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Helmholtz Centre for Environmental Research - UFZ
Department of System-Ecotoxicology

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Assessing the exposure and risks by pesticides in German small streams to derive recommendations for protection measures – On the basis of the Kleingewässermonitoring

ASSESSING THE EXPOSURE AND RISKS BY PESTICIDES IN GERMAN SMALL STREAMS TO DERIVE RECOMMENDATIONS FOR PROTECTION MEASURES - ON THE BASIS OF THE KLEINGEWÄSSERMONITORING

Von der Fakultät für Mathematik, Informatik und Naturwissenschaften der RWTH Aachen University zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften

vorgelegt von

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1. 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. In: *Water Research.*

2. Vormeier, P.; Liebmann, L.; Link, M.; Schäfer, R.B.; Schneeweiss, A.; Schreiner, V.C.; Weisner, O.; Liess, M. (2023): Temporal scales of pesticide exposure and risks in German small streams. In: *Science of the Total Environment*.

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4. Weisner, O.; Frische, T.; Liebmann, L.; Reemtsma, T.; Roß-Nickoll, M.; Schäfer, R.B.; Schäffer, A.; Scholz-Starke, B.; Vormeier, P.; Knillmann, S.; Liess, M. (2021): Risk from pesticide mixtures - The gap between risk assessment and reality. In: *Science of the Total Environment.*

5. Weisner, O.; Arle, J.; Liebmann, L.; Link, M.; Schäfer, R.B.; Schneeweiss, A.; Schreiner, V.C.; Vormeier, P.; Liess, M. (2021): Three reasons why the Water Framework Directive (WFD) fails to identify pesticide risks. In: *Water Research*.

6. Vormeier, P.; Liebmann, L.; Weisner, O.; Liess, M. (2023): Width of vegetated buffer strips to protect aquatic life from pesticide effects. In: *Water Research.*

The annexes referred to in the publications are not part of this doctoral thesis. For references see Chapter 15.

Table of Contents

Abbreviations

AA-EQS	Annual Average – Environmental Quality Standard
AC_{field}	Field-based Acceptable Concentration
ASPT	Average Score Per Taxon
BMWP	Biological Monitoring Working Party
DGM25	Digital Elevation Model 25 x 25 meter resolution
EC_{50}	Effect Concentration 50%
EDS	Event-driven sample
EFSA	European Food Safety Authority
EQS	Environmental Quality Standard
EPT	Ephemeroptera, Plecoptera and Trichoptera
ERA	Environmental Risk Assessment
ERO	Ecological Recovery Option
ESC	Ecological Status Class
FOCUS	Forum for the Co-ordination of pesticide fate models and their Use
FOD	Frequency Of Detection
GAM	Generalized Additive Model
KgM	Kleingewässermonitoring
LC-HRMS/MS	Liquid Chromatography - High Resolution tandem Mass Spectrometry
LC_{50}	Lethal Concentration 50%
LOD	Limit Of Determination
LOQ	Limit Of Quantification
LRC	Longitudinal Runoff Corridor
MAC-EQS	Maximum Acceptable Concentration – Environmental Quality Standard
MCR	Maximum Cumulative Ratio
NAP	National Action Plan for sustainable use of plant protection products
PEC	Predicted Environmental Concentration
PIR	Pesticide Input Ratio

PPDB	Pesticide Property Data Base
PPP	Plant Protection Product
PS	Priority Substance
RAC	Regulatory Acceptable Concentration
RBSP	River Basin-Specific Pollutant
RQ	Risk Quotient
SI	Saprobic Index
$SPEAR_{pesticides}$	SPEcies At Risk Index
TU	Toxic Unit
TWI	Topographic Wetness Index
UBA	Umweltbundesamt
VBS	Vegetated Buffer Strip
VIF	Variation Inflation Factor
WFD	Water Framework Directive
WWTP	Wastewater Treatment Plant

Abstract

Pesticides are an important pillar of food production today. The use of pesticides has increased in recent years as the world's population has grown and food production has increased. The large-scale application of pesticides in nature leads to pesticides also reaching non-target areas. Here, they can cause lasting damage to existing ecosystems and natural communities. One important pathway is the flushing of pesticide residues from agricultural fields into adjacent streams. It has been observed that the surface runoff leads to high concentrations of pesticides in surface waters and alters the community there. This raises the question on how exactly the input takes place and how risk mitigation measures such as vegetated buffer strips (VBS) can optimally retain pesticides in the field. These questions, among others, were the objectives of the "Kleingewässermonitoring" (KgM), in which small streams in agricultural environments were sampled on a large-scale basis in 2018 and 2019 using rainfall-related and grab samples.

We evaluated the pesticide exposure at the KgM-stream sections based on the results of six publications. The difference between rainfall-related samples and dry weather samples was examined at over 100 stream sections over Germany (publication 1, 2 & 3). We assessed the period of highest pesticide exposure to aquatic invertebrates and algae/aquatic plants (hereafter algae), by analysing real spray series and connected these to the seasonal and short-term exposure patterns of the KgM-samples (publication 2). We connected the observed exposure to the invertebrate community and evaluated the current environmental risk assessment (ERA) which is used during the approval process of pesticides (publication 3). Possible additional effects from toxic pesticide mixtures were assessed by cumulating the risk in the KgM-samples to invertebrates and algae (publication 4). We identified flaws in the monitoring program of the Water Framework Directive (WFD) to assess pesticide risks (publication 5). Finally, we investigated the effects of VBS along the KgM-stream sections in retaining pesticides (publication 6).

We found that the strongest factor influencing in-stream pesticide exposure is their application. Seasonal and short-term exposure peaks depend on the time of pesticide application (publication 2). In rainfall-related samples, not only concentrations are generally higher by a factor of 10 (90% percentile) (publication 1 & 3), but they are particularly higher during the seasonal peak in May/June (publication 1 & 2). The peak exposures significantly influenced the composition of the invertebrate community (publication 3). As a result, the current ERA is not sufficient to protect aquatic communities, as there are widespread exceedances of thresholds which are derived insufficiently (publication 3). The simultaneous occurrence of pesticide exposure and the resulting exceedance of the regulatory thresholds increases the risk by a factor of 3.2 (publication 4). The monitoring program of the WFD currently lacks event-related sampling, relevant analyte spectrum and missing availability of regulatory thresholds (publication 5). The presence of VBS could drastically minimize the risk if they were extended to an average width of 18 meter and if inputs from dry ditches were also reduced (publication 6).

The results of this thesis show that the general targets of the ERA are not achieved, because during the regulation process many sources of errors are present and partly unprotective thresholds are given. Not only that the exposure of pesticides is too high, but the recommendations of the risk mitigation measures are also flawed. The data of this thesis shows for the first time, that real-world VBS in their current form cannot adequately contain the exposure. This thesis gives profound guidance on how to monitor pesticides in streams, improve regulations of pesticides and design risk mitigation measures to better contain the input of pesticides through surface runoff. This could significantly reduce the input of pesticides and improve the conditions for aquatic organisms. In this way, the good ecological status stipulated by the WFD could be achieved in the long term.

1 Introduction

1.1 Pesticide use fuels insect decline

Intensively farmed arable land currently occupies 50% of the German land area (Statistisches Bundesamt, 2017). A greater intensification of agricultural production and transformation of unimpacted areas is forecasted to ensure food security for the growing world population (Handford et al., 2015). This intensive agriculture is based on the use of pesticides (Carvalho, 2017). The approval of active substances has increased only marginally in recent decades, while in the last 10 years, the number of approved products has increased (see Figure 1) (BVL, 2020). While pesticide application amounts were more or less stagnant in Germany for the past 25 years, the pesticides used tended to have a higher acute toxic potential to aquatic invertebrates and pollinators (Schulz et al., 2021). The use of pesticides can lead to unpredictable long-term effects on humans and the environment (Cardinale et al., 2012; Stehle and Schulz, 2015). Pesticides are designed to be biologically active and aim to kill various pests. However, pesticides also reach non-target environments and affect all kind of non-target species. The large-scale use of pesticides thus causes widespread ecological damage (Altieri, 2001; Matson et al., 1997). This is on important reason why we face a severe biodiversity crisis on insects both in terrestrial and aquatic ecosystems today (Albert et al., 2021; Raven and Wagner, 2021). The ongoing insect decline threatens our food supply, as our agricultural system relies on pollination and other ecosystem services provided by insects and intact ecosystems (Díaz et al., 2006; van der Sluijs, 2020). In particular, freshwater ecosystems that serve as drinking resource, habitat and breeding area are important in resisting the advancing biodiversity crisis on insects (Biggs et al., 2017). It can be assumed, that the biodiversity crisis will continue to worsen under the future conditions of climate change (Heino et al., 2009). At the same time, the impact of climate change is expected to increase the spread of pests and the use of pesticides (Chen and McCarl, 2001; Koleva and Schneider, 2009). Therefore, it is of crucial importance to provide freshwater ecosystems and biodiversity with the best possible opportunities to adapt to climate change (Capon et al., 2013).

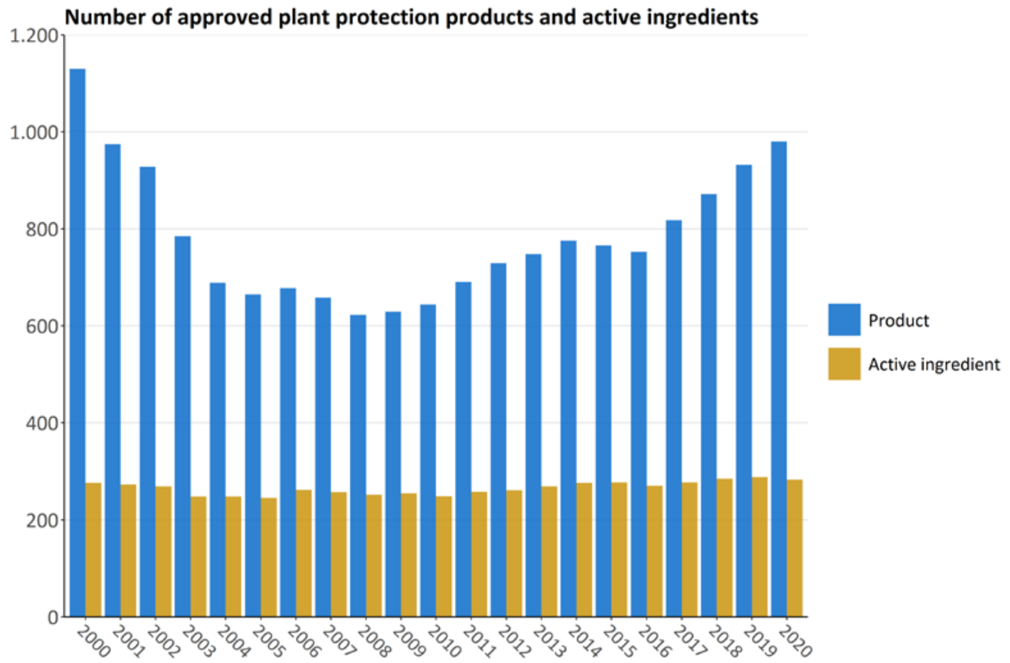

Figure 1: Number of plant protection product and active ingredient approvals from 2000 to 2020 (modified from BVL, 2020).

1.2 The state of surface waters

With the Water Framework Directive (WFD), the first Europe-wide directive on water protection was adopted by the European Parliament and the Council (European Union, 2000). Since 2000, the aim has been to achieve sustainable and environmentally compatible management of water resources. The WFD requires a good ecological and chemical status for surface waters and a good chemical and quantitative status for groundwater. Chemical and biological parameters are considered in the assessment of surface waters. For this purpose, thresholds are set for anthropogenic substances and certain requirements are placed on aquatic communities. Five status classes are distinguished, ranging from "very good" to "good", "moderate", "unsatisfactory" and "poor". The member states are responsible for implementing the WFD. In Germany, this was done by adapting the Surface Water Ordinance (OGewV) (BGBl, 2009).

The most recent status report of the 3rd management cycle of the WFD showed that almost 90% of German surface waters are not in a good ecological status (UBA, 2022a). Different stressors act on surface water bodies, while the agricultural sector has the biggest impact (see Figure 2). The monitoring of the WFD provides valuable data for the assessment of the ecological status of surface waters. However, this monitoring is limited to larger rivers and does not include small streams (catchment area < 10 km²) on a regular basis (Vehanen et al., 2020). Therefore, the status of small streams is not well assessed nor are the stressors that also affect these small water bodies (Rabiet et al., 2010; Stehle et al., 2013). Due to the missing assessment of the status of small streams, the impacts of the widespread use of pesticides on freshwaters in Germany is not comprehensively assessed (Lorenz et al., 2017). The poor ecological status in the larger surveyed water bodies could probably result from the poor status of smaller water bodies (Malaj et al., 2014). In order to better assess the relevance of small water bodies, their current status and the stressors affecting them must be systematically recorded.

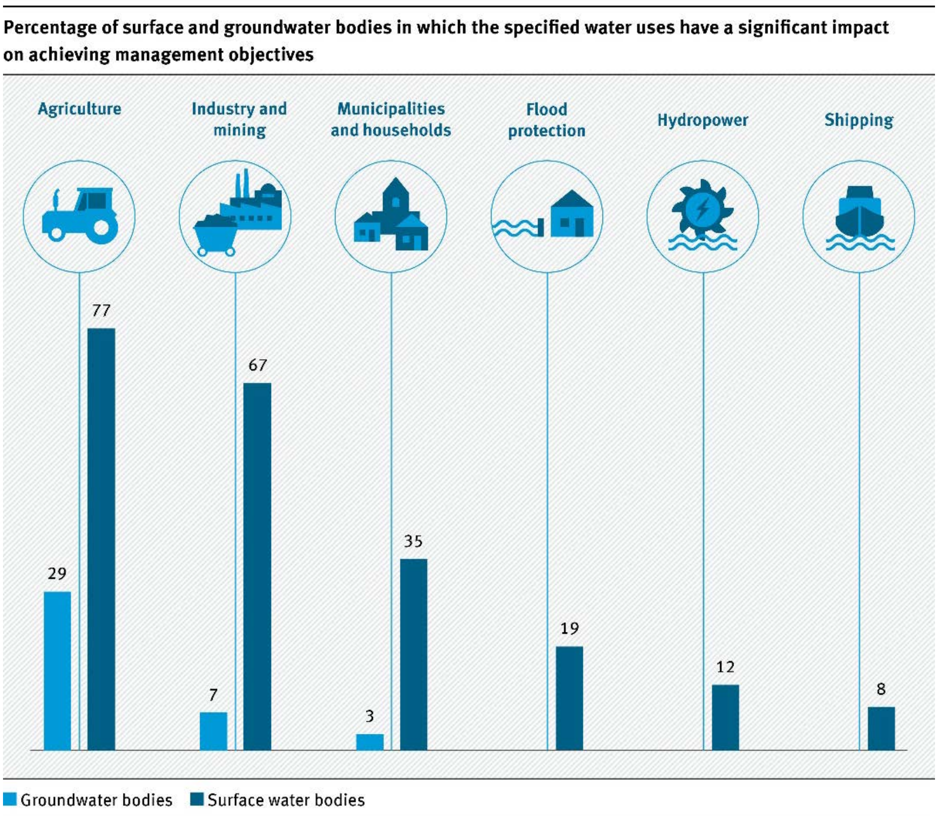

Figure 2: Impact of different stressors on surface and groundwater bodies in Germany from 2015 to 2021 (modified from UBA, 2022a).

1.3 The relevance of small streams and their exposure to pesticides

Small streams are hotspots for biodiversity in our landscapes (Biggs et al., 2017; Meyer et al., 2007). They not only serve as a refuge for breeding, but also provide many important ecosystem services that enables human and animal life (Ferreira et al., 2022). Although having a comparable short individual stream length compared to larger rivers, they contribute to about 70-80% to the overall stream network (Downing, 2012). With a comparably low discharge volume, small streams are in fact chemical fingerprints of their upper catchments and have only a low dilution capacity (Beketov et al., 2013; Kirchner et al., 2000; Wohl, 2017).

One major chemical stressor is diffuse input of pesticides from the agricultural sector (Liess et al., 1999). However, the assessment of this diffuse pesticide exposure to small streams faces certain problems (Lorenz et al., 2017). The pesticide exposure patterns in small streams heavily rely on weather conditions (Bundschuh et al., 2014). During dry weather background concentrations at comparably low water levels drive the pesticide exposure and are easy to measure by grab sampling (Sandin et al., 2018). However, short-term peak concentrations can occur during rainfall events, resulting from surface runoff from pesticide-treated fields. Applied to killing weeds, fungi and insect pests on agricultural fields, they can cause damage to aquatic organisms in water bodies (Liess and Schulz, 1999). This was already observed in previous studies, where the input of insecticides in small streams affected the invertebrate community (Liess and Ohe, 2005). By affecting the community structure, small streams are unable to perform their ecosystem services and this can have severe consequences for the whole ecosystem (Beketov et al., 2013; Schäfer et al., 2007). However, the influence of surface runoff is difficult to measure, as rainfall leads to very immediate water level rise and requires near real-time sampling (Jergentz et al., 2005). Automated sampling is needed to realistically capture this surface runoff influence (Lefrancq et al., 2017). This could also be done by using continuous sampling methods, but is even more elaborate than automated sampling methods.

Biological indicator systems are usually used to estimate impacts of certain stressors on stream communities. They assess how much the community is affected by a stressor (Liebmann et al., 2022). This is done by assessing the presence of known key species, as e.g. in the EPT indicator where Ephemera, Plecoptera and Trichoptera (EPT) larvae are counted, or by assessing the presence of certain traits. The most common used indicator to describe the impact of pesticides in streams is the $SPEAR_{pesticides}$ indicator (Liess and Ohe, 2005). Several recent studies were able to show the impacts of insecticides on the in-stream invertebrate community by using the $SPEAR_{pesticides}$ indicator (Hunt et al., 2017; Kuzmanović et al., 2016; Schäfer et al., 2011; Schäfer, 2019). The pesticide exposure and the resulting toxic effects to aquatic organisms can be described by the toxic unit approach (Sprague, 1969). However, the pressures on aquatic communities do not come from pesticides alone, but are linked to the occurrence of multiple stressors (Birk et al., 2020; Jackson et al., 2016; Ormerod et al., 2010). For example, inputs from urban sources can also occur in large quantities in small water bodies and potentially cause effects (Munz et al., 2017; Neale et al., 2020). A key to distinguish between the impacts of different stressors on the community is to assess these factors as holistically as possible (Rortais et al., 2017). However, the distinction from other stressors such as the influence of urban point sources, degraded hydromorphology, oxygen deficiency and other stressors has not yet been sufficiently quantified (Jackson et al., 2016).

1.4 The regulation of pesticides

1.4.1 Basics of pesticide risk assessment and pending issues

Pesticides are regulated on the basis of the environmental risk assessment (ERA). Before a pesticide is approved for application, their exposure in the environment and toxic effects are predicted (EFSA, 2013). To assess toxic effects of pesticides, usually effect concentrations (EC_{50} and LC_{50}) are derived from experimental laboratory or mesocosm studies. The effect concentration for the most sensitive organism tested is divided by an assessment factor to account for remaining uncertainties to derive a Regulatory Acceptable Concentration (RAC). As long as the in-stream concentration is below the RAC, there are no unacceptable effects on the aquatic community predicted to be caused by pesticides (European Commission, 2011). On the other hand, the environmental exposure after application is modeled based on exposure models such as FOCUS (FOCUS, 2001) or Exposit in Germany (UBA, 2017). The exposure model assumes one or multiple generic stream sections and includes the application quantity, physico-chemical properties, distance to streams, slope, crop type and effects of risk mitigation measures (Pereira et al., 2017). The result of the exposure modelling is the Predicted Environmental Concentration (PEC) and describes a concentration that enters agricultural field neighboring surface waters. In order for a pesticide to be approved for application, this PEC must not exceed the RAC, as this is expected to result in unacceptable effects on the aquatic community. If the PEC exceeds the RAC, the application quantity can be reduced or risk mitigation measure must be implemented to reach a PEC < RAC.

Conclusions for the ERA as to whether the exposure to a pesticide actually corresponds to the predicted exposure are drawn from the monitoring data collected. The measured concentrations are compared with RAC and environmental quality standards (EQS) retrospectively, EQS being the only legally binding thresholds (EFSA, 2010). The derivations of the two thresholds are based on the same methods (Pereira et al., 2017). However, for EQS there is a difference in the threshold exceedance assessment. When aggregating monitoring data for a year, EQS assessment distinguishes between annual average-EQS (AA-EQS), to account for long-term exposures, and maximum acceptable concentrations-EQS (MAC-EQS), to account for peak concentrations (Pereira et al., 2017). If these values are exceeded, reduction measures must be initiated at the water bodies. A discrepancy here is the comparison of exposure modelling of small water bodies with the comparison of concentrations and EQS of larger water bodies (Knauer, 2016). This results in a spatial offset of exposure and regulatory retrospective evaluation by the ERA.

Under ideal circumstances this ERA would result in a pesticide use which is in accordance with a good ecological status as no aquatic organisms are affected. However, recent studies demonstrated that FOCUS model predictions by the ERA tend to be not protective in predicting pesticide concentrations in small streams (Knäbel et al., 2012; Knäbel et al., 2014). Besides the inappropriate exposure models, there are other flaws in the current ERA (outdated assumptions, misrepresented dynamics of pesticide exposure, missing mixed exposure risk assessment) that could be responsible for the current poor ecological status in small streams (Topping et al., 2020). The flaws of the current ERA have been addressed in more detail in a previous related dissertation (Weisner, 2022).

1.4.2 Relevant risk mitigation measures

Risk mitigation measures are a requirement during the approval process of pesticides to reduce exposure in adjacent water bodies. Apart from the little-used mulch sowing method, the implementation of vegetated buffer strips (VBS) along the stream side is the most important risk mitigation measure to retain pesticides in surface runoff on the field (Rasmussen et al., 2011;

Reichenberger et al., 2007). As shown in Figure 3, the VBS acts as a filter strip through which the surface runoff flows and the solved pesticides contained in the sediment or surface runoff water are retained by the plants (Arora et al., 1996). There are two different definitions for VBS. While in ERA, buffer strips refer to the distance between the stream embankment edge and area where pesticides are applied on the agricultural field, we here refer to the distance between the stream embankment edge and the agricultural field due to practical limitations, as the additional buffer strip on the agricultural field from the ERA could not be determined.

Several studies have examined the positive effects of VBS on retaining pesticides in the field (Arora et al., 2003; Carluer et al., 2011; Cole et al., 2020; Lacas et al., 2005; Reichenberger et al., 2007; Zhang et al., 2010). A VBS with at least 5 m width on each side of a water body is required by law 38§ in the German Water Resources Act (with a few exceptions) (BGBl, 2009). However, studies have shown that a width of 5 m is not sufficient to protect aquatic organisms (Liu et al., 2008; Prosser et al., 2020). In addition, other factors such as the vegetation cover of the VBS and erosion rills on steep slopes can also impair the effectiveness of VBS (Bereswill et al., 2012; Dosskey et al., 2010; Lerch et al., 2017; Stehle et al., 2016). The influence of other factors in the catchment areas has also not yet been sufficiently investigated. Moreover, there has been no systematic recording of VBS width so far. Therefore, a comprehensive quantification of VBS, their effectiveness and correct usage of this risk mitigation measure is lacking.

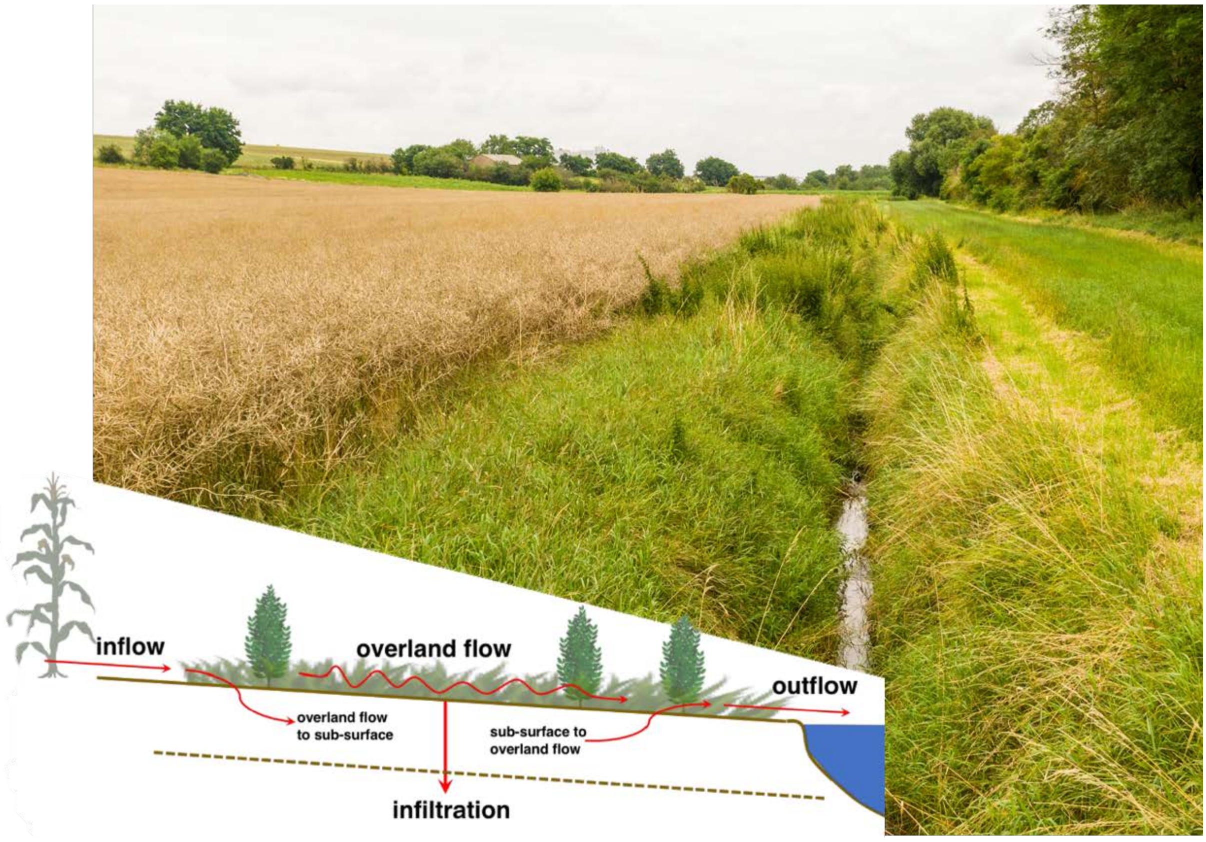

Figure 3: The pathways of pesticides and nutrients from treated agricultural fields through vegetated buffer strips into surface water bodies. Modified from Barfield et al. (1998) and Prosser et al. (2020). Photo by André Künzelmann.

1.5 Current monitoring of surface waters

The governmental monitoring of surface water bodies in Germany and the EU is mainly based on the WFD. It consists of two components: chemical monitoring to assess the occurrence of chemical substances and biological monitoring to determine the ecological status.

The chemical monitoring of surface waters consists typically of conducting grab samples once per month at a sampling site (European Union, 2000). Each sampling site is sampled at least once every three years (BGBl, 2016). Monthly sampling is carried out randomly and independently of the weather situation. The highly variable exposure patterns of pesticides (peak concentrations during rainfall events causing surface runoff), which depend on the weather situation (for more details see chapter 1.3), are not taken into account (Rabiet et al., 2010). The WFD relevant thresholds are Environmental Quality Standards (EQS). There is only a limited number of EQS for pesticides that are legally binding and, according to WFD criteria, lead to measures to improve the status of the water body if they are exceeded.

Biological monitoring includes the sampling of invertebrates, fish, diatoms and algae/aquatic plants. In the following we focus on invertebrates. Same as for the chemical monitoring, each site is sampled at least once every three years (BGBl, 2016). The invertebrates are sampled by kick sampling in subsections representative of the water body. This procedure is done according to the WFD (Meier et al., 2006). The invertebrates collected by the kick sampling are being determined at species level in the laboratory. The invertebrate community determined is assessed by indicator systems that allow for the estimation of the ecological status of the surface water (for more details see chapter 1.3). The various indicator systems provide information about the impairment by stressors and whether measures may have to be introduced at the water body to improve the ecological status.

Multiple studies demonstrated that the current monitoring by the WFD underestimates pesticide exposure in small streams (Bundschuh et al., 2014; Moschet et al., 2014; Ohe et al., 2011; Rabiet et al., 2010). On the one hand, small streams with smaller catchment size than 10 km² are not sampled on a regular basis (Lorenz et al., 2017; Szöcs et al., 2017), even though the pesticide burden to small streams is highest (see chapter 1.3) and on the other hand, no rainfall-related sampling is performed to capture short-term peak concentrations (Rabiet et al., 2010). Another underestimation is that the samples are not tested for sufficient pesticide active substances with corresponding thresholds (Moschet et al., 2014). So far, this underestimation of WFD monitoring of pesticide exposure has not been sufficiently investigated and only few concrete adaptation strategies have been identified.

1.6 Recent programs and activities

1.6.1 National Action Plan for sustainable use of plant protection products (NAP)

In October 2009, the European Parliament and Council adopted a law establishing a framework of action by the member states on the sustainable use of plant protection products (PPP) (Plant Protection Framework Directive) (European Parliament, 2009). Article 4 of this Directive 2009/128/EC stipulated that member states should develop a National Action Plan (NAP) for sustainable use of PPP. Here, quantitative targets, objectives, measures and timetables have been set to "reduce the risks and the impacts of PPP use on human health and the natural balance" (BMEL, 2013).

This was done in Germany on 10 April 2013 by the federal government, which adopted the NAP. In this context, the state of pollution and possible measures for small water bodies are now also given greater consideration. For the first time, not only the effects of PPP applications on small water bodies located directly in the agricultural landscape are to be investigated, but also current risk assessment is to be investigated and subsequent measures to reduce them.

In addition to the existing WFD (European Union, 2000), the Drinking Water Ordinance (European Union, 2020), the Groundwater Directive (European Union, 2006), the Directives on environmental quality standards (European Union, 2008) and national legislation (BGBl, 2009) is now being expanded to include the NAP, which aims to achieve sustainable use of plant protection products (Brinke et al., 2017). This is intended to counteract deterioration of the water bodies and enable improvement. One major goal of Germany’s NAP was to carry out a monitoring of small streams.

1.6.2 The “Kleingewässermonitoring” (KgM)

The monitoring of small streams (Kleingewässermonitoring - KgM) is commissioned by the German Environment Agency (UBA) to implement the goals of the NAP. This monitoring is being carried out in cooperation with the Institute of Environmental Sciences at the University of Koblenz-Landau (UKL) and the Helmholtz Centre for Environmental Research, Leipzig (UFZ). The aim of the project is to carry out a representative Germany-wide monitoring of the condition of small streams and to quantify their pesticide contamination with rainfall-related sampling.

The implementation of the NAP is divided into three sub-projects. The first two sub-projects have already been completed. The aim of the first sub-project was to set up an "Inventory to collect data on the pollution of small streams in the agricultural landscape". This was done to evaluate the condition of small water bodies through existing Germany-wide water body data. However, due to a lack of coherent data, no concrete statements could be made, especially with regard to the status of small streams (Brinke et al., 2017).

The second sub-project "Conception of a representative monitoring of the pollution of small streams in the agricultural landscape", focused on creating a concrete framework for a representative monitoring, which is to investigate the pollution of small streams in the agricultural landscape throughout Germany (Wick et al., 2019). In addition to the requirements for the sampling sites (catchment area < 30 km², agricultural share > 40 %, urban share < 5 %, good accessibility, no wastewater discharge from sewage treatment plants), a list of relevant pesticides for chemical analysis was established. In order to be able to measure not only the basic pollution of the water bodies with grab samples, but also rainfall-related inputs of pesticides, automatic rainfall-event-controlled sampling was also recommended (Wick et al., 2019). In addition to the chemical analysis, comprehensive monitoring of biological quality components, invertebrate and algal community and other abiotic parameters such as hydrological parameters, structural quality, nutrients and land use mapping should be carried out (Wick et al., 2019).

The present work was carried out as part of the third sub-project "Pilot study to determine the pollution of small streams in the agricultural landscape with pesticide residues". In order to implement the objectives of the NAP, this KgM-project was carried out based on the results of sub-project I (Brinke et al., 2017) and sub-project II (Wick et al., 2019). The monitoring is intended to provide findings that can be used to conduct future pesticide surveys on small streams on a regular basis. The field campaigns of sub-project III were conducted at over 140 streams from April to July in 2018 and 2019 (UBA, 2022b) (see figure 4). Grab- and automated event-driven sampling (EDS) during rainfall were conducted, which resulted in more than 1,000 chemical samples of the streams (Liess et al., 2021).

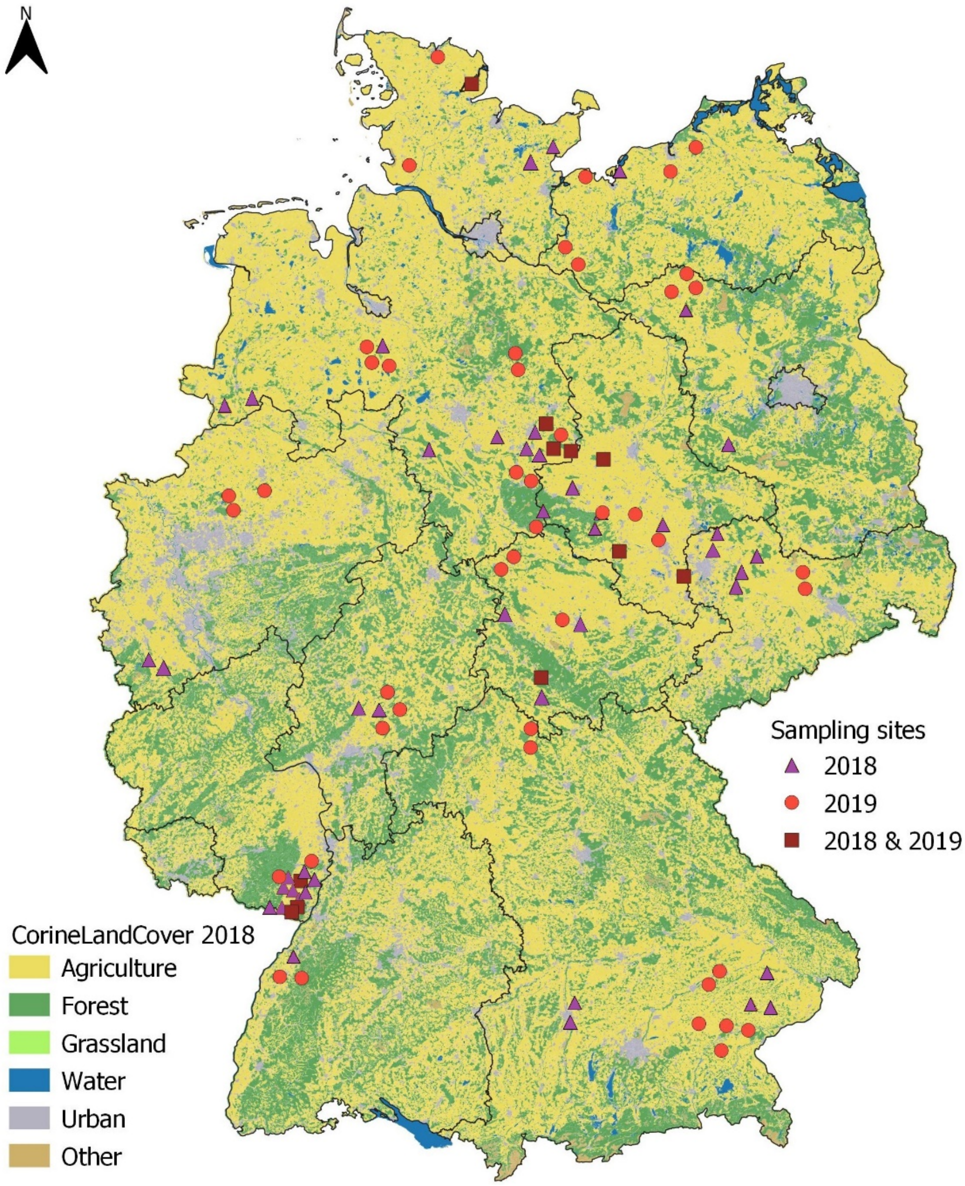

Figure 4: Map of the investigated sampling sites during the KgM-project in 2018 and 2019. Land use types were obtained from Copernicus Land Monitoring Service (2019).

1.7 Objectives of this thesis

The aim of this dissertation is to summarise the results of the KgM-project, to provide a more detailed description of current pesticide exposure, to make appropriate recommendations for the current ERA, and to evaluate current risk reduction measures such as VBS. The regulation of pesticides follows a cycle: the approval of pesticides, their application, their entry into water bodies and their monitoring. The results of the monitoring and observed effects are supposed to be used to draw conclusions for the approval process of the ERA. The associations of the publications used in this dissertation to the cycle are shown in the schematic figure 5. Since they are all based on monitoring in small streams, they are mentioned at the pesticide monitoring part combined. In the following, the specific objectives of each publication are outlined.

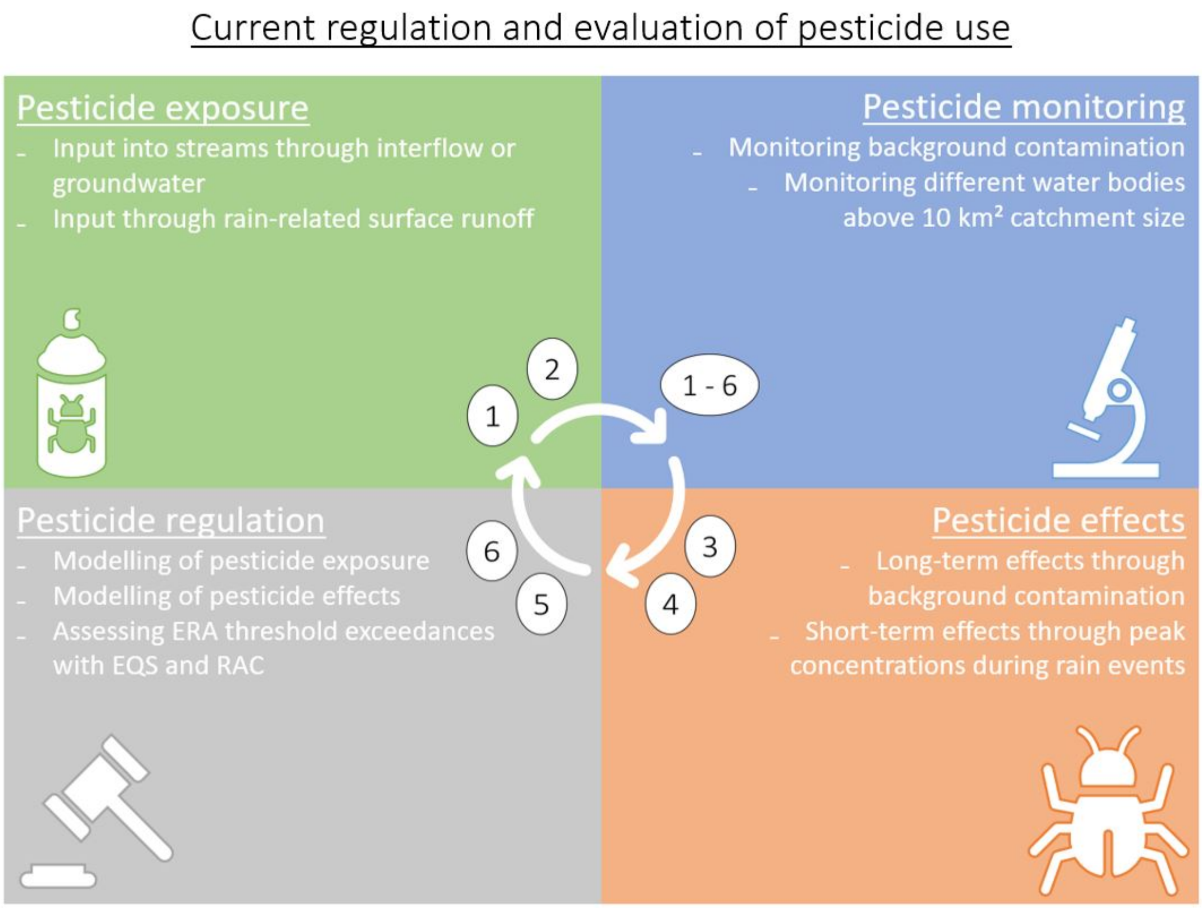

Figure 5: Schematic figure of the current cycle of pesticide use, effects, monitoring and regulation. The numbers imply the thematic affiliation of the different publications 1-6.

1 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. In: *Water Research.*

This publication answers the most obvious question of the KgM-project: whether rainfall events trigger surface runoff which lead to exposure peaks of pesticide concentrations. Further focus of the investigations is to what extend EDS concentrations exceed those of grab samples which are used in the official WFD monitoring. Besides this, this investigation connects the pesticide exposure patterns to the cultivated crop types in the catchments and analysis the exposure to relevant metabolites.

2 Vormeier, P.; Liebmann, L.; Link, M.; Schäfer, R.B.; Schneeweiss, A.; Schreiner, V.C.; Weisner, O.; Liess, M. (2023): Temporal scales of pesticide exposure and risks in German small streams. In: *Science of the Total Environment*.

As event-driven sampling is cost- and time intense this publication investigates the pesticide exposure in more detail to tailor event-related monitoring concepts. Especially the different temporal scales of short-term exposure peaks and monthly trends of exposure are examined. In order to draw conclusions about the origin of these entries, the results were compared with temporal trends of pesticide application data. However, the pesticide applications are based on a different data set independent of the KgM-data.

3 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. In: *Water Research.*

This publication unravels the effects of different stressors on the invertebrate community and to what extent RACs are exceeded. It also investigates which ecological endpoints are most suitable in describing the effects of pesticides on the invertebrate community. In addition, this study examines the extent to which the current risk assessment of pesticides underestimates actual exposure. Furthermore, a new protective field-based acceptable concentration (AC_{field}) threshold is derived on the basis of these findings.

