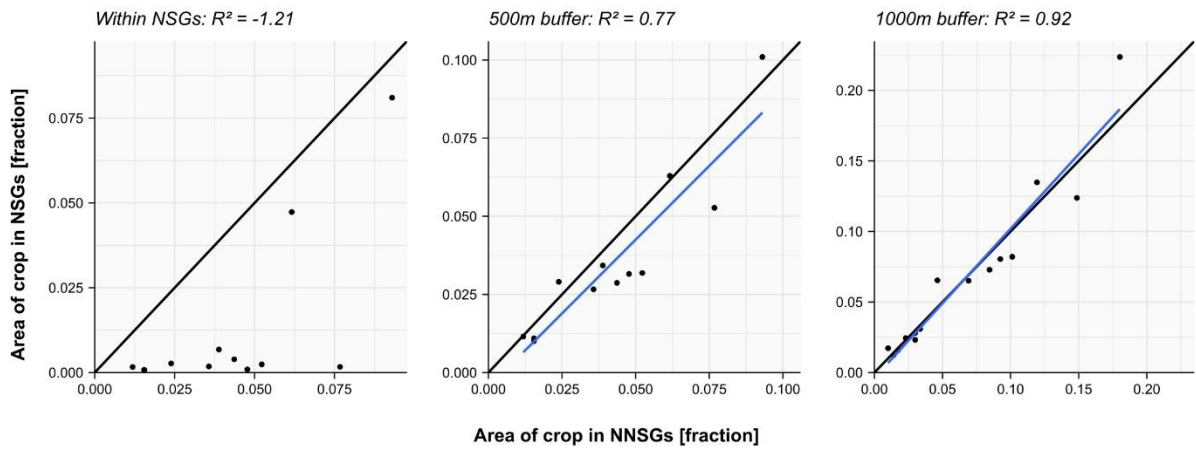


Figure S8: Comparison of the relative composition of agricultural crops ($n = 15$) between total Saxony (x-axis) and areas in or surrounding NCA (y-axis) for (a) the area within NCA, (b) the 500m directly surrounding NCA and (c) the 1000m directly surrounding NCA. The closer dots lay to the 1:1-diagonal, the more similar a specific crop-composition is between both systems (Saxony, NCA) as expressed by the R^2 in the title, which is based on a 1:1 diagonal line regression. Within NCA (left), no correlation exists as indicated by -1.21. The blue line depicts a linear regression with variable slope and intercept parameters.

1.6 SI Tables

Table S1: Corine Land Cover 2012 (CLC) categories originally found in the data with their respective CLC IDs. Simplified categories denote a condensed form of the original 44 CLC land use categories into 5 categories.

ID	CLC category name	Simplified category
1	Continuous urban fabric	urban
2	Discontinuous urban fabric	urban
3	Industrial or commercial units	industrial
4	Road and rail networks and associated land	industrial
5	Port areas	industrial
6	Airports	industrial
7	Mineral extraction sites	industrial
8	Dump sites	industrial
9	Construction sites	industrial
10	Green urban areas	urban
11	Sport and leisure facilities	urban
12	Non-irrigated arable land	agriculture
13	Permanently irrigated land	agriculture
14	Rice fields	agriculture
15	Vineyards	agriculture
16	Fruit trees and berry plantations	agriculture
17	Olive groves	agriculture
18	Pastures	agriculture
19	Annual crops associated with permanent crops	agriculture
20	Complex cultivation patterns	agriculture
21	Land principally occupied by agriculture ...	agriculture
22	Agro-forestry areas	forest
23	Broad-leaved forest	forest
24	Coniferous forest	forest
25	Mixed forest	forest
26	Natural grasslands	natural
27	Moors and heathland	natural
28	Sclerophyllous vegetation	natural
29	Transitional woodland-shrub	natural
30	Beaches dunes sands	natural
31	Bare rocks	natural
32	Sparsely vegetated areas	natural
33	Burnt areas	natural
34	Glaciers and perpetual snow	natural
35	Inland marshes	natural
36	Peat bogs	natural
37	Salt marshes	natural
38	Salines	natural
39	Intertidal flats	natural
40	Water courses	natural
41	Water bodies	natural
42	Coastal lagoons	natural

43	Estuaries	natural
44	Sea and ocean	natural
45	NODATA	no_data

Table S2: List of pesticides measured by the Saxon monitoring program attributed with their CAS-RN, RTL for aquatic invertebrates (RTL_{inv}), RTL for fish (RTL_{fish}), RTL for aquatic plants (RTL_{aplant}), pesticide type (PPP = plant protection product, biocide) and pesticide class (H = herbicide, I = insecticide, F = fungicide, META = metabolite).