4 Weisner, O.; Frische, T.; Liebmann, L.; Reemtsma, T.; Roß-Nickoll, M.; Schäfer, R.B.; Schäffer, A.; Scholz-Starke, B.; Vormeier, P.; Knillmann, S.; Liess, M. (2021): Risk from pesticide mixtures - The gap between risk assessment and reality. In: *Science of the Total Environment.*

This publication assesses the risks of pesticide mixtures in small streams. As pesticides are usually applied in mixtures by farmers, their exposure is versatile and usually different types occur at the same time. Since the previous ERA only evaluates the toxicity of individual substances, the question arises as to whether the simultaneous occurrence of pesticide mixtures could possibly lead to stronger effects that have gone unnoticed until now. Based on the risk-quotient approach (RQ), the effects of all pesticides in a sample are added up and RAC exceedances caused by the mixtures are identified.

5 Weisner, O.; Arle, J.; Liebmann, L.; Link, M.; Schäfer, R.B.; Schneeweiss, A.; Schreiner, V.C.; Vormeier, P.; Liess, M. (2021): Three reasons why the Water Framework Directive (WFD) fails to identify pesticide risks. In: *Water Research*.

One major goal of the KgM-project was to identify whether the current monitoring can assess the effects of pesticides on aquatic organisms. As most of the monitoring results from the WFD, this publication checks possible flaws in the WFD monitoring concept and how it could be improved to detect possible pesticide effects in the future.

6 Vormeier, P.; Liebmann, L.; Weisner, O.; Liess, M. (2023): Width of vegetated buffer strips to protect aquatic life from pesticide effects. In: *Water Research.*

Pesticide inputs to small streams are not only dependent on their application, but also on the possibility of retention by vegetated buffer strips (VBS). As these are used as a risk mitigation measure in the ERA, their effect must also be investigated in comparison to other environmental parameters. In this publication, the VBS were digitalised 3 km into the headwaters along all investigated stream sections in the KgM. The focus was to investigated the effectiveness of VBS, the influence of physico-chemical substance properties on the VBS retaining efficacy and how VBS should be designed to avoid thresholds exceedances such as the RAC or AC_{field}.

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2 Small streams - Large concentrations? Pesticide monitoring in small agricultural streams in Germany during dry weather and rainfall

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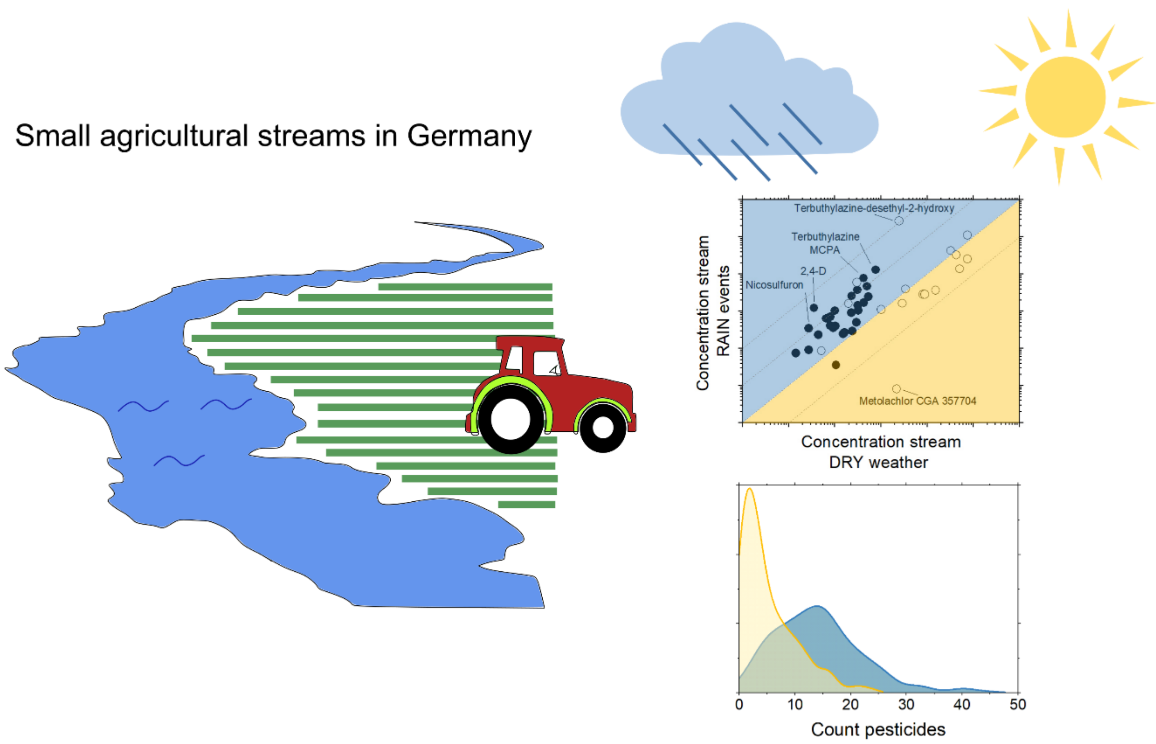
Abstract

Few studies have examined the exposure of small streams (< 30 km^2 catchment size) to agriculturally used pesticides, compared to large rivers. A total of 105 sites in 103 small agricultural streams were investigated for 76 pesticides (insecticides, herbicides, fungicides) and 32 pesticide metabolites in spring and summer over two years (2018 and 2019) during dry weather and rainfall using event-driven sampling. The median total concentration of the 76 pesticides was 0.18 µg/L, with 9 pesticides per sample on average (n = 815). This is significantly higher than monitoring data for larger streams, reflecting the close proximity to agricultural fields and the limited dilution by non-agricultural waters. The frequency of detection of all pesticides correlated with sales quantity and half-lives in water. Terbuthylazine, MCPA, boscalid, and tebuconazole showed the highest median concentrations. The median of the total concentration of the 32 metabolites exceeded the pesticide concentration by more than an order of magnitude. During dry weather, the median total concentration of the 76 pesticides was 0.07 µg/L, with 5 pesticides per sample on average. Rainfall events increased the median total pesticide concentration by a factor of 10 (to 0.7 µg/L), and the average number of pesticides per sample to 14 (with up to 41 in single samples). This increase was particularly strong for 2,4-D, MCPA, terbuthylazine, and nicosulfuron (75 percentile). Metabolite concentrations were generally less responsive to rainfall, except for those of terbuthylazine, flufenacet, metamitron, and prothioconazole. The frequent and widespread exceedance of the regulatory acceptable concentrations (RAC) of the 76 pesticides during both, dry weather and rainfall, suggests that current plant protection product authorization and risk mitigation methods are not sufficient to protect small streams.



2.1 Introduction

Most surface waters are subject to the input of anthropogenic chemicals. These may stem from discharges of treated municipal wastewater, combined sewer overflows, cooling waters, industrial wastewaters, and diffuse sources such as road runoff or agricultural fields (Wittmer et al., 2010). Pesticides applied to agricultural fields for the protection of crops can enter water bodies by surface runoff, subsurface drainage systems, groundwater inflow, and spray drift (Bundschuh et al., 2014; Leu et al., 2010; Liess et al., 1999). Important parameters that influence the extent of pesticide input into surface waters are weather, soil type, pesticide properties, and application method (Gramlich et al., 2018). Pesticides are biologically active compounds, and it is known for long that their input into surface waters can affect aquatic biota from single species to community level and the whole river ecosystem (Beketov et al., 2013; Liess and Ohe, 2005; Stehle and Schulz, 2015). The input of pesticides into surface waters is particularly high during the main application period in spring and summer and has been shown to increase during rain events (Leu et al., 2004; Szöcs et al., 2017).

In the European Union (EU), the first regulation of pesticide concentrations in aquatic compartments dates back to 1980 (The Council of the European Communities, 1979) and was directed to groundwater as an important source of drinking water, with a limit of 0.1 µg/L for any pesticide. With the Water Framework Directive (WFD) of 2000 pesticide concentrations in surface waters were regulated (European Parliament and Council of the European Union, 2020). To date, maximum allowable concentration Environmental Quality Standards (MAC-EQS) are derived for only 14 of the 463 pesticides that are currently approved in the European Union (European Commission, 2020).

According to the WFD, official monitoring programs are in place in the EU to surveil surface water quality. The ten major river basin districts in Germany have to be monitored representatively and sampled at defined intervals independent of weather conditions. Small basins with less than 100 km^2 are sampled less frequently than larger basins (Wick et al., 2019), and small catchments < 10 km^2 are not specifically considered by the WFD. However, small streams are important habitats, and, they comprise the majority of running waters. In Germany, for example, almost 2/3 of the total length of running waters is represented by small streams with catchment sizes < 10 km^2 (approx. 258 000 of 400 000 km) (Bundesamt für Naturschutz, 2004). Small agricultural streams are characterized by immediate proximity to agricultural fields and a low dilution capacity of field runoff by other waters compared to larger rivers further downstream (Szöcs et al., 2017). Such stream sections are denoted as "edge-of-field" surface waters in the EU-EFSA risk assessment of plant protection products for aquatic organisms (European Food Safety Authority, 2013). Small streams have been shown to be specifically exposed to high pesticide concentrations. Spycher et al. reported that risks in five small streams in Switzerland were underestimated by current monitoring strategies with low temporal resolution (Spycher et al., 2018). In Germany, a so-called National Action Plan on Sustainable Use of Plant Protection Products (NAP) was implemented that demands monitoring of the pesticide burden of small streams to better account for their risks (Federal Ministry of Food and Agriculture Germany, 2013).

The protection of the environment is a primary aim of pesticide regulation. For the approval of plant protection products in the EU, exposure models are used to derive predicted environmental concentrations (PEC) in surface waters for a given application. Furthermore, regulatory acceptable concentrations (RACs) of pesticides are derived for surface waters based on available effect data (European Food Safety Authority, 2013) to exclude “unacceptable effects on the environment” (European Parliament, Council of the European Union, 2009). RACs can vary between EU member states and are subject to change if new effect data become available. Eventually, only those applications of plant protection products are approved, for which the PEC remains below the RAC. Consequently, exceedances of RACs should not occur. Pesticide monitoring data can be used to check for compliance with the respective RACs and, thus, serve as reality check for the approaches established in the approval of plant protection products.

Such a reality check needs to consider also ecologically relevant and potentially critical situations and, thus, to include small agricultural streams and rainfall events. To become representative, such monitoring would also have to include a large diversity of settings in terms of catchment morphology, land use, and crops grown in the catchment and to cover diverse weather conditions.

In this study, pesticides were monitored at 105 sites in spring and summer of two years (2018, 2019) by a combination of sampling at regular intervals and event-driven sampling to account for rain events (Liess et al., 2021). This sampling combines high temporal resolution with high spatial coverage in Germany, resulting in > 800 samples processed in the same way to generate highly comparable concentration data for 76 pesticides and 32 metabolites, plus 4 indicator compounds to account for inputs from non-agricultural sources. This large set of monitoring data is interpreted involving information on the catchments (land use, crops growth) and the physico-chemical properties and use characteristics of the pesticides under study.

This work aims at answering the following questions: What are the concentration levels of pesticides and pesticide metabolites in small agricultural streams compared to larger ones during the period of pesticide application? How do rainfall events affect pesticide concentrations in these streams? Which pesticides reacted most sensitively to rainfall events? Can we explain the concentrations of pesticides based on their physico-chemical properties or their use characteristics? Can the concentrations be predicted from the fraction of agricultural land use in a catchment? How do pesticide metabolites compare to their parent compounds? Do pesticide concentrations during dry weather and rainfalls comply with the concentration levels derived during the approval of plant protection products? By answering these questions, this study aims at supporting risk assessment as well as risk management of pesticides, with a focus on small streams.

2.2 Materials and Methods

2.2.1 Sampling

In total, 886 samples were taken in small agricultural streams in spring and summer (April to July) of 2018 and 2019, covering the application period of intensive pesticide use (Wick et al., 2019). Sampling was carried out at 105 sampling sites in 103 streams, selected based on catchment size, high percentage of agricultural land use and expected low urban influence (Wick et al., 2019), distributed over twelve federal states in Germany (Figure S1). The mean catchment size was 17.6 km^2. The sampling strategy comprised regular grab water sampling (every third week), following the approach employed in monitoring according to WFD, and event-driven sampling to cover rain events. Of the regularly taken 551 samples, only 480 samples are included in this study. These were categorized as taken during dry weather (referred to as “DRY” hereafter), because less than 10 mm rainfall was reported on the day of sampling and no rainfall event was noted on this day or the day before. The event-driven sampling was conducted with automated samplers (MAXX TP5, Rangendingen, Germany) triggered by a rise of water level in the respective stream that corresponded to precipitation > 10 mm/day in the respective catchment (“RAIN”, 335 samples). Further details on sampling and site characteristics were provided in a previous publication (Liess et al., 2021).

2.2.2 Sample Preparation

The water samples were filtered with a disposable syringe filter (a combination of glass fiber filter and 0.45 µm regenerated cellulose acetate (Altmann Analytik, Munich, Germany)). One mL of the filtered sample was spiked with five isotope-labeled internal standards of the pesticides with very low RAC values of 0.00077 – 0.01 µg/L (spiking concentrations are provided in Table S2). Three further internal standards were added to check for instrumental performance.

The reference substances at purities of 98 % and higher were delivered from HPC Standards (Borsdorf, Germany) and Chemos (Altdorf, Germany) dissolved in acetonitrile at concentrations of 100 µg mL^{-1}. The dilutions to build the calibration curves were prepared in Milli-Q-water.

2.2.3 LC-MS/MS Analyses

In total, 76 pesticides (40 herbicides, 24 fungicides, and 12 insecticides), 32 pesticide metabolites, and 4 indicator compounds were analyzed in the water samples. The target analytes changed slightly from 2018 to 2019. Amino-bifenox acid, bifenox acid, and sulcotrione were excluded from the monitoring in 2019, whereas chlorantraniliprole (insecticide), hexamethoxymethylmelamine (HMMM, a marker for road runoff), and the metabolite R471811 of chlorothalonil (fungicide metabolite) were included.

The analyses were carried out with an LC-MS/MS system involving an Agilent 1290 infinity liquid chromatography system coupled to a QTrap6500+ tandem mass spectrometer equipped with an electrospray ionization (ESI) interface (Sciex) by direct injection of the aqueous samples and multiple-reaction-monitoring. Quantification was performed by external calibration in ultrapure water, except for the five analytes for which labelled internal standards were added (see above). Details on the analytical method, linear calibration range, and validation data are provided in the supplement information (Table S1 to S3).

2.2.4 Data Analysis

For data processing, the MultiQuant™ software version 3.0 (Sciex, Darmstadt, Germany) was used. While most of the pesticides were quantified with external calibration curves (concentration levels between 0.005 µg L^{-1} to 0.75 µg L^{-1}), those marked by low RACs (clothianidin, imidacloprid, thiacloprid, methiocarb, and fipronil) were quantified using the isotope-labeled internal standards listed in Table S2. The linear calibration range between the limit of detection (LOD) and 0.75 µg L^{-1} allowed the quantification of the DRY samples. If pollutant concentrations exceeded the linear calibration range, e.g., in RAIN samples, the quantification was carried out after repeated analysis injecting a smaller sample volume of 10 µL instead of 80 µL. All concentration data will be available under https://doi.org/10.1594/PANGAEA.931673 from the 30.09.2022 onwards.

Further data analysis was carried out with OriginPro 9.7.0.185 (OriginLab). The frequency of detection (FOD) was calculated based on values larger than the limit of quantification (LOQ, FOD_{LOQ}) as well as the limit of detection (LOD, FOD_{LOD}). For calculations of concentration ranges, values below the LOQ were also considered (specified where applicable). Quantiles were interpolated based on the method “empirical distribution with averaging” in Origin. K-means (k=5) clustering was conducted to group the pesticides and their metabolites based on the physico-chemical properties charge and $\log D_{ow}$ (pH=7.4, chemicalize (ChemAxon)), see results in Table S9 and Figure S8.

To test the explanatory power of different variables on the FOD_{LOQ} values, we conducted a correlation analysis (Spearman's rank correlation analysis) with the sales quantities per pesticide (Federal Office of Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, 2020)), half-life in water and soil (retrieved from the Pesticide Properties DataBase (University of Hertfordshire, 2020)), and the polarity ($\log D_{ow}$) values (chemicalize (ChemAxon)). A correlation was considered significant at $p < 0.05$. To further explore the relationship between pesticide concentrations and their physico-chemical properties and use characteristics, a multiple linear regression was performed followed by an ANOVA in OriginPro 9.7.0.185 (OriginLab).

Land use within the hydrological catchments of the studied streams was derived using the CORINE land cover data (Copernicus). All land use subtypes of the classes agriculture, forest, urban, and grassland were aggregated at class level, and the respective area share was calculated. Land use and cultivated crops were mapped in-situ within a buffer zone reaching 3 km upstream and 500 m to each streamside. The four main land use classes were not further differentiated and no pesticide application data for the grassland and forests were available. Thus, only the agriculture and urban land use are further discussed in this manuscript. Cultivated crops are displayed as the percentage of the agricultural area (Figure S9).

The land use data were tested for correlation (Spearman's correlation) with the measured concentration per site. For sites with different land use in 2018 and 2019, the years were considered separately, resulting in a total number of 119 observations. The median and mean values of the measured concentrations per compound at one site were calculated from all samples taken (DRY and RAIN, n = 815), and the correlation was tested for both values (Table S10). The five pesticides with the highest correlation coefficient are shown in Table S10a with the urban land use plus the marker substances and in Table S10b with the six most representative (area-wise) crops (wheat, corn, rape, barley, viticulture, sugar beet). Information on typically applied pesticides per crop for 2018 and 2019 was provided by the Julius-Kühn Institute (Julius-Kühn Institute).

2.3 Results and Discussion

2.3.1 Frequency of Detection and Concentration Ranges of the Monitored Pesticides

A total of 815 samples from 105 sampling sites in 103 small agricultural streams taken in spring and summer of 2018 and 2019 are considered in this study (480 samples denoted as "DRY"; 335 samples denoted as "RAIN") (Figure S1). They were analyzed for 76 pesticides (40 herbicides, 24 fungicides, and 12 insecticides) and 32 pesticide metabolites. Pesticide selection was based on previous monitoring data, use, and ecotoxicity (Wick et al., 2019).

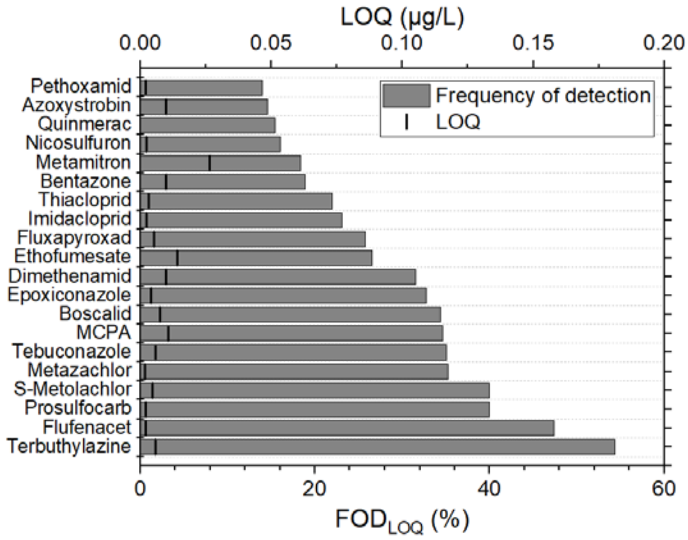


Figure 6: Frequency of detection (FOD_{LOQ}) of pesticides in all samples (n = 815) at agricultural sites for the 20 parent substances with the highest FOD_{LOQ} (grey bars). Straight bold lines represent the LOQ value (average value of LOQ 2018 and 2019). For full all data, please refer to Table S4.

In this set of > 50,000 concentration data, terbuthylazine, flufenacet, prosulfocarb, and S-metolachlor showed the highest frequency of detection (FOD_{LOQ}) of > 40% (Figure 8, complete list in Table S4). Considering all values above the LOD, these compounds were detected in more than 64% of all samples (Table S4). FODs of all pesticides correlated with their sales quantity for Germany ($r_s = 0.51$, $p < 0.001$) and, less pronounced, with their half-lives in water ($r_s = 0.28$, $p < 0.05$) (Figure S2) but not with their half-lives in soil, their water solubility or polarity expressed as logD_{ow}. The latter finding may point to the fact that pesticides can be exported from the agricultural field in dissolved form as well as particle-bound during runoff events. Their application amounts and persistence, then, remain as the decisive factors for the occurrence in agricultural streams. A multiple linear regression (Figure S3) also confirmed the significance of the sales quantity and the half-lives in water, explaining 32% of the variance. This agrees to a previous study for one defined catchment that also found the applied amount as the major determinant of occurrence in adjacent streams, although for a lower number of pesticides (Kreuger and Törnqvist, 1998). The FOD_{LOQ} in this study with 815 samples were comparable or higher than those reported in an earlier study in Germany, based on samples taken between 2005 and 2015 as part of the regular monitoring according to the WFD (Szöcs et al., 2017). Higher FODs are presumably partially due to lower LOQs in this study compared to regular monitoring. Much higher FODs were found for picoxystrobin (factor 45), prosulfocarb, and bromoxynil (factor 16 and 13). Lower FODs were recorded for dimethachlor and isoproturon (factor 11 and 5 lower FODs than Szöcs et al., respectively, Figure S4). The latter may be explained by the fact that the approval of isoproturon ended in 2016 in the EU and that the sales quantity of dimethachlor declined by a factor of 6 between this earlier study and the years 2018 and 2019 (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, 2020).

The pesticides detected most frequently were also often those determined with the highest median concentration (Figure 9). Terbuthylazine was detected in concentrations ranging up to 0.56 µg/L (95 percentile, median = 0.0056 µg/L, Figure 9). Other predominant pesticides in this study were MCPA (95 percentile = 0.38 µg/L, median = 0.0035 µg/L), boscalid, tebuconazole, S-metolachlor, dimethenamid, flufenacet, and fluxapyroxad (95 percentile = 0.041 µg/L, median = 0.0013 µg/L). Also, for the concentrations determined in the streams, a significant correlation with sales quantity ($r_s = 0.46$, $p < 0.001$) and half-lives in water ($r_s = 0.26$, $p < 0.001$) was found (Figure S6).

For 45 pesticides, concentrations measured here could be compared with governmental monitoring data for the years 2018 and 2019 of two Federal States in Germany; these data have a higher share of larger streams (Figure 10). Pesticide concentrations of this study were significantly higher (Kolmogorov-Smirnov test, $p < 0.05$, p-values in Table S5) for 28 (federal state A) and 25 (federal state B) of the 45 pesticides. The higher concentration may be attributed to i) the small stream sizes of this study, ii) the high number of RAIN samples or iii) the collection of samples only during the application period of most pesticides. However, within the 105 sites of this study, a decrease of pesticide concentrations with increasing catchment size was not visible ($r_s = 0.082$, $p = 0.40$ for the mean and $r_s = 0.051$, $p = 0.61$ for the median). This may be due to the limited span of catchment sizes (from 9 km^2 to 19 km^2 for the 25 – 75 percentile). Higher concentrations in smaller streams have been reported previously, for example in a study on 42 Danish streams in three size classes with > 1000 samples analyzed. This increase was particularly pronounced for the peak concentrations (95 percentiles) (Lorenz et al., 2017).

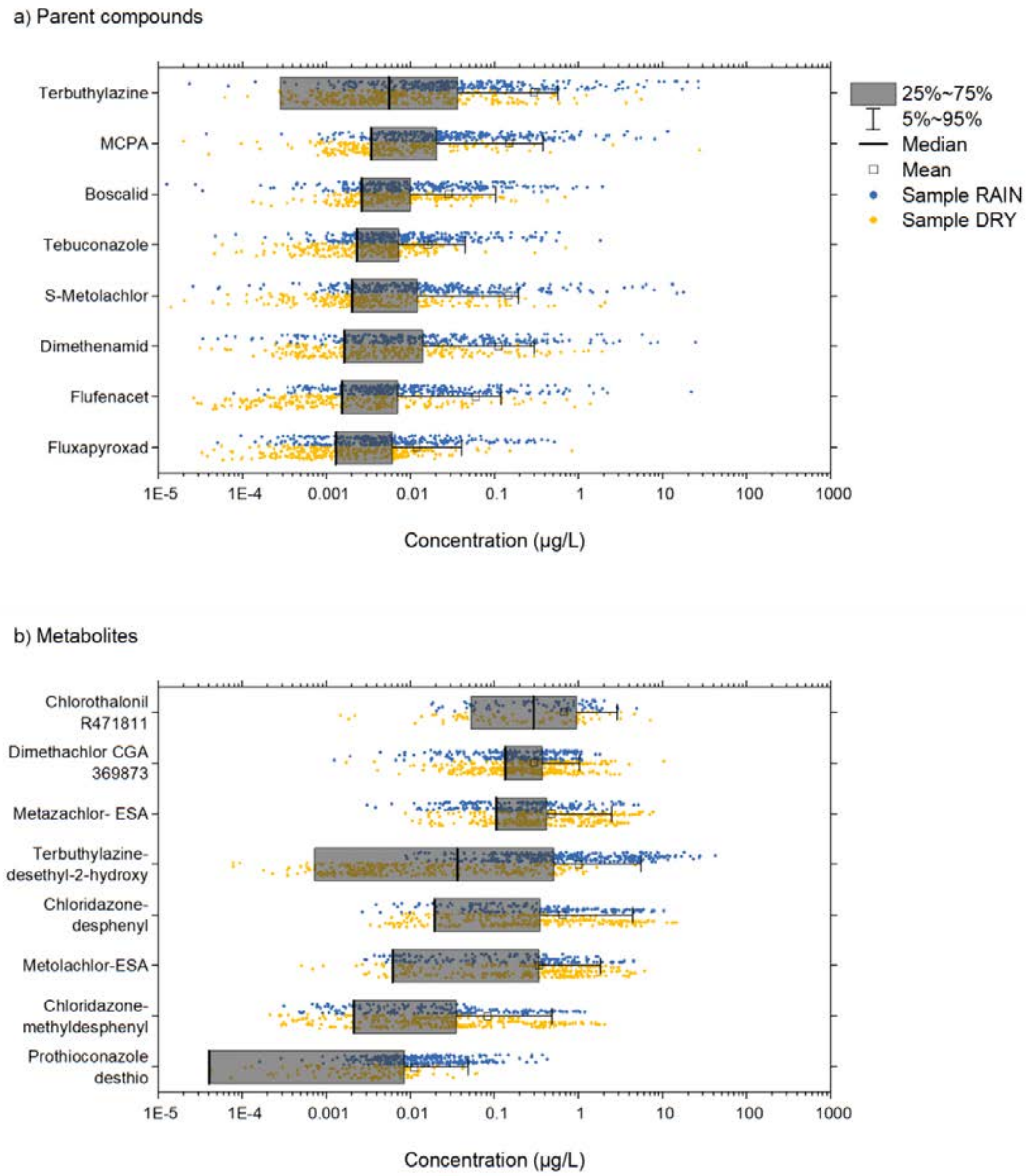


Figure 7: Boxplots and underlying data of the eight compounds with the highest median values in the whole sample set (n = 815) for a) the parent substances and b) the metabolites. Colors indicate the weather conditions at sampling:

yellow (DRY) and blue (RAIN). Due to the logarithmic scale, only data > 0 are displayed. Values between LOD and LOQ were considered.

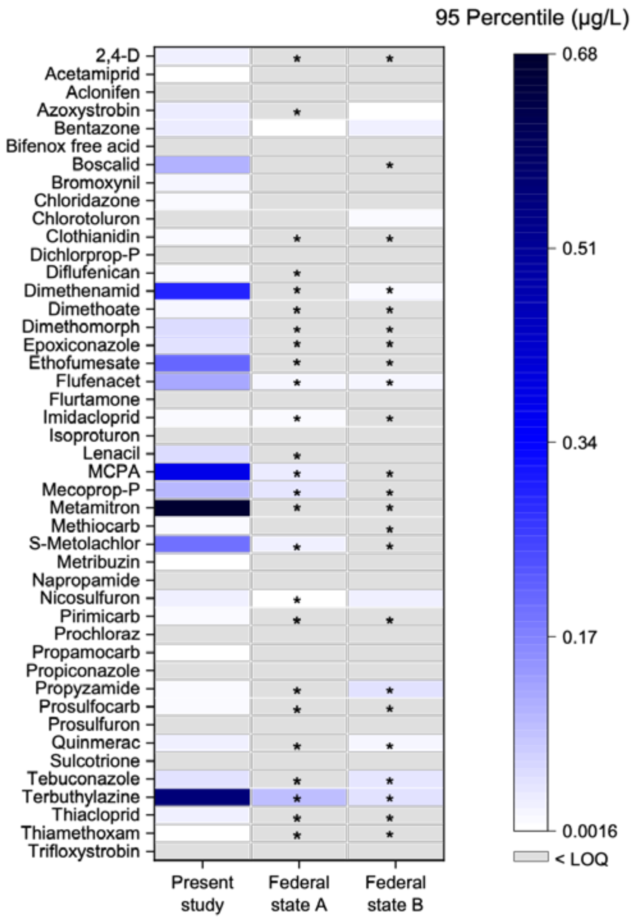


Figure 8: Comparison of the 95 percentile concentrations for selected pesticides of the present study (n = 815) and samples taken in two Federal states in Germany in 2018 and 2019 as part of the monitoring for the WFD (range of n = 98-1,680 for Federal State A and n = 241-516 for Federal State B). Displayed are the pesticides that were measured in the present study and monitored in both Federal States. Limits of quantification (LOQ) did not differ by more than a factor of 10 between this study and the two states. Data only above the LOQ were available from one Federal state, thus for percentile calculation values below the LOQ in all three datasets were set to zero. * indicates a significantly different distribution of the measured concentration data from the present study ($p < 0.05$, Kolmogorov-Smirnov test, see for exact p-values Table S5).

Taken together, the FOD data (Figure 8) and the concentration data (Figure 10) for the 76 pesticides at the 105 sites confirm the previous notion that pesticide concentrations in small agricultural streams of catchment sizes of 10 km^2 and below are not well reflected in the official monitoring program (Leu et al., 2004; Szöcs et al., 2017). It should be noted in this context that the small streams make up almost 2/3 of the running water in Germany (Bundesamt für Naturschutz, 2004).

The formation of metabolites from parent pesticides is inevitable for non-persistent pesticides (Fenner et al., 2013). These metabolites are often more polar as well as mobile and can also be more persistent (Gassmann et al., 2013). Monitoring of pesticide metabolites supports the understanding of pesticide fate in agricultural systems. The median of the total concentration of the 32 metabolites included in this study was 2.4 µg/L and, thus, exceeded the total pesticide concentration by a factor of 13. Given the comparatively low number of metabolites, the concentration of all metabolites must be even higher. The most frequently detected metabolites in this study originate from chlorothalonil (FOD_{LOQ} 94%), terbuthylazine, chloridazon, and dimethachlor (FOD_{LOQ} > 40%; Figure S5). The chlorothalonil metabolite R471811 was only recently reported as the predominant pesticide metabolite in groundwater and surface water samples from Switzerland (Kiefer et al., 2020). Two other studies reported chloridazon-desphenyl in between 43% and 77% of surface water samples in Germany and with mean concentrations comparable to this study (Buttiglieri et al., 2009; Szöcs et al., 2017). Dimethachlor CGA 369873 was previously described as an emerging metabolite in German ground and surface waters in 2013, but at a much lower concentration (median 0.02 µg/L for groundwater and surface water vs. 0.14 µg/L in the present study) (Reemtsma et al., 2013).

The mean concentrations of some metabolites were two to three orders of magnitude higher than their parent compounds (factor 89 – 530) for metazachlor-ESA, chloridazon-desphenyl, and dimethachlor CGA 369873 (Table S6). In single samples, the concentration of metazachlor-ESA, metolachlor-ESA, terbuthylazine-desethyl-2-hydroxy, and chloridazon-desphenyl even exceeded their parent compounds by a factor > 1000 (Table S6). Chloridazon-desphenyl was previously detected in higher concentrations than chloridazon in the Hesse region, Germany, in 2007 (Buttiglieri et al., 2009).

The so-called 'relevant metabolites' are of the highest regulatory concern, as these still cause pesticidal, toxic, or ecotoxicological effects (European Commission, 2003). Among the monitored metabolites, the two metazachlor metabolites BH 479-11 and BH 479-9 (6% and 0.1% FOD_{LOQ}) and terbuthylazine-desethyl-2-hydroxy (40% FOD_{LOQ}) are classified as 'relevant metabolites' (Banning et al., 2019; LAWA, 2019). Furthermore, the European Food Safety Authority (EFSA) recently recommended considering all metabolites of chlorothalonil as 'relevant metabolites' (Kiefer et al., 2020). Chlorothalonil R471811 (median = 0.29 µg/L) was also classified as relevant in Switzerland (Bundesamt für Landwirtschaft, 2020). Especially, the high FODs of the relevant metabolites of chlorothalonil and terbuthylazine raise concern.

It should be noted, however, that due to their mobility and persistence, also "non-relevant" metabolites in surface water can affect water quality and the downstream use of surface water, e.g., as a resource for drinking water via bank filtration.

2.3.2 Effects of Rainfall on the Frequency of Detection and Concentrations

Previous studies, more limited in pesticide number or spatial extent than this study, have shown that pesticide concentrations in streams can be strongly elevated during rainfall compared to those found during dry weather due to inputs by surface runoff, macropore flow, or subsurface drainage (Chow et al., 2020; Liess et al., 1999). The large number of sites, samples, and pesticides of this study allows evaluating the effects of rain events on pesticide export in more detail.

The total concentration of pesticides in surface waters drastically increased during rainfall events (Figure 11a): the median increased by one order of magnitude, from 0.072 µg/L to 0.70 µg/L from DRY to RAIN, and the 95 percentile from 1.7 to 24 µg/L. Correspondingly, also the number of pesticides per sample drastically increased: the maximum of the frequency distribution shifted from two pesticides per sample in the DRY sample set to 14 pesticides per sample in the RAIN sample set (Figure 11c), and the mean number of quantified pesticides (concentration > LOQ) increased from 5.2 pesticides per sample to 14 in RAIN samples. For the detected pesticides (concentration > LOD) the number increased from 16 in the DRY to 31 in the RAIN sample set. In single RAIN samples, more than 40 different pesticides occurred above their LOQ.

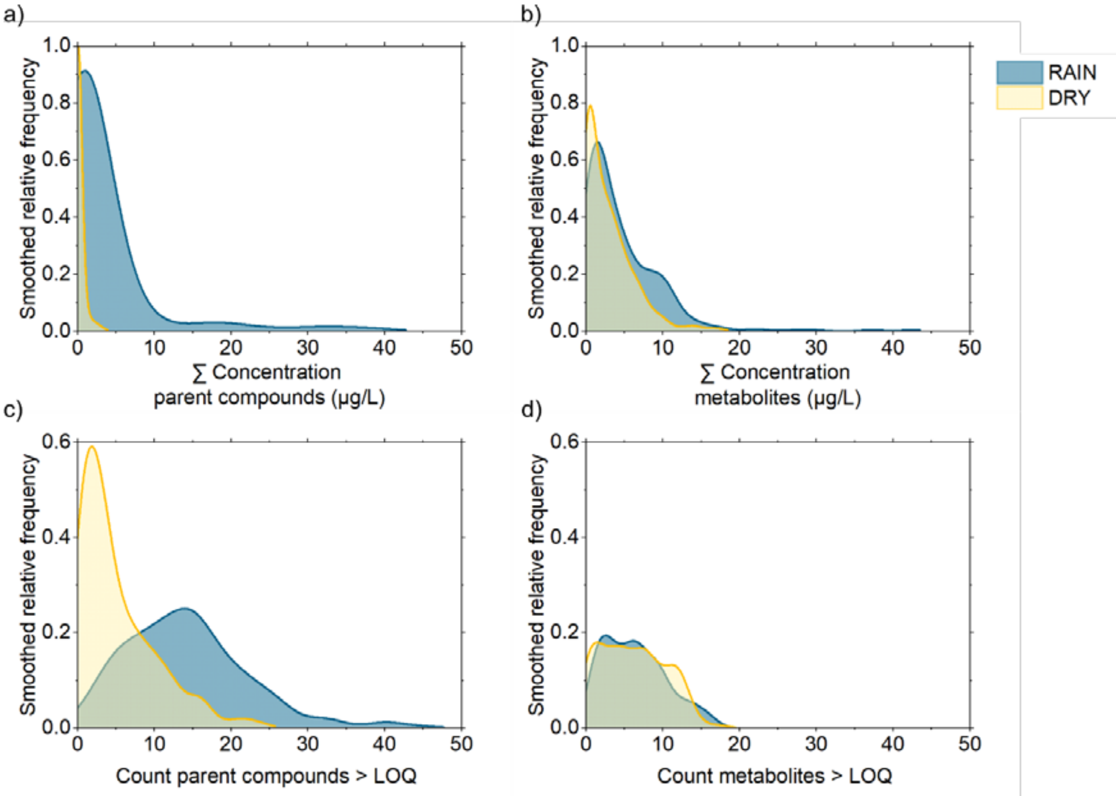


Figure 9: Smoothed (kernel) relative frequency for DRY (yellow, n = 480) and RAIN (blue, n = 335) samples of a) summed concentration of the 76 parent compounds per sample (5 samples with a summed concentration up to 96 µg/L are out of scale), b) summed concentration of the 32 metabolites per sample, and c) the total number of parent compounds and d) of metabolites per sample detected above the limit of quantification (LOQ).

Correspondingly, the mean FOD_{LOQ} for the 76 parent pesticides tripled from 6.9% in the DRY samples to 18.6% in the RAIN samples. Thus, rainfall events lead to a drastic increase in both pesticide concentration and pesticide number in the small agricultural streams. This matches with a study on 10 Danish streams, in which an increase of the total pesticide concentration from 0.19 µg/L at base flow to 1.8 µg/L at storm flow was recorded (Rasmussen et al., 2015).

For a quantitative description of pesticide dynamics, it would be useful to calculate rainfall-dependent pesticide fluxes (Wittmer et al., 2010). However, the continuous recording of water fluxes in the streams, as necessary for that purpose, was not feasible at the 105 sites under study. As this study was directed to studying the effects of pesticide export on stream water quality, concentrations are more important than fluxes.

The more frequent detection of higher concentrations of pesticides during rainfall events is already seen in Figure 9 (RAIN, blue data points). However, this trend is not equally strong for all pesticides, as visible by comparing the 75 and 90 percentiles of the RAIN and DRY samples for the 76 pesticides (Figure 12 and S5). As data for 105 sampling sites are included in this comparison, these values were expected to be largely independent of site characteristics and mainly relate to physico-chemical properties or use characteristics of the pesticides.

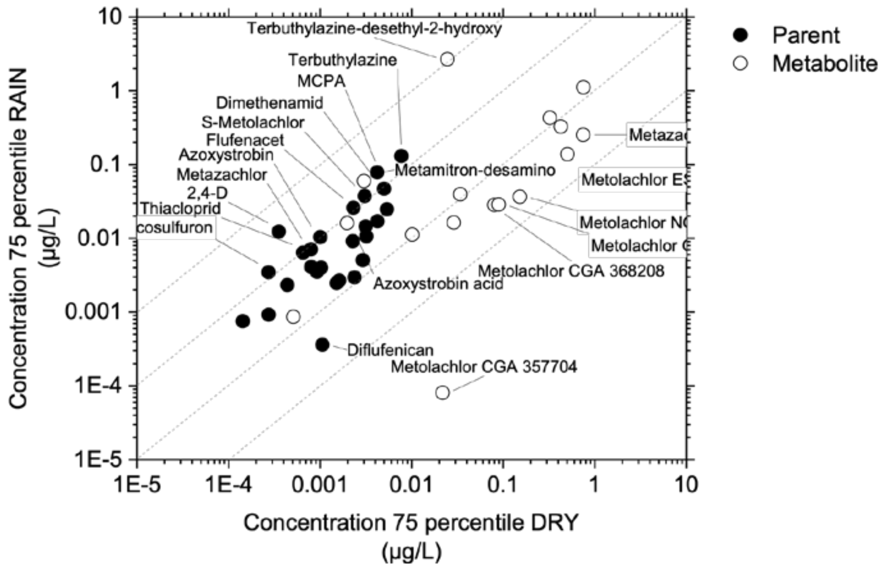


Figure 10: Comparison of the 75 percentile concentrations of pesticides and metabolites in RAIN samples (n = 335) versus the DRY samples (n = 480).

The pesticides 2,4-D, terbuthylazine, flufenacet, metamitron, trifloxystrobin, MCPA, ethofumesate, pirimicarb, nicosulfuron, methiocarb, fluroxypyr, S-metolachlor, mecoprop-P, dimethenamid, isoproturon, thiacloprid, and azoxystrobin exhibited strongly elevated concentrations during rainfall (> one order of magnitude) for both, 75 and 90 percentiles compared to dry weather (Figure 12, S7 and Table S7). These are mainly substances of medium polarity (average logD_{ow} 2.2) and either neutral or with a single negative charge (Tables S8 and S9, Figure S8). The same is true, however, for the compounds that do not show a clear increase during rain events (Figure 12), like bentazone, clothianidin, and diflufenican (Table S7).

Contrary to the parent pesticides, the summed concentrations of metabolites and the number of detected metabolites per sample were hardly affected by rainfalls (Figure 11b and d). The formation of metabolites by (bio-) transformation takes time, so that their occurrence in surface waters is less directly linked to the application period of their parent substances. Correspondingly, the concentrations of the metabolites have been shown to be less influenced by rainfall events and to be largely exported via subsurface as previously shown for the metabolites of dimethenamid, atrazine, and metolachlor in a headwater catchment (Gassmann et al., 2013).

Nevertheless, marked differences between RAIN and DRY samples were also visible for individual metabolites (Figure 12, S5, and Table S7). For example, the metabolites terbuthylazine-desethyl-2-hydroxy, flufenacet thiadone, metamitron-desamino, and prothioconazole-desthio exhibited one to two orders of magnitude elevated concentrations during rainfall.

Conversely, the metabolites metolachlor CGA 357704 and metazachlor BH 479-12 were much less concentrated (one order of magnitude) in surface waters during rain events (Figure 12). These two metabolites of metolachlor and metazachlor are no primary metabolites but are formed only in later stages of the degradation processes (Reemtsma et al., 2013). It is reasonable to assume that their formation requires longer periods of time, which are not available when surface runoff occurs shortly after pesticide application, but when pesticides and their metabolites infiltrate into the soil. These metabolites may, therefore, reach surface water by groundwater exfiltration (Kern et al., 2011). During rainfall, the increased surface runoff dilutes the fraction of groundwater in the surface water and, therefore, the concentration of these metabolites may decrease. The very high polarity of the two metabolites ($\log D_{ow}$ -4 to -5 and a negative charge of -2) supports the notion that they may be transported via groundwater, as sorption to organic as well as inorganic soil constituents should be negligible for such compounds.

The effects of rainfall events on the concentrations of pesticides and their metabolites in small agricultural streams are illustrated for selected compounds at selected sites in Figure 13. The concentrations of flufenacet, metamitron, MCPA, 2,4D, terbuthylazine, and metazachlor strongly increased during rainfall after application. The magnitude of this increase is clearly larger than for their metabolites (except terbuthylazine-desethyl-2-hydroxy). The terbuthylazine metabolite largely exceeded the concentration of its parent compound (1-3 orders of magnitude; Figure 13d). However, also the metabolites flufenacet thiadone and metamitron-desamino showed increased concentrations during rainfall events (Figure 13a, b). In contrast, the concentrations of the metabolites of S-metolachlor were not increased (Figure 13f). These concentration profiles highlight the strong fluctuation in concentrations evoked by rainfall events in small agricultural streams.

Site-specific RAIN/DRY ratios for the sum of all pesticides may be used to elaborate on catchment characteristics that support pesticide export into surface water, such as slope, the distance between fields and the water body, or the presence of subsurface drainage systems. In addition, the consideration of site-specific concentration ratios of certain metabolite/parent pairs may also provide information on the preferred transport pathway at that site. Such an extended data analysis may help identify characteristics of catchments that are critical for pesticide export and, in this way, point to options on how to reduce this export into surface waters.

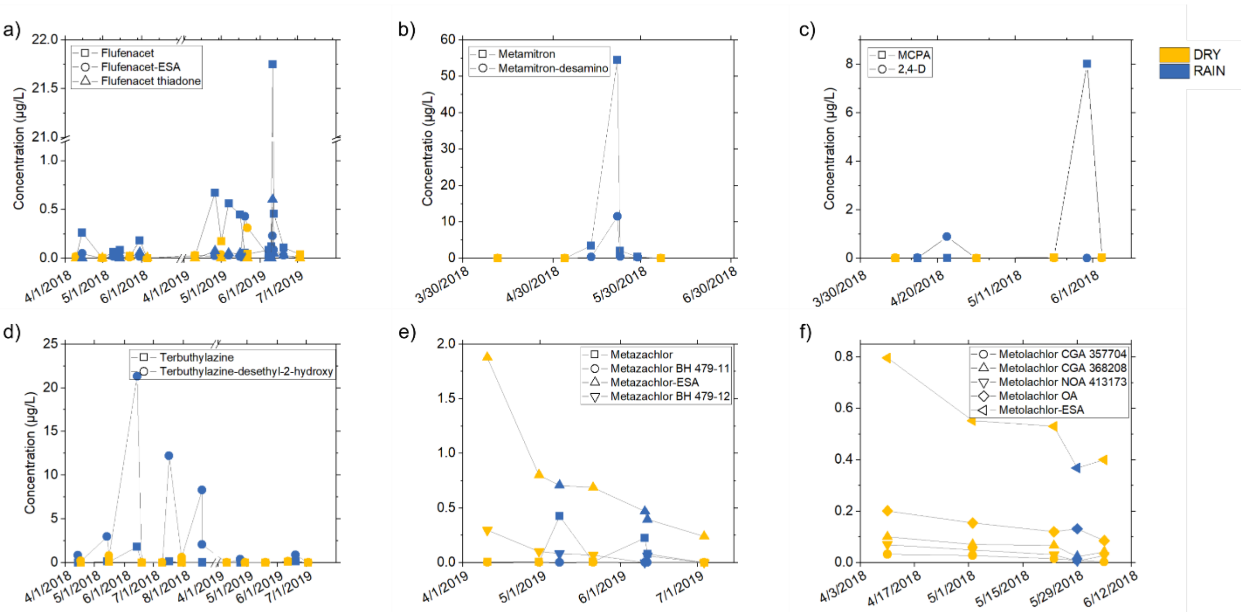


Figure 11: Concentration-time profiles for six groups of pesticides/metabolites at selected sites: a) flufenacet and its metabolites ESA and thiadone, b) metamitron and metamitron-desamino, c) MCPA and 2,4-D, d) terbuthylazine and terbuthylazine-desethyl-2-hydroxy, e) metazachlor and three metabolites BH 479-11, ESA, and BH 479-12 and f) the five metabolites of S-metolachlor CGA 357704, 368208, 413173, OA and ESA. Colors indicate the weather classification of the water samples as “DRY” (yellow) and “RAIN” (blue).

2.3.3 Influence of Land Use on Pesticide Concentrations

The land use in the 105 catchments under study was categorized into four main groups: agriculture, forest, urban, grassland (Figure S9), and the agricultural area, then further divided into crop groups. The six most representative crops (largest grown area) of agricultural land use were wheat, corn, rape, barley, vineyard, and sugar beet (Figure S9).

Many pesticides showed a significant Spearman's correlation ($p < 0.05$, Table S10) with the main crop types grown in the respective catchment: e.g., the concentrations of the herbicides ethofumesate, quinmerac, and the fungicide epoxiconazole moderately correlated with the percentage of wheat in the respective catchment (r_s = 0.54 – 0.46, 102 sites), the herbicide S-metolachlor, terbuthylazine, and nicosulfuron with corn (r_s = 0.37 – 0.28, 91 sites). The herbicides propyzamide, diflufenican, and the fungicide dimoxystrobin correlated moderately positive with areas where rape (r_s = 0.47 – 0.38; 90 sites) and the herbicides diflufenican and flurtamone where barley (r_s = 0.47 – 0.29, 85 sites) was grown. For the vineyard areas, a correlation with the fungicides metrafenone, boscalid, and dimethomorph was found (r_s = 0.79 – 0.51; 20 sites), the herbicides metamitron, ethofumesate, lenacil, quinmerac, and chloridazon correlated with the area where sugar beet was grown (r_s = 0.60 – 0.42, 47 sites). Many of these pesticides were also listed for the respective crop by the so-called "PAPA-survey" in Germany, which collects pesticide application data from selected agricultural farms for 2018 and 2019 (Julius-Kühn Institute). These results outline the strong link between the agricultural activity in a catchment and the occurrence and concentration of pesticides in the respective stream. Hence, agricultural practice is the key to reduce pesticide concentrations in small streams. Also, the practice in the past years may affect present pesticide loads in streams (Rasmussen et al., 2015).

A few pesticides could be linked to urban activities. To account for input from sources other than agriculture, a few indicator compounds were also monitored, such as the pharmaceutical diclofenac and the corrosion inhibitor benzotriazole for municipal wastewater discharges and hexamethoxymethylmelamine (HMMM) for road runoff (Alhelou et al., 2019; Reemtsma et al., 2010; Seitz and Winzenbacher, 2017); (Table S10). HMMM (r_s = 0.56 – 0.61) and the phenoxyacid herbicides mecoprop P (r_s = 0.42 – 0.49) and, although weaker, 2,4-D (r_s = 0.27 – 0.35) showed a significant correlation with the percentage of the urban area but not with the agricultural area. These are pesticides that are especially used as a weed killer in urban areas and are also described as an indicator for urban runoff waters (Jekel et al., 2015; Raina et al., 2011). Benzotriazole and diclofenac also correlated with urban land use (r_s = 0.24 – 0.38) but weaker than HMMM.

2.3.4 Frequency of RAC Exceedances

The RAC value represents the environmental concentration below which no unacceptable effects on the environment are expected in regulation. The approval of plant protections aims at avoiding RAC exceedances by requiring the farmers to implement certain risk management measures such as keeping distances to water bodies. However, a companion paper analyzing the monitoring data of this study shows that RAC-exceedances are frequently occurring in small agricultural streams especially during rainfall and outlined the consequences for aquatic invertebrate communities (Liess et al., 2021).

A comparison of RAC exceedances separately for the DRY and RAIN samples is performed here to assess the relevance of rain events (Table 1). For the set of 480 samples taken during dry weather conditions, RACs were exceeded 143 times in 23% of the samples and at 50% of the sites. The situation became significantly worse during rainfall events (335 samples): then, a total of 448 RAC-exceedances were recorded (on average 1.3 times per sample) in 60% of the samples (Figure S10) and at 73% of the 105 sites. In other words, only 27% of the sampling sites at small agricultural streams were left without a RAC-exceedance during rainfall during the sampling period (April-July) in two years.

These data show that RAC exceedances in small streams occur widely and frequently. Although rain events are especially critical, RAC exceedances also occur frequently during dry weather: in that phase, only 50% of the sites covered in this study did not show a RAC exceedance. Overall, this suggests that streams of small size are especially susceptible to RAC-exceedances, amplified by rain events.

The almost systematic exceedance of RACs questions the approval process of plant protection products that aims at preventing such exceedances. Three factors may explain the discrepancy between the regulatory aim and agricultural reality: a) some assumptions underlying the models for predicting environmental concentrations are too optimistic so that PEC modeling systematically underestimated the real environmental concentrations (Bach et al., 2017; Knäbel et al., 2012), b) the risk management measures that should be taken in agricultural practice are either not taken or have less benefit than expected, with the consequence that concentrations in agricultural streams exceed the PECs on a broad scale, and c) the application of a plant protection product that was approved several years ago for a certain culture fails to comply with more recently derived (lower) RACs of the respective pesticide (Liess et al., 2021).

The RAC-exceedances encountered in this study also outline the importance of an adequate post-approval monitoring. This would inform to which extent the real-world situation agrees to the predictions made in the approval or plant protection products.

The insecticides thiacloprid, clothianidin, and fipronil were the three compounds with the most frequent RAC exceedances during dry weather as well as during rainfall events (Table 1). The RACs for thiacloprid, clothianidin, and fipronil are in the low ng/L or even pg/L range (0.007 – 0.00077 µg/L) as these insecticides are also highly toxic to aquatic insects. It appears generally challenging to comply with such low RACs. The EU has reacted with a ban on clothianidin and thiacloprid field applications (approval expired in 2019 and 2020). Therefore, a decline in the concentrations of these two compounds in surface waters may be observed in the future. The approval of the insecticide fipronil as seed treatment expired in 2017 already. Its frequent RAC exceedances observed in 2018 and 2019 may be due to the stock of fipronil in the agricultural soils remaining from its previous application (previously recommended amount of 10 kg/ha in potato). The importance of legacy pesticides for current streamwater quality has been outlined earlier (Rasmussen et al., 2015). Alternatively, the ongoing use of fipronil as a biocide and as a veterinary product may explain these findings; this option is corroborated by the correlation of fipronil concentrations with the percentage of urban land use in this study (r_S = 0.39; Table S10). The use of fipronil as veterinary flea products was recently suggested to cause elevated concentrations in rivers in England (Perkins et al., 2021).

Furthermore, the carbamate pesticide methiocarb and the neonicotinoids imidacloprid and thiamethoxam (approval expired in the EU in 2019 and 2020), the herbicides lenacil, terbuthylazine, metolachlor, and nicosulfuron, also exceeded their RAC-value in up to 5% of the samples taken during rain events (Table 1). Frequent exceedance of the RACs by neonicotinoids has been recognized earlier (Casado et al., 2019; Szöcs et al., 2017).

Beyond the 76 pesticides selected for this study, another 387 pesticides are approved in the EU (European Commission, 2020); furthermore, not all crop cultures could be covered representatively by the 815 samples of this study. Therefore, further pesticides than those listed in Table 1 and Tables S11 may lead to RAC exceedances.

It may seem obvious to reduce the pesticide burden of agricultural streams by reducing the application of those pesticides with frequent RAC exceedances (Table 1) and recommending using pesticides with a similar application domain but a lower number of exceedances. However, such a strategy may eventually lead to an increasing frequency of RAC exceedance for the substitute with overall little if any positive effect on the pesticide burden of agricultural streams (Boyd, 2018). Consequently, more holistic approaches have been proposed to reduce the environmental burden of pesticide application (Topping et al., 2020).

2.4 Conclusions

- The total median pesticide concentration at 105 sites in small agricultural streams (median catchment size 13 km^2) in Germany in spring and summer 2018 and 2019 was 0.18 µg/L. This concentration was considerably higher than recorded during governmental monitoring, according to the WFD.
- The local agricultural use was linked to the pesticide concentration in the streams.
- Current official monitoring strategies in Germany underestimate the input of pesticides into small streams.
- Across all sites, the FOD_{LOQ} was highest for terbuthylazine, flufenacet, prosulfocarb, S-metolachlor, and metazachlor; for the 76 pesticides of the study, FODs correlated with their sales quantity and aqueous half-lives.
- Rainfall induced a strong increase of pesticide concentration by a factor of 10 in the small streams compared to dry weather to a median total concentration of 0.7 µg/L. Also, the average number of quantified pesticides increased to 14 per sample. Concentration increase with rainfall was strongest for 2,4-D (factor 35), MCPA, and terbuthylazine (factor 17).
- Pesticide metabolites occurred in much higher concentrations at dry weather than their parent compound (total median 2.0 vs. 0.07 µg/L) but were, in general, less affected by rain events.
- RAC exceedances in small agricultural streams are frequent and widespread. They are very high during rainfall events but do also occur frequently during dry weather at 50% of the sites. This outlines that the present approval of plant protection products fails to ensure compliance of pesticide concentrations in small agricultural streams.

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3 Temporal scales of pesticide exposure and risks in German small streams

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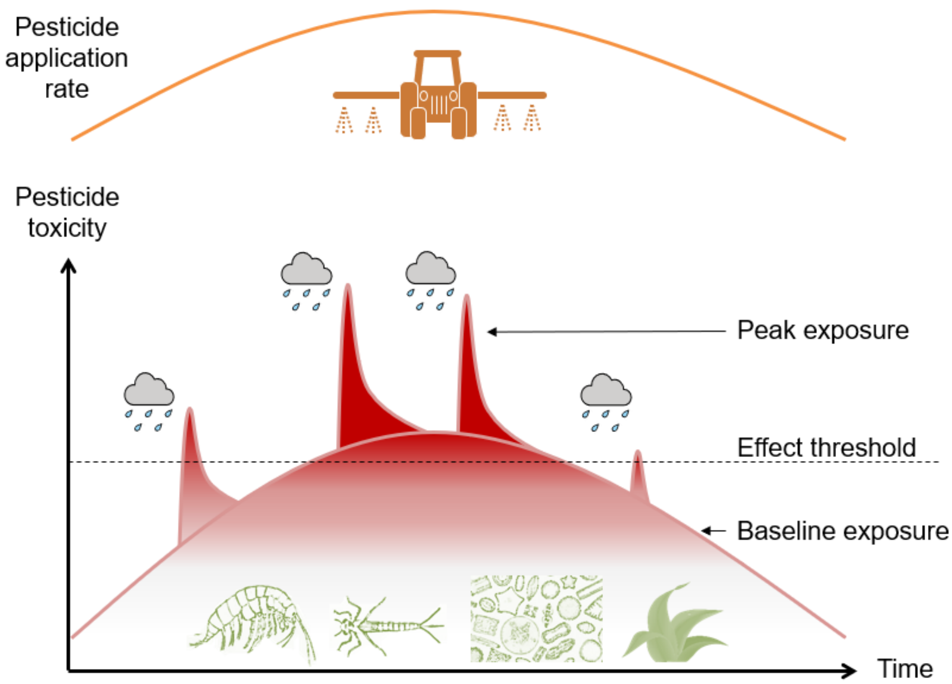
Abstract

Following agricultural application, pesticides can enter streams through runoff during rain events. However, little information is available on the temporal dynamics of pesticide toxicity during the main application period. We investigated pesticide application and large scale in-stream monitoring data from 101 agricultural catchments obtained from a Germany-wide monitoring from April to July in 2018 and 2019. We analysed temporal patterns of pesticide application, in-stream toxicity and exceedances of regulatory acceptable concentrations (RAC) for over 70 pesticides. On a monthly scale from April to July, toxicity to invertebrates and algae/aquatic plants (algae) obtained with event-driven samples (EDS) was highest in May/June. The peak of toxicity towards invertebrates and algae coincided with the peaks of insecticide and herbicide application. Future monitoring, i.e. related to the Water Framework Directive, could be limited to time periods of highest pesticide applications on a seasonal scale. On a daily scale, toxicity to invertebrates from EDS exceeded those of grab samples collected within one day after rainfall by a factor of 3.7. Within two to three days, toxicity in grab samples declined compared to EDS by a factor of ten for invertebrates, and a factor of 1.6 for algae. Thus, toxicity to invertebrates declined rapidly within 1 day after a rainfall event, whereas toxicity to algae remained elevated for up to 4 days. For six pesticides, RAC exceedances could only be detected in EDS. The exceedances of RACs coincided with the peaks in pesticide application. Based on EDS, we estimated that pesticide exposure would need a 37-fold reduction of all analysed pesticides, to meet the German environmental target to keep RAC exceedances below 1% of EDS. Overall, our study shows a high temporal variability of exposure on a monthly but also daily scale to individual pesticides that can be linked to their period of application and related rain events.



3.1 Introduction

Organisms in agricultural streams are exposed to pesticides (Liess and Schulz, 1999; Wolfram et al., 2019; Zubrod et al., 2019). This exposure alters species composition of aquatic communities, such as aquatic invertebrates and algae/aquatic plants (hereafter algae) (Beketov et al., 2013; Boxall et al., 2013; Liess and Ohe, 2005; Rydh Stenström et al., 2021; Schäfer, 2019). The pesticide exposure typically increases minutes to hours after rain events due to surface water runoff (Doppler et al., 2012; Liess et al., 1999; Wittmer et al., 2010). To capture peak concentrations, water samples need to be taken associated with rain events (La Cecilia et al., 2021; Lefrancq et al., 2017; Rabiet et al., 2010). However, currently global pesticide monitoring including the European Water Framework Directive (WFD), Australian National Water Quality Management Strategy and the US Clean Water Act is mainly conducted using grab samples independent of rainfall induced surface water runoff, but following a regular schedule (Australian Government, 2018; European Union, 2000; U.S. Environmental Protection Agency, 1972). Recent studies provided detailed information that rain event-related pesticide exposure peaks exceed those of grab samples (Bundschuh et al., 2014; Chow et al., 2020; Halbach et al., 2021; Lefrancq et al., 2017; Weisner et al., 2022). For example, the pesticide toxicity measured in weather-independent grab samples underestimated the toxicity of event-driven samples (EDS) after rainfall approximately 30-fold (Lefrancq et al., 2017). Pesticide concentrations in grab samples and to a higher extent in EDS frequently exceeded threshold levels such as regulatory acceptable concentrations (RACs) above which unacceptable effects on non-target organisms (lethal doses for invertebrates or growth inhibition for algae) may occur (European Food Safety Authority (EFSA), 2013). A related study on German agricultural streams reported RAC exceedances for 59% of EDS and 26% of grab samples (Liess et al., 2021a). This is in contrast to the German target for 2023 that 99% of EDS in a year have no RAC exceedance (BMEL, 2013).

While RACs are derived as part of the authorisation of pesticides, a post-registration monitoring that validates relevant concentrations is lacking (Vijver et al., 2017). However, the WFD-related monitoring of the ecological quality of streams and rivers is not suitable to identify pesticide effects (Weisner et al., 2022). This pesticide monitoring under the WFD is neither tailored to capture event-related exposure peaks after rainfall nor to account for the potential seasonality of pesticide exposure (Weisner et al., 2022). The period to assess concentrations with respective environmental quality standards (EQS) averages the whole year and thus underestimates periods with high application rates and associated exposure (LAWA-AO, 2019). This issue is addressed by considering an annual maximum concentration. Still this would be insufficient to appropriately determine the risk to aquatic organisms from pesticides, because the analyte spectrum of the WFD is limited and relevant compounds are missing (Moschet et al., 2014; Weisner et al., 2022). Regarding monthly trends, defined as increased or decreased exposure over several months, studies have shown increased exposure to herbicides in individual catchments during the application period (Chow et al., 2020; Doppler et al., 2014; Leu et al., 2004b; Rabiet et al., 2010). This also relates to regulatory threshold exceedances, as Szöcs et al. (2017) found elevated proportions of RAC exceedances from April to June based on the analysis of grab sample monitoring data from German streams. However, the question remains whether a monthly trend of pesticide exposure for a wide range of pesticides can be delineated at a larger scale and with EDS, which would inform the design of monitoring programmes. In an accompanying study, Halbach et al. (2021) quantified pesticide occurrence and RAC exceedances for dry weather and rainfall periods and analysed patterns of individual pesticides. Here, we reanalyse these data, complemented by additional data sets, regarding trends on different temporal scales (i.e. monthly and daily) of estimated pesticide toxicity to invertebrates and algae and on how this translates into RAC exceedances. We focused on differences between EDS and grab samples to identify the extent of underestimation by the WFD monitoring and how toxicity changes on short-term scale in grab samples conducted within 3 days after an EDS. Besides contributing to the improvement of monitoring strategies including that of the WFD, our findings can also inform the design of experimental studies regarding field conditions (e.g. exposed life stages and exposure duration).

We aimed to identify the (i) monthly patterns of the toxicity towards freshwater invertebrates and algae (ii) performance of grab samples compared to EDS on monthly and daily scale, (iii) monthly trends of RAC exceedances and possible implications for future pesticide monitoring and (iv) the influence of the amount of rainfall on the magnitude of event-related peak exposure.

3.2 Material and methods

3.2.1 Study area

A total of 124 stream sections were sampled across Germany between April and July in 2018 as well as in 2019 (Liess et al., 2021b). They were distributed over twelve federal states (see SI Fig. 1). The catchments showed a large gradient of agricultural land use and different crop types (SI Fig. 2). We defined small agricultural streams as streams with a width between 0.5 and 5 m, with a catchment size mostly between 5 and 25 km² (average 18.7 km²) and with more than 20% agricultural land use in the catchment (see SI Fig. 3). The stream sections were checked for representativeness to all other German catchments that fulfil the site selection requirements (for more details see Weisner et al. (2022)). Due to the lack of heavy rainfall events, the required minimum agricultural land use of 20% (n = 22) or technical defects (n = 1), samples were lacking from 23 sampling sites (total = 124), resulting in 101 sampling sites. We provide the number of all sites investigated (n = 124) for purpose of transparency. Based on the results of a previous study (Bunzel et al., 2014), the type of land use and crop types was mapped in-situ 3 km upstream of the sampling sites within a 500 m-wide corridor to each side to capture the area of possible runoff (longitudinal runoff corridor, LRC) (see SI Fig. 2 A and 2 B) (Schriever et al., 2007). For most sites, we ensured the absence of point sources (wastewater treatment plants (WWTPs), farm drains, etc.) in the catchments to minimize chemical input from non-agricultural uses. Only at eleven of the 101 sampling sites, small WWTP (< 3000 population equivalents) were present upstream. Analyses of the data, however, showed that sites without upstream WWTPs also contained micropollutants from urban sources including markers of untreated wastewater (Neale et al., 2020) and that sites with and without the presence of small upstream WWTPs exhibited similar toxicity to invertebrates (Liess et al., 2021a). We reanalysed this data for the toxicity to algae and could also observe similar conditions between sites with and without WWTPs (see SI Fig. 4). This suggests that the pesticide toxicity towards aquatic invertebrate and algae of the present data set was not dominated by compounds of urban sources, which is in agreement with a study on Swiss streams (Munz et al., 2017). Another study on Swiss streams (catchment size 25 km²) concluded that biocide and pesticide concentrations from urban sources are as high as agricultural pesticides (Wittmer et al., 2010; Wittmer et al., 2011). However, in comparison to the stream sections studied here, the catchment area had several WWTPs that were the source for the majority of the biocide input. For certain active ingredients like mecoprop, biocide and pesticide loads from urban sources can exceed the load from the usage in agriculture.

3.2.2 Sampling design and chemical analysis

Water samples (n = 833) were obtained from mid-April to mid-July in 2018 and 2019 during the main application period of pesticides (Liess et al., 2021a). Samples were taken with two different techniques:

Grab samples (n = 521) were taken regularly every three weeks in accordance with the guidelines of the WFD (European Union, 2000). Additionally, EDS (n = 312) were taken with automatic sampling devices (MAXX TP5, Rangendingen, Germany) triggered by a water-level rise of more than 5 cm (adapted to the stream profile). Each EDS (on average 3 EDS were sampled per stream section and year) comprised a total of 40 subsamples that were taken continuously every five minutes (40 mL) for three hours and 20 minutes resulting in a mixed sample (Liess et al., 2021a). Samples were cooled in the samplers to 4°C, transported to the laboratory and analysed within two days. All water samples were subjected to target analysis with an LC-MS/MS (details see Halbach et al. (2021)). The analyte spectrum comprised 11 insecticides, 26 fungicides and 38 herbicides (for full list see SI Tab. 1). The herbicide glyphosate and the insecticide group of pyrethroids were excluded, as they could not be incorporated into the analytical method.

3.2.3 Toxicity assessment for freshwater communities

To estimate the toxicity of pesticides in water samples, concentrations of pesticides were converted into toxic units (TU) (Sprague, 1969). In detail, all measured concentrations c of the monitored chemicals in a sample were divided by the concentration where half of laboratory organisms exhibited an effect, termed effect concentration 50 (EC_{50}) (see equation 1).

$$TU = log_{10}\left(\frac{c_i}{EC_{50_i}}\right) \qquad (1)$$

We then calculated the maximum toxicity, the TUmax, for each sample based on the calculation of Liess and Ohe (2005) (see equation 2).

$$TU_{max} = \max(TU) \qquad (2)$$

All EC_{50} values were selected according to the criteria and date of the download of Liess et al. (2021a) (most sensitive taxon) and taken from the Pesticide Property Data Base (Lewis et al., 2016) and the United States Environmental Protection Agency Ecotoxicology (U.S. Environmental Protection Agency, 2019). To assess the toxicity of pesticides to invertebrates, the acute EC_{50} (48-96 hours) value of the most sensitive taxon, in this case either Daphnia magna or Chironomus sp. was chosen per pesticide (see SI Tab. 1). The toxicity of pesticides to freshwater algae or plants (hereafter referred to as algae toxicity) was evaluated with effect values from the acute 7-day biomass tests for aquatic plants (EC_{50}, Lemna gibba) and acute 72-hour growth inhibition tests for freshwater algae (EC_{50}, mostly Raphidocelis subcapitata and Scenedemus subspicatus; for full list see SI Tab. 1). The toxicity between invertebrates and algae is not directly comparable, as the endpoints of the EC_{50} values are different. While the short-term mortality is used as an endpoint for invertebrate tests, developmental parameters such as growth rate and biomass production are used as endpoints in the algae and aquatic plant tests, which can be more sensitive to pesticide exposure.

3.2.4 Application scheme and rainfall data

Pesticide application data was provided by the private institute for sustainable land management (INL Halle, Germany). The data set consists of 889 application schemes for different crop types that were present in the LRC of our stream sections (for list see SI Fig. 2). The application schemes were monitored in Germany and Austria during the years 2007 to 2015. Despite a missing temporal or spatial link between the spray series and the stream monitoring data, we consider the general temporal patterns to be sufficiently representative for our purpose. For a further description of the data set see Weisner et al. (2021) and Knillmann et al. (2021). We only used applications that reflected the crops present in the investigated catchments in our analyses (see SI Fig. 2). Using the respective application scheme data, the relative rate of applications was calculated per week by dividing the weekly amount of applications (kg/ha) by the total amount of applied pesticides for each pesticide type (see equation 3). The weekly amount of applications was averaged regardless of the crop type, as these were distributed rather heterogeneously in the catchments and, in most cases, without a single dominating crop type.

$$relative\ application\ rate_a = \left(\frac{weekly\ amount\ of\ application_a}{total\ amount\ of\ application_a} \times 100\right) \quad (3)$$

a = pesticide type (herbicides, fungicides and insecticides)

An application is defined as a field spray event where one or multiple pesticides may be present in a tank mix. We considered the data sets of the application rate and measured pesticide data compatible for our purpose, as they were sampled during several years.

The daily amount of rain was obtained from interpolated radar weather data from the German weather service (DWD) in 2018 and 2019 with a spatial resolution of 1 km² in the LRC (Deutscher Wetterdienst (DWD), 2020; Rauthe et al., 2013).

3.2.5 Data analysis

3.2.5.1 Monthly exposure variations

Time series of the relative application rate and toxicity data were compared with generalized additive models (GAM) to identify periods of similar change. The non-linear modelling was performed in R with the “mgcv-package” (version 1.8-31) (Wood, 2017). Calculation of related confidence intervals to identify periods of significant increase or decrease were done according to Simpson (2014). The model for the relative application rate and toxicity data contained weekly and daily aggregated values of applications (n) and toxicity, respectively. For toxicity, the three most toxic pesticides per sample and each for invertebrates and algae were used, as they reflect the maximum toxicity exposure and are thus robust to outliers.

3.2.5.2 Daily exposure variations

We compared the maximum toxicity (TU_{max}) for invertebrates and algae in EDS and grab samples, both related to the same period of time, using two-sided unpaired t-tests. The data fulfilled assumptions for normal distribution and variance homogeneity. For this comparison, we focused on EDS and corresponding grab samples (1-3 days delay after EDS) from May and June (2018 and 2019) as these were carried out during the period of highest pesticide applications (based on the results from chapter 3.1.1). We also examined the decrease in toxicity in grab samples collected 4-10 days after a rain event compared to EDS collected during the corresponding rain event.

3.2.5.3 Comparison between exposure and regulatory protection goals

We assessed the compliance with the German National Action Plan (NAP) for sustainable use of pesticides goal that limits RAC exceedances to 1% of all EDS taken over the period of one year (BMEL, 2013). In our data set, 1% corresponds to 9 EDS. We assumed that outside of our period of investigation (August to March), no RAC exceedance would have occurred (312 EDS in four months, considering 12 months = 936 EDS, 1% = 9 EDS) to not overfit the number of exceedances and to be conservative. 9 EDS with one RAC exceedance would still comply with the goal of the German NAP (BMEL, 2013). If a tenth sample exceeded the RAC, the goal set in the German NAP would be missed. We assessed the factor of German NAP exceedance of the tenth EDS by sorting the concentration to RAC ratio in descending order. This approach considers the maximum necessary reduction of all RAC-exceeding substances together. Substances that are less likely to exceed the RAC have a lower factor of exceedance.

3.2.5.4 General information on data processing, visualisation and availability

Data were processed using the software R (version 4.0.2) (R Core Team, 2020). All diagrams were generated with the R package “ggplot2” (version 3.3.2) (Wickham, 2009). All data concerning the stream sampling is publicly available under Liess et al. (2021b).

3.3 Results & Discussion

3.3.1 Monthly trends of pesticide toxicity between April and July

3.3.1.1 Translation from pesticide application and aquatic exposure

We used existing application data and the large scale in-stream monitoring data with event-related samples to compare monthly trends in pesticide application and aquatic exposure. For all pesticide groups, the relative application rate in the years from 2007 to 2015 increased significantly from the beginning of March to May and decreased until August (Fig. 1A). While herbicide and fungicide applications peaked in mid-May, most insecticides were applied in June. A lower but longer lasting peak was observed for fungicide use than for herbicide and insecticide use. The relative application rate of fungicides increased by at least 5% for two months, while this was only three weeks for insecticides and herbicides.

Comparing the monthly application trends to the in-stream toxicity in the years 2018 and 2019, the maximum invertebrate toxicity in EDS increased by a factor of 7.9 from April (-3 TU_{max}) to the end of May (-2.1 TU) (Fig. 1B). The peak of in-stream invertebrate toxicity is lagging two weeks behind the application peak of insecticides and fungicides, but with a similar temporal trend of increase and decrease. This indicates a direct temporal link between the application and the resulting maximum toxicity to invertebrates in EDS at larger scales. Toxicity to invertebrates is mainly driven by insecticides and to a lesser extent by fungicides. After the peak in May, toxicity to invertebrates decreased 10-fold by July (-3.1 TU). The range of toxicity values and 5th and 95th percentiles are shown in SI Fig. 5. The 5th and 95th percentiles of EDS are generally above those of grab samples.

The algae toxicity in EDS increased by a factor of 5 from May (-2.1 TU) to the beginning of June (-1.4 TU) (Fig. 1C). The peak in algae toxicity lags three weeks behind the herbicide application peak (mid of May). This is due to annual differences between 2018 and 2019 (see SI Fig. 6), where algae toxicity was not as pronounced in 2019 as in 2018, which is additionally shifted to mid-June with a lag of 3 weeks. The weekly average rainfall was similar between May and June in 2018 and 2019, through the distribution differed with stronger concentration towards May and June in 2019 (see SI Fig. 7).

Our results match those of previous studies, where a direct temporal link between the application of pesticides and the exposure peaks in single catchments was found (Hladik et al., 2014; Leu et al., 2004b; Vryzas et al., 2009). A recent study showed that within 10 days 69% of the applied amount of two pesticides was transported from soil to surface water in one catchment (Commelin et al., 2022). Our large-scale data with many spatially distributed catchments showed a similar temporal linkage between application and resulting toxicity. In addition, we found that the monthly trend in algae toxicity between

May and June for the investigated measuring period matched the monthly trend of invertebrate toxicity (Fig. 1B and C). A large part of the increased pesticide exposure could be covered by EDS obtained from May to June, as all three pesticide groups have their exposure peak during this period in Europe. However, the exposure resulting from pesticide application can vary depending on the region, application techniques and climatic conditions (O'Brien et al., 2016). In a recent study conducted in Australia in a catchment in the dry tropics, the highest pesticide concentrations were determined during rainfall-induced runoff following the sugarcane growing season from July to December (O'Brien et al., 2016). Based on this study and our results, we conclude that pesticide exposure peaks are temporally linked to the maximum application period, though this likely varies with crop and climatic region and the pattern may be very different in regions with dry growing seasons.

3.3.1.2 Differences between monthly trends of EDS and grab samples

Throughout the complete measurement period from April to mid-July, toxicity to invertebrates and algae in EDS was on average a factor of 10 and 6.3 higher than in grab samples, respectively. Grab samples showed weaker change in toxicity to invertebrates and algae from April to May/June than EDS (Fig. 1B und C). Previous studies also found higher pesticide exposure in late-spring to summer from gab samples (Smiley et al., 2014; Szöcs et al., 2017). However, our results indicate that the grab sample toxicity to invertebrates and algae remained relatively stable during the study period.

The risk posed to invertebrates could be underestimated since pyrethroids, a class of insecticides widely used in Germany, were not measured. An analysis of the application rate of pyrethroids indicates that pyrethroids showed a similar application pattern compared to other insecticide groups (SI Fig. 8). We therefore expect a similar period of exposure (May/June) for pyrethroids.

3.3.1.3 Monthly trends on substance level

For invertebrates, the neonicotinoids thiacloprid (27.4%) and imidacloprid (12.4%) were most frequently among the three most toxic pesticides per EDS (see SI Fig. 9). Both insecticides were also the most dominant toxicity drivers in EDS in Germany during each of the investigated months (April to July). The respective temporal patterns can be seen in detail for each pesticide in SI Tab. 2 displayed as weekly averaged toxicity divided by the total toxicity.

The most toxic pesticides to algae were flufenacet (19.4%), terbuthylazine (16.6%) and dimethenamid-P (12.2%). All three pesticides exhibited a similar level of toxicity in Germany during the investigation period from April to July. For the monthly trend of each pesticide see SI Fig. 10 and 11.

The summed toxicity (TU_{sum}) according to the approach of concentration addition (Loewe and Muischnek, 1926) is strongly determined (69% across all samples for invertebrates) from the most toxic pesticide per sample (Liess et al., 2021a). Accordingly, the TU_{sum} and TU_{max} for each sample exhibit a strong association for both invertebrates ($R^2 = 0.98$) and algae ($R^2 = 0.96$) in EDS and grab samples. Moreover, in an analysis of multiple data sets on small agricultural streams, the TU_{sum} and TU_{max} exhibited a very similar explanatory power for ecological responses (Liess and Ohe, 2005; Schäfer et al., 2013). Thus, we used the TU_{max} as the most parsimonious descriptor of toxicity.

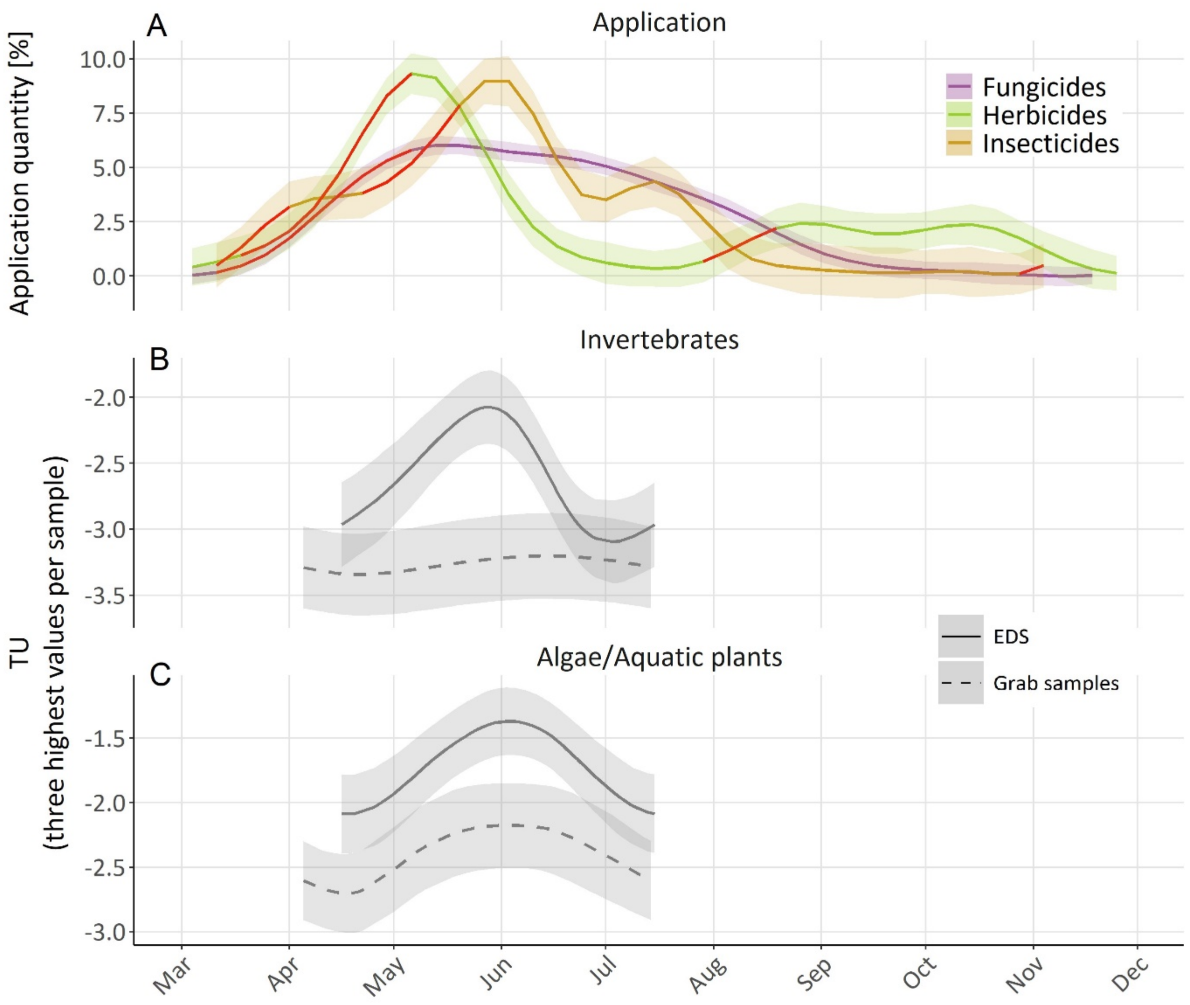


Figure 12: Time series of the relative application rate (%) (A) and the three highest logarithmic toxic units per sample (TU) (pesticides) for (B) invertebrates and (C) algae/aquatic plants (algae). The generalised additive model with 0.95 confidence interval of the application rate and toxicity towards invertebrates and algae show the temporal patterns for herbicides, fungicides and insecticides in case of applications (A) and grab and event-driven samples (EDS) at 101 stream sections for toxicity (B and C). (A): The relative application rates are expressed as application rate (kg/ha) per week for each field divided by the annual total number of applications per field. Red lines indicate seasons of significant increase of applications. (B) and (C): The curves for toxicity show aggregated values from the two campaign years 2018 and 2019. For the difference in toxicity between the two sampling years 2018 and 2019 see SI Fig. 6. The dashed curves represent grab samples while the solid line shows EDS.