Substance	CAS-RN	RTL _{inv}	RTL _{fish}	RTL _{aplant}	Pesticide type	Pesticide class
1,3-Dichlor-2-propyl-2,3-dichlor-1-propylether	59440-90-3	-	-	-	PPP	META
2,4,5-T	93-76-5	-	-	-	PPP	H
2,4-D	94-75-7	1342	1000	270	PPP	H
2,4-DB	94-82-6	-	-	-	PPP	H
2,6-Dichlorobenzamid	2008-58-4	-	-	-	PPP	META
2-Methyl-4,6-Dinitrophenol	534-52-1	-	-	-	PPP	I
3,4-Dichloranilin	95-76-1	-	-	-	PPP	META
3-Hydroxy Carbofuran	16655-82-6	-	-	-	PPP	META
Acetamiprid	135410-20-7	498	1000	-	PPP	I
Acetochlor	34256-82-1	-	-	0.059	PPP	H
Acetochlorsäure	184992-44-4	-	-	-	PPP	META
Acetochlorsulfonsäure	187022-11-3	-	-	-	PPP	META
Aclonifen	74070-46-5	9.52	6.7	0.67	PPP	H
Alachlor	15972-60-8	100	18	96.6	PPP	H
Alachlorsulfonsäure	142363-53-9	-	-	-	PPP	META
Aldrin	309-00-2	-	-	-	PPP	I
Ametryn	834-12-8	-	-	-	PPP	H
AMPA	1066-51-9	-	-	-	PPP	META
Atrazin	1912-24-9	10	45	1.1	PPP	H
Atrazin, 2-Hydroxy	2163-68-0	-	-	-	PPP	META
Avermectin B1a	65195-55-3	-	-	-	PPP	I
Azinphos-ethyl	2642-71-9	-	-	-	PPP	I
Azinphos-methyl	86-50-0	0.0022	-	-	PPP	I
Azoxystrobin	131860-33-8	1.3	4.7	9.8	PPP	F
Benalaxyl	71626-11-4	5.9	37.5	240	PPP	F
Bensulfuron-methyl	83055-99-6	-	-	-	PPP	H
Bentazon	25057-89-0	1325	-	490	PPP	H
Bifenox	42576-02-3	6.6	-	0.0175	PPP	H
Bifenthrin	82657-04-3	0.0011	0.001	-	PPP	I
Biphenyl	92-52-4	17	-	130	PPP	F
Bis(1,3-dichlor-2-propyl)ether	59440-89-0	-	-	-	PPP	META
Bis(2,3-dichlor-1-propyl)ether	7774-68-7	-	-	-	PPP	META
Bixafen	581809-46-3	-	0.95	9.7	PPP	F
Boscalid	188425-85-6	53.3	27	375	PPP	F
Bromacil	314-40-9	-	-	-	PPP	H
Bromocyclen	1715-40-8	-	-	-	PPP	I
Bromoxynil	1689-84-5	125	230	11.8	PPP	H
Carbendazim	10605-21-7	1.5	0.19	770	PPP	F
Carbofuran	1563-66-2	0.094	1.8	650	PPP	I
Chlordan	57-74-9	-	-	-	PPP	I
Chlorfenvinphos	470-90-6	-	-	-	PPP	I

Chloridazon	1698-60-8	1320	413	60	PPP	H
Chloroxuron	1982-47-4	-	-	-	PPP	H
Chlorpyrifos-ethyl	2921-88-2	0.001	0.25	53	PPP	I
Chlortoluron	15545-48-9	670	77	2.4	PPP	H
Clomazon	81777-89-1	5.7	155	3400	PPP	H
Clothianidin	210880-92-5	0.21472	-	5500	PPP	I
Cyanazin	21725-46-2	-	-	-	PPP	H
Cyazofamid	120116-88-3	-	5.6	2.7	PPP	F
Cypermethryn	52315-07-8	0.000053	0.0283	-	PPP	I
Cyprodinil	121552-61-2	0.33	13.5	122	PPP	F
DEET	134-62-3	-	-	-	PPP	I
Deltamethrin	52918-63-5	0.0000017	-	-	PPP	I
Demeton (O+S)	8065-48-3	-	-	-	PPP	I
Demeton-S-methyl	919-86-8	-	-	-	PPP	I
Demeton-S-methylsulfon	17040-19-6	-	-	-	PPP	I
Desethylatrazin	6190-65-4	-	-	-	PPP	META
Desethylterbutylazin	30125-63-4	-	-	-	PPP	META
Desethylterbutylazin-2-Hydroxy	66753-06-8	-	-	-	PPP	META
Desisopropylatrazin	1007-28-9	-	-	-	PPP	META
Desmetryn	1014-69-3	-	-	-	PPP	H
Desphenyl-Chloridazon	6339-19-1	-	-	-	PPP	META
Diazinon	333-41-5	-	2.7	640	PPP	I
Dichlorprop	120-36-5	-	-	18000	PPP	H
Dichlorvos	62-73-7	0.19	5.5	5280	PPP	I
Dicofol	115-32-2	-	-	7.5	PPP	I
Dieldrin	60-57-1	-	-	-	PPP	I
Diflufenican	83164-33-4	-	0.985	0.025	PPP	H
Dimethachlor	50563-36-5	85.4	39	0.217	PPP	H
Dimethachlorsäure	1086384-49-7	-	-	-	PPP	META
Dimethachlorsulfonsäure	1231710-75-0	-	-	-	PPP	META
Dimethenamid	87674-68-8	120	26	6.2	PPP	H
Dimethenamidsulfonsäure	205939-58-8	-	-	-	PPP	META
Dimethoat	60-51-5	20	302	9040	PPP	I
Dimethomorph	110488-70-5	-	34	100	PPP	F
Dimoxystrobin	149961-52-4	0.394	0.434	1.7	PPP	F
Disulfoton	298-04-4	-	-	-	PPP	I
Diuron	330-54-1	11	67	0.1	PPP	H
Endosulfan, alpha	959-98-8	-	-	-	PPP	I
Endosulfan, beta	33213-65-9	-	-	-	PPP	I
Endrin	72-20-8	-	-	-	PPP	I
Epoxiconazol	133855-98-8	86.9	31.4	0.43	PPP	F
Esfenvalerat	66230-04-4	0.27	0.001	0.65	PPP	I
Ethofenprox	80844-07-1	0.012	0.027	-	PPP	I
Ethofumesat	26225-79-6	135.2	109.2	390	PPP	H
Etrimfos	38260-54-7	-	-	-	PPP	I
Fenhexamid	126833-17-8	1050	13.4	-	PPP	F
Fenitrothion	122-14-5	-	-	-	PPP	I