3.3.2 Daily changes of pesticide toxicity

A complete risk assessment of pesticides that considers all exposures causing effects on the aquatic community, requires consideration of short-term (daily) pesticide peaks associated with surface water runoff. We investigated the short-term differences of invertebrate and algae toxicity between EDS and grab samples conducted within 1-3 days of the respective EDS (Fig. 2). In total, 51 grab samples (9.8% of all grab samples) were collected on days following EDS (after 1 day n = 19, after 2-3 days n = 32) during May and June. Overall, we detected decreasing toxicity with increasing time after a rain event (Fig. 2). The toxicity to invertebrates was statistically significantly higher in EDS compared to grab sample toxicity 1 day (3.7 times more toxic, $p = 0.009$) and 2-3 days (10 times more toxic, $p < 0.001$) after the respective EDS. For algae, toxicity was only statistically significantly higher in EDS than grab samples taken 2-3 days (1.6 times more toxic, $p = 0.036$) after the respective EDS. Toxicity to algae in grab samples taken 1 day after the EDS was 1.3 times lower than the toxicity in EDS, but not statistically significantly (p = 0.067). The decrease in toxicity estimated from grab samples levelled off 4-10 days after the rainfall event. Recent studies using event-related sampling have found that peak exposure in small streams lasts only a few hours (Carpenter et al., 2019; La Cecilia et al., 2021). The difference between the toxicity of the EDS and the following grab sample toxicity (1 - 3 days) was larger for invertebrates compared to algae. A similar decrease in herbicide concentrations over 2 to 3 days after a rain event was observed in a previous study in one catchment with atrazine, dimethenamid and metolachlor (Leu et al., 2004a). Additionally, Leu et al. (2004a) observed that all three pesticides had similar temporal occurrence patterns. They concluded, that the transport is dominated by the transport dynamic of surface runoff and/or of preferential flow and to lesser extent by the different chemical properties of the three herbicides. Additionally, drainages could influence the pesticide exposure pattern after rain events. A study from Denmark showed that after a rain event drainage led to a longer elevated exposure of the herbicide bentazon compared to the analysed fungicides and insecticides (Kronvang et al., 2004). Another study from Australia showed that drainage delayed the maximum concentration of different herbicides in streams up to 4 days after a rainfall event (20 mm) (Tran et al., 2007). Indeed, we also found that the insecticide exposure and toxicity to invertebrates declined more rapidly after a rain event compared to herbicides/algae. The pesticide toxicity shown is estimated based on different laboratory test systems (acute and chronic) (see details in section 2.3). According to the aquatic guidance document (European Food Safety Authority (EFSA), 2013), concentrations must not exceed the RAC at any time, regardless of the origin of the underlying experimental data. Our results show that pesticide exposures to invertebrates in small streams reaches its maximum at the day of the rain event with a subsequent rapid decline within a short time period (< 24 h). Although this time span is shorter than acute laboratory experiments (48-96 h), several studies have demonstrated a close association between TU's from EDS and ecological responses (Liess et al., 2021a; Schäfer et al., 2013; Schäfer, 2019). This indicates that event-related sampling is required to detect potential influences of pesticide toxicity on the invertebrate community.

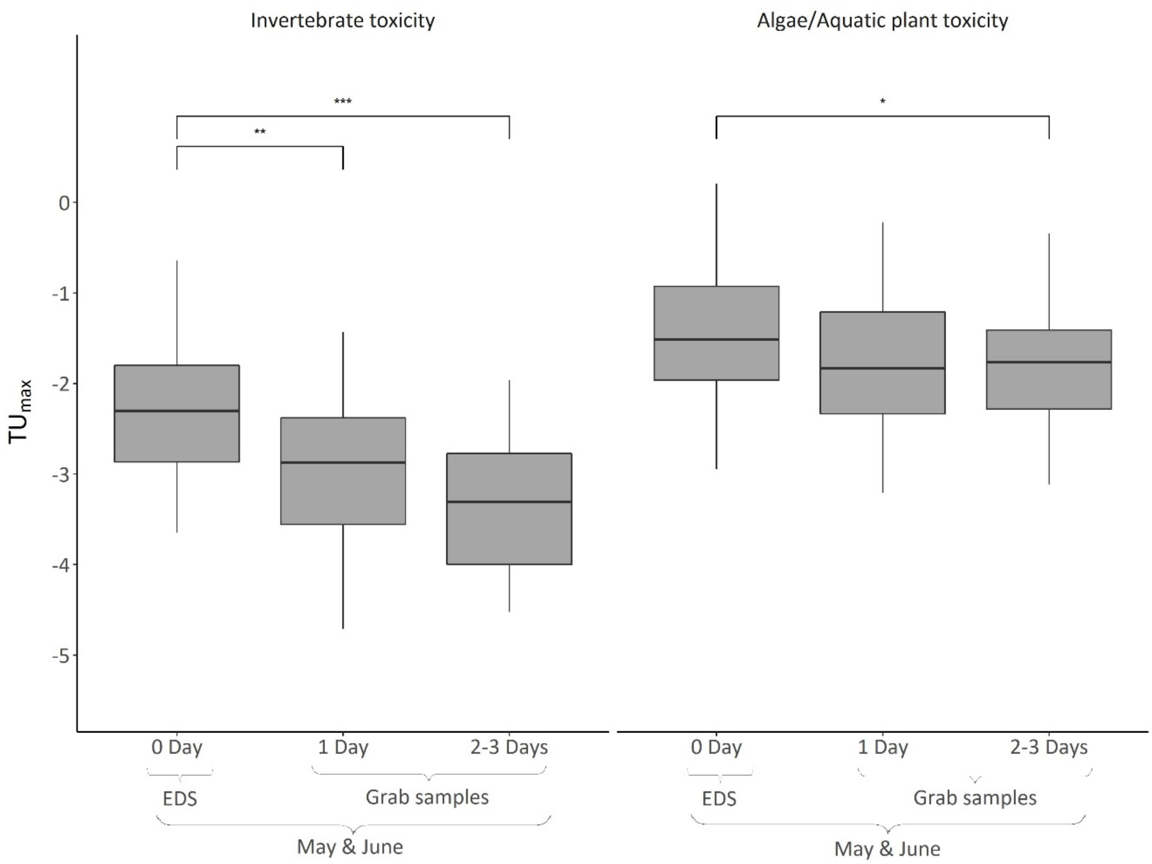


Figure 13: Comparison of TU_{max} per sample of event-driven samples (EDS) (n = 51) and grab samples for invertebrates and algae/aquatic plants. Grab samples were taken 1 day (n = 19) and 2-3 days (n = 32) after the corresponding EDS. The main pesticide application period (May and June) as identified from Fig. 1. Two-sided unpaired t-test (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$) were performed between each sampling method and corrected for multiple testing using the Bonferroni correction. The toxicity to invertebrates and algae was derived from different test systems (for more details see chapter 2.3 Toxicity assessment for freshwater communities).

3.3.3 Monthly trend of RAC exceedances

RAC thresholds were introduced as limits that should not be exceeded by pesticide concentrations and are assumed to then be sufficiently protective for aquatic organisms based on protection goals. It has been found that they are frequently exceeded, but the temporal variability of exceedances has not been studied. Therefore, we analysed how temporal pesticide exposure dynamics translate into RAC exceedances in order to give future monitoring programmes orientation (Fig. 3). 37 of the 76 investigated pesticides exceeded their RAC at least once during the two sampling periods in 2018 and 2019. Averaging for all pesticides analysed, EDS revealed 2.7 times more RAC exceedances (insecticides = 2.1, fungicides = 2.3 and herbicides = 3.8) than grab samples. For the RAC exceedances of grab samples see SI Fig. 12. Same as for the monthly and daily peaks in toxicity, EDS revealed 2.6 times more RAC exceedances compared to grab samples.

We found the highest average rate of RAC exceedances in May EDS with 1.6 per sample (April = 0.8, June = 1.2 and July = 0.9). 57% of all pesticide-specific RAC exceedances occurred in May coinciding with the trend of toxicity and application. Concerning pesticide-specific RAC exceedances, between April and July, small agricultural streams as analysed in this study experienced on average 4.3 RAC exceedances, showing that unacceptable effects from the perspective of regulators on the environment may occur

(European Food Safety Authority (EFSA), 2013). However, the RAC is derived using data of the most sensitive test organism for which data is available. Given potential differences in data availability and sensitivity to a specific compound, this means that the RACs can reflect different test organisms and test types (i.e. chronic and acute). While this suggests against inferring comparable ecological impacts directly from RAC exceedances, previous studies have found a strong link between RAC exceedances and impacts on invertebrates (Liess et al., 2021a). Only eleven pesticides exceeded the RAC in April, we found 30 pesticides with exceedances in May, 24 in June and nine in July. Significantly more RAC exceedances were found for insecticides (n = 274) compared to herbicides (n = 106) and fungicides (n = 23) (p = 0.002 for herbicides and p = 0.009 for fungicides).

For six pesticides, only EDS enabled to detect RAC exceedances (Fig. 3). These were the herbicides and fungicides 2,4-D, cyazofamid, ethofumesat, flufenacet, mecoprop-p and metamitron (details in SI Tab. 1). 45% of RAC exceedances were caused by the pesticide group of neonicotinoids. The neonicotinoids acetamiprid, clothianidin and thiamethoxam were measured in higher concentrations in May and June. Thiacloprid and imidacloprid could be detected even 4 times more often in May EDS compared to EDS from April, June or July. RAC exceedances of neonicotinoids occurred at most sites (71%). These results show that neonicotinoid exposure is widespread across our sampling sites. However, in our comparison of in-stream pesticide concentrations and RAC values, it should be noted that the RAC values are based on varying test species and durations (2 - 434 days, for more details see SI Tab. 1). This is a potential source of error as some aquatic organisms may be less affected by exposure to the in-stream pesticide concentrations, which are a result of a 200-minute sampling. These in-stream concentrations are compared with RAC values derived from tests with a duration of several days (median test duration = 14 days). Nevertheless, according to the aquatic guidance document (European Food Safety Authority (EFSA), 2013), concentrations must not exceed the RAC at any time, regardless of the origin of the underlying experimental data. For more information about individual pesticide occurrences and concentration levels see Halbach et al. (2021).

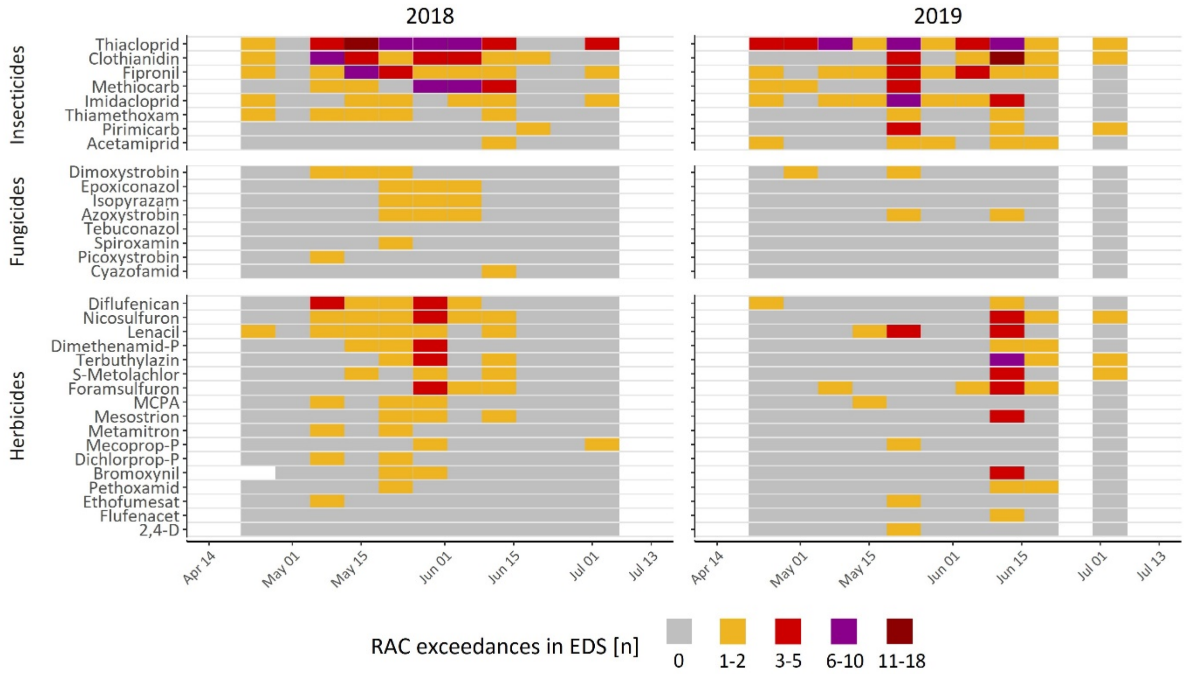


Figure 14: Weekly averaged number of regulatory acceptable concentration (RAC) exceedances in event-driven samples (EDS) per pesticide during the two sampling periods in 2018 and 2019 at 101 stream sections. For RAC exceedances of grab samples see SI Fig. 12. Only pesticides with at least one RAC exceedance are listed. The coloured squares represent the measured number of exceedances of the respective week. The Y-axis is grouped by pesticide class and sorted in descending order by the summed number of exceedances per pesticide.

3.3.4 Comparison between reality and the goals of the German national action plan (NAP) for pesticides

According to the aims of the German NAP for sustainable use of pesticides, 99% of EDS should remain below the RAC until 2023 (BMEL, 2013). Using our data which exhibited frequent RAC exceedances, we estimated the required reduction of pesticide concentrations in streams to comply with the goals of the German NAP. Assuming that no RAC exceedance occur during the off-season (August to March), 9 EDS above the RAC would be allowed to comply with the NAP-goal (further details see chapter 2.5.3). We assessed whether our 10 highest ratios of concentrations from EDS to RACs exceed 1, i.e. are above the RAC (Fig. 4). Given, that the tenth highest concentration to RAC ratio exceeded the RAC by a factor of 37, related pesticide concentrations in our streams would need to be reduced by a factor of 37 to meet the aims of the German NAP. For the given pesticide spectrum, this factor indicates the reduction that would be necessary to achieve only 1% of EDS above RAC. The size of this factor was driven by substances with very frequent RAC exceedances such as neonicotinoids. At the same time, other substances would have to be reduced by a lower factor in substance-specific terms. It should be noted that this calculation is to a certain extent dependent on the peak values of the statistical population. However, this is also due to the ambitious goal of the German NAP that 99% of the samples in a year should be free from RAC exceedance.

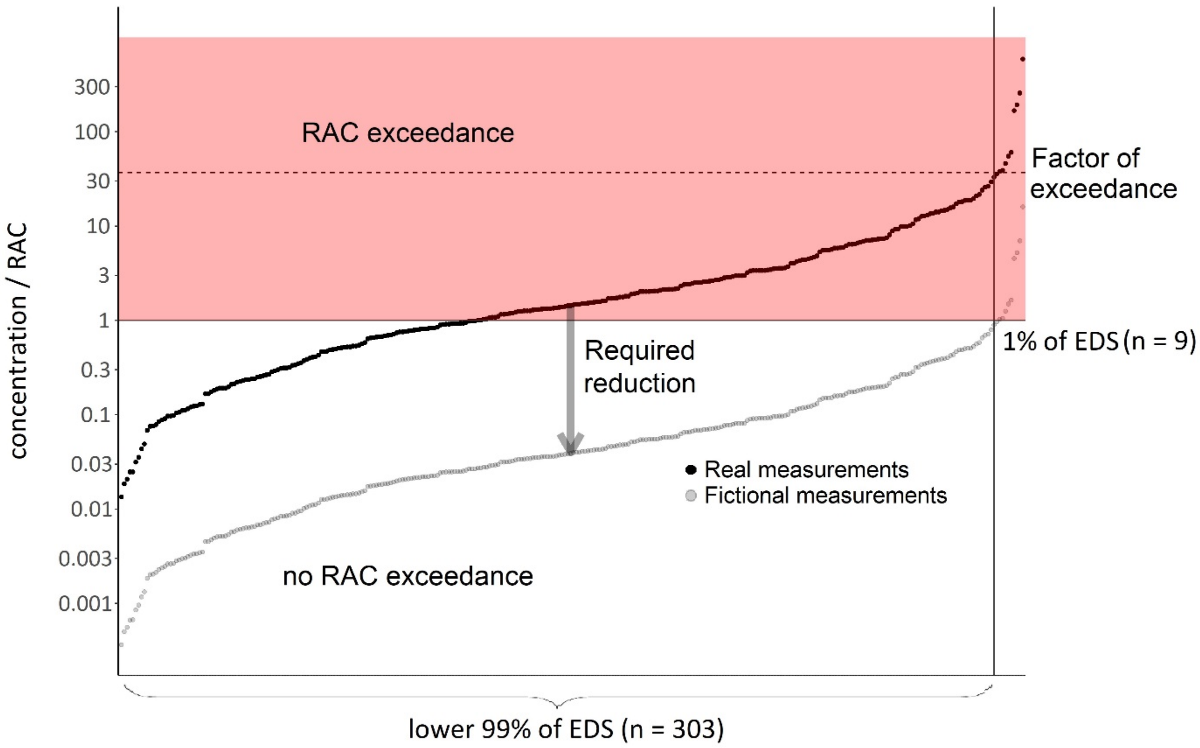


Figure 15: Distribution of the maximum concentration to regulatory acceptable concentration (RAC) ratios per sample of all event-driven samples (EDS) (n = 312). Only the ratio of maximum concentration to RAC per sample was used, independent of the substance. Ratios (>1) falling in the red area represent RAC exceedances. Given that 60.6% of EDS (n=189) have exceeded the RAC (red area), the chemical status of small freshwater streams in Germany therefore fails to meet the German National Action Plan (NAP) for sustainable use of pesticides goal. The dashed line indicates the factor of exceedance for the German NAP protection goal at the Y-axis.

3.3.5 Influence of precipitation amount on in-stream toxicity of pesticides

We did not observe an increase of toxicity in EDS with increasing amount of precipitation (invertebrates $p = 0.26$, $R^2 = 0.005$; algae $p <= 0.001$, $R^2 = 0.05$) but found that events with less than 15 mm of precipitation can already lead to median TU_{max} values of -2.6 for invertebrates and -1.8 for algae (SI Fig. 13). This toxic pressure is ecologically relevant at least for invertebrates, where unacceptable changes in the community already occur above a log TU_{max} of -3.3 (Liess et al., 2021a). We also did not find a positive correlation between the amount of precipitation (> 15 mm) and pesticide concentrations of insecticides ($p = 0.42$, $R^2 <= 0.005$), herbicides ($p = 0.001$, $R^2 <= 0.005$) or fungicides ($p = 0.69$, $R^2 <= 0.005$). In contrast, previous studies found a positive correlation between the volume of surface runoff and the amount of rain (Nearing et al., 2005; Schulz, 2004). Although overland transport of pesticides in the particulate phase is a substantial transport pathway (Commelin et al., 2022), hydrological conditions such as increased soil moisture prior to a small rain event (< 15 mm) play a major role in the mobilisation of pollutants (Rabiet et al., 2010). Previous studies have shown that overland transport caused the formation of a temporary pool of pesticides in the saturated zone, which was then sufficiently large to maintain elevated concentrations through leaching over longer periods of time and reactivation by subsequent minor events (Leu et al., 2004a; Louchart et al., 2001). Another reason for the missing correlation between pesticide exposure and amount of precipitation may be the fact that our precipitation data represented interpolated radar data, which has been shown to insufficiently quantify small-scale heavy rainfall events (Sokol et al., 2021).

3.3.6 Recommendations for the Water Framework Directive (WFD) monitoring

The monitoring of the Water Framework Directive (WFD) aims to characterise the chemical and ecological quality of various waters. This monitoring of streams fails to adequately assess the risk from pesticides for reasons such as lacking event-related sampling, too narrow analyte spectrum and missing availability of regulatory thresholds (Weisner et al., 2022). Regarding agricultural pesticides, our results indicate for Germany and regions with comparable climate conditions and cultivation practices that time and cost-intensive EDS could be limited to the main application period to adequately assess the highest pesticide exposure to invertebrates and algae, though for individual compounds and where fungicides drive the overall toxicity the maximum may occur outside of this window. The optimal time period for event-related sampling of application peaks can vary depending on the crop, application technique, region and climatic conditions and should be adjusted accordingly. While we likely covered the period in Germany that is most relevant for pesticide toxicity towards invertebrates (Szöcs et al., 2017), in particular for algae toxicologically relevant exposure peaks may occur before and after winter, given intensive herbicide use in this period. Overall, pesticide risk assessment with the RAC or environmental quality standards is only reliable if all relevant exposure is characterised appropriately with EDS during the major application periods (e.g. April to July for insecticides and risks to invertebrates). Pesticide risk assessment with the RAC value or environmental quality standards can only be fully comprehensive with EDS obtained during this period (April to July). Also rain events with lower precipitation (< 15 mm) can cause high pesticide exposures during the main application period in May/June and should also be monitored with EDS.

3.4 Conclusion

For 101 agricultural streams in Germany, we observed highest pesticide toxicity to invertebrates and algae/aquatic plants (algae) during end of May to mid-June. This temporal pattern coincides with the main pesticide application period and maximum in-stream pesticide toxicity and was largely independent of the amount of precipitation measured in the catchment. Regarding daily changes in pesticide toxicity, already two to three days after a rainfall event, grab sampling underestimated the peak pesticide toxicity by average factors of up to 10 for invertebrates. Sampling exposure peaks during the main application period or during individual crop-related application peaks is hence indispensable to appropriately characterise pesticide-related ecological risks. Mandatory collection and public access to pesticide application data would strongly improve our ability to relate application and exposure data and to establish models that can predict exposure peaks. Considering that governmental monitoring currently only requires one grab sample per month, an underestimation of the actual pesticide exposure is very likely. Event-driven sampling in addition to grab sampling is required to account for short-term concentration maxima. The provided temporal patterns of pesticide exposure and toxicity may help future monitoring programs to adapt to monthly and daily trends of pesticide toxicity and focus measures to reduce pesticide exposure. Current in-stream exposure, including neonicotinoids, would have to be reduced by a factor of 37 to meet the German National Action Plan target of 99% of a year's event-related samples without a RAC exceedance.

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4 Pesticides are the dominant stressors for vulnerable insects in lowland streams

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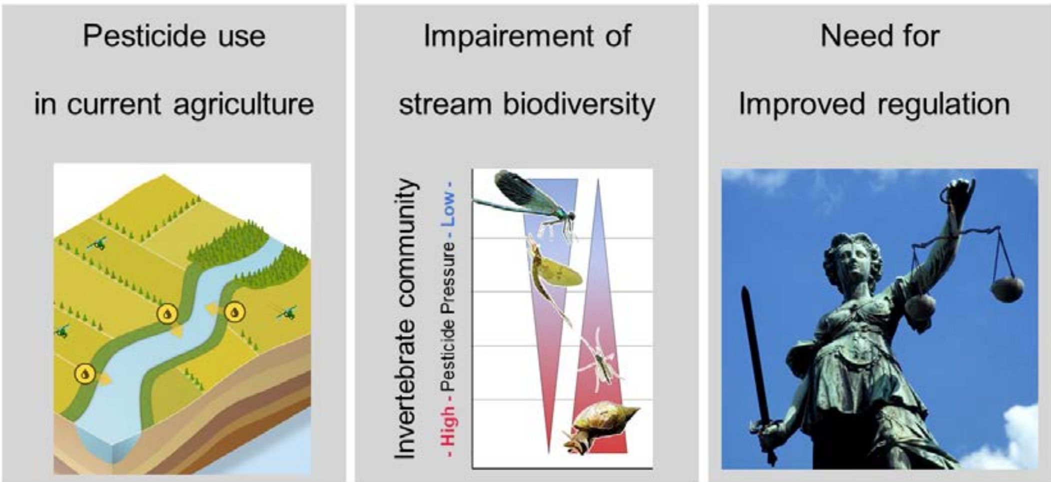
Abstract

Despite elaborate regulation of agricultural pesticides, their occurrence in non-target areas has been linked to adverse ecological effects on insects in several field investigations. Their quantitative role in contributing to the biodiversity crisis is, however, still not known. In a large-scale study across 101 sites of small lowland streams in Central Europe we revealed that 83% of agricultural streams did not meet the pesticide-related ecological targets. For the first time we identified that agricultural nonpoint-source pesticide pollution was the major driver in reducing vulnerable insects in aquatic invertebrate communities, exceeding the relevance of other anthropogenic stressors such as poor hydro-morphological structure. We revealed that the current authorisation of pesticides, which aims to prevent adverse effects, underestimates the actual ecological risk as (i) measured pesticide concentrations exceeded current regulatory threshold levels in 81% of the agricultural streams investigated, (ii) for several pesticides the inertia of the authorisation process impedes the incorporation of new scientific knowledge and (iii) existing thresholds of invertebrate toxicity drivers are not protective by a factor of 5.3 to 40. To provide more reliable thresholds, the authorization process needs to include monitoring-derived information on pesticide effects at the ecosystem level. Here, we derive thresholds that ensure a protection of the invertebrate stream community.



4.1 Introduction

The ongoing biodiversity crisis is caused by a variety of anthropogenic stressors including pesticides (Agency, 2015). However, great uncertainty remains about the respective contribution of various stressors to ecosystem degradation. This debate also relates to agricultural pesticides as some investigations have identified strong impacts of nonpoint-source pesticide pollution on streams in Australia (Beketov et al., 2013), Europe (Beketov et al., 2013; Liess and Von Der Ohe, 2005), North America (Chiu et al., 2016) and South America (Hunt et al., 2017) while others only identified comparatively low impacts of pesticides (Noges et al., 2016). Accordingly, the question remains how severe the effects of pesticides are compared to other stressors and, more specifically, at which concentrations ecosystem effects occur and which species and functional parameters are affected. Only with this knowledge it is possible to prioritize and manage stressors effectively.

The regulatory authorisation of agricultural pesticides is supposed to prevent unacceptable effects in the environment. For example in Australia, the EU and the US, an extensive test-system based assessment scheme to protect communities in non-target aquatic ecosystems has been established (Australian Environment Agency, 2009; EFSA, 2013; US Goverment, 2004). This regulatory framework is based on the concept of scaling the effect of individual pesticides in single-species test systems or model ecosystems to the effect in the ecosystem. On this basis, pesticide concentrations are determined at which damage to aquatic communities can be excluded. However, the natural and anthropogenic stressors present in the ecosystem are not systematically included. Nor has there been any validation of the prediction of ecosystem effects to date.

In this investigation we therefore performed a monitoring in a large geographical area that allows us to quantify all relevant anthropogenic stressors with high temporal resolution. Additionally, we identified the stream invertebrate community as a measure of ecological quality. On this basis, we aimed (i) to model the relative contribution of environmental variables determining the occurrence of aquatic invertebrates and to attribute measured pesticide pressure to ecological status, (ii) to evaluate the protectivity of the aquatic pesticide risk assessment and (iii) to derive evidence-based thresholds for the effects of pesticides considering the presence of additional stressors relevant to the ecosystem.

4.2 Materials and Methods

4.2.1 Site Selection

A total of 101 stream sections distributed over Germany were sampled in April and July for 2018 and 2019 (see map in SI Figure 1), 11 sites were monitored both years. The initial selection comprised 124 stream sections, however, we omitted those stream sections that were affected by drought (lack of flow, drying out) or where the automatic rain Event-Driven Samplers (EDS) did not function (EDS: SI chapter 3). The catchment areas of the monitoring sites were characterized by a gradient of agricultural land use (agricultural land cover in hydrological catchment 0- 100%) and less than 5% of urban areas to focus on agricultural diffuse source pollution. 86 streams were located in agricultural environments (agricultural land cover in hydrological catchment > 20%, referred to as “agricultural” streams) whereas 15 streams were located in areas with less agricultural influence (agricultural land cover in catchment < 20%, see SI chapter 1 for land use analyses). Catchment sizes were generally below 30 km² to represent small lowland streams where those with a catchment greater than 10 km^2 (n = 60) correspond to the reporting requirements of the WFD (Commision, 2000); stream sections with a catchment size of less than 10 km^2 (n = 41) corresponding to the "edge-of-field" surface waters of the EU-EFSA risk assessment of plant protection products for aquatic organisms (EFSA, 2013). Detailed site characteristics are listed in Tab. SI 1.

4.2.2 Water Sampling and Chemical Analyses

Streams were sampled from April to July in 2018 and 2019 during the main application period of pesticides in spring and early summer for most crops (Szöcs et al., 2017). During this time period grab samples (n = 520) were taken regularly in a three-week cycle. This sampling method followed the monthly sampling in governmental monitoring practices under the WFD regardless of weather conditions. EDS samples (n = 320) were taken with automated (MAXX TP5, Rangendingen, Germany) and bottle samplers (Liess and Von Der Ohe, 2005) in order to capture runoff-induced exposure peaks associated with heavy rainfall (Liess et al., 1999), (see Figure SI 5). Small streams with agricultural catchment area are subject to short-term water level rise (Liess and Von Der Ohe, 2005) with the occurrence of storm events exceeding approximately 10 mm/d (Schulz et al., 1998). EDS sampling was triggered by a rise of water level of more than 5 cm so that waves did not trigger the sampling and every runoff event could be captured. (further details see SI chapter 3). The total of 840 samples of both field campaigns 2018 (n = 411) and 2019 (n = 429) were analyzed for pesticides, trace elements and nutrients.

For pesticide analysis, water samples were filtered and analyzed via direct injection into LC-MS/MS without enrichment by multiple-reaction-monitoring (Reemtsma et al., 2013) (details see SI chapter 4). The target analysis tested for 75 pesticides and 33 pesticide metabolites. Pyrethroid insecticides and the herbicide Glyphosate were not included due to analytical limitations. The compound selection was established by prioritization according to active substance-related sale quantities, the consideration of current environmental quality standards (EQS) and the regulatory acceptable concentrations (RAC) (Brinke et al., 2017), (see Tab. SI 2).

To test for further urban toxicants, the samples of 2018 were additionally subjected to LC-HRMS/MS screening analytics (details see SI chapter 6). This screening analyses tested for 257 substances, which were grouped into 16 compound classes including pharmaceuticals, industrial chemicals, rubber additives, stimulants, corrosion inhibitors, plastic additives, sweeteners, biocides, UV filters, bitterns, repellents, per- and polyfluorinated compounds, food ingredients, surfactants, dyes and flame retardants (see Tab. SI 4).

The concentrations of trace elements (arsenic, cadmium, copper, zinc, lead, mercury) were analyzed in water samples using Agilent's ICP-MS 8000 Triple Quad. At the site the samples were pre-filtered (20 ml, 0.45 µm) for arsenic, cadmium, copper, zinc, lead, while mercury samples were bottled unfiltered in a stabilizing solution of nitric acid and potassium dichromate.

4.2.3 Scaling Concentrations for Toxicity

Concentrations of pesticides and trace elements were converted to invertebrate toxicity by calculating Toxic Units (TUs), where measured substance concentrations are normalized to their respective LC_{50} in acute standard laboratory test systems (Sprague, 1969). These LC_{50} values were derived from *Daphnia magna* or *Chironomus sp.* whose acute sensitivity, when considering a wide range of organic toxicants, is approximately equal or slightly less than the acute sensitivity of many insects (Morrissey et al., 2015; von der Ohe and Liess, 2004). For the TU calculation, the LC_{50} of the most sensitive species was considered and retrieved from the Pesticide Property Data Base (PPDB) and in few cases the US EPA ECOTOXicology knowledgebase, if the PPDB lacked respective data (see Tab. SI 3), (Lewis et al., 2016). In case no experimental data was available (0% of target analytes, 57% of non-target analyte LC_{50} values, mostly urban contaminants also including rubber additives as street-runoff indicators), Quantitative

Structure Activity Relationship (QSAR)-derived effect concentrations were used to estimate TUs (Busch et al., 2016).

Pesticide peak exposure (TU_{max}) in streams toxic to invertebrates was determined by the maximum single substance insecticidal toxicity measured (Liess and Von Der Ohe, 2005) (TU_{max}, see Tab. SI 1). Extending this calculation method, we identified that exceptionally toxic samples, that are highly unusual in the exposure profile of the respective stream, did not reflect the ecological situation ($SPEAR_{pesticides}$) and were therefore not considered in the TU_{max} calculation. These exceptional exposure peaks, encountered in 20% of streams (n = 20), were defined by a TU_{max} exceeding the mean TU_{max} of the five subsequent samples (ranked by TU_{max}) by a factor of more than 100. An inclusion of exceptionally high single pulses led to a weaker correlation between the toxic pressure and the ecological effect on vulnerable species ($SPEAR_{pesticides}$) (R^2 = 0.34 versus R^2= 0.43 with and without high pulses considered). The authors are not aware of studies that have identified the reduced significance of an exceptionally high toxicant pulse compared to many, significantly lower pulses. In contrast, the great ecotoxicological significance of several successive toxicity pulses was recognized; the "culmination“ of low-dose pesticide effects (Liess et al., 2013). Analogously, the typical peak pesticide mixture toxicity (TU_{sum}) was determined by summing all individual substance TUs detected in a sample. To assess regulatory thresholds, pesticide concentrations were also scaled by the RACs instead of the LC_{50} values (see SI chapter 11). The toxicity of urban toxicants was determined in the same way as for pesticides (see Tab. SI 4). The toxicity of trace elements was calculated using literature LC_{50} values (Liess et al., 2017; Tsui and Wang, 2005), see Tab. SI 3). Here, the local maximum of summed TUs (TU_{sum}) including all trace elements per sample is considered in the multiple linear regression.

4.2.4 Further Abiotic Parameters

Ortho-phosphate, nitrate, nitrite and ammonium concentrations were determined in all grab and EDS samples using either colorimetric tests by "Visicolor" (MColortest, Merck KGaA; Darmstadt, Germany) or a UV spectrophotometer (PF-12 and visocolor ECO tests, Machery-Nagel, Düren, Germany) in 2018 and a UV spectrophotometer (DR 1900, Hach Lange GmbH; Düsseldorf, Germany) in 2019. Furthermore, total phosphorus (TP) and total nitrogen (TN) contents of all water samples were analyzed (ICP-MS 8800 Triple Quad from Agilent). Oxygen, temperature, water level was continuously measured throughout the sampling period from April to June in a 3-minute interval using multi-parameter probes (LogTrans7-compact measuring system SENSOdive CTDO2, UIT; Dresden, Germany and O2-Log3055-INT and CTD3100-10 Logger, Driesen+Kern, Bad Bramstedt, Germany). PH was measured with every grab samples using pH-meter (Greisinger G 1500, Regenstauf, Germany and Xylem Analytics WTW Multi 3620 IDS Set G, Weilheim, Germany). The continuous discharge was derived from a stage-discharge relation calculated based on manually measured reference values for flow velocities and water depth for a subset of 31 streams. Hydromorphology was recorded in-situ according to the official procedure by the German Länderarbeitsgemeinschaft Wasser (LAWA) quantifying all hydromorphological criterions required under the WFD. These include among others meandering of the watercourse, variation in stream depth and width as well as riparian conditions (Commision, 2000). Additionally, bed habitat structure described the presence of potential holding substrate for invertebrates (Gieswein et al., 2017). This parameter represents the combined fraction of coarse particulate organic matter, plants, debris and stones > 2 mm in the stream bed. See SI chapter 2 for site-specific data and variable aggregation.

4.2.5 Invertebrate Sampling

Benthic macroinvertebrates were sampled at the beginning of June towards the end of the main pesticide application period for most crops and therefore suitable for ecological effect identification

(Liess and Von Der Ohe, 2005) (SI Invertebrate list). Standardized multi-habitat sampling(Meier et al., 2006) as prescribed under the WFD ensured comparable observations. A 50 m long section of each stream was divided into its substrate types on a percentage basis. A total of 20 subsamples (100%) were subdivided into frequencies of the occurring substrate types (smallest unit 5%). Each unit (5%) was sampled by kick sampling ten times using a net with a surface of 0.0625 m^2 and a mesh size 0.5 mm. Sampled organisms were separated the from coarse organic debris using a column sieve set, preserved in 90% ethanol, and later determined in the laboratory generally down to the lowest taxonomic level possible under the binocular. The invertebrate determination level, abundance and occurrence at sampling sites is provided in the SI chapter 8.

4.2.6 Biological Metrics of Invertebrates

We applied a wide range of biological indicator systems to assess the ecological effects of the stressors measured. Some of the invertebrate based indicators selected were developed to unspecifically respond to stressors. These are taxa number, number of insect taxa, insect and EPT% biomass- estimated using average taxa body volumes approximated by simple geometries (cylinder, ellipsoid, rotational ellipsoid or cone depending on taxon body shape) and a density of 1.06 g/mL (SMIT et al., 1993), Shannon taxa diversity (Shannon and Weaver, 1949), proportion of ephemeroptera, plecoptera and trichopteran (Lenat, 1988), Ecological Status Class (ESC) as multimetric index applied under the WFD considering individual indicators for morphological structure, organic pollution and acidification (Commision, 2000), the biological monitoring working party (BMWP) index and the Average Score Per Taxon (ASPT) indicating general water quality(Armitage et al., 1983), the Fauna Index (Lorenz et al., 2004) and the 3 functional diversity components richness, divergence and evenness (Mason et al., 2005) considering the traits body size, feeding type, locomotion and aquatic stages (Schmidt-Kloiber and Hering, 2015; Usseglio-Polatera et al., 2000). As indicators responding to specific stressors we included the $SPEAR_{pesticides}$ (Liess and Von Der Ohe, 2005) index that relates to the toxic pressure of pesticides on invertebrates and can be calculated with an online tool (https://systemecology.de/indicate/) and the Saprobic index related to the organic pollution that is linked to oxygen deficiency (Kolkwitz and Marsson, 1909; Rolauffs et al., 2013).

We defined the desired ecological status related to pesticides as for other invertebrate metrics under the WFD; with 4 boundaries separating the 5 even quality classes equal EQR (Ecological Quality Ratio) values of 0.8, 0.6, 0.4, and 0.2 (EU Commission, 2008) and classified the resulting ecological status into the usual 5 quality classes ranging from “high” to “bad” related to $SPEAR_{pesticides}$ (for details of approach and classes see SI chapter 9).

4.2.7 Statistical Analyses

All statistical analyses were performed with the statistical software R (version 3.6.1, (R-Core Team, 2019)). Multiple linear regression was performed with all predictors for each of the above listed biological metrics of invertebrates. These include: pesticide pressure, dissolved oxygen, hydromorphology, bed habitat structure, pH, ortho-phosphate, nitrate, nitrite, ammonium, total phosphorus, total nitrogen, flow velocity, temperature, rubber additive concentration, discharge, urban toxicity, metal toxicity, stream width and stream depth (see Tab. SI 1). All predictors were checked for homoscedasticity and normality, some of which were log-transformed if necessary. Different aggregations for individual predictors were investigated to explain all biological indicators by single linear regressions. Those yielding highest coefficients of determinations compared to other aggregations were chosen (details see SI chapter 2). If parameters were only available for a subset of streams (rubber additive concentration, discharge and urban contaminants toxicity) regression analyses was reduced to the respective stream section subset.

Intercorrelation of environmental parameters was tested using the variance inflation factor (VIF). Parameters with VIF-scores greater than two were omitted. The selection of the total model was carried out by an automated forward model selection analysis and the Akaike Information Criterion (stepAIC, R-package "MASS")(Venables and Ripley, 2002). The total model is composed of significant parameters only and the explained variance is given by the adjusted R^2. The contribution of each significant parameter to the total explained variance was evaluated with the metric approach "lmg", which uses R^2 for the evaluation (Hierarchical Partitioning (Chevan and Sutherland, 1991), R-package "relaimpo" (Grömping, 2006)).The visualisation of the data and linear regression models were performed in R using ggplot2 (Wickham, 2016).

4.3 Results and Discussion

4.3.1 Assessment of Anthropogenic Stressors

4.3.1.1 Determining Relevant Anthropogenic Stressors

The 101 streams selected are a representative cross-section of small lowland streams in Central Europe (see SI chapter 1). They cover a wide gradient of agricultural pollution, include 11 small wastewater treatment plants (WWTPs) with less than 3000 population equivalents and a number of diffuse domestic discharges identified by wastewater markers. We used multiple linear regression to identify those anthropogenic stressors that determine invertebrate community composition (see SI chapter 3 for stressor distribution, chapter 8 for invertebrates sampled). Stressors with the highest explanatory power were (i) pesticide toxic pressure during exposure peaks, (ii) oxygen deficiency and (iii) poor hydromorphology (Figure 14). Stressors showing no or only minor associations with invertebrate-related endpoints include urban toxicants such as pharmaceuticals, heavy metals, and street run-off. Agricultural pesticides, related to the substance of the peak exposure events with the highest exposure to effect concentration ratio, the TU_{max} (maximum TU), were on average 91 times more toxic than urban contaminants (related to the sum of all toxicants (TU_{sum}) 76 times more toxic). We also found that TUs measured at 11 stream sections with WWTPs were similar to those without WWTPs (SI chapter 7) comparable to a study related to WWTP in Switzerland (Munz et al., 2017). Agricultural nonpoint-source pesticide pollution was thus identified as a major driver of invertebrate community composition in the ecosystems under investigation (see chapter 3.3.3.2. on the ecological processes of the low-concentration effects of pesticides).

Non-additive interactions between stressors were investigated limited to relevant stressor combinations so as not to reduce statistical power. These were interactions between those stressors already known to act synergistically: toxicants and water temperature (Arambourou and Stoks, 2015; Verheyen and Stoks, 2020) and oxygen deficiency (Ferreira et al., 2008; Gupta et al., 1983; Van der Geest et al., 2002). We also added the remaining stressor that proved to be relevant for many of the ecological endpoints; the deficiency of morphological structure. Interactions between these three stressor combinations were all additive; none resulted in measurable antagonistic and synergistic ecological effects. Other investigations yielded comparable results for the minor relevance of interactions (Birk et al., 2020; Gieswein et al., 2017) explaining them with community adaptation processes which reduce non-additive stressor interactions (Romero et al., 2019).

4.3.1.2 Assessment of Ecological Endpoints

Ecological endpoints best responding to the measured anthropogenic stressors were: (i) the $SPEAR_{pesticides}$ index, identifying the degradation of invertebrate communities by pesticide toxicity (Liess and Von Der Ohe, 2005), (ii) the proportion of vulnerable insects %EPT (Ephemeroptera, Plecoptera, Trichoptera), identifying the general degradation of the community (Lenat, 1988) and (iii) the saprobic index, identifying the oxygen deficiency (Kolkwitz and Marsson, 1909) (Figure 14). Other common indicators of community disturbance were only marginally associated with any of the anthropogenic stressors quantified, namely the BMWP and ASPT (Armitage et al., 1983). Also the Ecological Status Class (ESC) for the biological quality element invertebrates under the EU water framework directive (WFD) (Völker et al., 2016) seems unable to reflect anthropogenic stressor effects in small lowland streams. An extended list of endpoints and their association to stressors is displayed in Figure 14.

Our results show that indicators of function were only marginally associated with any of the anthropogenic stressors quantified. These include invertebrate biomass, taxa number and also diversity indices as functional richness, evenness and divergence (Mason et al., 2005). Similar results were revealed for other small lowland streams (Voß and Schäfer, 2017). The weak association of anthropogenic stressors and several indicators of function is likely due to compensatory processes (Frost et al., 1995). Obviously such “integrating endpoints” that describe a system in its entirety (i.e. total abundance or biomass) are subject to compensatory processes and therefore respond less to stressors compared to "differentiating endpoints" (Liess and Foit, 2010). The loss of sensitive species may be compensated through tolerant species (Dornelas et al., 2019). Accordingly, "differentiating endpoints" that include structural community measures and can reflect declines of the fraction of vulnerable taxa – increased by competitive processes between taxa (Liess et al., 2013) – show strong associations with stressors. These measures describe biological systems by grouping its elements (individuals and populations) according to contrasting traits (Liess and Foit, 2010). Examples are the endpoints $SPEAR_{pesticides}$, %EPT, and the Saprobic index that differentiate community composition according to the vulnerability of taxa towards pesticides, general stressors or oxygen depletion. It follows that measures describing the community without reference to competitive processes, the “integrating endpoints” such as total invertebrate biomass, taxa number and the Shannon index are not capable of indicating anthropogenic stress. It is precisely the exclusive use of integrating endpoints that carries the risk of overlooking actual stressor effects and signs of ecological degradation. One example is a recent comprehensive meta-study that reported an increase in freshwater insect abundances over the last decades, based only on integrating endpoints (Klink et al., 2020). Accordingly, total biodiversity without reference to contrasting traits such as size, longevity or sensitivity may not be a sensitive indicator of global change.

4.3.1.3 Characterization of the Agricultural Pesticide Pollution

In terms of pesticide toxic pressure, regular grab samples, mainly taken during base-flow conditions, revealed a background contamination with an average of 17 detected pesticides and 10 pesticide metabolites per sample, whereas event-driven sampling (EDS) revealed an increased average of 31 pesticides and 11 metabolites per sample. Pesticide concentrations (95% percentiles) sampled by EDS events exceeded grab sample derived background concentrations by a factor of 54 on averaging, with a median of 6.3. A detailed overview of the detected pesticides and their concentrations is reported in the SI chapter 4.

Figure 16: Relative importance of stressors for biological endpoints - multiple linear regression to determine the explained variance, R^2 (numbers below dots). Significance levels $p < 0.05$*; < 0.01**; < 0.001***. Red dots indicate a deterioration of the biological endpoints with increasing stress, blue dots an improvement.

Pesticides contributing dominantly to the toxic pressure of peak events on invertebrates included the neonicotinoids thiacloprid (mean share of TU_{sum} = 46.6%), imidacloprid (9.5%) and clothianidin (3.6%) as well as the biocide fipronil (9.9%) and the carbamates methiocarb (5.1%) and pirimicarb (4.8%). These 6 pesticides drove the invertebrate toxicity in 91.3% of the peak exposure events when considering the pesticide with the highest exposure to effect concentration ratio, the TU_{max}. On average, TU_{max} accounted for 69% of the invertebrate mixture toxicity assuming concentration addition (TU_{sum}). Accordingly, we show that the pesticide causing the highest toxic pressure out of the complex mixture of numerous pesticides is a good proxy of the total toxic pressure from a peak event. This was also confirmed by the linear regression depicted in Figure 16A which showed no improved association between the toxic pressure and $SPEAR_{pesticides}$ when using TU_{sum} instead of TU_{max} (both R^2 = 0.43). This finding matches previous studies, which compared the relevance of the dominant compound to the mixture for the environmental impact of pesticides in agricultural streams (Knillmann et al., 2018; Liess and Von Der Ohe, 2005; Schäfer et al., 2007). Here it is necessary to recognize that the dominant compound in each event can be a different one. Several such pesticide peak exposure pulses with at least a tenth of the TU_{max} occurred on average 3.7 times per site and sampling period.

4.3.2 Current Risk Assessment Underestimates Exposure and Effects of Pesticides

4.3.2.1 Exceedances of Regulatory Acceptable Concentrations (RACs)

The authorisation of a pesticide requires that its regular application results in an environmental exposure below the safe level for non-target organisms within the ecosystem (EFSA, 2013). Exposure models are applied to derive predicted environmental concentrations (PEC). The level of exposure considered to be safe is determined in a tiered approach identifying regulatory acceptable concentrations (RAC) for each pesticide. Our monitoring-based findings show that these regulatory requirements (PEC < RAC) are often not met in reality:

The measured environmental concentration (MEC) was higher than the predicted environmental concentrations (MEC > PEC, Figure 15B). For 11 out of 16 pesticides that frequently exceeded RACs (selection see Tab. SI 2) we observed PECs being exceeded in more than 1% of EDS samples (Figure 15B).

The RACs in place during the monitoring were exceeded in the majority of streams (Figure 15A). Even pesticides no longer approved at the time of the investigation (2018, 2019) were present in concentrations above their RAC (SI Tab. 2). At least one exceedance of a RAC was detected in the 81% of sites in catchments with agricultural land use exceeding 20% (Figure 15A). More than 5 RAC exceedances within one sampling period were identified in 41% of agricultural streams. EDS with a total n = 296 from agricultural streams revealed RAC exceedances in 59%, grab samples with a total n = 440 in 26% of samples. This is similar to the results obtained by the most comprehensive meta-study to date, which found that 45% of the 1566 cases of measured insecticide concentrations in EU surface waters exceeded their respective RACs (Stehle and Schulz, 2015). On the substance level, 37 pesticides and 2 metabolites exceeded their RAC (Figure 15B, for the 20 pesticides with most exceedances, Tab. SI 2 for all substances). Moreover, in this current investigation we identified 41% of the 17 streams with less than 20% of agricultural land use where RACs were still exceeded. 4 out of 7 streams without any agriculture or known point sources within their catchment showed RAC exceedances of 3 pesticides (imidacloprid, clothianidin, fipronil; see Figure SI 4A). Although the authorisation of spray applications for 3 neonicotinoids had already expired in 2019, similar high exceedances as in 2018 were measured (clothianidin, imidacloprid, thiamethoxam).

4.3.2.2 Reasons for Non-Compliance with Regulatory Thresholds

For the 20 pesticides that most frequently exceeded the RACs, the following potential reasons for non-compliance with the regulatory thresholds were identified (Figure 15B and SI chapter 4).

- For 11 of these pesticides PECs were exceeded, possibly either due to unauthorised application rates, faulty exposure modelling, failure to consider multiple applications in the river basin, or overestimation of the predicted effectiveness of risk reduction measures (thiacloprid, terbuthylazin, nicosulfuron, lenacil, diflufenican, thiamethoxam, S-metolachlor, foramsulfuron, dimethenamid-P, pirimicarb, mesotrione).
- Due to regulatory updated effect information after pesticide approval the RAC has been lowered for 8 pesticides after approval of available products. However, this updated effect information does not have an impact on the already authorised products on the marked. This leads to the situation, that products are available for use even if the expected PEC is above the updated RAC and an authorisation would not have been granted (EU Commission, 2011). However, due to the inertia of the risk assessment practice where re-evaluation is generally intended only every 10 to 15 years, this incorporation of new knowledge had not been performed for several products containing the pesticides thiacloprid, clothianidin, methiocarb, imidacloprid, thiamethoxam, acetamiprid, dimoxystrobin and bromoxynil.
- The measured environmental concentrations of 2 pesticides exceed their RAC without having a PEC assigned as authorisation assumed that there is no discharge into streams. For methiocarb, no PEC run-off was modelled due to the exclusive use as seed treatment. Although this assumption has proven wrong years ago, the new assessment practice in place did not have an impact on authorized products already on the market. fipronil on the other hand is only approved for biocidal and veterinary use and therefore has no PEC for agricultural use assigned.

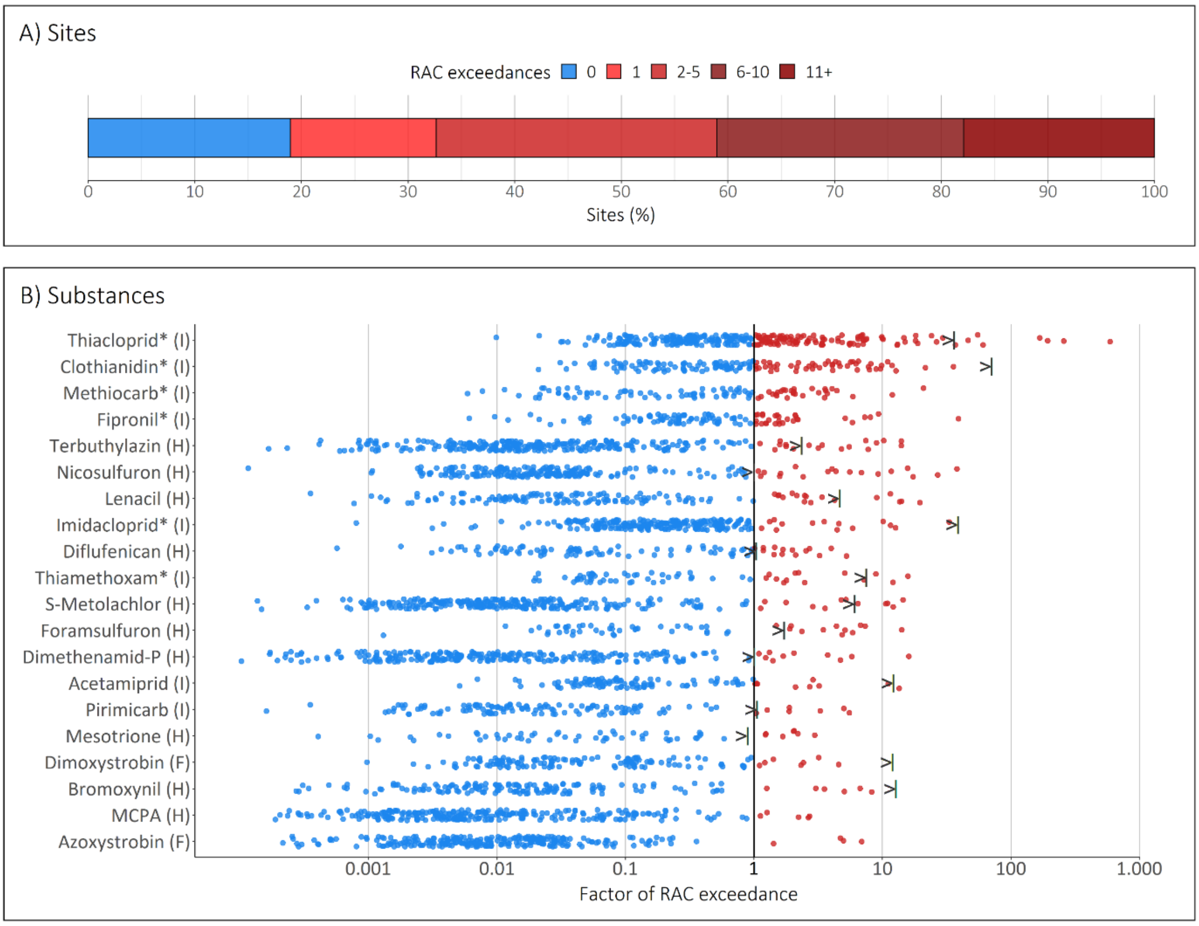


Figure 17: Measured exceedances of regulatory acceptable concentrations (RAC), Event-driven samples (EDS) from streams with >20% agricultural land use within the catchment. A) RAC exceedances per site and year (n = 95). No exceedances in 19% of sites, 1 in 14%, 2-5 in 23%, and more than 11 in 18%. B) Substance-related RAC exceedances in EDS samples (n = 296) of RACs for those 20 pesticides with most exceedances. Regulatory approval of marked (*) substances expired by December 2020. The ratio of predicted environmental concentrations (PEC) to the respective RAC including risk mitigation measures is shown by black ">|" symbols. For MCPA and Azoxystrobin no single PEC value could be identified.

4.3.2.3 Contradiction to the Pesticide Regulation and the Water Framework Directive (WFD)

The environmental situation as revealed in the current investigation related to agricultural streams shows an impairment of vulnerable populations, represented by a reduction of the SPEAR$_{\text{pesticides}}$ index. This situation does not comply with the Regulation (EU) 546/2011 that states "Member States shall ensure that use of plant protection products does not have any long-term repercussions for the abundance and diversity of non-target species." (EU Commission, 2011). This also contradicts the requirements of the EU regulation 1107/2009 that pesticides must not exert "unacceptable effects on the environment" considering "particularly contamination of surface waters," with regards to "non-target species" and "impact on biodiversity and the ecosystem" (EU Parliament, 2009). As required by the European parliament, no authorization to pesticides shall be granted "unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact on the viability of exposed species ... occurs" (EU Commission, 2011). Whereas unacceptability is defined within the specific protection goal for the "ecological threshold option" as "negligible population-level effects" on the "most sensitive populations". "The term negligible is used since it is difficult to demonstrate that no effect is occurring" (EFSA, 2013). Furthermore, the responsible authorities themselves are questioning the extent to which these environmental protection requirements are being implemented in practice. For example, the European Court of Auditors noted "limited progress in measuring and reducing risks" of plant protection products (European Court of Auditors, 2020). Furthermore, the German Federal Environment Agency (UBA) criticizes "the current intensity of chemical plant protection in Germany as ecologically unsustainable and thus threatening the achievement of key targets of environmental protection and nature conservation policies" (Frische et al., 2018).

The Water Framework Directive (WFD) also requires a good chemical status of water bodies by not exceeding Environmental Quality Standards (EQS). The respective exceedances of these thresholds point a similar picture, see SI chapter 10 and SI Table 2.

4.3.3 Deriving Protective Thresholds for Pesticides

4.3.3.1 Deriving the Acceptable Concentration (AC_{field})

The extensive dataset generated here allows to identify field-based safe concentrations at which no unacceptable adverse ecological effects on invertebrate communities are expected, the field validated Acceptable Concentration (AC_{field}). For the first time, this enables a validation of regulatory effect thresholds. The AC_{field} is based on 3 components: (i) the indicator system $SPEAR_{pesticides}$, (ii) an identification of the desired ecological status related to pesticides, (iii) the quantification of the uncertainty of the exposure-effect relationship.

(i) As a specific biological indicator, we applied the $SPEAR_{pesticides}$ index that uses pesticide-specific traits (pesticide sensitivity, generation time, migration ability, presence during the time of contamination) characterising the aquatic invertebrate community to establish a link between test-system based toxicity (LC_{50}; *D. magna, C. riparius*) and ecological impact (Liess and Von Der Ohe, 2005). The index responds primarily to toxic pressure and is largely independent of other environmental factors as shown earlier (Knillmann et al., 2018; Liess et al., 2008) and also here (Figure 14). The approach has been successfully applied in various geographical regions including Europe (Knillmann et al., 2018; Schäfer et al., 2012), Australia (Burgert et al., 2011) and South America (Hunt et al., 2017) enabling a widespread adoption of the presented approach.

(ii) To define the ecological status related to pesticides we derived an EQR (Ecological Quality Ratio) following the respective EU-WFD procedure (EU Commission, 2008) and as detailed within the methods section and the SI chapter 9. The respective quality classes are indicated in Figure 16A, where the boundary between a "good" and "moderate" status was set to a $SPEAR_{pesticides}$ value of 0.6 resulting in 83% of agricultural streams that did not reach the pesticide related ecological targets.

(iii) The uncertainty of the exposure-effect relationship is quantified by the variance of the relationship (Figure 16A). Causes for this variance are likely to include site-specific environmental factors and their interaction with pesticides as well as inaccurate exposure and effect assessment. The linear regression between toxic pressure (TU_{max}) and community response ($SPEAR_{pesticides}$) intersects the transition between the "good" and "moderate" quality class at a log TU_{max} of -3.27, identifying the threshold where 50% of sites below the regression line fail to meet a "good" ecological quality for invertebrates (Figure 16A). To establish a reliable ecosystem-based exposure-effect relationship we assume that all the variance observed is not related to the effects of pesticides but to other factors. This approach will considerably underestimate the true impact of pesticides. Accordingly, the $SPEAR_{pesticides}$ benchmark for an acceptable ecological status is reduced by the variance observed and should therefore be considered a conservative indicator of pesticide exposure (1.645σ corresponding to a one-sided confidence level of 95%, see Figure 16A, line *a*). Thus, a log TU_{max} of -3.27 marks the toxic pressure at which only 5% of sites will show an unacceptable $SPEAR_{pesticides}$ with a 95% confidence level (Figure 16A & B, line $b_{5\%}$). With this framework we consider the pesticide effects and as well as the related variability existing in the field and transform an adaptive cause-effect relationship of toxic pressure ($SPEAR_{pesticides}$) into a benchmark-related ecological cause-effect relationship (95% of streams protected), termed the AC_{field}. Accordingly, the threshold value for a pesticide that adversely affects invertebrates equals the substance-specific acute LC_{50} divided by an extrapolation factor of about 2000 (AC_{field} see Tab. SI 2). This measure describes the typical short-term exposure of primarily invertebrate-toxic pesticides at which no adverse effect on the invertebrate community is expected in 95% of the streams. The relationship displayed in Figure 16B additionally allows to identify the toxic pressure of a pesticide that relates to any percentage of streams affected.

The approach presented here presupposes that the extrapolation factor from the laboratory-based LC_{50} to the field-effect is similar for all pesticides. Only then is it possible to include all peak loads to derive a common extrapolation factor, regardless of the dominant pesticide in a given mixture. The exceptionally good association between toxic pressure (TU) and invertebrate response ($SPEAR_{pesticides}$) for an ecological context shows that this assumption can obviously be made. Furthermore, pesticides that do not cause the highest toxicity are also contributing to the overall ecological impact. As for other environmental factors, for the ecological assessment they are considered as a constant effect-determining factor that is included in the extrapolation factor. The good correlation identified in Figure 16A indicates that these assumptions are valid for the majority of the pesticides investigated. Nevertheless, significant deviations from this rule may occur in individual cases, so that the AC_{field} values are merely an indication of the ecological potency of a toxicant. With this restriction in mind a prospective assessment of the ecosystem impact of new pesticides is possible. Accordingly, this approach integrates prior knowledge into the derivation of ecologically effective concentrations in a similar way as other studies have based the probability of occurrence of taxa on habitat suitability (Vermeiren et al., 2020) and toxicant concentration (Liess and Von Der Ohe, 2005). The AC_{field} allows an effect assessment for a pesticide on the basis of the other pesticides typically present in agricultural streams. Therefore, the AC_{field} can only be compared with the RAC when considering that RAC values were derived without taking into account the presence of other pesticides.

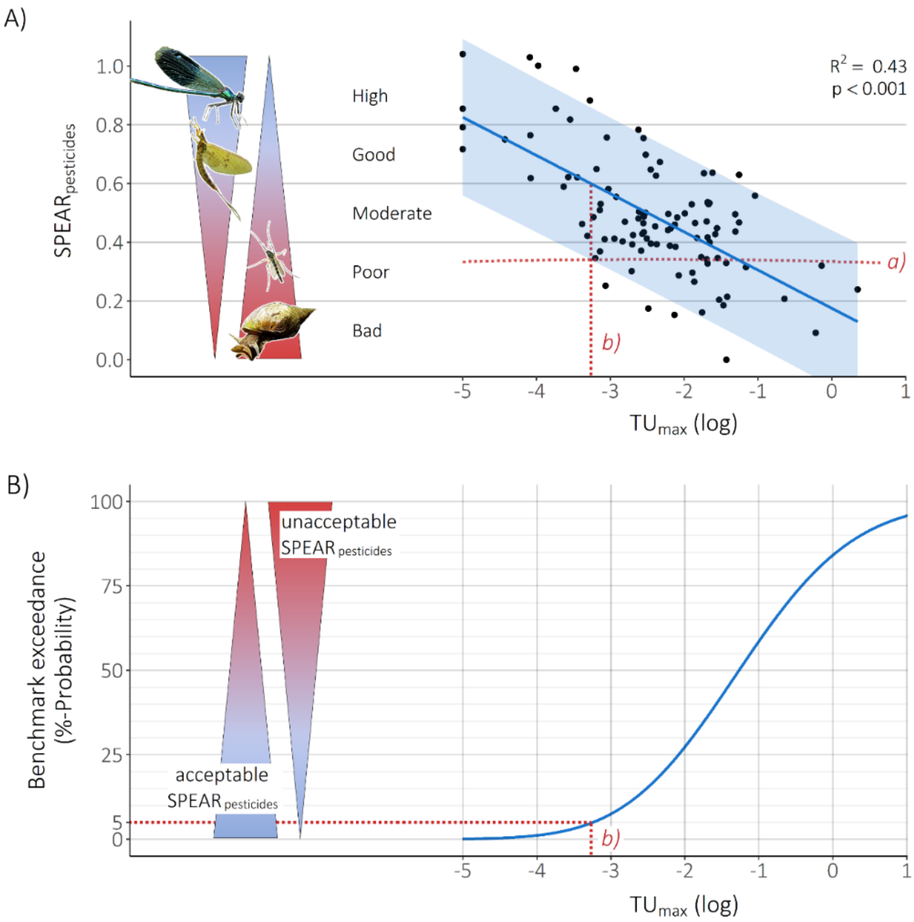


Figure 18: Field-based adaptive (A) and benchmark-related (B) cause-effect relationship for pesticides. A) Adaptive cause-effect relationship of toxic pressure (TU_{max}) and ecological effect ($SPEAR_{pesticides}$) observed in the 101 streams. The blue band corresponds to the 90% prediction interval. Line $a_{95\%}$ depicts the $SPEAR_{pesticides}$ benchmark to identify unacceptable pesticide effects with a confidence of 95% ("good"-"moderate" benchmark reduced by 1.645σ of the linear regression). Line $b_{5\%}$ represents the log TU_{max} threshold of -3.27, where 5% of streams show an unacceptable ecological status according to $SPEAR_{pesticides}$ with a confidence of 95%. B) Benchmark-related ecological cause-effect relationship: Resulting probability of exceeding the $SPEAR_{pesticides}$ benchmark as a function of TU_{max}.

The AC_{field} that is available for 22 primarily invertebrate-toxic pesticides identifies an extrapolation factor related to acute LC_{50} values of about 2,000 protecting 95% of streams; a factor exceeding the acute regulatory Tier 1 "assessment factor" (100) by 20. To protect 99% of streams the respective extrapolation factor would amount to 18,000, a log TU_{max} of -4.25 (Figure 16B). However, the exposure to RAC ratio was found to explain $SPEAR_{pesticides}$ equally well as the exposure to LC_{50} ratio (R^2=0.44 versus R^2=0.43, see Figure SI 8A). This shows that the RAC values are related to the ecological effect as shown in the cause-effect relationship in Figure SI 8A. Nonetheless, their compliance would cause unacceptable effects in 14% of agricultural stream sections; 86% would be protected (Figure SI 8B). To protect 95% or 99% of streams, respectively, the RAC for invertebrate-toxicity driving pesticides (SI chapter 11) required an additional assessment factor of 5.3 or 40.2. It must to be taken into account that these results refer primarily to the pesticides with the greatest RAC exceedances. These include particularly 4 different neonicotinoids as well as fipronil, methiocarb and terbuthylazine (Figure SI 4).

4.3.3.2 Mechanisms for the Observed Low-Concentration Effects of Pesticides

We hypothesize the following ecological processes as the reason for the high field sensitivity of vulnerable species and the associated increased extrapolation factor identified here:

- The multitude of pesticides present in the streams may not only result in additive effects (Loewe and Muischnek, 1926) but also in a synergistic increase of pesticide toxicity due to the presence of additional toxicants that may exceed the additive effects by a factor of up to 660 as identified in laboratory investigations (Liess et al., 2020) or by an increase of single-substance toxicity by more than one order of magnitude as identified in field investigations (Rydh Stenström et al., 2021).
- Environmental stressors may act synergistically when acting in concert. Examples include the combined effects of nutrients, suspensions and temperature frequently producing synergistic effects on abundance at the population level of periphyton communities (Piggott et al., 2015) and the combined effects of nutrients, suspensions and chloride inducing invertebrate drift in streamside mesocosms (Beermann et al., 2018). Additionally, stressors such as predator pressure, competition and suboptimal environmental conditions may increase the sensitivity of populations to pesticides by a factor of up to 100 as revealed in microcosm (Liess et al., 2016) and mesocosm studies (Liess and Beketov, 2011).
- Repeated insecticide pulses leading to multiple exposure of individuals within a generation (within a spray season for annual species), increases the impact compared to a single insecticide pulse (Wiberg-Larsen et al., 2021). Also repeated pesticide pulses leading to multiple exposure of populations between generations (between spray seasons for annual species), increases the impact compared to a single insecticide pulse and may result in a multigenerational culmination of low-concentration effects (Liess et al., 2013).

The effect-determining factors and their related processes described here are generally not considered in the aquatic risk assessment. Thus, neither for individual-based lower-tier studies nor for mesocosm-based higher-tier studies effect-determining factors are taken into account that are comparable in their expression with the field. Calibration of existing assessment factors by means of traditional higher-tier studies has been successfully carried out (Brock et al., 2016; Rico et al., 2019), but does not allow prediction of pesticide effects in the field. We therefore suggest to calibrate the assessment factors applied in pesticide regulation integrating field-based findings. For example, a relevant candidate for such an exercise is the insecticide chlorantraniliprole, a pesticide that may replace the widely used neonicotinoids and could therefore gain high relevance in the near future (Schmidt-Jeffris and Nault, 2016). For chlorantraniliprole the RAC is a factor of 50 higher than the respective AC_{field}. Accordingly, regular authorities could review the derivation of the current RAC in order to avoid future environmental problems with this pesticide.

4.4 Conclusions

- In this study of 101 small lowland stream sections, we revealed for the first time the prime relevance of agricultural pesticide pressure for the composition of invertebrate communities.
- The diversity and number of vulnerable species was already reduced at very low pesticide concentrations, so that most of agricultural streams did not meet the pesticide-related ecological targets.
- We revealed that the current authorisation of pesticides underestimates the actual ecological risk, as measured pesticide concentrations exceeded current regulatory threshold levels in most of the agricultural streams and even existing thresholds were not protective for invertebrates.

- By including monitoring-derived information on pesticide effects within the ecosystem we identified pesticide threshold concentrations that will ensure a protection of the invertebrate stream community.
- Future research should extend this concept developed here to other groups of aquatic organisms such as amphibians, fish, plant and fungi communities, and also to terrestrial ecosystems. This identification of field validated Acceptable Concentrations for the ecosystem (**AC**$_{field}$) can then be used to review the existing thresholds of the Pesticide Risk Assessment (R**AC**) and the Water Framework Directive (M**AC**-EQS).

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5 Risk from pesticide mixtures - The gap between risk assessment and reality

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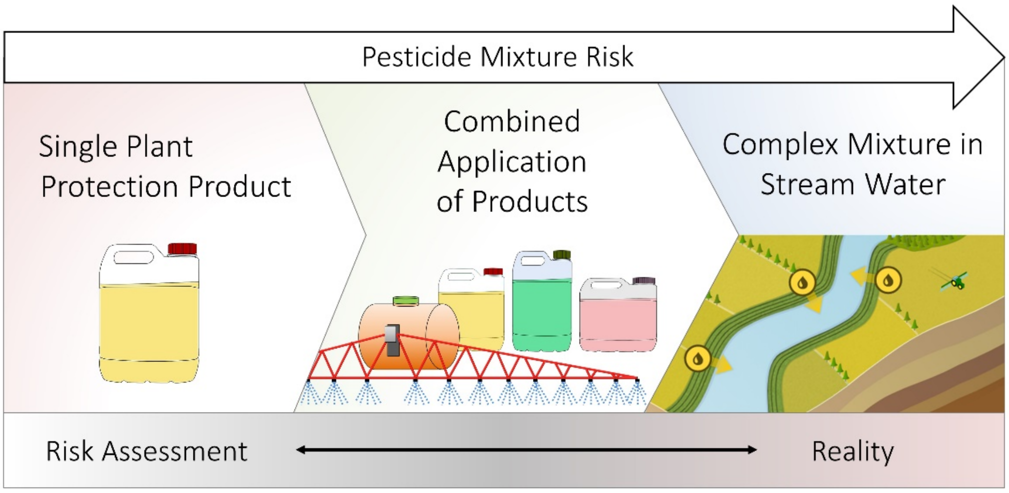
Abstract

Pesticide applications in agricultural crops often comprise a mixture of plant protection products (PPP), and single fields face multiple applications per year leading to complex pesticide mixtures in the environment. Restricted to single PPP, the current European Union PPP regulation, however, disregards the ecological risks of pesticide mixtures. To quantify this additional risk, we evaluated the contribution of single pesticide active ingredients to the additive mixture risk for aquatic risk indicators (invertebrates and algae) in 464 different PPP used, 3,446 applications sprayed and 830 water samples collected in Central Europe, Germany. We identified an average number of 1.3 different pesticides in a single PPP, 3.1 for complete applications often involving multiple PPP and 30 in stream water samples. Under realistic worst-case conditions, the estimated stream water pesticide risk based on additive effects was 3.2 times higher than predicted from single PPP. We found that in streams, however, the majority of regulatory threshold exceedances was caused by single pesticides alone (69% for algae, 81% for invertebrates). Both in PPP applications and in stream samples, pesticide exposure occurred in repeated pulses each driven by one to few alternating pesticides. The time intervals between pulses were shorter than the 8 weeks considered for ecological recovery in environmental risk assessment in 88% of spray series and 53% of streams. We conclude that pesticide risk assessment should consider an additional assessment factor to account for the additive, but also potential synergistic simultaneous pesticide mixture risk. Additionally, future research and risk assessment need to address the risk from the frequent sequential pesticide exposure observed in this study.



5.1 Introduction

A total of 466 pesticide active ingredients, referred to as pesticides in the following, are currently approved for use in plant protection of the various agricultural crops within the EU (European Commission, 2021). In Germany alone, 288 different pesticides were approved ingredients in 932 plant protection products (PPP) in 2019 (UBA). PPP application schemes, referred to as spray series, comprise multiple applications per field and year, where multiple PPP are frequently applied simultaneously, which in turn often contain a mixture of pesticides. Consequently, manifold pesticide residues occur in the different environmental compartments, resulting in complex environmental pesticide mixtures (Schreiner et al., 2016; Silva et al., 2019; Stehle and Schulz, 2015b).

Small streams with agricultural catchments face particularly diverse and ecologically relevant pesticide pollution (Knauer, 2016; Stehle and Schulz, 2015a; Szöcs et al., 2017). In a Germany-wide monitoring of more than 100 lowland streams, Liess et al. (2021a) and Halbach et al. (2021) confirmed the widespread occurrence and ecological relevance of pesticides in streams on a large scale. The adjacency to agricultural fields in combination with a limited dilution capacity makes streams particularly receptive to an agricultural input of pesticide residues. These enter the water bodies via rain-induced runoff, drainage and spray drift (Jong et al., 2008; Liess et al., 1999). The respective contribution of each pathway to the total input depends on site-specific parameters and pesticide properties; however, runoff is most likely to cause peak concentrations in typical agricultural catchment scenarios (Liess and Schulz, 1999). Especially after rainfall, streams thus represent a reservoir for recent pesticide applications within their catchments. Multiple studies have reported an increased risk due to pesticide mixtures occuring in these aquatic environments and stressed their adverse potential (Gustavsson et al., 2017; Schreiner et al., 2016; Vallotton and Price, 2016).

The current European environmental risk assessment (ERA) of pesticides, however, considers almost exclusively single applications of single PPP on a single crop (European Union, 2009; Frische et al., 2014; Frische et al., 2018; Northern Zone, 2018; Topping et al., 2020). More precisely, this means that the ERA accounts for the mixture in a single PPP, which is a formulation of one or more pesticides and additives to improve the PPP's properties such as solubility for example. If at all, PPP applications with one or more PPPs at the same time are only considered in rare cases where application mixtures of several PPPs are specifically registered as such and listed on the label of use with a clear name and dose rate. However, the ERA of PPP currently provides no concept to address all unknown PPP application mixtures, spray series and, more importantly, unintended pesticide mixtures present in the environment. To our knowledge, no country or region in other parts of the world considers the risk due to simultaneous pesticide mixtures in the environment within the authorisation or risk mitigation of PPPs.

This is problematic following the widely acknowledged assumption that exposure to multiple pesticides as a consequence of intensive PPP use represents a major disregarded ecological risk and a contribution to the biodiversity decline (Backhaus and Faust, 2012; Brühl and Zaller, 2019; Hayes et al., 2006; Silva et al., 2002). This assumption is often supported by studies testing equitoxic mixtures, in which all components contribute equally to the toxicity of the mixture based on a consistent measurement endpoint (Altenburger et al., 2000; Backhaus et al., 2000; Silva et al., 2002). Especially under such conditions, the combined effect of the mixture significantly exceeds respective single substance effects. Accordingly, the guidance documents defining principles for the ERA generally acknowledge the need to also consider possible effects due to other chemicals already present in the environment (EFSA, 2009, 2013). However, the aquatic guidance states that "a thorough analysis of PPP usage practices in major crops […] is not yet available" and assumes that "observed effects are, in many cases, related to the effects of one or two [pesticides]". The disregard of multiple PPP exposure in the ERA is reasoned by a lacking systematic analysis of and harmonized concept how to consider real-world PPP usage practices and environmental exposure patterns (Ctgb, 2021; Garthwaite et al., 2015).

In this study, we address this knowledge gap by comparing comprehensive monitoring data sets on (i) real-world PPP applications and (ii) measured concentrations in surface waters also considering peak exposure scenarios. This allows the gap between the pesticide mixture risk considered by PPP authorisation and the actual environmental risk to be quantified. In addition, the combined dataset provides insight how often agricultural fields and streams face exposure pulses of such mixtures. We therefore aim to (i) estimate and compare the risk considered under the single PPP-oriented ERA with the risk of pesticide mixtures present in the field, (ii) evaluate stream water pesticide mixtures in the light of regulatory threshold levels, (iii) characterise environmental pesticide mixture composition and identify pesticides driving mixture risk and (iv) quantify the sequential pesticide exposure due to serial applications on fields and recurring inputs in streams.

5.2 Material and Methods

5.2.1 General Approach

In order to compare the risk considered under the single PPP-oriented ERA with the risk of pesticide mixtures present in the field, we quantified the risk of pesticide mixtures in single PPP, PPP applications (=single spray event of one or several PPP) and water samples taken from agricultural streams. For this, we reviewed a large dataset of real-world PPP spray series comprising applied PPP and their components for common crop types. On the basis of the amount of pesticides applied, we modelled the surface water exposure as performed within the European environmental risk assessment (ERA) for individually sprayed PPP as well as combined PPP applications and estimated the resulting risk in surface waters for invertebrates and algae/macrophytes. Under real world conditions, the pesticide mixtures in surface waters are expected to show a different toxicity than estimated by exposure modelling based on single PPP applications. Most importantly, off-site transportation, parallel PPP applications on adjacent fields and degradation of pesticides result in spatially and temporally integrated environmental mixtures. In addition to the modelled pesticide exposure, we therefore analysed measured pesticide concentrations in agricultural streams and compared these with the modelled exposure of the reported PPP applications. The spray series and stream monitoring data we jointly analyzed originate from different projects and are temporarily divergent. Although the water samples were collected in 2018-2019, we expect them to match the spray series data from 2007-2015 in terms of applied and environmental pesticide toxicity given that application intensities remained stable (Julius Kühn-Institut, 2020). Single pesticide or PPP authorisations were withdrawn and new substitutes entered the market while toxicity ratios in environmental mixtures are likely to remain unchanged. The reported PPP applications and monitored streams do not cover the same hydrological catchments but are from the same geographical region.

5.2.2 Pesticide Application Data & Exposure Modelling

The pesticide application data were obtained from the INL – "Privates Institut für Nachhaltige Landbewirtschaftung" Halle, Germany, and compiled as part of the COMBITOX project (FKZ 3715 63 407 0) (Knillmann et al., 2019). The dataset included 889 real-world spray series from the years 2007-2015 (see Supporting Information/SI Figure 1). A total of 229 different pesticides were applied on twelve different crops including different cereals, oilseed rape, potato, sugar beet, vine, and apple (see substance and crop list in SI). The 24 farms and 175 fields are mostly located in different agricultural regions in Germany and a few in neighbouring Austria that were also included due to comparable climatic conditions and the fact that both countries fall under the Central Zone for the registration of PPP.

Each spray series in the dataset describes a sequence of plant protection and plant growth regulation measures over one growing season. In each case, this covers the time from sowing (arable crops) or from leaf development (permanent crops) to harvest. One application within a series is defined as the total of all measures applied on one specific day and field. Each application is characterised by the PPP used, the pesticide(s) in the PPP, the application rate (e.g. in kg/ha) and the date of application. The application frequencies of the spray series analysed were congruent with the strongly aggregated, but publicly available pesticide statistics of the Julius Kühn-Institut for each crop type (see SI Table 1) (Julius Kühn-Institut). Therefore, we expect that the dataset on spray series well reflects the agricultural practice in recent years. To avoid bias from seasonal variability, only data from PPP applications sprayed in the stream sampling period (April until mid-July, n = 3,446) were compared with the water samples.

We modelled the predicted environmental concentrations in surface water on the basis of the amounts of pesticide applied. Exposure modelling is used to account for the pesticides' physico-chemical properties driving their tendency to enter surface waters. For this, we used FOCUS, the official model for estimating pesticide exposure at EU level (FOCUS, 2012). We performed FOCUS Step 2 calculations (unavailable case-specific data would be required for Step 3 and 4) limited to the most relevant entry pathways, runoff and drainage, to ensure comparability with measured peak concentrations after rainfall (Huber et al., 2000; Liess and Schulz, 1999). In the model, we accounted for plant interception reducing pesticide loads in the soil, depending on the culture and its stage during application (EFSA, 2014). As assumed in FOCUS models, the residues of each application are washed out by a defined rainfall after partially degrading in soil for 4 days. The physico-chemical properties of the pesticides applied required for the calculations were retrieved from the Pesticide Properties DataBase (PPDB, experimental data) and the US EPA EPI Suite (modelled data), where experimental data was prioritised (Lewis et al., 2016; US EPA, 2015). Model parameters are described in more detail within the SI. Depending on the application scenario (e.g. treated culture, growth stage, slope of field, seasonality), PPP may only be sprayed under "mandatory conditions of use". This may include maintaining untreated buffer strips along surface waters. As this information was not available, surface water concentrations were modelled without accounting for conditions of use. This may have resulted in higher concentrations than modelled in the actual ERA.

5.2.3 Stream Water Pesticide Sampling

The information on stream water pesticide concentrations were collected as part of the "Kleingewässermonitoring", a Germany-wide monitoring of small streams (FKZ 3717 63 403 0) (Helmholtz-Centre for Environmental Research - UFZ, 2020). The monitoring involved several stakeholders as it was supported by the German Federal Environment Agency (UBA), regional water authorities and also advised by regional agricultural authorities. See Liess et al. (2021a) and Halbach et al. (submitted 2021) for a description of sampling methods and a detailed discussion of measured pesticide concentrations and observed ecological effects. In brief, this study focused on a sub-selection of 103 agricultural streams where agriculture made up at least 20 % of land cover in the hydrological catchment (Copernicus Land Monitoring Service, 2019). A total of 830 water samples were taken from the beginning of April to mid-July in 2018 and 2019. Pesticide applications are most frequent during this period, so that peak concentrations are most likely to occur (SI Figure 2). Upstream catchments were mostly smaller than 30 km^2 (mean = 17 km^2, max = 267 km^2) and characterised by a gradient of agricultural influence (agricultural land cover ranged from 22-100%, mean = 74.5%, excluding forestry). Settlements and other urban land covers accounted for less than 5% in the majority of stream catchments (see SI for catchment characteristics).

The sampling was carried out in two different ways to capture (i) background concentrations under dry weather conditions and (ii) rainfall-driven peak concentrations. To sample the continuous background concentrations, grab samples were taken in a regular, 3-week cycle (n = 518). To sample rainfall-driven peaks, we used automatic sampling devices triggered by a water level increase resulting in sampling during or directly after rainfall. These event-driven samples (EDS, n = 312) are of high ecological relevance, capturing transient, short-term peak concentrations of pesticides in surface waters, which have been shown to especially affect stream communities and relate to biological effects (Liess and Schulz, 1999). All stream water samples were analysed for 74 pesticides and 33 pesticide metabolites using LC-MS/MS (see substance list and analytical details in SI). The selection of analytes was based on (i) pesticide use data in relation to its toxicity, (ii) substances occurring in elevated concentrations in previous monitoring programs and (iii) compatibility with a multi-substance method for chemical analysis (Wick et al., 2019). We thus assume that we have captured the main proportion of pesticide toxicity. All data are publicly available in Liess et al. (2021b).

5.2.4 Toxicity Calculations

The Toxic Unit (*TU*) concept was applied to estimate the toxicity of a substance and of mixtures in the environment (Sprague, 1971). Predicted and measured substance concentrations c_i were normalised to their respective EC_{50} – the concentration that causes a defined effect in 50% of test organisms. Hence, the toxicity of substance *i* described as TU_i is defined as

$$TU_i = \frac{c_i}{EC_{50_i}} \quad (1)$$

The mixture component resulting in the highest environmental toxicity yields the highest *TU*-value, the TU_{max}:

$$TU_{max} = max_{i=1}^{n} \frac{c_i}{EC_{50_i}} \quad (2)$$

We also aimed to predict which pesticides drive stream water toxicity by modelling surface water concentrations of the monitored PPP applications and identifying pesticides applied causing the TU_{max}. Toxicity drivers were defined as pesticides predicted to cause a log TU_{max} > -4 in at least 1% of applications. We then validated our predicted toxicity drivers to those pesticides causing a log TU_{max} > -4 in at least 1% of event-driven stream water samples.