Fenoprop	93-72-1	-	-	-	PPP	H
Fenpropidin	67306-00-7	-	19	0.57	PPP	F
Fenpropimorph	67564-91-4	22.4	23	32.7	PPP	F
Fenthion	55-38-9	-	-	-	PPP	I
Fenuron	101-42-8	5020	2040	-	PPP	H
Fluazifop-P-butyl	79241-46-6	5.3	13.1	51	PPP	H
Flufenacet	142459-58-3	309	21.3	0.2	PPP	H
Fluopicolide	239110-15-7	-	3.6	2.9	PPP	F
Fluopyram	658066-35-4	-	-	232	PPP	F
Fluoxastrobin	361377-29-9	0.604	4.35	35	PPP	F
Fluquinconazole	136426-54-5	-	13.4	1.4	PPP	F
Flurtamone	96525-23-4	130	66.4	1.41	PPP	H
Flusilazol	85509-19-9	-	12	640	PPP	F
Flutriafol	76674-21-0	670	330	65	PPP	F
Fluxapyroxad	907204-31-3	11	2.9	40	PPP	F
Glufosinat	51276-47-2	-	-	147	PPP	H
Glyphosate	1071-83-6	400	380	444	PPP	H
HCH, alpha	319-84-6	-	-	-	PPP	META
HCH, beta	319-85-7	-	-	-	PPP	META
HCH, delta	319-86-8	-	-	-	PPP	META
HCH, epsilon	6108-10-7	-	-	-	PPP	META
HCH, gamma (Lindan)	58-89-9	0.063	0.029	2.7	PPP	I
Heptachlor	76-44-8	-	-	2.7	PPP	I
Heptachlorepoxyd	1024-57-3	-	-	-	PPP	META
Heptenophos	23560-59-0	-	-	-	PPP	I
Hexazinon	51235-04-2	-	-	1.45	PPP	H
Imidacloprid	138261-41-3	0.341	2110	1000	PPP	I
Ioxynil	1689-83-4	-	-	2.7	PPP	H
Irgarol	28159-98-0	24	8.6	0.23	Biocide	AL
Irgarol M1	30125-65-6	-	-	-	Biocide	META
Isodrin	465-73-6	-	-	-	PPP	I
Isoproturon	34123-59-6	5.8	180	1.3	PPP	H
Isopyrazam	881685-58-1	-	0.258	-	PPP	F
Isoxaben	82558-50-7	-	-	1.1	PPP	H
Kresoxim-methyl	143390-89-0	1.86	1.9	6.3	PPP	F
lambda-Cyhalothrin	91465-08-6	0.000019	0.00078	0.5	PPP	I
Lenacil	2164-08-1	-	-	0.77	PPP	H
Linuron	330-55-2	58.1	67	2.25	PPP	H
Malathion	121-75-5	0.0072	-	410	PPP	I
MCPA	94-74-6	-	-	15.2	PPP	H
MCPB	94-81-5	-	-	-	PPP	H
Mecoprop	93-65-2	-	2400	4020	PPP	H
Metalaxyl	57837-19-1	-	9.6	42	PPP	F
Metaldehyd	108-62-3	-	750	7590	PPP	M
Metamitron	41394-05-2	57	-	38	PPP	H
Metazachlor	67129-08-2	330	85	0.23	PPP	H
Metazachlorsäure	1231244-60-2	-	-	-	PPP	META

Metazachlorsulfonsäure	17290-62-2	-	-	-	PPP	META
Metconazol	125116-23-6	-	21	170	PPP	F
Methabenzthiazuron	18691-97-9	-	-	3.3	PPP	H
Methiocarb	2032-65-7	0.077	6.5	82	PPP	I
Methobromuron	3060-89-7	-	-	-	PPP	H
Methoxychlor	72-43-5	-	-	-	PPP	I
Methyldesphenyl- Chloridazon	17254-80-7	-	-	-	PPP	META
Metolachlorsäure	152019-73-3	-	-	-	PPP	META
Metolachlorsulfonsäure	171118-09-5	-	-	-	PPP	META
Metoxuron	19937-59-8	-	-	-	PPP	H
Metribuzin	21087-64-9	490	746	0.79	PPP	H
Mevinphos	7786-34-7	-	-	-	PPP	I
Mirex	2385-85-5	-	-	-	PPP	I
Monolinuron	1746-81-2	-	-	240	PPP	H
N,N-Dimethylsulfamid	3984-14-3	-	-	-	PPP	META
Napropamid	15299-99-7	54	66	23.7	PPP	H
Nicosulfuron	111991-09-4	900	657	0.17	PPP	H
o,p-DDD (o,p TDE)	53-19-0	-	-	-	PPP	META
o,p-DDE	3424-82-6	-	-	-	PPP	META
o,p-DDT	789-02-6	-	-	-	PPP	META
Omethoat	1113-02-6	-	-	-	PPP	I
Oxadiazon	19666-30-9	-	-	-	PPP	H
Oxadixyl	77732-09-3	-	-	-	PPP	F
p,p-DDD (p,p TDE)	72-54-8	-	-	-	PPP	META
p,p-DDE	72-55-9	-	-	-	PPP	META
p,p-DDT	50-29-3	-	-	-	PPP	I
Parathion-ethyl	56-38-2	0.0011	0.17766	-	PPP	I
Parathion-methyl	298-00-0	0.00126	-	-	PPP	I
Penconazol	66246-88-6	-	-	9.6	PPP	F
Pencycuron	66063-05-6	-	-	-	PPP	F
Pendimethalin	40487-42-1	1.47	1.96	0.38	PPP	H
Pentachlorphenol	87-86-5	-	-	8	PPP	I
Pethoxamid	106700-29-2	32.8	21.9	0.4	PPP	H
Phoxim	14816-18-3	-	-	-	PPP	I
Picolinafen	137641-05-5	-	-	0.018	PPP	H
Picoxystrobin	117428-22-5	0.057	0.5	23	PPP	F
Pirimicarb	23103-98-2	0.17	790	14000	PPP	I
Prochloraz	67747-09-5	7.7	12	0.55	PPP	F
Prometryn	7287-19-6	14	55	0.2	PPP	H
Propamocarb	24579-73-5	-	968	30100	PPP	F
Propazin	139-40-2	-	-	88	PPP	H
Propiconazol	60207-90-1	102	26	900	PPP	F
Propoxur	114-26-1	0.10868	62	-	PPP	I
Propyzamid	23950-58-5	39	-	2.1	PPP	H
Prosulfocarb	52888-80-9	5.1	8.4	4.9	PPP	H
Pyraclostrobin	175013-18-0	-	0.06	172	PPP	F
Pyrimethanil	53112-28-0	29	105.6	120	PPP	F

Quinmerac	90717-03-6	-	868	9600	PPP	H
Quinoxifen (5,7-dichloro-4-(p-fluorophenoxy)quinoline)	124495-18-7	-	-	-	PPP	F
Sebutylazin	7286-69-3	-	-	-	PPP	H
Simazin	122-34-9	11	900	4	PPP	H
Simazin, 2-Hydroxy	2599-11-3	-	-	-	PPP	META
S-Metolachlor	87392-12-9	14	12.3	1.7	PPP	H
Spiroxamin	118134-30-8	-	71.3	0.13	PPP	F
Sulcotrion	99105-77-8	-	2270	5.1	PPP	H
Tebuconazol	107534-96-3	4.6	44	14.4	PPP	F
Terbutryn	886-50-0	-	-	0.24	PPP	H
Terbutylazin	5915-41-3	1.67	22	1.2	PPP	H
Terbutylazin, 2-Hydroxy	66753-07-9	-	-	-	PPP	META
Thiacloprid	111988-49-9	0.0912	197	9670	PPP	I
Thiamethoxam	153719-23-4	0.3409	-	-	PPP	I
Thifensulfuron-methyl	79277-27-3	-	-	-	PPP	H
Tolclofos-methyl	57018-04-9	0.89	6.9	78	PPP	F
Tolyfluanid	731-27-1	1.9	0.16	-	PPP	F
trans-Chlordan	5103-74-2	-	-	-	PPP	I
Triadimenol	55219-65-3	510	174	960	PPP	F
Triallat	2303-17-5	0.91	9.5	1.3	PPP	H
Tribenuron	106040-48-6	-	-	-	PPP	H
Trichlorfon	52-68-6	0.0001	-	-	PPP	I
Triclosan-methyl	4640-01-1	-	-	-	Biocide	META
Trifloxystrobin	141517-21-7	-	0.15	1.74	PPP	F
Trifluralin	1582-09-8	2.45	0.88	1.22	PPP	H
Tritosulfuron	142469-14-5	-	-	2.6	PPP	H

Table S3: Information about NCA, their respective surface water monitoring stations (MKZ), their size (area in km²), percent land uses (grassland, small woody features, agriculture and others) based on data from Blickensdörfer⁸ and the date of the latest changes to the nature conservation area regulation (RVO date).