To evaluate and quantify the risk caused by pesticide mixtures, we applied the Concentration Addition (CA) approach (Loewe and Muischnek, 1926), that has proven predictive power and is the recommended default for the ERA mixture toxicity assessment (Altenburger et al., 2000; EFSA Scientific Committee et al., 2019; Rodney et al., 2013). Following CA, the total toxicity of the mixture TU_{mix} is calculated by adding together the *TU*s of all the individual mixture components *i*:

$$TU_{mix} = \sum_{i=1}^{n} \frac{c_i}{EC_{50_i}} \quad 3$$

Other approaches such as Independent Action (IA) require more data and have led to less conservative predictions when comparing predicted and observed laboratory experiment effects, with some exceptions where mixtures explicitly consisted of dissimilarly acting toxicants (Backhaus et al., 2000; Bliss, 1939).

*TU*s were calculated for the organism groups of aquatic invertebrates (AI) and algae/aquatic plants (AP) (for EC_{50} values see SI Table 4). Given their sensitivity to pesticides, surrogate species of these groups are ecotoxicological standard test species and therefore provide a high data availability (SI Table 5). We considered mortality for AI and growth rates or biomass for AP as effect measures considered for the EC_{50}. These ecotoxicity data were retrieved from the PPDB database (Lewis et al., 2016). Data assigned a quality criterion equal to or less than 2 was discarded to exclude unverified data from unknown sources.

Mixture risk was also evaluated from a regulatory perspective by applying regulatory acceptable concentrations (RACs). These are defined as surface water concentrations that, if not exceeded, are assumed to ensure no unacceptable effects on the environment. RACs were retrieved from the German Federal Environment Agency (Umweltbundesamt – UBA) and reflect the state of regulation during the stream monitoring period (see SI Table 4) (European Union, 2009; UBA). By analogy with *TU*s, risk quotients (*RQ*s) relate a measured concentration to the respective RAC instead of to EC_{50} in the case of the *TU*, and indicate whether a single pesticide (RQ_{max}) or the mixture (RQ_{mix}) pose an unacceptable risk from a regulatory point of view ($RQ > 1$).

$$RQ_{max} = max_{i=1}^{n} \frac{c_i}{RAC_i} \quad 4$$

$$RQ_{mix} = \sum_{i=1}^{n} \frac{c_i}{RAC_i} \quad 5$$

Each RAC is based on the effect concentration observed for the most sensitive organism group for a particular pesticide and an assessment factor to account for the uncertainty when predicting field effects from experimental data. Hence, a pesticide RAC may relate to either AI, AP or fish. RQ_{mix} values were calculated separately for the organism groups AI and AP by only summing up *RQ*s of pesticides with RAC values for these groups. AI represented the most sensitive organism group of 22 pesticides analysed in this study (12 insecticides, 8 fungicides, 3 herbicides, see substance list in SI). AP represented the most sensitive organism group of 36 analysed pesticides (34 herbicides, 2 fungicides).

Finally, the maximum cumulative ratio (*MCR*) allows to identify the contribution of a single compound to the mixture by comparing the additive toxicity of the mixture with the highest toxicity of a single component (Price and Han, 2011):

$$MCR = \frac{Toxicity\ of\ the\ mixture}{Highest\ toxicity\ of\ single\ mixture\ component} \quad 6$$

The *MCR* thus estimates the factor by which the mixture is more toxic than the highest single pesticide toxicity in terms of *TU*s. The *MCR* was calculated for the mixtures in (i) a PPP (MCR_{PPP}), (ii) an application (MCR_{app}) and (iii) water samples (MCR_{sample} and MCR_{RAC}, see equations 7-10). The *MCR* of a mixture is generally different for the endpoints AI and AP due to the deviating EC_{50} values. To generalise across the organism groups AI and AP, we calculated the arithmetic mean of the organism group-specific *MCR*s.

PPP

$$MCR_{PPP} = \frac{TU_{mix}\ of\ PPP}{TU_{max}\ of\ PPP} \quad 7$$

Application

$$MCR_{app} = \frac{TU_{mix}\ of\ application}{TU_{max}\ of\ application} \quad 8$$

Water samples

$$MCR_{sample} = \frac{TU_{mix}\ of\ sample}{TU_{max}\ of\ sample} \quad 9$$

$$MCR_{RAC} = \frac{RQ_{mix}\ of\ sample}{RQ_{max}\ of\ sample} \quad 10$$

All calculations were performed using the statistical software R (version 3.5.1), all plots were created using the “ggplot2” R package (version 3.2.0) (R Core Team, 2017; Wickham, 2009b).

5.3 Results and Discussion

5.3.1 Quantifying the Increased Risk Posed by Pesticide Mixtures

We estimated the toxicity of pesticide mixtures in single plant protection products (PPP), PPP applications and water samples. By calculating the Maximum Cumulative Ratio (*MCR*), we assessed and compared the pesticide mixture risk in these mixture categories. Regardless of the mixture category, the MCR generally increased with the number of mixture components (Figure 17). Conversely, the fewer pesticides a mixture contained, the more its risk was driven by a single component (low *MCR*). Details of the investigated mixture categories are given below:

Single PPP - The PPP that were sprayed during the main application period from April to mid-July (n = 464) contained a mean of 1.3 different pesticides (min = 1, max = 4, Figure 17 - Single PPP). 30% (n = 138) of PPP consisted of at least two pesticides. PPP applied in apple cultures generally contained fewer pesticides (mean = 1.1), whereas PPP used to treat sugar beet and cereals were more likely to contain a mixture of pesticides (mean = 1.5). PPP mixtures showed a mean MCR_{PPP} of 1.1 (10^{th} percentile = 1, 90^{th} = 1.2, Figure 17).

Single application - The PPP applications (n = 3,446) of one or several PPP at a timepoint contained a mean of 3.1 pesticides (min = 1, max = 12) and 2.2 PPP (min = 1, max = 7). In 80% (n = 2751) and 73% (n = 2513) of applications, multiple pesticides or PPP were applied simultaneously. Cereals and sugar beet in particular were characterised by the highest number of pesticides per application (mean = 3.3 and 4.3, Figure 17 - Single application). Apple and oilseed rape cultures exhibited the lowest number of pesticides per application (mean = 2 and 2.2, respectively). Pesticide mixtures in applications revealed a mean MCR_{app} of 1.3 (10^{th} = 1, 90^{th} = 1.9). Apple and rape applications were on average 1.1, cereals 1.3 and sugar beet 1.8 times more toxic than the most potent mixture component.

Stream water - Pesticide mixtures detected in the streams, by comparison with the other mixture categories, were far more complex containing a mean of 17 (27 including metabolites) detected pesticides in grab samples (n = 518) and 30 (42 including metabolites) in event-driven samples (EDS) (n = 312) taken during rainfall induced exposure peaks (Figure 17 - Stream water). A maximum of 57 pesticides was detected in a single EDS. Hence, we detected almost twice as many pesticides in an average EDS compared with the common grab sample and ten times as many as sprayed in an application. Pesticide mixtures detected in EDS were on average 2.2 times more toxic than the most potent pesticide alone (MCR_{sample}, 10^{th} = 1.5, 90^{th} = 3.1, including measured metabolites). In 69% of the grab samples (n = 360) and 43% of EDS (n = 133), a single pesticide caused a higher toxicity than all other detects in combination ($MCR_{sample} < 2$). During exposure peaks, an increased MCR_{sample} of 2.7 was shown for aquatic plants/algae (AP), whereas a minor impact of the sampling method was found for aquatic invertebrates (AI) with an MCR_{sample} of 1.7. In the grab samples, the mean MCR_{sample} yielded 1.8 (10^{th} = 1.1, 90^{th} = 2.5) and was comparable for AI and AP. Especially for AP, mixtures thus become more relevant during rain-induced exposure peaks as more pesticides occur in relatively high concentrations and contribute to the overall risk.

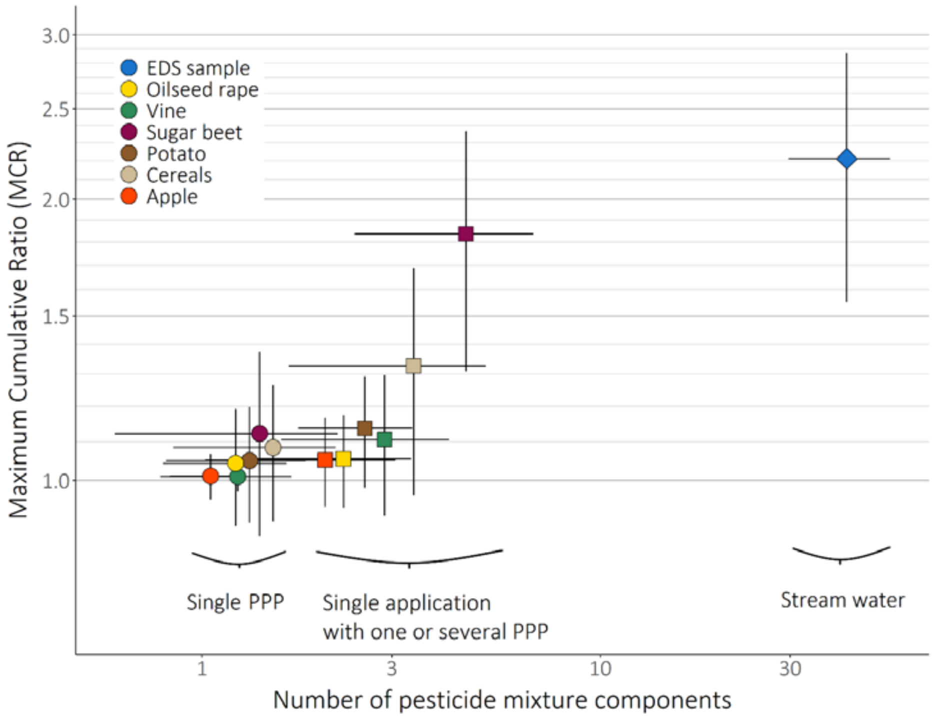


Figure 19: ***MCR*s of different mixture categories against the number of pesticide mixture components: Culture-specific Plant Protection Products (PPP) applied (MCR_{PPP}, circle), applications (MCR_{app}, square) and EDS stream water samples (MCR_{sample}, diamond, including metabolites). Data points represent mean values and bars display the respective standard deviation.**

Generally, the additional risk by mixtures in stream water was not associated with the total estimated pesticide toxicity: The logarithmic TU_{mix} exhibited no correlation with the MCR_{sample} (for AI: $R^2 = 0.01$, $p < 0.005$; for AP: $R^2 = 0.01$, $p < 0.005$). Even at low toxic pressure, where the number of detected compounds decreased, the MCR_{sample} remained relatively constant. This suggests that the *MCR* calculation was largely unaffected by analytical constraints in terms of limits of quantification. Furthermore, no influence of the hydrological catchment size on the MCR_{sample} was observed ($R^2 < 0.01$, $p = 0.05$, area log-transformed). Within the limited gradient of studied catchment sizes, we therefore observed the pesticide mixture risk in different-sized stream or river systems to be comparable. Our findings match those of Vallotton and Price (2016) who derived slightly higher MCR_{sample} values from 2.4 to 2.85 for pesticide mixtures in grab samples from US American surface waters. Accordingly, Gustavsson et al. (2017) found MCR_{sample} values for AI and AP in weekly samples from Swedish small agricultural streams ranging from 2.22 to 2.86, which were constant across streams of different catchment sizes. Regional differences in PPP use and climate conditions impact the spectrum of mixture components and their environmental fate. Nevertheless, comparable pesticide contamination of surface waters has been observed in several other parts of the world, including Africa (Ganatra et al., 2021), Australia (Burgert et al., 2011), France, Finland (Schäfer et al., 2007) and South America (Hunt et al., 2017). Therefore, despite varying mixture components, we expect the risk due to simultaneous pesticide mixtures in the environment to be comparable wherever similar agricultural practices are followed.

The *MCR* values increased from PPP to single applications and water samples indicating a stepwise increase of the pesticide mixture risk. In a first step, application practices combining multiple PPP lead to enhanced mixture risk. In a second step, pesticide residues of these sequential applications from numerous fields featuring different crops with varying PPP treatments within the catchment area enter streams resulting in more complex pesticide cocktails. As the authorisation of PPP is performed at single PPP level, the respective ERA only considers mixtures as represented by the MCR_{PPP}. In the environment, however, pesticide risk is on average twice as high when considering mixtures assuming concentration addition ($MCR_{sample} \approx 2 \times MCR_{PPP}$). We consider the 95th percentile of the event-driven sampling MCR_{sample} of 3.4 to reflect realistic worst-case pesticide mixture conditions. A factor of 3.2 would thus be required to extrapolate from single PPP risk to environmental pesticide mixture risk ($3.4 \approx 3.2 \times MCR_{PPP}$) to cover mixture risk in 95% of observed peak exposure scenarios.

This extrapolation factor relies on the assumption of additive effects from pesticide mixtures, which is recommended as default in the ERA mixture toxicity assessment (EFSA Scientific Committee et al., 2019). While the effects of most mixtures of pesticides were shown to be additive, specific pesticide combinations greatly exceeded the additive effect predictions, i.e. acted synergistically (Cedergreen, 2014). Synergistic combinations may also involve a pesticide and other pollutants like metals or antifoulants. In addition, synergisms were exacerbated when organisms were exposed to additional environmental stress, such as food limitation (Liess et al., 2016; Shahid et al., 2019). In the case of synergistic combinations, the proposed additive mixture extrapolation factor of 3.2 still underestimates the actual ecological effect.

5.3.2 Pesticide Mixtures in the Light of Regulatory Thresholds

Single PPP are generally regulated in such a way that the modelled peak concentrations remain, often only marginally, below predicted ecological threshold levels ($RQ_{max} < 1$). In the field, multiple pesticides may co-occur in concentrations close to their regulatory acceptable concentration (RAC). In combination, mixture components may then accumulate to exposure levels jointly posing an unacceptable risk to aquatic organisms ($RQ_{mix} > 1$) (Junghans et al., 2019).

We therefore assessed the likelihood of pesticides individually or jointly (sum of components primarily affecting the same organism group) causing threshold exceedances in EDS (n = 312). RAC exceedances already by single pesticides for AI and AP were detected in 53% and 18% of EDS, respectively ($RQ_{max} > 1$, see Figure 18). Adding up the risk from all mixture components affecting either AI or AP, the exceedances in EDS increased to 66% and 26% ($RQ_{mix} > 1$). On the one hand, this shows that AI, in particular, are frequently subject to RAC-exceeding pesticide concentrations. On the other hand, 81% (AI) and 69% (AP) of joint RAC exceedances were due to single pesticides, though several samples revealed MCR_{RAC} values greater than 4 or 5. The MCR_{RAC} resulted in a mean value of 1.6 (10^{th} = 1.0, 90^{th} = 2.2) for AI reflecting a 63%-contribution of a single pesticide to the RQ_{mix}. For AP, the mean MCR_{RAC} of 2.4 (10^{th} = 1.3, 90^{th} = 3.6) reflected a 42%-contribution of the dominant pesticide to the RQ_{mix} and affirmed the increased mixture risk for AP compared with AI. Rather than through the joint action of many individual mixture components, exceedances of regulatory thresholds are primarily caused by single pesticides in high concentrations. Nevertheless, the frequent exceedances of regulatory thresholds by single pesticides alone is further aggravated by the joint toxicity of mixtures in the stream water samples.

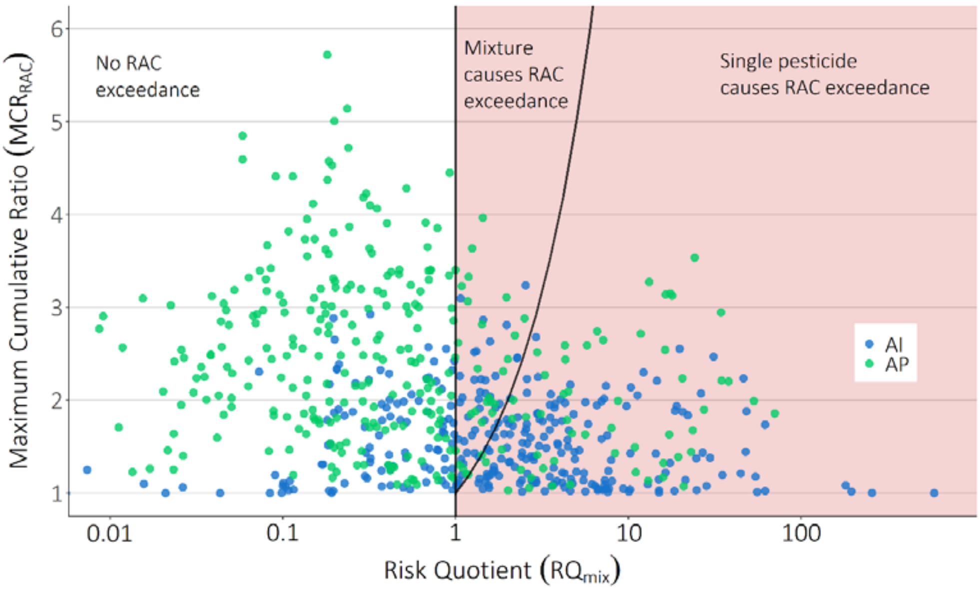


Figure 20: The additive concentration-RAC quotient (RQ_{mix}) indicating regulatory threshold exceedance and respective Maximum Cumulative Ratio (MCR_{RAC}) derived separately for aquatic invertebrates (AI, blue dots) and aquatic plants/algae (AP, green dots) of each event-driven stream water sample (n = 312). Log RQ_{mix} values ≤ 0 represent samples not exceeding the RAC (34% for AI, 74% for AP). Log RQ_{mix} values > 0 represent samples exceeding the RAC (within red shaded area). Dots between the black lines represent samples that exceed the RAC only as a mixture (13% for AI, 8% for AP). Dots to the right of the curved, black line represent samples where single substances already exceed the respective RAC (53% for AI, 18% for AP).

To derive the RQ_{mix} of a sample, all RQs of pesticides affecting the same organism group (AI or AP) were cumulated. This approach may underestimate the actual ecological risk as (i) indirect pesticide effects may enhance the sensitivity of another organism group and increase the overall risk faced by the aquatic ecosystem (Edge et al., 2020; Fernández et al., 2015), (ii) pesticides primarily affecting one organism group may still adversely affect other organisms (Misaki et al., 2019) and (iii) pesticides primarily affecting organisms omitted from our analysis (e.g. fish) additionally contribute to the mixture risk. This RQ_{mix} approach, however, relies on laboratory-based effect concentrations and can thus only estimate the actual ecological risk in the field.

5.3.3 The Variable Dominance of Single Pesticides

Both the low *MCR* values and the regulatory threshold exceedances described above indicate that the main contribution to the toxicity of a mixture could be largely attributed to a single pesticide. However, the identity of these pesticides was found to vary spatio-temporally: 55 different pesticides and 3 pesticide metabolites of the 107 analytes were dominant and ecotoxicologically relevant (log $TU_{max} > -4$) for AI or AP in at least one stream water sample. 21 different pesticides and 1 metabolite were dominant in at least 1% of the samples (see SI Table 4). Previous studies confirmed that pesticide mixture risks in aquatic ecosystems are driven by 1 to very few alternating compounds that vary among sites (Gustavsson et al., 2017; Liess and Ohe, 2005; Liess and Schulz, 1999; Markert et al., 2020; Stenström et al., 2021; Vallotton and Price, 2016). The dominance of single pesticides in the monitored PPP applications implies similar conditions in agricultural fields. This marks a departure from the many studies investigating the effect of mixtures, in which the individual components equally contribute to mixture risk (Altenburger et al., 2000; Backhaus et al., 2000; Silva et al., 2002). Assessing the risk of these equitoxic mixtures proved the combined effect of mixture components in principle, but does not reflect the observed toxic imbalance of components in the environment and thus overrates pesticide mixture relevance. Laboratory toxicity tests assessing the effects of mixtures should consider this toxic imbalance of components for an improved simulation of environmental conditions. For pesticide monitoring programs, the variable spectrum of dominant substances observed here suggests a broad set of analytes to be measured ideally comprising all pesticides applied in a stream's catchment area.

We further assessed whether pesticides that were identified to drive stream water toxicity can be predicted based on the spray series data. Our exposure modelling led to 27 pesticides causing a log $TU_{max} > -4$ in at least 1% of monitored applications (see SI Table 2). However, only 5 of these matched the subset of the 21 pesticides identified as drivers in real water samples. The other 22 pesticides were not identified as drivers in the water samples (n = 9 pesticides) or were absent from the list of analytes (n = 13). Therefore, identification of pesticide toxicity drivers using our application data was limited. Reasons for this may be (i) the changing spectrum of PPP and mitigation measures applied over the years so that the time interval of several years between the monitoring of spray series and streams limits the comparability and (ii) the lack of location information for the monitored applications: We expect that georeferenced spray series data on catchment-scale are needed to account for locally specific cultures shaping mixture patterns. To enhance our predictive capacity of environmental mixtures, more precise knowledge about the timing and localisation of PPP applications is required.

5.3.4 The Frequency of Recurring Exposure Pulses

The mixtures identified in this study represent one-time snapshots of environmental conditions, but over the longer term, the investigated pesticide exposure pulses occur repeatedly. The ERA of pesticides requires that "populations of short-cyclic water organisms" and "species with contrasting life cycle traits (i.e. longer generation time) are able to completely recover in the time available between the exposure events" (Environmental Recovery Option – ERO) (EFSA, 2013). This is at least questionable according to the monitored spray series where an average field faced more than 1 application per month during our stream monitoring period from April to mid-July (Figure 19). In 30% (n = 266) and 75% (n = 670) of analysed spray series, a follow-up application was sprayed less than 7 or 24 days after the previous application. Especially for crops with high application frequency such as apple (mean = 20 times per season, see SI Table 1), potato (10), and vine (8), it can be assumed that application intervals are too short to allow non-target organisms to fully recover or for pesticide residues to degrade. The agricultural streams also encountered a mean of 2.5 and up to 10 exposure pulses resulting in RAC exceedances during the sampling period (Figure 19). In 88% of spray series and 53% of streams, such pulse intervals were, at least once, shorter than 8 weeks – the time period after exposure in which recovery renders adverse effects acceptable under the ERO in the ERA (EFSA, 2013).

Especially vulnerable species are often characterised by generation times of six months or longer clearly exceeding exposure pulse intervals (Liess and Ohe, 2005). Individual-, population-, and community-level effects can accumulate within a single generation (Wiberg-Larsen et al., 2020) and culminate over multiple generations (Liess et al., 2013). Indirect effects (e.g. competition) further increase pesticide sensitivity and can delay recovery from pulse exposure (Dolciotti et al., 2014; Foit et al., 2012; Knillmann et al., 2012). Conversely, species and whole communities have been seen to recover from single pulses and even acquire tolerance to toxic pressure to a certain degree (Beketov et al., 2008; Shahid et al., 2018). Hence, complex and partly contradictory processes determine the effect of sequential exposure and its prediction is therefore challenging. This in turn complicates risk assessment, where no general concept has yet been identified to account for sequential exposure and this uncertainty is translated into assessment factors that lack robust validation.

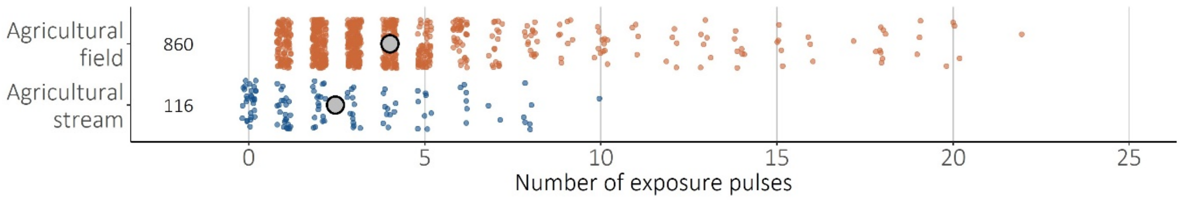


Figure 21: Number of exposure pulses from April to mid-July (stream monitoring period) for agricultural fields and streams. Orange points reflect the number of applications per field (n = 860), blue points reflect number of samples showing a RAC exceedance (RQ_{max} > 1) per stream (n = 116). Grey points depict respective means.

5.4 Conclusion

While PPP are considered mostly individually in the process of authorisation, we found them to occur almost exclusively as a mixture in the environment. 73% of PPP applications already featured a mixture of multiple PPP and stream water samples exhibiting the pesticide use footprint of an entire catchment revealed a mean of 30 detected pesticides. However, we revealed that environmental pesticide mixtures are mostly dominated by one, but alternating, pesticide. Assuming additive effects of mixture components and realistic worst-case conditions, the simultaneous pesticide mixture risk in the environment exceeds the estimated single PPP toxicity by a factor of 3.2. However, uncertainties remain concerning the validity of the additive effect of mixtures under environmental conditions disregarding any potential synergistic interactions. The proposed factor also does not account for the observed sequential pesticide exposure, where the high frequency of pesticide applications and recurring inputs into surface waters most likely exacerbate the ecological risk. Our findings imply that both the simultaneous mixture risk as well as the sequential pesticide exposure represent typical field conditions and hereby confirm concerns described by EFSA's aquatic guidance document stating that "assessing risks for individual PPPs for their use in crop protection programmes characterised by intensive PPP use (e.g. simultaneous use of PPPs with similar mode of action in tank mixtures or their repeated use)" may be "uncertain". The ERA of pesticides thus needs to consider simultaneous and sequential exposure. Further research is needed to estimate the environmental relevance of mixture component interactions (synergism and antagonism) under realistic conditions and to elaborate concepts enabling a quantification of the additional ecological risk due to sequential exposure. This study therefore provides one piece of the puzzle to narrow the gap between prospective single PPP-oriented risk assessment and reality.

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6 Three reasons why the Water Framework Directive (WFD) fails to identify pesticide risks

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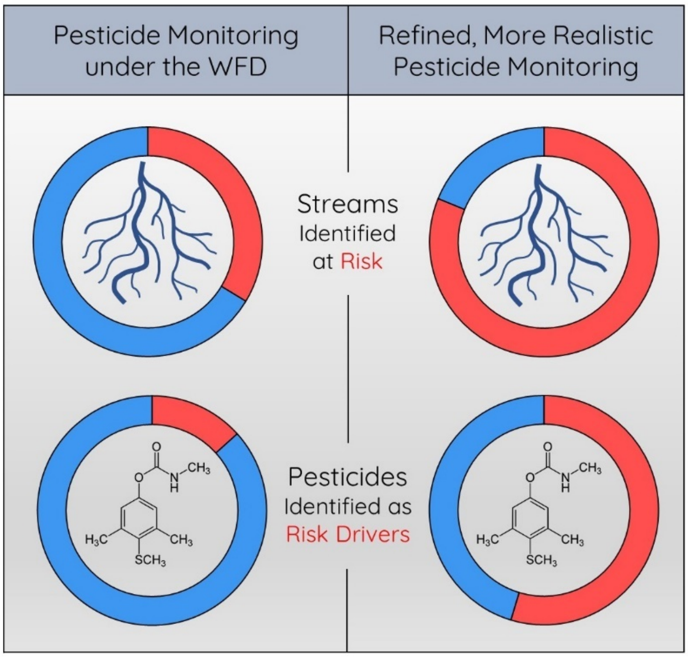
Abstract

The Water Framework Directive (WFD) demands that good status is to be achieved for all European water bodies. While governmental monitoring under the WFD mostly concludes a good status with regard to pesticide pollution, numerous scientific studies have demonstrated widespread negative ecological impacts of pesticide exposure in surface waters. To identify reasons for this discrepancy, we analysed pesticide concentrations measured in a monitoring campaign of 91 agricultural streams in 2018 and 2019 using methodologies that exceed the requirements of the WFD. This included a sampling strategy that takes into account the periodic occurrence of pesticides and a different analyte spectrum designed to reflect current pesticide use. We found that regulatory acceptable concentrations (RACs) were exceeded for 39 different pesticides at 81% of monitoring sites. In comparison, WFD-compliant monitoring of the same sites would have detected only eleven pesticides as exceeding the WFD-based environmental quality standards (EQS) at 35% of monitoring sites. We suggest three reasons for this underestimation of pesticide risk under the WFD-compliant monitoring: (1) The sampling approach - the timing and site selection are unable to adequately capture the periodic occurrence of pesticides and investigate surface waters particularly susceptible to pesticide risks; (2) the measuring method - a too narrow analyte spectrum (6% of pesticides currently approved in Germany) and insufficient analytical capacities result in risk drivers being overlooked; (3) the assessment method for measured concentrations - the protectivity and availability of regulatory thresholds are not sufficient to ensure a good ecological status. We therefore propose practical and legal refinements to improve the WFD's monitoring and assessment strategy in order to gain a more realistic picture of pesticide surface water pollution. This will enable more rapid identification of risk drivers and suitable risk management measures to ultimately improve the status of European surface waters.



6.1 Introduction

Since its implementation in the year 2000, the Water Framework Directive (WFD, 2000/60/EG) has served as the legal basis for EU member states to protect their surface waters (European Union, 2000). It requires member states to achieve and maintain a good status of all lentic and lotic waters. To have good status, a surface water must exhibit both a good chemical and a good ecological status. However, the latest results on the status of European surface waters submitted by the member states reveal that at least 35% of surface waters fail to achieve a good chemical status and 51% show an insufficient ecological status (moderate, poor or bad) (EEA, 2018).

The drivers made responsible for this poor status mainly include the occurrence of ubiquitous, persistent, bioaccumulative and toxic substances (uPBTs), morphological degradation and high nutrient loads (BMUB/UBA, 2016; EEA, 2018). Pesticides, on the contrary, are broadly represented in the WFD list of analytes but cause only 0.4% of surface waters to fail to achieve a good chemical status according to the monitoring data from the 2nd river basin management plan (Mohaupt et al., 2020). This contradicts numerous studies which observed that pesticides frequently exceed regulatory acceptable concentrations (RACs) (Stehle and Schulz, 2015b; Szöcs et al., 2017) and even pose a greater threat to European surface water ecology than any other pollutant class (Malaj et al., 2014; Wolfram et al., 2021). Pesticides have been shown to impair surface water fauna and flora within Europe (Beketov et al., 2013; Larras et al., 2017; Liess et al., 2021a; Liess and Ohe, 2005; Schäfer et al., 2011), but also worldwide, for example in Africa (Ganatra et al., 2021), Australia (Burgert et al., 2011; Wood et al., 2019) and North and South America (Chiu et al., 2016; Hunt et al., 2017). These contrasting results suggest that the current monitoring and assessment methods used in compliance with the WFD result in an underestimation of the actual pesticide risk.

The WFD surface water monitoring strategy focuses on larger rivers while catchments are surveyed less frequently if <100 km^2 or only in exceptional cases if <10 km^2 (Szöcs et al., 2017; Wick et al., 2019). The chemical and ecological status of European small streams is therefore largely unknown. This is problematic because small headwater streams play a decisive role in large-scale overall ecological condition and biodiversity, as they make up two thirds of the entire river network (BfN, 2021; Meyer et al., 2007). Small stream ecosystems are considered biodiversity hotspots, offering diversified habitats for numerous animal, plant, algae and fungi species, and act as recolonization sources for impaired downstream reaches (Liess and Ohe, 2005; Orlinskiy et al., 2015). Such streams have also been shown to be particularly susceptible to agricultural diffuse pesticide pollution, often being located in direct proximity to agricultural fields while lacking the capacity of larger waters to dilute pesticide inputs (Schulz, 2004; Stehle and Schulz, 2015b; Szöcs et al., 2017). These inputs are mostly due to rainfall-induced surface runoff transporting pesticide residues from fields into adjacent streams, resulting in short-term concentration peaks (Liess et al., 1999). For these reasons, there is growing global concern about the chemical and ecological quality of small rivers, which is also reflected in more recent monitoring programs focusing on small streams such as the Regional Stream Quality Assessment (RSQA) in the US (https://webapps.usgs.gov/RSQA/#!/) or the NAWA SPEZ in Switzerland (https://www.eawag.ch/en/research/water-for-ecosystem/pollutants/nawaspez).

Among other objectives, the German National Action Plan (NAP) for the Sustainable Use of Plant Protection Products addressed this blind spot in WFD monitoring, specifically requiring representative monitoring of small surface waters in agricultural catchments with an area of <10 km^2 (BMEL, 2013). Consequently, a uniquely comprehensive monitoring campaign of 124 small streams designed to adequately characterise pesticide pollution was carried out in 2018 and 2019 throughout Germany, Central Europe (see project homepage under www.ufz.de/kgm). Apart from the focus on small streams, its strategy comprised (i) event-driven sampling (EDS) to capture transient pesticide peak concentrations in addition to WFD-compliant regular grab sampling, (ii) an analyte spectrum based on current pesticide use statistics, which differs from the WFD pesticide analytes, and (iii) the consideration of additional pesticide surface water thresholds beyond those listed for the purposes of the WFD. On the basis of this stream monitoring, Liess et al. (2021a) confirmed the frequent occurrence of pesticides in ecologically harmful concentrations generally exceeding regulatory thresholds. Additionally, they linked ecological status to pesticide pressure and proposed protective pesticide thresholds relying on field observations. Further, Halbach et al. (2021) quantified the periodic occurrence of pesticides following rain events in these streams and compared measured concentrations with those recorded during the routine WFD monitoring of two German federal states. The present study now uses this stream monitoring data to evaluate the WFD's pesticide monitoring strategy. Therefore, we compared the results of the surface water assessment of our refined stream monitoring approach against a WFD-compliant approach of the same monitoring sites. In this way, we aim to evaluate the WFD's ability to detect pesticide risks in surface waters, identify reasons for divergent results where they exist, and propose refinements to improve the WFD's pesticide monitoring strategy.

6.2 Material and Methods

6.2.1 Pesticide Monitoring under the WFD – the Current Situation

Under the WFD, EU member states monitor three different categories of sites: (i) Surveillance monitoring sites, where all the WFD quality elements (ecological, hydromorphological, chemical and physico-chemical) are normally assessed. In Germany, the extensive surveillance monitoring network comprises about 260 sites mostly located in larger rivers. (ii) Operational monitoring sites are more abundant (>13,000 in Germany), but require a limited monitoring effort restricted to the assessment of quality elements known to react most sensitively in a water body. This operational monitoring therefore depends on the locally specific pressure situation. (iii) Investigative monitoring sites to locate and assess causes of water pollution that make a surface water fail to achieve a good status (Arle et al., 2016).

The WFD monitoring of pesticides is involved in both the chemical and the ecological status assessment. To classify a surface water's chemical status, all EU member states regularly measure 45 priority substances (PS) or substance groups listed in the WFD and implemented in German law by the Surface Water Ordinance (BGBl, 2016 Annex 8). The list of PS contains 23 pesticides (see supplementary information - SI Table 1). As part of the ecological classification, each EU member state is also obliged to identify pollutants of regional or local importance, the river basin-specific pollutants (RBSP). In Germany, the list of RBSP comprises 67 substances, 44 of which are pesticides (BGBl, 2016 Annex 6). Both PS and RBSP are assigned legally binding environmental quality standards (EQS) reflecting concentration levels below which it is assumed that the aquatic environment and human health are protected. If a single PS or RBSP exceeds an EQS, the chemical status is classified as "not good" or the ecological status is downgraded to less than "good" (at most "moderate"), respectively. In contrast to PS, RBSP must be monitored if "discharged in significant quantities". Monitoring frequencies are legally defined in that PS are measured twelve times per year at least once every three years (operational monitoring) or six years (surveillance monitoring), while RBSP require monitoring four to 13 times per year at least once every three years (operational monitoring) or six years (surveillance monitoring) (BGBl, 2016 Annex 10).

6.2.2 Monitoring Design Used in this Study

The information on stream water pesticide concentrations was collected as part of a Germany-wide monitoring campaign of 124 small lowland streams in 2018 and 2019. The monitoring strategy was described in detail by Liess et al. (2021a) and only a short summary is provided here.

This study focused on a subset of the complete monitoring dataset by considering lowland streams (i) within agricultural catchments, i.e. those with > 20% agricultural land cover within the catchment (Copernicus Land Monitoring Service, 2019) and (ii) where rainfall event-driven sampling (EDS, see below) could be carried out. This subset comprised 91 agricultural streams, of which ten were monitored in both 2018 and 2019. These ten streams are analysed individually for each year, as weather conditions and/or crop types in the catchments differed between the years. The hydrological catchments of these small streams were mostly <30 km^2 (mean = 19 km^2) with an agricultural land cover ranging from 22% to 100% (mean = 75%). Although the selection of agricultural stream monitoring sites and respective catchments showed a higher percentage of agricultural land cover than average German small stream catchments, we estimate the level of pesticide pollution to be representative for German agricultural streams in general (see SI – Representativity analysis). Urban land cover accounted for less than 5% in the majority of stream catchments (see SI Figures 1 & 2).

The streams were sampled from the beginning of April to mid-July, covering the intense application period of pesticides in early summer (Szöcs et al., 2017; Weisner et al., 2021). The samplings were carried out in two different ways: (i) Grab samples (n = 450) were taken on a regular, three-week cycle comparable to the monthly samplings performed under the WFD. Grab sampling was thus carried out irrespective of weather and discharge conditions. (ii) Additionally, the streams were sampled directly after rainfall assumed to cause surface runoff (EDS, n = 312) using automatic sampling devices collecting time-integrated composite samples triggered by a significant water level increase (for details see SI). In total, an average of 4.5 grab samples and 3.1 EDS samples was collected per site.

All water samples were cooled below 4°C during sampling and transport and analysed within four days for 75 pesticides and 33 pesticide metabolites using LC-MS/MS (see SI for substance list and Halbach et al., 2021 for the analytical method). The selection of pesticide analytes was compiled from a prior study by Wick et al. (2019), taking into account (i) a pesticide's current use statistics in relation to its toxicity, (ii) measured concentrations in previous monitoring programmes and (iii) its compatibility with a multi-substance method for chemical analysis. The selected analyte spectrum overlapped with the list of PS and RBSP for two and 22 pesticides, respectively (see SI Table 2). Pyrethroid insecticides and the herbicide glyphosate are expected potential risk drivers for aquatic ecosystems that were omitted due to analytical limitations. Nonetheless, we consider that the analyte spectrum covered the majority of ecotoxicologically relevant pesticides at the time.

6.2.3 Pesticide Surface Water Thresholds

We applied three different types of pesticide surface water thresholds to assess the ecological relevance of measured concentrations: the WFD-based EQS, the regulatory acceptable concentrations (RAC) derived during the authorisation of plant protection products containing the pesticides (UBA, 2019) and the field-based acceptable concentrations (AC_{field}) (Liess et al., 2021a).

The EQS values were taken from the list of PS and German RBSP according to the Surface Water Ordinance (BGBl, 2016 Annex 6/8). To account for the duration of exposure, there are two different EQS under the WFD: (i) the annual average-EQS (AA-EQS) covering long-term effects normally derived on the basis of chronic toxicity data, and (ii) maximum acceptable concentration-EQS (MAC-EQS), which covers short-term effects normally derived on the basis of acute toxicity data (European Commission, 2018). AA-EQS are therefore used to assess time-averaged, long-term concentration levels, while MAC-EQS are used to assess short-term peak concentrations. AA-EQS were available for 24 pesticides (three insecticides, three fungicides, 18 herbicides) and MAC-EQS were available for ten of these 24 pesticides (two insecticides, one fungicide, seven herbicides). When comparing MAC-EQS to the RAC and AC_{field}, we also considered pesticides that are listed as RBSP in other EU member states (see SI Table 2, EEA, 2021) and/or were not included in the stream monitoring analyte spectrum (see SI Table 3).

The RACs as thresholds derived within the environmental risk assessment of plant protection products were obtained from UBA (2019). As each plant protection product containing a specific pesticide (= active ingredient) requires (re-)authorisation prior to use, RACs were available for all pesticides analysed (n = 75, eleven insecticides, 25 fungicides, 39 herbicides). The metabolites methiocarb sulfoxide and prothioconazole-desthio are also assigned a RAC due to their elevated ecotoxicological potential. The RACs applied in this study reflect the regulatory status when monitoring was carried out in 2018 and 2019. Individual RACs may have been adjusted in the meantime as the plant protection products may have been reauthorised taking new scientific knowledge into account. Both RAC and MAC-EQS assess concentration maxima but originate from different legal frameworks and differ in terms of the definition of the protection goal and the precise derivation approach. If a MAC-EQS is exceeded then counteractive measures must be initiated, while compliance with RACs is not legally required.

The AC_{field} was derived on the basis of field observations by Liess et al. (2021a) by linking a stream's peak exposure to its ecological status as reflected by the invertebrate community. This threshold aims for 95% of streams to show a good or high ecological status in terms of the invertebrate-based indicator $SPEAR_{pesticides}$, which responds specifically to pesticide pressure. An AC_{field} was only assigned to the 22 pesticides (eleven insecticides, eight fungicides, three herbicides) for which freshwater invertebrates were considered the most sensitive organism group according to UBA (2019) (referred to in this article as primarily invertebrate-toxic pesticides from here). In contrast to the EQS or RAC, this threshold incorporates other environmental stresses present in the field that interact with pesticide toxicity (e.g. other pesticides, nutrients, temperature or competition). All thresholds are listed in SI Table 2.

6.2.4 Evaluation of Risk Indicated by Threshold Exceedances

Exceedances of the RAC, MAC-EQS and AC_{field} were determined by comparing the measured concentration c_i of pesticide or pesticide metabolite *i* to the relevant threshold. A threshold exceedance is indicated by a risk quotient (*RQ*) greater than 1:

$$RQ = \frac{c_i}{Threshold_i} \qquad 21$$

To determine exceedances for the AA-EQS, the average of all measured concentrations of the pesticide *i* is divided by the threshold:

$$RQ_{AA-EQS} = \frac{mean\ c_i}{AA\text{-}EQS_i} \qquad 22$$

In the WFD-compliant assessment, the monthly sampled concentrations are commonly averaged over an entire year and then compared to the AA-EQS (LAWA-AO, 2019). Since the stream samples of this study were taken only during the period of intense pesticide application, our averaging period only ranged from April to July. This limited averaging period may result in a higher risk than if considering the year as a whole, which would include months with no or reduced pesticide application, particularly in winter (Weisner et al., 2021). However, unlike in practice, the WFD guidance document also explicitly advises that averaging periods should be shorter than a year when episodic exposure is known, which will also be discussed below (see chapter 3.1) (European Commission, 2018). Therefore, we also considered a best-case scenario including hypothetical measurements in which no pesticides were detected in the months when no samplings took place and calculated annual average concentrations following the German guidance (see SI and LAWA-AO, 2019). All calculations were performed using the statistical software R (version 3.5.1) and all plots were created using the R package "ggplot2" (version 3.2.0) (R Core Team, 2018; Wickham, 2009a).