NCA	MKZ	Area in km ²	Grassland	Small woody features	Agriculture	Other	RVO date
C 02	OBF32705	1.54565785	14	19.1	0.82	66.08	25.11.1997
C 102	OBF32750	2.58547474	21.61	11.07	0.45	66.86	20.03.2015
C 102	OBF32751	2.58547474	21.61	11.07	0.45	66.86	20.03.2015
C 103	OBF44800	0.83720499	20.15	37.32	4.82	37.7	29.06.2015
C 13	OBF38205	4.16416641	0	0.03	0	99.97	06.03.2003
C 30	OBF41710	1.07991369	0	0	0	100	30.03.1961
C 35	OBF51401	1.11197679	0.15	0.38	0	99.47	08.08.1938
C 48	OBF40631	6.12129397	0.67	0.47	0.01	98.86	27.02.2008
C 60	OBF33713	5.38587216	1.52	5.26	4.09	89.14	22.02.1995
C 72	OBF40675	3.14139593	0	0.47	0	99.53	15.02.2010
C 90	OBF49501	2.61343026	44.77	27.41	0.63	27.2	13.06.2007
C 90	OBF49502	2.61343026	44.77	27.41	0.63	27.2	13.06.2007
C 90	OBF49511	2.61343026	44.77	27.41	0.63	27.2	13.06.2007
C 93	OBF32206	2.84263089	14.6	28.02	7.5	49.87	19.12.2000
D 04	OBF30940	3.60729884	6.44	1.11	0.09	92.36	24.02.2020
D 103	OBF30200	2.83279029	3.13	3.61	0.07	93.19	18.11.2003
D 103	OBF30205	2.83279029	3.13	3.61	0.07	93.19	18.11.2003
D 115	OBF30955	0.21635948	0.42	2.12	0	97.46	16.11.2020
D 13	OBF24803	20.1253894	8.01	2.85	4.06	85.08	17.02.2011
D 13	OBF25708	20.1253894	8.01	2.85	4.06	85.08	17.02.2011
D 13	OBF25709	20.1253894	8.01	2.85	4.06	85.08	17.02.2011
D 13	OBF25711	20.1253894	8.01	2.85	4.06	85.08	17.02.2011
D 33	OBF29450	0.59121882	2.8	9.76	0.14	87.3	30.03.1961
D 50	OBF04700	5.14027873	26.49	6.9	0.5	66.11	16.12.2011
D 50	OBF05400	5.14027873	26.49	6.9	0.5	66.11	16.12.2011
D 78	OBF27701	17.0889522	7.88	2.72	1.2	88.19	07.03.1995
D 89	OBF28800	68.9122124	1.46	0.96	0.04	97.54	01.10.1996
D 89	OBF29003	68.9122124	1.46	0.96	0.04	97.54	01.10.1996
D 92	OBF05910	1.94027865	16.79	10.61	0.65	71.94	11.11.1997
D 93	OBF21900	131.445976	4.63	4.24	3.42	87.71	18.12.1997
D 93	OBF23804	131.445976	4.63	4.24	3.42	87.71	18.12.1997
D 93	OBF23808	131.445976	4.63	4.24	3.42	87.71	18.12.1997
D 93	OBF24389	131.445976	4.63	4.24	3.42	87.71	18.12.1997
L 44	OBF49332	41.0963099	2.01	3.5	0.76	93.72	06.03.2000
L 45	OBF55660	5.90753454	17.12	7.09	0.23	75.57	13.06.2000
L 53	OBF40451	2.50749393	0.23	3.7	2.39	93.68	23.05.1997
L 54	OBF16072	4.71807321	55.58	7.22	17.72	19.48	30.10.1997
L 59	OBF47500	14.6344248	45.34	17.28	6.19	31.19	20.12.2001

Table S4: Overview statistics of the metabolite-parent ratio for the 31 available pairs. UPA and NCA denote the number of available metabolite-parent ratios as well as the p-value and effects size from the Mann-Whitney-*U* test.

CAS (Metabolite)	Metabolite	CAS (parent)	Parent	UPA	NCA	p-value	effect size
95-76-1	3,4-Dichloraniline	330-54-1	Diuron	7	0	NA	NA
1066-51-9	AMPA	1071-83-6	Glyphosate	805	4	0.328	1.557
187022-11-3	Acetochlor sulfonic acid	34256-82-1	Acetochlor	4	0	NA	NA
184992-44-4	Acetochlor acid	34256-82-1	Acetochlor	4	0	NA	NA
2163-68-0	2-hydroxy-atrazine	1912-24-9	Atrazine	236	0	NA	NA
6190-65-4	Desethylatrazine	1912-24-9	Atrazine	4236	22	0.135	1.053
30125-63-4	Desethylterbutylazine	5915-41-3	Terbutylazine	3041	32	0.203	1.215
66753-06-8	2-hydroxy-desethylterbutylazine	5915-41-3	Terbutylazine	309	2	0.9	1.106
1007-28-9	Desisopropylatrazine	1912-24-9	Atrazine	1170	25	< 0.001	2.344
6339-19-1	Desphenyl-chloridazon	1698-60-8	Chloridazon	330	1	0.128	0.1
1231710-75-0	Dimethachlor sulfonic acid	50563-36-5	Dimethachlor	216	0	NA	NA
1086384-49-7	Dimethachlor acid	50563-36-5	Dimethachlor	48	0	NA	NA
205939-58-8	Dimethenamid sulfonic acid	163515-14-8	Dimethenamid	601	4	0.113	0.418
319-84-6	HCH, alpha	58-89-9	Lindane	2006	7	0.121	0.639
319-85-7	HCH, beta	58-89-9	Lindane	1557	3	0.151	0.494
319-86-8	HCH, delta	58-89-9	Lindane	234	0	NA	NA
6108-10-7	HCH, epsilon	58-89-9	Lindane	98	0	NA	NA
1024-57-3	Heptachlorepoxyde	76-44-8	Heptachlor	17	0	NA	NA
30125-65-6	Irgarol M1	28159-98-0	Irgarol	80	0	NA	NA
17290-62-2	Metazachlor sulfonic acid	67129-08-2	Metazachlor	1615	9	0.785	1.217
1231244-60-2	Metazachlor acid	67129-08-2	Metazachlor	1353	7	0.562	1.388
17254-80-7	Methyl-desphenyl-chloridazon	1698-60-8	Chloridazon	193	0	NA	NA
171118-09-5	Metolachlor sulfonic acid	87392-12-9	Metolachlor	1338	12	0.925	1.129
152019-73-3	Metolachlor acid	87392-12-9	Metolachlor	510	2	0.197	0.363
2599-11-3	2-hydroxy-simazine	122-34-9	Simazine	479	9	0.787	1.265
66753-07-9	2-hydroxy-terbutylazin	5915-41-3	Terbutylazine	1936	16	0.389	0.925
53-19-0	o,p-DDD (o,p TDE)	50-29-3	p,p-DDT	251	10	0.754	1.107
3424-82-6	o,p-DDE	50-29-3	p,p-DDT	38	1	0.1	0
789-02-6	o,p-DDT	50-29-3	p,p-DDT	368	6	0.346	1.424
72-54-8	p,p-DDD (p,p TDE)	50-29-3	p,p-DDT	846	13	0.081	1.548
72-55-9	p,p-DDE	50-29-3	p,p-DDT	181	2	0.248	0.138

2 References

1. Wolfram, J.; Stehle, S.; Bub, S.; Petschick, L. L.; Schulz, R., Water quality and ecological risks in European surface waters–Monitoring improves while water quality decreases. *Environ. Int.* **2021**, *152*, 106479.
2. Landesamt für Umwelt, Landwirtschaft und Geologie,, Gewässergütedaten Sachsen. In <https://bit.ly/3KuOdpc>, 2022.
3. Kynetec, FarmTrak - Pesticide active ingredient tonnages applied in Saxon agriculture from 2004 - 2020. In 2021.
4. Schulz, R.; Bub, S.; Petschick, L. L.; Stehle, S.; Wolfram, J., Applied pesticide toxicity shifts toward plants and invertebrates, even in GM crops. *Science* **2021**, *372*, (6537), 81-84.
5. Wolfram, J.; Stehle, S.; Bub, S.; Petschick, L. L.; Schulz, R., Meta-Analysis of Insecticides in United States Surface Waters: Status and Future Implications. *Environ. Sci. Technol.* **2018**, *52*, (24), 14452-14460.
6. Lewis, K. A.; Tzilivakis, J.; Warner, D. J.; Green, A., An international database for pesticide risk assessments and management. *Human and Ecological Risk Assessment: An International Journal* **2016**, *22*, (4), 1050-1064.
7. Buah-Kwofie, A.; Humphries, M. S., The distribution of organochlorine pesticides in sediments from iSimangaliso Wetland Park: ecological risks and implications for conservation in a biodiversity hotspot. *Environ. Pollut.* **2017**, *229*, 715-723.
8. Blickensdörfer, L., Schwieder, Marcel, Pflugmacher, Dirk, Nendel, Claas, Erasmi, Stefan, & Hostert, Patrick National-scale crop type maps for Germany from combined time series of Sentinel-1, Sentinel-2 and Landsat 8 data (2017, 2018 and 2019). (accessed August 20th, 2021).