6.3 Results and Discussion

6.3.1 Reason #1 – Sampling Pesticides

Here we discuss the time and sites to sample surface waters for pesticides. Firstly, the WFD sampling frequencies and intervals must be regarded as unsuitable with respect to the seasonal application of pesticides and their event-related input. The rainfall event-driven sampling (EDS) used in our refined monitoring approach captured on average 8.3 times higher pesticide concentration peaks (95th percentile) compared to common grab sampling as performed under the WFD (see Figure 25 and SI Table 2) (Halbach et al., 2021; Liess et al., 2021a). For the metabolites analysed, EDS concentration peaks exceeded the relevant grab sample concentration on average by a factor of 3.8. EDS detected higher total pesticide concentrations compared to grab sampling in 80% of streams (n = 81).

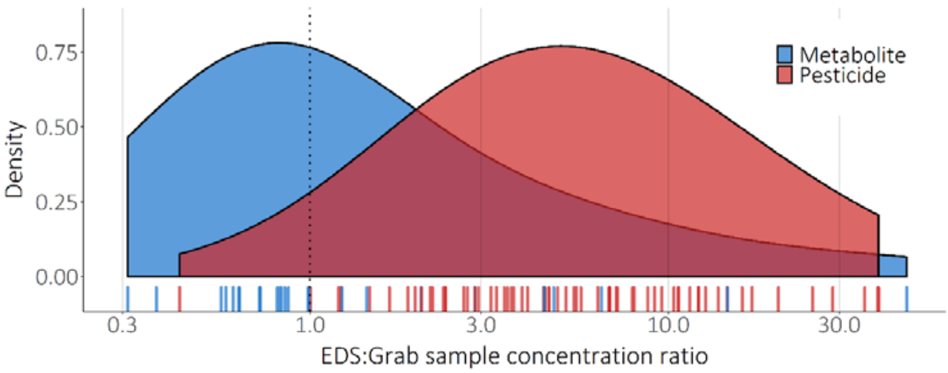


Figure 22: Smoothed distribution of ratios of measured concentration peaks (95th percentile) from event-driven sampling (EDS) and grab sampling for monitored pesticides (red, n = 63 substances) and pesticide metabolites (blue, n = 25) on a logarithmic scale. Vertical lines at the bottom show the single, compound-specific ratios. Pesticides and metabolites not shown revealed 95th percentiles of 0 in EDS (n = 3), grab samples (n = 8) or both (n = 9, see SI Table 2).

As a consequence, EDS increased the probability that an exceedance of the maximum acceptable concentration environmental quality standard (MAC-EQS) would be detected by a factor of four: respective exceedances were identified in 3% (n = 16) of grab samples and 12% (n = 35) of EDS samples. Restricting our analysis to grab sampling caused 16 of the 30 streams with MAC-EQS exceedances to go unnoticed. EDS was thus indispensable to adequately monitor pesticide toxicity peaks as shown in multiple studies (Bundschuh et al., 2014; Lorenz et al., 2017; Rasmussen et al., 2017). It is these peak concentrations that were shown to determine the ecological status of a surface water (Liess et al., 2021a; Ohe et al., 2011; Schäfer et al., 2012). By investigating the concentration differences depending on weather conditions, Szöcs et al. (2017) and Halbach et al. (2021) confirmed the periodic occurrence of pesticides in surface waters on runoff-relevant days. However, WFD-compliant grab sampling following a regular schedule coincided with such runoff-relevant days in only 7% of samplings, minimizing the likelihood of capturing relevant concentration peaks (rainfall >10 mm/d). Norman et al. (2020) and Spycher et al. (2018) found that regular grab sampling needed to be performed at a high frequency of 12 – 24 hours to capture transient peaks adequately. For optimal cost benefit, we therefore recommend supplementing the usual grab sampling with EDS sampling during the main period of pesticide application and following rainfall events. This can also be performed with less elaborate methods than automated sampling devices, for example simple bottle samplers (Liess and Ohe, 2005).

The monthly WFD samplings also cover periods outside the growing season when no relevant pesticide inputs are expected. Accordingly, the assessment of chronic exposure through compliance with annual average-EQS (AA-EQS) involves averaging all monthly measurements for the entire year (LAWA-AO, 2019). However, pesticide application frequencies peaking in April-May (Weisner et al., 2021) were shown to directly relate to measured toxicity peaks in streams in April-June (Liess et al., 1999; Spycher et al., 2018). The current AA-EQS assessment under the WFD thus causes a downscaling of time-averaged concentrations which conceals exceedances of AA-EQS. This is in contrast to the WFD guidance explicitly stating that "when the exposure pattern for a substance is known to be episodic e.g. many pesticides, the averaging period may be a shorter period than a year" (European Commission, 2018). So far, this guidance has been disregarded in practical implementation. The scheduling of sampling and the corresponding averaging period for the AA-EQS assessment thus need to account for the substance-specific, periodic occurrence of pesticides. For larger rivers, the timing of sampling may be of less relevance as pesticide exposure may occur in flattened peaks as inputs from different tributaries arrive successively.

Secondly, the selection of sampling sites currently monitored under the WFD is biased, resulting in unrepresentative estimations of the status of surface waters and contributing to the underestimation of pesticide risk. Wolfram et al. (2021) estimated a median catchment area of 238 km^2 of European surface waters monitored under the WFD, while the median catchment area of the natural river network is less than 20 km^2. Small streams are thus underrepresented in the WFD monitoring site selection while being particularly susceptible to pesticide pollution (Lorenz et al., 2017; Schulz, 2004; Stehle and Schulz, 2015b; Szöcs et al., 2017). This especially concerns small waters with catchments of <10 km^2, which are completely omitted from regular WFD monitoring and are not required to achieve good status despite making up approximately two thirds of the entire river network (BfN, 2021). For these, we observed the same concerning level of pesticide pollution: the number of RAC exceedances detected between streams with catchments of >10 km^2 (n = 65) and <10 km^2 was comparable (n = 36, Wilcoxon rank sum test, p = 0.6). We therefore recommend that the current monitoring performed in the context of the WFD be shifted more towards small water bodies (30-100 km^2) and even include smaller waters with catchments of <10 km^2.

6.3.2 Reason #2 – Measuring Pesticide Contamination

In this section, we discuss issues related to the chemical analysis following water sampling. Firstly, we found the spectrum of pesticide analytes to be measured under the WFD to be outdated and inconsistent. All 108 pesticides and metabolites detected in this study were chosen on the basis of their expected environmental relevance (see chapter 2.2). However, only 24 of the 75 detected pesticides are subject to mandatory monitoring under the WFD and assigned an EQS (two priority substances (PS) and 22 river basin-specific pollutants (RBSP), see SI Table 2). Accordingly, WFD-compliant monitoring of the 101 streams identified eleven pesticides that exceeded their EQS if only grab samples were counted, or 16 if EDS were included. We also found that pesticides not listed in the WFD occurred in ecologically relevant concentrations, with 31 pesticides and one metabolite (grab samples only) or 37 pesticides and two metabolites (EDS included) exceeding the regulatory acceptable concentrations (RACs, see SI Table 2). For aclonifen and metazachlor, the EQS but not the RAC was exceeded. By contrast, 31 RAC exceeding pesticides were identified that would have gone unnoticed in WFD monitoring (see SI Figure 4). Of the ten pesticides most frequently found in concentrations exceeding their RAC, only three are included in the WFD spectrum of analytes. None of the four pesticides that most frequently caused RAC exceedances - thiacloprid, clothianidin, methiocarb and fipronil (∑ = 54% of RAC exceedances) - are listed as a PS or RBSP. These results are supported by Tsaboula et al. (2016) who identified 71 pesticides that required monitoring based on a multi-criteria prioritisation in a large Greek river basin while only small fractions of 13 and 6 pesticides were PS and RBPS, respectively. Accordingly, Moschet et al. (2014) found that when measurements were restricted to pesticides listed as PS in a Swiss stream monitoring campaign, 80% of threshold exceedances remained undetected.

This significantly influences the status classification of surface waters. WFD-compliant pesticide monitoring would yield a good status for 65% (n = 66) of the streams investigated in this study (see Figure 26). Only in 12% (n = 12) of streams, more than one pesticide exceeding the EQS would have been detected. By including EDS samples and RACs to assess additional pesticide analytes, only 19% (n = 19) of streams were found to achieve good status with respect to pesticides. Almost two thirds of the streams (64%, n = 65) exhibited at least two RAC-exceeding pesticides. WFD-compliant monitoring and assessment therefore failed to detect the unacceptable pesticide risk (RAC exceedance) for 57% of agricultural streams and 72% of the pesticides. Consequently, the list of analytes to be monitored under the WFD by far does not include the majority of environmentally relevant pesticides.

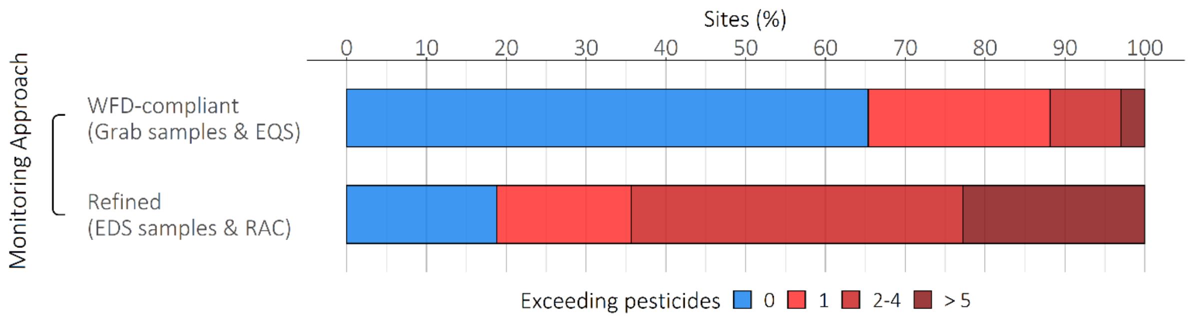


Figure 23: Fraction of sites with good status (blue = no threshold exceedance) and failing to achieve good status (shades of red = threshold exceedances) due to pesticides depending on the type of assessment. The WFD-compliant assessment is limited to grab samples and pesticides with an assigned EQS and found 65% of agricultural streams to have a good status with respect to pesticides. When EDS samples and a wider spectrum of pesticides were included, only 19% of streams were found to have a good status with respect to pesticides.

At the same time, we found that approximately three quarters (n = 49) of pesticides considered under the WFD were no longer approved for use in Germany (EU Pesticides Database of the European Commission, as of July 2021). In contrast, of the 301 different pesticides currently approved for use in Germany, only 6% (n=18) are subject to mandatory analysis under the WFD. Previous investigations have already emphasized that prioritization, monitoring and assessment mostly cover long-known substances while those of emerging concern remain disregarded (Brack et al., 2017; Heiss and Küster, 2015). Thiacloprid, for example, was responsible for 25% of RAC exceedances showing the highest rate of exceedances in our study. Thiacloprid, along with other neonicotinoids, was placed on the so-called Watch List, which brings together candidates for an updated list of PS, in 2015. In 2020, however, its use for plant protection was banned across the EU (European Commission, 2020). Not yet listed as a PS, thiacloprid has probably already peaked in terms of environmental relevance. In the stream monitoring campaign, clothianidin, methiocarb and fipronil were also often measured in concentrations exceeding the RAC. These substances have not been monitored under the WFD and were also banned for plant protection in recent years. Nevertheless, substitutes (e.g. anthranilic diamides like chlorantraniliprole (Schmidt-Jeffris and Nault, 2016)) will fill the emerging gap, and if the aim is to avoid unexpected ecological consequences environmental concentrations must be monitored directly when a compound is used in significant amounts. The list of WFD pesticide analytes and the corresponding EQS must therefore respond more rapidly to the continuously changing spectrum of pesticides applied and relevant in the environment. The Watch List needs to be updated before the candidate substance's environmental relevance peaks. This could be achieved by monitoring a wide range of pesticides in a representative selection of agricultural surface waters and through regular dialogue with pesticide regulators familiar with the dynamics of the current-use pesticide spectrum. For now, we recommend that environmental authorities in charge of monitoring extend the mandatory analyte spectrum to include pesticides currently used (e.g. on the basis of sales quantities as published by the BVL for Germany) or identified as drivers of risk in this study (see SI Table 2). To classify measured concentrations when EQS are not available, we suggest using the AC_{field} (for invertebrate toxic pesticides, Liess et al., 2021a) and the RAC (for pesticides primarily affecting other organism groups, UBA, 2019) to assess concentration maxima. The Swiss Ecotox Centre has also derived chronic and acute quality standards for many pesticides not assigned an AA- or MAC-EQS following the official guidance (Oekotoxzentrum, 2021), that may not provide sufficient protection, though (see Reason #3 below).

Furthermore, the spectrum of RBSP to be measured by an EU member state involves two deficiencies: (i) Increasing the monitoring effort and extending the RBSP spectrum involves additional costs for monitoring and possible risk mitigation measures. By providing less monitoring data, the obligation to initiate such measures can be circumvented, thus penalising ambitions to protect the environment. (ii) Under the WFD, RBSP are monitored in a certain surface water if they were considered beforehand to be "discharged in significant quantities". Whether an RBSP is "discharged in significant quantities" in a specific water body and needs to be integrated in routine WFD monitoring is difficult to evaluate reliably as long as the RBSP is not measured. Monitoring capacities for almost 10,000 WFD water bodies in Germany alone are limited and do not allow all pollutants "discharged in significant quantities" to be precisely identified in advance. Meanwhile, continuous changes in agricultural use and pesticide application schemes make it more difficult to monitor relevant RBSP (Arle et al., 2016). Moreover, the WFD does not define what "significant quantities" are, with the result that different interpretations prevail in the EU member states. We therefore support the integration of RBSP monitoring into the chemical status assessment as proposed by Brack et al. (2017). The separate assessment of PS for chemical status and RBSP for determining ecological status unjustifiably implies different monitoring intensities and complicates the interpretation of the effect of chemicals on the ecological status. The proposed integration would also have the positive side effect of harmonizing monitoring ambitions, as all EU member states would monitor the same list of RBSP assigned harmonized EQS. To take into account regional differences in pollution patterns and risk drivers, EU member states might omit analytes of negligible concern for their region or river basin. Such a negligible concern would have to be convincingly demonstrated on a regular basis by representative measurements, pesticide sales and application quantities or exposure modelling.

In addition to the insufficient analyte spectrum, analytical capacities hinder measuring the pesticide contamination. Several pesticides are so toxic for aquatic organisms that their acceptable concentrations in the water phase are below common analytical limits of detection. This partly concerns legacy compounds like heptachlor and dichlorvos, but also current-use neonicotinoid and pyrethroid insecticides. The AA-EQS for imidacloprid and cypermethrin, for example, are only 2 ng/L and 80 pg/L – concentrations too low to be quantified by the commissioned laboratories in the WFD monitoring (Jarosch, 2018; Moschet et al., 2014; Rösch et al., 2019; Weißbach and Stricker, 2020). EQS exceedances may therefore remain unmeasured, raising the question of how to adequately monitor such toxic compounds and whether their use is generally justifiable when the resulting risk cannot be reliably assessed.

6.3.3 Reason #3 – Assessing Pesticide Effects

Here, we address the assessment of potential ecological consequences of measured concentrations by applying regulatory thresholds. Firstly, we raise concerns regarding the capacity of current regulatory thresholds to adequately assess pesticide risk. We compared the absolute values of MAC-EQS (including other member states' RBSP) with the German RACs and the field-based acceptable concentrations (AC_{field}, Liess et al., 2021a), all of which aim to assess acute pesticide risks.

RAC and MAC-EQS values differed for 29 of the 31 analysed pesticides that are assigned both thresholds, but were on average comparable (log-transformed paired t-test, $p = 0.4$). All four pesticides that are assigned MAC-EQS and AC_{field} values, imidacloprid, dimethoate, pirimicarb and ethofumesate, exhibit a MAC-EQS greater than the respective AC_{field} by a mean factor of 16 (geometric mean, min = 2.4, max = 195, see Figure 27). The RAC exceeded the corresponding AC_{field} values for 90% (n = 20) of compared pesticides. RACs were significantly higher than AC_{field} values (log-transformed paired t-test, $p<0.001$) by a mean factor of 4.2 (geometric mean, n = 22, min = 0.04, max = 56.5). Consequently, applying the mostly lower AC_{field} classified more streams as being at risk than the EQS or RAC, showing 96% (n = 97) of agricultural streams as failing to achieve good status (see SI Figure 4).

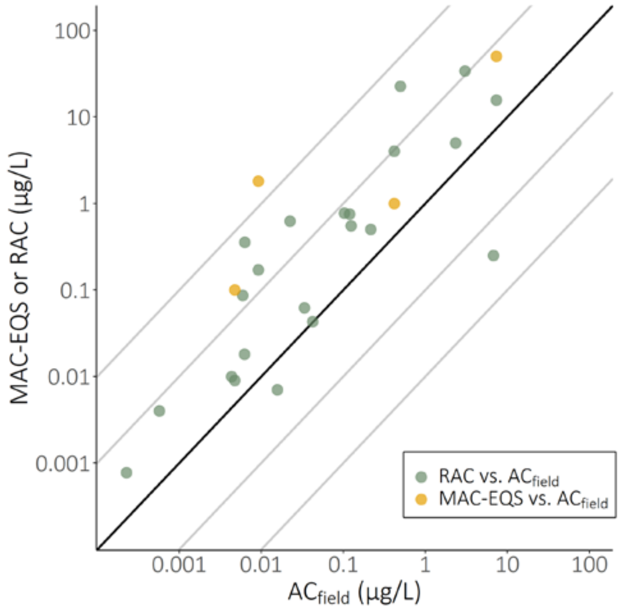


Figure 24: Comparison of the acceptable concentrations for pesticides derived from field observations (AC_{field}, Liess et al., 2021a) with those provided in the WFD (maximum acceptable concentration environmental quality standard - MAC-EQS) or from pesticide risk assessment (regulatory acceptable concentration - RAC). Each dot represents one pesticide for which the AC_{field} and either the MAC-EQS (orange) or the RAC (olive) is available. A dot on the black bisectrix indicates equal values for the AC_{field} and the MAC-EQS/RAC. Dots above or below the black bisectrix indicate a lower or higher AC_{field} compared to the MAC-EQS/RAC, respectively. Grey lines indicate value differences in orders of magnitude. The average deviation from the AC_{field} was 16 for the four MAC-EQS and 4.2 for the 22 RACs (geometric mean).

The general comparability of absolute values of MAC-EQS and RAC and the divergence from the AC_{field} are largely due to the differing assessment factors (AFs) applied in the respective threshold derivation. MAC-EQS and RAC rely on comparable or partly equal AFs aiming to account for the uncertainties relating to the transferability of effects from artificial test systems to the field. To extrapolate from acute toxicity tests to the field for invertebrates for example, the guidance for the derivation of MAC-EQS and RAC propose AFs of 100 (EFSA, 2013; European Commission, 2018). While AFs of MAC-EQS and RAC are generally based on estimations derived from artificial test systems, the AF determined for the AC_{field} is calibrated to pesticide effects observed in the field. Following this approach, Liess et al. (2021a) determined an AF for acute toxicity tests of almost 2,000 required to protect vulnerable species in the field, resulting in the mostly lower AC_{field} values. This insufficiency of current AFs is supported by several other studies relating pesticide concentrations to effects on invertebrates under field conditions. Significant shifts in stream invertebrate communities were demonstrated at concentrations of one 100th of the concentration causing 50% of organisms to display effects in acute toxicity tests (Knillmann et al., 2018; Liess and Ohe, 2005; Münze et al., 2017; Ohe et al., 2011). Schäfer et al. (2012) found that the relative abundance of sensitive species decreased by 27% - 61% with an AF of 100 and estimated that an AF of 1,000 - 10,000 was required to avoid pesticide-related effects. In addition, the richness of invertebrate families was found to decrease in the field when concentration maxima exceeded levels equalling one tenth of regulatory thresholds (Beketov et al., 2013; Stehle and Schulz, 2015a). In contrast to these field investigations, an AF of ten to 100 was estimated as sufficient to extrapolate from single species acute toxicity tests to multi-species micro- and mesocosms (Brock and van Wijngaarden, 2012; van Wijngaarden et al., 2015). These test systems, however, fail to realistically represent environmental conditions and to account for factors that increase the sensitivity of organisms in the field. These include the joint toxicity of co-occurring pesticides (Weisner et al., 2021), additional environmental stress

(Beermann et al., 2018), complex trophic interactions leading to indirect effects (Miller et al., 2020), delayed effects appearing after the runtime of the test (Rasmussen et al., 2017), sequential pesticide exposure (Wiberg-Larsen et al., 2020) and the insensitivity of commonly studied biological metrics (Liess and Beketov, 2011).

All these investigations indicate that regulatory thresholds are too high to protect aquatic ecosystems. This inadequacy of regulatory thresholds is also supported by an extreme variability between EQS for a single RBSP in different EU member states (when national RBSP overlap) with divergences amounting to as much as a factor of 100,000 despite a common guideline for the derivation of thresholds (Arle et al., 2016). This is despite an absence of evidence that effect thresholds vary by such magnitude across geographic regions. Instead, this underlines the regulatory uncertainty when predicting effect thresholds from experimental data. Further efforts are therefore needed to validate regulatory thresholds based on field observations – also for AA-EQS and considering groups of organisms other than invertebrates. For many pesticides, algae, plants or fish are the first organism groups to show effects (Leblanc, 1984) but still lack a suitable bioindicator for pesticide stress, which is required to validate the relevant regulatory thresholds and AFs.

Besides the question whether EQS are protective enough, we raise concerns regarding the availability of MAC-EQS to assess concentration maxima. For the 24 pesticides to be analysed both under the WFD in Germany and in our study, only ten are assigned a MAC-EQS. However, the remaining fourteen pesticides also showed a periodically increased occurrence following rain events (mean EDS:Grab sample concentration ratio = 9.5, see Figure 25). The guideline theoretically requires that exposure duration be taken into account, since “exposure may also occur intermittently for short periods e.g. coinciding with storm events” (European Commission, 2018), but once again, the implementation has so far disregarded this requirement.

In conclusion, there is strong evidence that compliance with current regulatory thresholds does not ensure a good ecological status in the field. We therefore recommend the use of AC_{field} values validated by field observations for invertebrate-toxic pesticides. However, a field-based validation of MAC-EQS for pesticides primarily affecting organism groups other than invertebrates as well as AA-EQS in general is lacking. The comparability of status assessments throughout the EU and the coherence of initiation of risk-reducing strategies requires an EU-wide harmonization of EQS for pesticides and other RBSP. Furthermore, there is no logical reason to separately define divergent pesticide thresholds for acceptable concentration maxima, as for the RAC under Regulation (EC) No 1107/2009 and the EQS under the WFD. Following the recommendations of Brack et al. (2017) and Schäfer et al. (2019), coexisting legal frameworks should thus be more interconnected where their scopes overlap in order to harmonize protection goals.

6.3.4 Our Findings in the Light of EU-Wide Results

Even if pesticide risk drivers are expected to vary locally due to differing cropping patterns and pest pressures, pesticide pressure and related ecological risks were found to be comparable for surface waters across European regions despite differences in agricultural use intensities (Schreiner et al., 2021; Stehle and Schulz, 2015b; Wolfram et al., 2021). We thus assume that our findings quantifying pesticide risk are generally transferable to other regions beyond our German study area. However, our results differ distinctly from EU-wide WFD-compliant assessments. By applying the RAC, we found 81% of the streams investigated to be at risk due to pesticides (see Figure 26). RACs were exceeded in 38% (n = 38) of streams by herbicides and in 75% (n = 76) of streams by insecticides. An EU-wide assessment of WFD monitoring data covering the period 2007 to 2017 found only 5% to 15% and 3% to 8% of surface waters failing to achieve a good status due to herbicides and insecticides, respectively (Mohaupt et al., 2020). This discrepancy is partly rooted in our focus on surface waters in the agricultural landscape. More importantly, we conclude that the discrepancy in results is due to the issues associated with the WFD monitoring strategy as outlined above, which apply to all EU member states.

6.4 Conclusions

- WFD sampling, chemical analysis and assessment of measured concentrations are insufficient to identify pesticide risks in surface waters. As a consequence, the chemical status of surface waters is overestimated and the contribution of pesticides to the ecological status is underestimated under the WFD.
- We propose legal and practical adjustments that would enable refined and more realistic WFD pesticide monitoring. This will (i) help explain and narrow the gap between the chemical and ecological status of surface water bodies also requiring the consideration of suitable ecological indicators that respond to pesticide pressure and (ii) implement an adequate pesticide post-registration monitoring that enables a shift in the prospective pesticide risk assessment from non-validated exposure and effect predictions to actual environmental exposure and protective thresholds. As shown in this study, current governmental monitoring under the WFD is only of very limited use for such validation as critical pesticides and threatened surface waters remain undetected. Following the polluter pays principle, the European Parliament has already suggested in the plant protection products regulation that the additional costs for specific pesticide monitoring could be (co-)financed by plant protection product manufacturers.
- Early identification of risk drivers and immediate feedback to pesticide regulators is key to reducing the proportion of surface waters that fail to achieve a good chemical and ecological status. 20 years after the implementation of the WFD, the failure to come closer to meeting the envisaged good status for European surface water bodies underlines the necessity to substantially improve the monitoring and assessment strategy.

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7 Width of vegetated buffer strips to protect aquatic life from pesticide effects

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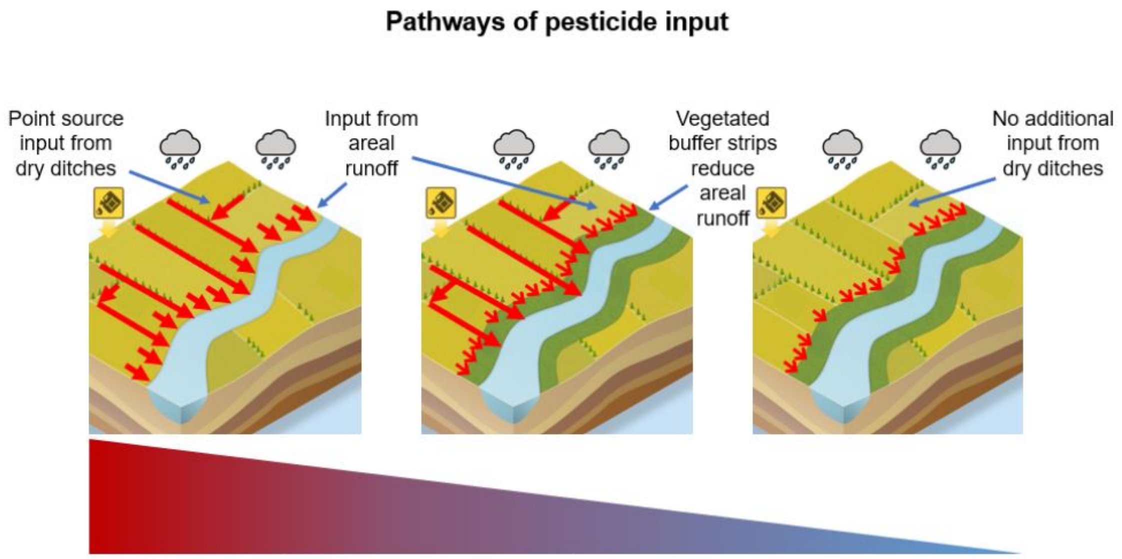
Abstract

Vegetated buffer strips (VBS) are an effective measure to retain pesticide inputs during rain events. Numerous studies have examined the retention effects of VBS on pesticides. However, no study has addressed on a large scale with event-related peak concentrations how wide the VBS should be to avoid ecological impacts on aquatic life. Here, we investigated for 115 lowland stream sections in Germany the relevance of environmental and physico-chemical parameters to determine the in-stream pesticide concentration and their ecological risks. Based on peak concentrations related to rain events with precipitation amount resulting in VBS relevant surface runoff for 30 of the 115 investigated stream sections (25 to 70 mm/d), we demonstrated that the average width of VBS was the main parameter (R^2 = 0.38) reducing the pesticide input ratio, indicating a relevant proportion of surface runoff contributing to the total in-stream pesticide concentrations. Additionally, dry ditches within agricultural fields increased pesticide input (R^2 = 0.31). Generally, substances classified as slightly mobile were better retained by VBS than mobile substances. Other factors including slope, land use and vegetation cover of VBS had only a minor influence. We assessed the ecological risk of in-stream pesticide concentrations by quantifying exceedances of regulatory- (RAC) and field-validated acceptable concentrations (AC_{field}). We then translated this ecological risk into protective VBS width by calculating the quotient of in-stream concentration and threshold (RQ). We estimate that a VBS width of 18 m is sufficient to meet the $RQ_{ACfield}$ protection goal for 95% of streams. The presence of dry ditches increased the protective VBS width to 32 m. In current agricultural practice, however, 26% of the water stretches investigated do not comply with the prescribed 5 m VBS. An extension of the VBS area to 18 m would demand 3.8% of agricultural land within the catchments. A 50% reduction in pesticide use, as required by the European green deal, would still result in 39% (RAC) and 68% (AC_{field}) of event-related samples being exceeded. Consequently, we see the extension of the VBS width as the most efficient measure to sustainably reduce pesticide concentrations in small streams.



7.1 Introduction

Small streams are exposed to ecologically relevant pesticide concentrations due to rainfall-related runoff from adjacent agricultural fields (Liess et al., 1999; Liess and Schulz, 1999). Vegetated buffer strips (VBS) located between the stream channel and the agricultural field are an important measure to reduce diffuse pesticide input from direct runoff (Reichenberger et al., 2007). The positive effect of VBS in retaining pesticides has been shown in many review studies (Arora et al., 2010; Cole et al., 2020; Lacas et al., 2005; Prosser et al., 2020; Reichenberger et al., 2007; Zhang et al., 2010). However, there is an ongoing debate about the magnitude of the retention capacity for pesticides (Bereswill et al., 2013; Carluer et al., 2011; Davies et al., 2008; Donohue et al., 2006; Fitzpatrick et al., 2001; Lorenz et al., 2022; Wang et al., 1997). Despite the numerous studies available, few have quantified the effects of VBS on the in-stream pesticide concentration of small agricultural streams (Bereswill et al., 2013; Dunn et al., 2011; Rasmussen et al., 2011). For example, the minimum VBS width proved to be the best measure to explain the maximum toxicity of 20 pesticides in 14 Danish lowland streams (Rasmussen et al., 2011). The authors conclude that a 6.6 m-wide VBS would be sufficient to keep in-stream pesticide concentrations at a low risk level for invertebrates (< -3 log toxic unit). A meta-analysis of 80 studies regarding the most effective VBS width in terms of cost-benefit estimation (Liu et al., 2008) established that a 10 m-wide VBS would be the optimum width to retain pesticides – without, however, quantifying the degree of reduction. Currently, a VBS width of 5 m is prescribed for second-order streams with a catchment area larger than 10 km² according to §38a of the German Water Resources Act (BGBl, 2009). This objective was also set out in the National Action Plan for Plant Protection Products (NAP) (BMEL, 2013). However, there is no scientific consensus as to whether a width of 5 m is sufficient to protect in-stream communities.

The effectiveness of VBS is dependent not only on their width but also on their vegetation cover, the longitudinal continuity of the VBS and the physico-chemical properties of the pesticides to be retained (Cole et al., 2020; Dosskey et al., 2010; Liu et al., 2008; Prosser et al., 2020; Reed and Carpenter, 2002). In addition to the characteristics of the VBS itself, other catchment factors also determine the exposure to pesticides in a stream, such as soil, erodibility, length and slope of the adjacent field and the presence of erosion rills and dry ditches (Bereswill et al., 2012; Dabrowski et al., 2002; Hilary et al., 2021; Schönenberger et al., 2022; Schriever et al., 2007; Schriever and Liess, 2007; Stehle et al., 2016; Wohlfahrt et al., 2010). On-field erosion rills, in particular (for definition see chapter 2.4.5), can affect the VBS retention efficacy (Bereswill et al., 2012; Stehle et al., 2016). This has been shown in viniculture-dominated catchments, where pesticide concentrations in erosion rills positively correlated with in-stream pesticide concentration and buffer strips had no reducing effect (Bereswill et al., 2012; Ohliger and Schulz, 2010). However, the influence of dry ditches compared to on-field erosion rills has also not yet been sufficiently quantified.

We evaluated the influence of VBS and other catchment characteristics as well as the physico-chemical properties of the pesticides applied on in-stream pesticide contamination in 115 small streams distributed across Germany by means of event-related samples. During the period of highest pesticide contamination (April to July), we investigated (i) the relevance of VBS width and additional environmental factors on the in-stream pesticide concentration and (ii) the influence of physico-chemical properties on the in-stream occurrence of pesticides. We further estimated a protective VBS width to comply with risk assessment thresholds and mitigate ecological risks (RQ) of pesticides to small agricultural streams.

7.2 Material and Methods

7.2.1 Study area

We investigated 115 small streams in mainly agricultural environments (> 20% agricultural cover, average 59%) during the main pesticide application period from April to July in 2018 and 2019 (see map SI Fig. 1). The streams were distributed across Germany. The average catchment size was 12 km², varying from 1.8 km² to 70 km². The stream channel width varied between 0.5 m and 5 m (details are shown in SI Tab. 2). The influence of urban areas (average 6.7% of urban land cover) was minimised to the best of our knowledge to ensure that the stream did not flow through urban areas. Thus, point source discharges from wastewater treatment plants (WWTP) were reduced. For a further detailed description of the influence of urban point sources on the streams, see elsewhere (Liess et al., 2021).

Thirty stream sections fulfilled the requirements of the occurrence of at least one event-driven sample with a minimum of 25 mm/d and a maximum of 70 mm/d (maximum amount of rain for one EDS) of rainfall prior to the pesticide sampling. This site selection (n = 30) was used for the analysis in the results. We excluded two sites from the analysis as they were characterized by a 250 times lower in-stream pesticide concentration than other comparable sites. Organic farming in the catchment area could be one reason for this exceptionally low pesticide exposure. Statistically, sites were identified as outliers by Cook's distance.

7.2.2 Sampling design, chemical analysis and substance selection

Event-driven water samples (EDS, n = 318) were obtained from mid-April to mid-July in 2018 and 2019 during the main pesticide application period (Liess et al., 2021). For this we used an automatic sampling device to collect samples as soon as the water level rose by more than 5 cm (Maxx, Rangendingen, Germany). After initiating the sampling, a composite sample of 40 subsamples was obtained every 5 minutes (40 mL) for 3 hours and 20 minutes. Samples were cooled to 4°C on site and analysed within two days. Additionally, we obtained grab samples every three weeks according to the guidelines of the Water Framework Directive (WFD) (European Union, 2000). Grab samples were treated in the same way as the EDS and analysed for 11 insecticides, 26 fungicides and 38 herbicides with an LC-HRMS/MS (for further details see Halbach et al. (2021)). Substances were only included in the analysis if their use was authorised in Germany at the time of sampling (see list of substances SI Tab. 1). Approval statuses were obtained from the Federal Office of Consumer Protection and Food Safety (BVL) (BVL, 2022).

The average application rates for the selected pesticides were derived from the BVL (BVL, 2022) (see SI Tab. 1). We retrieved the pesticide application rate data from 1998 to 2018 for each substance and aggregated them by the median for each pesticide application. Application rates were not available for desaminometamitron, fipronil (not approved since 2016), fludioxonil and propyzamide. We then normalised the pesticide water concentration with the application rate for each substance to calculate a pesticide input ratio (PIR) for each stream section (see equation 1) (see SI Tab. 3).

$$Pesticide\ input\ ratio\ (PIR) = \sum_{i=1}^{n} \frac{concentration\ (\mu g/L)_i}{application\ rate\ (g/ha)_i} \qquad (1)$$

7.2.3 Ecological risk assessment

We estimated ecological effects on non-target organisms by comparing the in-stream pesticide concentration with existing regulatory and ecological effect thresholds. For this purpose, we used the RAC and AC_{field} (Liess et al., 2021; Umweltbundesamt (UBA), 2019). Regulatory acceptable concentrations (RACs) were introduced to prevent unacceptable effects of pesticide exposure on non-target organisms. Within this framework, no long-term adverse effects on the freshwater populations are anticipated as long as the threshold values are not exceeded (Umweltbundesamt (UBA), 2019). However, a recent extensive nationwide study in Germany showed that generally the existing RAC thresholds for insecticides and fungicides are not protective (Liess et al., 2021). Instead, they introduced a field-validated acceptable concentration AC_{field} that provides a protective threshold to achieve "good" ecological quality for 95% of streams. Limited to effects on the invertebrate community, the AC_{field} only covers insecticides and fungicides for which invertebrates represent the most sensitive organism group (for a full list see Liess et al. (2021)). The in-stream pesticide concentration was converted to a risk quotient (RQ) with these two thresholds. For this purpose, the concentration is divided by the threshold value. We defined three ecological risks based on the different thresholds: (1) the maximum concentration to threshold ratio with RAC values RQ_{max} (see equation 2), (2) the $RQ_{ACfield}$, by replacing RAC values with AC_{field} values if available (see equation 3), and (3) the RQ_{sum} with RAC values only to account for the summed risk of all pesticides present in a sample (see equation 4). For acetamiprid, bromoxynil, clothianidin, dimoxystrobin, foramsulfuron, imidacloprid, lenacil, methiocarb, s-metolachlor, terbuthylazin, thiacloprid and thiamethoxam we used the predicted environmental exposure concentration (PEC) values instead of post-regulated RAC values. This is due to the fact that a lower post-regulated RAC value identified during an update within the risk assessment process is in many cases not associated with an updated PEC. Such a delay in risk assessment of exposure and effect applied to 12 of the 16 most ecologically relevant pesticides at the time of the study. As a result, the post-regulated RAC values are often too low and the authorised application rates result in in-stream concentrations that substantially exceed the post-regulated RAC by factors of up to 5 and would, in our analyses, result in biased wide VBS widths to retain pesticides. Therefore, in these cases, the authorised application rates and resulting PECs don’t match the post-regulated RACs anymore. No authorisation would normally (in an optimal, non-inert risk assessment) be issued under these conditions. We here aim to derive a theoretically protective VBS width that neglects this inertia-problem of the risk assessment. To avoid that this imbalance of PEC and RAC is carried out on the backs of the VBS width, we consider the PECs instead. We also expect that PECs were close to the initial RACs before these RACs were post-regulated, since post-regulation already led to RACs smaller than the respective PECs. We therefore consider the PECs as a suitable approximation of initial RACs to derive a protective VBS width. See section 3.5 for a derivation of protective VBS width where post-regulated RACs were not exchanged by PECs.

$$RQ_{max} = \max_{i=1} \frac{c_i}{RAC_i} \qquad (2)$$

$$RQ_{AC_{field}} = \max_{i=1} \frac{c_i}{AC_{field_i}} \qquad (3)$$

$$RQ_{sum} = \sum_{i=1}^{n} \frac{c_i}{RAC_i} \qquad (4)$$

RQ = risk quotient

i = substance

c = concentration

RAC = regulatory acceptable concentration

AC_{field} = field-validated acceptable concentration

7.2.4 Quantification of catchment variables

7.2.4.1 Vegetated buffer strips

Vegetated buffer strips (VBS) were identified on each side of the streams with transects at 100 m intervals up to 3 km upstream of the sampling site (see SI Fig. 2). Information was obtained with QGIS (version 3.2.1) from aerial photography with field observation from every site. Transect lines (metres) were drawn from the stream channel towards the adjacent agricultural field. The maximum transect width for digitalisation was 150 m, as no input of pesticides is expected via the path of surface runoff from more distant fields. The widths were averaged for each transect of the stream. All transects were averaged for each stream section. In addition, a weighted width was calculated by dividing the maximum distance of the transects by the distance of each individual transect. Transects close to the sampling sites were thus given a greater weighting, as these are considered more relevant to explaining in-stream pesticide concentrations. The vegetation of the VBS was mapped during the 2018 and 2019 monitoring campaign. All data regarding VBS and catchment factors is listed in SI Tab. 2.

It is important to note that an intended pesticide-untreated area in the agricultural field (required by the terms of use of specific PPP and acting as an extension to the VBS) is not regarded as part of the VBS width. In addition, the potential misconduct of farmers who have applied pesticides on the VBS could not be traced.

7.2.4.2 Land use

The agricultural land use in the catchment was mapped 3 km upstream and in a 500 m wide longitudinal runoff corridor (LRC) to each site. Several studies have demonstrated that the impact of pesticide input through runoff is greatest within this area (Bunzel et al., 2014; Schriever et al., 2007). The characteristics of land use within the catchments investigated was highly diverse, covering a large gradient of environmental factors. Details on land use and crop types are listed in SI Fig. 3.

The loss of agricultural land due to a possible extension of the VBS area was calculated with QGIS. The land use data used for this analysis were obtained from the national digital landscape model (Basis-DLM, 2018) (ATKIS, 2018). A buffer area of 18 m and 5 m was calculated around the watercourses in the catchments (see SI Fig. 4). Subsequently, these areas were subtracted from each other. At each stream section, the agricultural land within this buffer area was summed and divided against the percentage of total agricultural land in the watershed.

7.2.4.3 Precipitation

The daily precipitation amounts were obtained from radar information collected by the German Weather Service in 2018 and 2019 with a spatial resolution of 1 km² (Deutscher Wetterdienst (DWD), 2020).

7.2.4.4 Slope

Slope data was processed by digital elevation model (DGM25) (EFA, 2013) with a resolution of 25 x 25 m. The slope of the field adjacent to the stream was calculated by the extended distance between the end of the transect (see SI Fig. 2) and the next highest location on the adjacent field DGM25.

7.2.4.5 Erosion rills and dry ditches

The number of erosion rills (i.e., concentrated flow paths on the field leading directly to the bank of a tributary (Bruce et al., 1975; Cooper et al., 2004)) was determined by calculating the Topographic Wetness Index (TWI) using the "SAGA flow accumulation tool" in ArcGIS (version 10.6) (SAGA, 2018). This approach determines the accumulating water from neighbouring cells and calculates the TWI value according to the slope of the catchments. This allows raster datasets to be created that show lines along streams indicating the potential for surface runoff and its flow direction. The number of dry ditches (i.e. canalized channels next to fields or roads that do not carry water during dry periods (Bereswill et al., 2013)) were mapped from aerial photography and supplemented by on-site field observations (Bereswill et al., 2013).

7.2.4.6 Soil

Information on the soil types occurring in the catchment areas of the stream sections was obtained from soil maps (BÜK1000) (BGR, 1998). Since several soil types occur in most catchments and thus no uniform value could be determined for the statistical evaluations, the soil types were classified according to their vulnerability to erosion by rainfall. This approach assumes that the input of pesticides from surface runoff is greater from soils that erode faster than from less erodible soils. For this purpose, the soil erodibility factor K of the general soil erodibility equation was used (Schwertmann et al., 1990; Wischmeier and Smith, 1978). This factor describes how easily soil material is washed from the aggregate structure. The K-factor was determined for each field and averaged for the LRC of a stream section.

7.2.5 Data analysis

7.2.5.1 Comparison of pesticides in terms of their physico-chemical properties

We compared the differences between physico-chemical property classes (mobility, solubility and persistence) of the measured in-stream pesticide concentrations divided by their average application rate. For this comparison we used an adapted classification system for substances obtained from the Pesticide Property Data Base (PPDB) (Lewis et al., 2016). Kfoc values were used instead of Koc values because they were available for more pesticides. Persistence is represented by water-sediment and water phase DT50soil values according to PPDB (for values see SI Tab. 1).

7.2.5.2 Procedure of linear regression models and multiple linear regressions

VBS width and in-stream pesticide concentration were correlated using log-transformed regression models. All regressions were performed using the linear ordinary least squares approach from the “stats” package (v4.0.2) in RStudio (R Core Team, 2020). To evaluate the influence of VBS width and the number of dry ditches on the logarithmic risk quotient, a model consisting of both variables was constructed.

Multiple linear regression analysis was performed to evaluate the influence of catchment factors on the average in-stream pesticide concentration divided by the average application rate (µg/L) and the corresponding risk quotients (RQ_{max}). All predictors were validated for homoscedasticity and normality, and were log-transformed if necessary. These include: average VBS width, agriculture in the LRC, average slope (%), number of erosion rills, number of dry ditches, dominant soil type in the LRC, the average erodibility (K-factor) per LRC, catchment size and the average field size per LRC (see SI Tab. 2). Intercorrelation of environmental parameters was tested using the variance inflation factor (VIF). Parameters with VIF scores greater than two were omitted. Since the sample number of 30 is too small to test all 6 parameters simultaneously, the correlations between the variables were tested iteratively. The selection of the total model was carried out by an automated forward model selection analysis and the Akaike Information Criterion (stepAIC, R package "MASS", v7.3.51.6) (Venables and Ripley, 2007). The total model is composed of significant parameters only and the explained variance is given by the adjusted R^2. The contribution of each significant parameter to the total explained variance was evaluated with the metric approach "lmg", which uses R^2 for the evaluation (Hierarchical Partitioning, R package "relaimpo", v2.2.3) (Grömping, 2006).

7.2.5.3 General information on data processing, visualisation and availability

All data was processed using the software R (v4.0.2) (R Core Team, 2020). The diagrams were created with the “ggplot2” package in R (Wickham, 2009). The stream monitoring data for this publication is available under Liess et al. (2021) (data publication via the data publisher PANGAEA, DOI: https://doi.org/10.1594/PANGAEA.931673).

7.3 Results and Discussion

7.3.1 Effectiveness of vegetated buffer strips width in retaining pesticides

We investigated the effect of vegetated buffer strips (VBS) on the PIR at 115 stream sections. For this we restricted the selection of stream sections to those that received considerable runoff associated with a minimum rainfall of 25 mm/d (n = 30). With this approach, we avoid including weak precipitation events in the assessment that did not cause a runoff event. Subsequently we found a negative correlation between the average width of VBS and the PIR of insecticides ($R^2 = 0.45$, $p \leq 0.001$), fungicides ($R^2 = 0.27$, $p = 0.003$) and herbicides ($R^2 = 0.43$, $p \leq 0.001$) (see figure 1A). The PIR also correlates negatively with the PR of all pesticides combined ($R^2 = 0.25$, $p \leq 0.001$). The explained variance between PIR and the average VBS indicates that a relevant proportion of surface runoff contributes to the total in-stream pesticide concentrations. On average, two such events with more than 25 mm/d and less than 70 mm/d of precipitation occurred in the catchments at the selected stream sections in 2018 and 2019 (90th percentile = 4 events, n = 115), representing a relevant input of pesticides to small streams. The average width of VBS transects per stream section correlated best with the PIR (on average $R^2 = 0.38$). The correlation between VBS width and PIR was lower for events below 25 mm/d ($R^2 = 0.08$). The correlation between the PIR and other VBS aggregations was lower. The median VBS width (on average $R^2 = 0.19$), minimum VBS width (on average $R^2 = 0.18$) and VBS width weighted to distance between transects and sampling site (on average R^2 = 0.08) correlated less strongly than the average width. The PIR value of herbicides (log -0.5) was 25 times higher than that of insecticides and fungicides (log -1.9) at the site with most narrow VBS width. This is due to the generally higher in-stream herbicide concentrations.

The average width of VBS covered a large gradient over the 30 selected stream sections (see SI Fig. 5). In Germany, most pesticides must be applied at least 10 m from water bodies. 26% of the digitalised VBS transects (n = 1,808) were narrower than the 5 m prescribed by the German Water Resources Act §38a and NAP (see figure 1B). This could potentially lead to inputs of pesticides through runoff. At present, the review of the VBS width by state authorities is carried out only on a random basis. It is therefore possible for large-scale violations of these prescribed VBS widths to occur.

The vegetation cover of the VBS was dominated by grass (66.8%), but also includes trees (19.2%), shrubs (10.9%), tracks (2.7%) and flowering strips (0.3%) (see SI Fig. 6). However, the different vegetation covers did not correlate with the PIR (on average for insecticides, fungicides and herbicides, R^2 = 0.1). One previous investigation obtained similar results, indicating no significant difference in retaining pesticides between grassed VBS and other types of vegetation cover (Lowrance et al., 1997). However, numerous studies have investigated the influence of the vegetation cover and found that the plant community and density of the VBS is also important for the quantity of pesticides entering the streams (Lyons et al., 2000; Pätzold et al., 2007; Prosser et al., 2020; Schmitt et al., 1999). This lack of information about vegetation cover (e.g., its density) may explain why we did not find differences in pesticide retention among the vegetation covers studied here.

Regarding the efficacy of the VBS width, our study revealed similar results to a study involving 14 small streams in Denmark (Rasmussen et al., 2011). Here, the authors identified a correlation (R^2 = 0.37, $p <$ 0.01) between average width and summed measured in-stream pesticide toxicity (TU) which is similar to the correlation found here (average R^2 = 0.31). In their investigation, the minimum width revealed the best association with TUmax (R^2 = 0.66) (Rasmussen et al., 2011). In contrast, our results showed for over 70 pesticides on a large spatial scale that the average width correlates better to the in-stream pesticide concentration than the minimum VBS width. By digitalising fewer transects (every 500 m), Rasmussen et al. (2011) may have missed actual VBS width minima along the stream sections, with the result that the minimum width corresponds better to the average width. This indicates the importance of a high spatial resolution when describing VBS width in order to include the influence of VBS width minima. Our results also suggest that using VBS minima alone is not sufficient to obtain an accurate prediction.

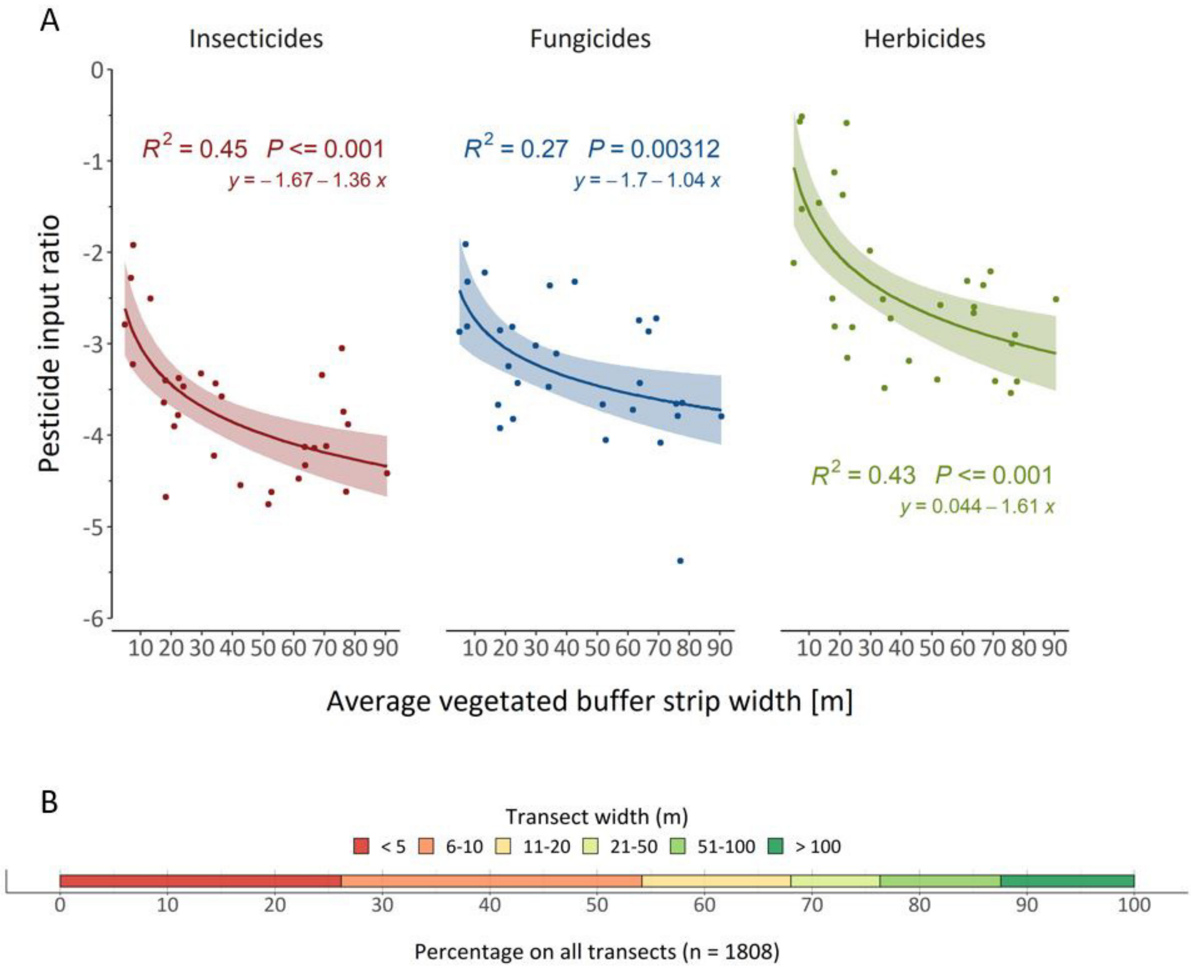


Figure 25: (A) Comparison of log-transformed linear regression models between the pesticide input ratio (average concentration / application rate per sample) and the average vegetated buffer strip (VBS) width at a stream section. The points show stream sections that had at least one event-driven sample (EDS) with more than 25 mm/d and less than 70 mm/d of precipitation. Confidence bands represent the regression model and are also log-transformed. Only substances that were approved at the time of sampling were used for the aggregation of insecticides, fungicides and herbicides (see chapter 2.2). (B) Percentage of all transects (digitalised VBS) grouped by their width.

7.3.2 The influence of physico-chemical properties on the effectiveness of vegetated buffer strips

We investigated the effectiveness of vegetated buffer strips (VBS) in retaining substances as a function of their physico-chemical properties by classifying the substances according to their mobility, solubility and persistence. The analyses revealed that substances that are moderately to very mobile (log Kfoc 1500 - 0.01 L/kg) can pass the VBS more easily, as indicated by their higher PIR in EDS at sites with narrow VBS (R^2 = 0.34, $p < 0.001$ “log Kfoc 1500 - 150 L/kg” and R^2 = 0.31, $p = 0.001$ “log Kfoc < 150 L/kg”; see figure 2). In contrast, there was no correlation between the average VBS width and substances that are only slightly mobile (log Kfoc > 1500 L/kg). These substances contain mainly fungicides and few herbicides (see classification SI Tab. 1). Water solubility (mg/L) and the persistence (DT50Soil) of the substances showed no influence on the pesticide input ratio.

Substances that have lower mobility and higher sediment adsorption capacity can be better retained by VBS. These substances are bound to organic particles and are retained by the vegetation cover of the VBS (Dunn et al., 2011; Habibiandehkordi et al., 2017; Prosser et al., 2020; Schmitt et al., 1999). For more details on the effect of vegetation cover on PIR see section 3.1. The lack of correlation for slightly mobile substances is possibly due to the complete trapping of slightly mobile hydrophobic pesticides in even narrow VBS, while very mobile pesticides can easier pass narrow and even wider VBS widths being dissolved in the surface runoff water. Our study illustrates the pesticide retaining effect of VBS for slightly mobile pesticides for an additional 16 substances on a large scale (see SI Tab. 1). For mobile substances we were able to show that these substances needed broader VBS than slightly mobile substances.

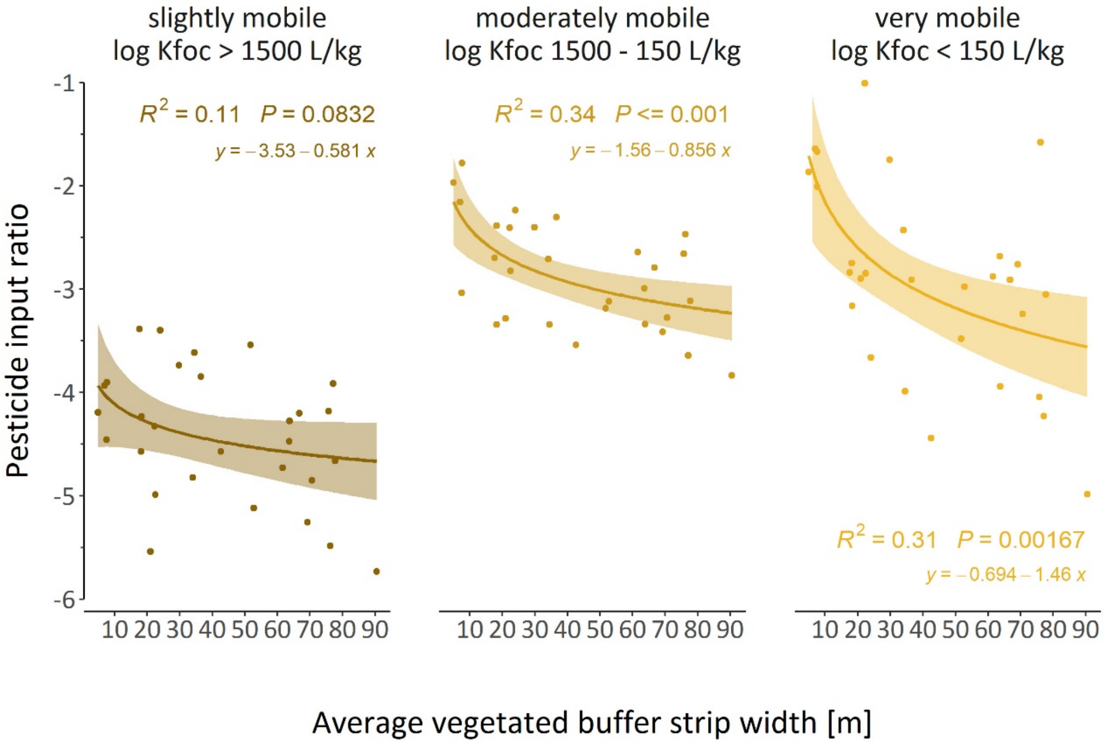


Figure 26: Comparison of log-transformed linear regression models between the pesticide input ratio (average concentration / application rate per sample) and the average vegetated buffer strip (VBS) width at a stream section. The points show stream sections that had at least one event-driven sample (EDS) with more than 25 mm/d and less than 70 mm/d of precipitation. Only substances that were approved at the time of sampling were used (see chapter 2.2). Substances were classified according to their mobility (log Kfoc L/kg, slightly mobile = > 1500, moderately mobile = 1500 - 150 and very mobile < 150).

7.3.3 Effectiveness of vegetated buffer strips in preventing RAC exceedances

Vegetated buffer strips (VBS) reduce runoff-related pesticide exposure. However, the pesticides entering water bodies are characterised by a strongly contrasting toxicity. We assessed the ecotoxicological relevance of substances entering the stream by comparing the in-stream concentrations with the regulatory (RAC) and field-validated (AC_{field}) effect thresholds. We considered three different protection goals (RQ_{max}, $RQ_{ACfield}$ and RQ_{sum}, see chapter 2.3). Logarithmic RQ values greater than 0 indicate an unacceptable risk (EFA, 2013). To estimate protective VBS widths, we modelled the relationship between average VBS widths and the RQ (log) per stream section and intersected the logarithmically transformed linear models at the zero line on the Y-axis (see Figure 3). RQ_{max} revealed required VBS widths of 18.6 m for algae. RQ_{sum}, which takes into account the summed risk caused by pesticides that occur together and affect the same organism group, revealed 31.6 m for algae (see values in table 1). Compared to invertebrates and fish, protective widths were widest for algae with respect to RQ_{max} and RQ_{sum}. To exclude unacceptable effects on the invertebrate community in 95% of streams and avoid a summed RAC exceedance by multiple substances, a VBS width of 10 m would be needed instead of 5 m. Invertebrates were best protected by the $RQ_{ACfield}$ protection goal for 95% of the streams (25.5 m). Compared to the regulated width of 5 m in Germany, by implementing a VBS width of 25.5 m we could lower the risk ($RQ_{ACfield}$) by a factor of 8 $RQ_{ACfield}$ for invertebrates, 2 for fish and 4 for algae due to the exponential relationship between risk and VBS width. It is noteworthy that these protective VBS widths include all point source inputs (dry ditches, etc.) and are therefore wider than a VBS width that would only retain runoff (see Section 3.4).

Table 1: Results of the protective VBS width modelling for different protection goals. More protection targets are listed in SI Fig. 7.

Risk quotients	RQ_{max}		RQ_{sum}		$RQ_{ACfield}$	
Protection targets	50%	95%	50%	95%	50%	95%
Invertebrates	5 m	8.5 m	6.7 m	10.3 m	19.6 m	25.5 m
Fish	0.5 m	2.5 m	1.2 m	4 m	-	-
Algae/Aquatic plants	12.6 m	18.6 m	23 m	31.6 m	-	-

Attempts to derive protective VBS widths that retain pesticides have already been made by several investigations. However, these usually refer to pesticide load reductions in percent and are difficult to translate into ecologically relevant concentrations (Dunn et al., 2011). In studies, the protective VBS width ranges from 6.6 m to 15 m (Liu et al., 2008; Prosser et al., 2020). Through the present investigation we were the first to develop an approach to derive a protective VBS width in compliance with the pesticide risk assessment under field conditions. Our proposed protective VBS widths are considerably higher than the 5 m prescribed by law and the 6.6 - 15 m suggested in the literature. Pesticide entries can still occur, but they should not exceed any RAC or AC_{field}, depending on the protection goal. However, the current risk assessment seems to underestimate the risk to fish as their maximum RQ is 7.6 times lower than that of invertebrates. Another recent study also found that pesticides in small to medium-sized streams pose a risk to fish and that pesticide risk may be underestimated (Werner et al., 2021).

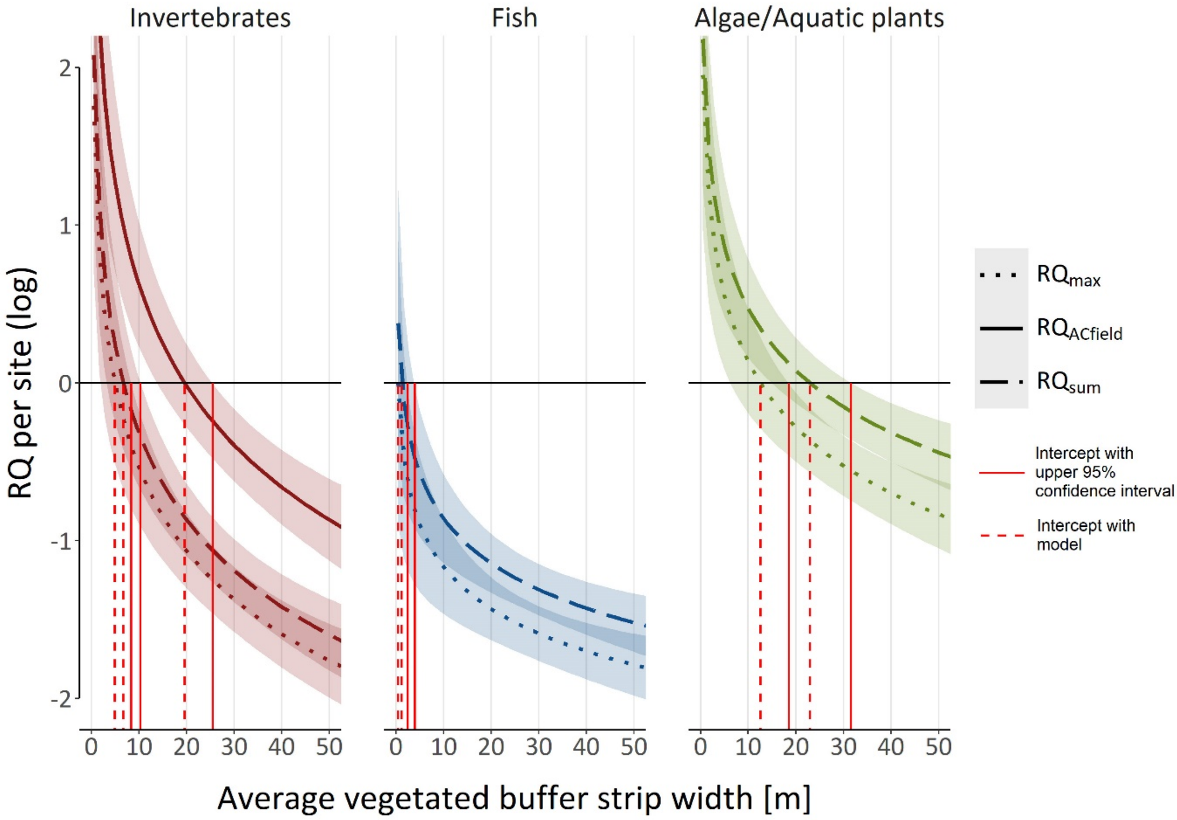


Figure 27: Comparison of logarithmic transformed (RQ values) linear regression models between the different risk quotients (RQ_{max}, $RQ_{ACfield}$ and RQ_{sum}) per site and the average vegetated buffer strip (VBS) width. The models are divided into the three endpoints of invertebrates (red), fish (blue) and algae/aquatic plants (green). Only samples with more than 25 mm/d and less than 70 mm/d of precipitation prior to the event-driven sample (EDS) were used. The red vertical lines show the intercepts at which the model crosses the zero line on the axis (red dashed) and the upper confidence interval (red solid). The values exceed the RAC when the model crosses the zero line of the y-axis.

7.3.4 Drivers for the effectiveness of vegetated buffer strips and proposed protective widths

Effective retention of pesticides by VBS requires evaluation of the factors that increase pesticide inputs to the streams. The protective VBS widths identified in Section 3.3 are likely to be too large because these results include pesticide input promoting factors. We therefore investigated the average slope, dominant soil type, number of erosion rills, average field size, erodibility of soils (K-factor) and the influence of dry ditches. A multiple linear regression revealed that dry ditches were found to correlate positively with the RQ_{max} (insecticides, R^2 = 0.35; fungicides, R^2 = 0.2 and herbicides, R^2 = 0.57), while the average VBS width was the only parameter that negatively correlated with the RQ_{max} (see SI Fig. 8). Other variables such as slope, agricultural cover in the catchment, vegetation cover, average field size, erosion rills, erodibility, soil type and catchment size were not significant and of minor influence < R^2 = 0.1. The full model of the multiple linear regression for RQ_{max} was highest for algae (R^2 = 0.61), followed by invertebrates (R^2 = 0.35) and fish (R^2 = 0.3). The R^2 of the full models for RQ_{sum} (invertebrates, R^2 = 0.33; fish, R^2 = 0.34 and algae, R^2 = 0.59) and $RQ_{ACfield}$ (invertebrates, R^2 = 0.24; fish, R^2 = 0.28 and algae, R^2 = 0.46) were smaller to a minor extend (see SI Fig. 8). However, multiple regressions have the disadvantage that as the number of observations decreases, the possibility of testing explanatory variables also decreases. Hence, all combinations of explanatory variables were tested consecutively.

The unexplained proportion of variance in all analyses shown in this study are generally exceeding 50%. This is partly due to the complex off-site transport processes of pesticides or to missing factors in the multifactorial analyses, such as the presence of drainage systems in the catchment areas. Drainage systems can change the input patterns of pesticides during rain events and, in the worst case, VBS can be bypassed (Brown and van Beinum, 2009). This should be taken into account when considering these results and could be the subject of future research.

We combined the effects of VBS width and dry ditches in one model to evaluate the protective width of VBS with and without the presence of dry ditches. There were only weak correlations (r = 0.7) between dry ditches or VBS width and any other variables (see SI Fig. 9). If dry ditches were optimally managed in the catchments and there would be no input from this source, then the most protective VBS width could be reduced by 7.7 m from 25.5 m ($RQ_{ACfield}$, invertebrates 95%) to 17.8 m ($RQ_{ACfield}$, invertebrates 95%). This reduction potential is even higher for herbicides. Here, the protective VBS width could be reduced by 20.4 m from 23 m (RQ_{sum}, algae 50%) to 2.6 m (RQ_{sum}, algae 50%). Most herbicides are very mobile (log Kfoc ≤ 150 L/kg). Due to their physico-chemical properties, these substances are therefore transported more quickly to the dry ditches and flow rapidly into the nearest receiving waters. Compared to algae and invertebrates, the reduction potential for fish is small (0.6 m, RQ_{sum} 50%), as in general their risk is low (see figure 3). We therefore propose an overall protective VBS width of 18 m to protect all vulnerable aquatic organisms and to avoid AC_{field} and RAC exceedance for 95% of the streams. A widening of the VBS from 5 m to 18 m would result in an average reduction in agricultural land of 3.8 % in the catchments studied here.

Until now, no study has shown such an extensive influence of dry ditches on the in-stream pesticide concentration. Previous studies have found a contribution of dry ditches to in-stream pesticide contamination (Bereswill et al., 2013; Ohliger and Schulz, 2010; Stehle et al., 2016). However, they observed more pesticide inputs coming from erosion rills (Bereswill et al., 2012). We did not observe a pesticide input-enhancing effect from on-field erosion rills on lowland streams. This could be due to the comparably steep slopes investigated by Stehle et al. (2016) (on average 8.4%) (the average slope in the catchments investigated here was 2.5%).

7.3.5 Deficiencies of the regulatory pesticide approval process

The regulatory approval of pesticides is designed to avoid long-term effects on aquatic life (European Food Safety Authority (EFSA), 2013). To ensure this, predicted environmental concentrations (PECs) are modelled for specific pesticide applications to assess whether the estimated in-stream concentrations are below the regulatory thresholds (RAC). A protective VBS width as derived in this study is therefore directly dependent on the application rates resulting from the pesticide authorisation process. Based on new scientific findings, pesticides may be classified as more toxic and RAC values are downregulated without a new authorisation process being initiated until the original authorisation expires. However, these new scientific findings are not reflected in the previously established exposure models, nor in the application rates which would require corresponding downregulation (Brühl and Zaller, 2019; Liess et al., 2021). Pesticides are thus treated with double standards by risk assessment; the application is based on old knowledge leading to high in-stream concentrations which are then compared to thresholds of new standards resulting in many threshold exceedances. If we want to implement a protective VBS width under current authorised pesticide application rates and RAC values, the calculated protective VBS width for algae would be 47.3 m (RQ_{sum}). Application rates adapted to the current scientific findings could therefore avoid many threshold exceedances and would also result in a more moderate protective VBS width. The proposed European green deal calls for a 50% reduction in pesticide use by the year

2030 (European Commission, 2022). Assuming that a 50% reduction in pesticide use would result in a 50% reduction in pesticide concentrations in the stream, 39% (RAC) and 68% (AC_{field}) of the EDS samples would still have exceedances (see SI Tab. 4). Since 95% of the RAC and AC_{field} exceedances could be avoided by expanding the VBS width, we consider this approach to be more effective than a 50% reduction in use.

7.4 Conclusion

- We conclude that the width of vegetated buffer strips (VBS) is the most efficient factor for pesticide retention and revealed an average width of 18 m to prevent ecological effects in 95% of stream sections.
- Pesticide retention is higher for slightly mobile substances. Moderately mobile to mobile substances are more likely to bypass the VBS and require a broader VBS.
- A wide VBS does not automatically protect streams as VBS can be bypassed by dry ditches, which positively correlate with the pesticide input ratio (on average R^2 = 0.31).
- If the current VBS adjacent to agricultural streams were increased to 18 m, an additional 3.8% of agricultural land would have to be transformed to protect in-stream communities.

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8 Discussion

8.1 Existing problems with pesticides in small streams

8.1.1 Insufficient monitoring of pesticide exposure

Governmental monitoring is designed to measure pesticide exposure in our waters and to assess effects on the aquatic community. The occurrence of pesticide exposure is highly variable over time and the measured values are compared with thresholds such as the Regulatory Acceptable Concentration (RAC) or Environmental Quality Standard (EQS). If monitoring does not capture the full range of exposure, risks from pesticides will be underestimated as peak concentrations lead to effects in the aquatic community (Liess and Ohe, 2005; Moschet et al., 2014).

Current knowledge and gaps

Challenges in the assessment and monitoring of pesticide exposure were previously observed (Fox et al., 2021). Especially, the lacking event-driven monitoring was in the centre of recent investigations (Bundschuh et al., 2014; Liess et al., 1999; Lorenz et al., 2017; Rabiet et al., 2010; Stehle et al., 2013). Previous studies focused on the underestimation of rainfall-related pesticide inputs by grab sampling. The underestimation by grab sampling resulted in about 10 to 30 times higher concentration for different pesticides in event-related samples compared to grab sampling concentrations (Lefrancq et al., 2017). The detection of lower pesticide concentrations is therefore a systematic error due to the non-use of event-related measurement methods (Liess and Ohe, 2005). This also leads to a significantly lower rate of exceedance of thresholds and thus to an underestimation of the risk from pesticides (La Cecilia et al., 2021; Lorenz et al., 2017). However, even if much of the recent research investigated the underestimation of pesticide risk by governmental monitoring, important lessons remain that could be used to adjust future monitoring approaches. For example, studies tend to focus only on a small number of investigated small streams or have a narrow range of analytes. This limits the extent to which general statements can be made on a large spatial scale and for different pesticides. Also, the temporal coverage of pesticide exposure over the course of a spraying season has not yet been sufficiently investigated. Following important questions remain unanswered: Is the underestimation by grab sampling observed over a monthly trend and are there possibly pesticide exposure peaks that are particularly worth measuring?

Contributing results to narrowing the knowledge gap

The holistic assessment of multiple stressors on aquatic communities in the KgM-project allows for the first-time general conclusions to be drawn for the governmental monitoring concept. In our studies, we found a similar underestimation of the risk of pesticides to small streams by grab sampling. We also observed on average 10 times higher pesticide concentrations in event-driven samples (EDS) compared to grab samples (see publications 1 & 3). Even if the grab samples were conducted within one day after the rainfall event, the toxicity to invertebrates and algae was 10 times lower compared to the corresponding EDS (see publication 2). We have observed the underestimation of grab samples compared to EDS for many different pesticides and widespread sampling sites (see publication 2, Annex). In addition, we found that the most frequent peak exposures in small streams occur in May and June, during a main pesticide application phase (see publication 2). Moreover, our analyses showed further gaps in the governmental monitoring that lead to a lack of holistic coverage of pesticide exposure (see publications 5). These consist, of a too small substance spectrum and too few legally binding thresholds (EQS) for the risk assessment. All these flaws in the monitoring lead to a systematically underestimation of the pesticide exposure.

8.1.2 Vast pesticide exposure and predicted effects on the aquatic community

An urgent question of the KgM-project was to find out whether the current agricultural practice leads to in-stream pesticide concentrations that adversely affect aquatic communities. For this purpose, pesticide exposure in small streams was assessed in detail using EDS and grab samples and correlated with biological samples of the invertebrate community. The pesticide concentrations were translated into toxic units (TU) to assess their effects.

Current knowledge and gaps

Effect-causing in-stream pesticide concentrations were observed in previous studies (Bundschuh et al., 2014; Liess and Ohe, 2005; Schäfer et al., 2013; Schäfer, 2019). High TU values were observed in many small streams on a widespread basis (Liess and Schulz, 1999; Schäfer et al., 2007; Schäfer et al., 2012). The exceedance of RAC values was also observed on a widespread basis. 40% of grab sample concentrations exceeded their RAC in a Germany-wide survey (Szöcs et al., 2017). However, pesticide exposure and related RAC exceedances have not been compared with the ecological quality components and effects on aquatic communities with EDS on such a wide scale as in the KgM-project. In addition, conclusions as to why this widespread pesticide exposure occurs have not yet been clearly established. Until now, there is no field-based threshold to show when pesticide exposure causes effects on the aquatic community. This however is needed, to not just predict effects from pesticides, but to subsequently adjust the ERA of pesticides (Vijver et al., 2017).

Contributing results to narrowing the knowledge gap

Our results showed that pesticide exposure to small streams is widespread and ubiquitous in dry weather grab samples, but also in particular in EDS during rainfall events. In grab samples we observed on average concentrations above -4 log TU toxic to invertebrates, while concentrations and TU values in EDS were on average -2.5 log TU (see publications 1 and 3, UBA, 2022). This results in a significantly increased toxic potential of EDS, which affects aquatic communities. Overall, the pesticide exposure resulted in 26% RAC exceedances in grab samples and 59% RAC exceedances in EDS (see publication 3). At 80% of the sampling site at least one RAC exceedance was observed (see publication 3). It can be concluded, that pesticide exposure in small streams is not only significantly increased during rainfall events, but to a lesser extent already occurs during dry weather in concentrations where effects on the community are to be expected (see publication 1, 2 and 3). In addition, we defined a new field-based threshold AC_{field} to assess pesticide effects on the invertebrate community (see publication 3). With this threshold it is now possible to evaluate the ERA with field-based measurements. We concluded, that current RACs are too high and not protective enough.

Similar to the larger water bodies covered by the WFD monitoring (see chapter 1.2), the ecological status of the water bodies in the KgM-project is mostly in an unsatisfactory condition (see publication 3). 88,4% of the streams had an ecological quality class according to the WFD between the classes unsatisfactory and poor (UBA, 2022). A strong link between the failure to achieve good status according to WFD and the effects of pesticide exposure could be demonstrated (see publication 3). This largely explains the poor ecological status for invertebrates of the small streams while other stressors such as hydromorphology, oxygen deficiency, hydrological parameters or nutrients were shown to play a minor role. A spill-over effect on the larger WFD-monitored streams (> 10 km²) studied cannot be excluded (Lorenz et al., 2017).

8.1.3 Failings of the environmental risk assessment of pesticides

The ERA is designed to protect non-target terrestrial and aquatic ecosystems from the input and effects of pesticides. However, the vast pesticide exposure found in streams during the KgM-project indicates that the current ERA has certain flaws which result in widespread high pesticide exposure and unacceptable effects on aquatic communities. In the following, these flaws are structured into three main flaws consisting of (i) single substance ERA, (ii) risks by mixtures and (iii) risk mitigation measures.

i We found that there are time gaps between the risk assessment of substances and the regular process of exposure modelling and risk analysis, so that both processes are not up to date with the latest scientific knowledge. Due to the consideration of new knowledge, the RACs have partly been updated and the threshold was lowered. As a result, 11 substances were authorised for uses leading to a predicted exceedance of the RAC. Now, in-stream pesticide concentrations of old application rates are being compared with new lower RAC values. This automatically leads to threshold exceedances, which in turn indicates effects (see publications 3 and 6). Additionally, for Fipronil and Methiocarb no exposure modelling was done in general. Both pesticides were majorly contributing to the observed RAC exceedances. One reason for this can be found in the change of responsibilities. For Fipronil, after the expiry of their authorisation, the pesticides are no longer to be used in agriculture, but in domestic use such as horticulture. Although it was last allowed to be used in agricultural use in 2016, we still find it today in concentrations exceeding RAC. This is either because domestic use is too high and leads to severe environmental exposure, or because the substance is so persistent that the agricultural use at that time is still in effect today. Obviously, these inconsistencies lead to pesticides not being properly regulated, exceeding thresholds and leading to avoidable effects on aquatic communities.

ii In addition to flaws by assessing the single pesticide risk, mixtures are not assessed by the current ERA at all. Both the applications on different fields in the catchments with different crops and the mixing in the tank of several pesticides lead to a mixture being introduced into an adjacent water body (Schreiner et al., 2016; Silva et al., 2002; Stehle and Schulz, 2015). Laboratory analysis have shown that pesticide mixtures can act synergistically and even enhance the effects from pesticides to aquatic communities (Cedergreen, 2014; Pérez et al., 2013; Shahid et al., 2019). When only assuming additive effects, we observed that under realistic worst-case conditions, the risk posed by pesticide mixtures is 3.2 times higher than for the single pesticide risk assessment (see publication 4). Future consideration of the additional impact of mixtures is necessary to better explain the effects that occur on the aquatic communities.

iii Risk mitigation measures, in this context vegetated buffer strips (VBS) (Drinking Water Resources Act §38a) (BGBl, 2009), aim to protect streams from high pesticide inputs. Mostly a width of 5 metres is prescribed for VBS to be protective. However, recent studies already observed that 5 metres are not protective enough and suggest widths of 6.6 to 15 metres (Liu et al., 2008; Prosser et al., 2020). Our result suggests a width of 18 metres in order to avoid RAC or AC_{field} exceedances for 95% of the investigated streams (see publication 6). This extension of a VBS width can only be protective if the input from dry ditches along the stream side, which contain surface runoff water during rainfall events (≥ 25 mm/d), is also reduced accordingly. An additional area of 3.8% of the agricultural land in the catchments would be converted to VBS. At present, there is no such derivation of protective VBS widths in the current ERA. Even though this seems as a large area for additional VBS, this could efficiently protect our streams from pesticide effects.

8.1.4 Lacking habitat quality for recolonization due to deficient hydromorphology

Although we have been able to show that pesticide exposure is currently the major stressor and most frequently affect invertebrate communities in agricultural streams (see publication 3), good habitat quality and sufficient hydromorphological structures are essential for invertebrates to recolonize on a permanent basis (Urbanič et al., 2020). Hydromorphological structures are not only important for providing habitats for invertebrates, but are also an indirect factor influencing the substrate diversity and the self-purification of streams for organic chemicals and nutrients (Elosegi et al., 2010; Villeneuve et al., 2018; Wyżga et al., 2012). If pesticide exposure can be reduced in the long term and the chemical status improved so that potentially more species-rich invertebrate communities could re-establish themselves, the habitat conditions must also be created for this to happen (Seidel et al., 2021).

During the KgM-project we assessed the hydromorphology of the small streams in agricultural landscapes and observed 77% of the streams to have an unsatisfactory to poor hydromorphology according to the criteria of the WFD (UBA, 2022). For re-establishing good habitats and hydromorphological structures in streams often renaturation measures are realised. It must be noted that renaturation of water bodies is very planning, cost and time intensive. The renaturation measures usually take place in isolated sections of water bodies and the chances of ecological status improvement of these measures could only partially achieve long-term success (Lorenz et al., 2018). Studies have also shown that the self-dynamic development of watercourses usually has many advantages for successful watercourse development (Muhar et al., 1995). Measures aimed at improving water body structure and ultimately habitat quality for aquatic invertebrates and achieving good status according to the WFD, should be based on self-dynamic stream development on a wide scale compared to small-scale interventions. To achieve this, more space should be provided for the small streams overall. This results in a synergy with the expansion of the areas for VBS for pollutant retention.

8.2 Solutions for improvement and achieving the NAP goals

8.2.1 Overarching and NAP goals to protect small streams

The KgM-project has impressively shown the chemical and biological situation of small streams in Germany under the current conditions of our agriculture and the ERA (UBA, 2022). At about 101 representatively distributed monitoring sites with over 1,000 stream samples we showed, that structural quality, ecological- and chemical status are all in generally poor condition due to high pesticide exposure (see publication 1, 2 and 3). Two main objectives can be derived from the results presented in this dissertation and existing problems with pesticides in small streams. By pursuing these two goals, the NAP's objectives can also be achieved. The main goals of the NAP consist of having less than 99% of EDS with an RAC exceedance, no EQS exceedances, 30% reduction of pesticide use, riparian strips with a width of at least 5 metres and effective buffer strips at all surface waters (BMEL, 2013).

1. The large widespread impacts of pesticides on aquatic communities are due to errors in pesticide risk assessment and monitoring. Pesticides are applied at too high rates (toxic in

surface waters) and too close to water bodies, as approved by the ERA, resulting in excessive overall pesticide exposure. As a result, important ecosystem services by small streams will be jeopardised in the future and the initial situation for insect mortality will not be improved but worsened. The aim here should be to adapt the ERA and monitoring concept of the WFD in such a way that future pesticide exposures do not cause effects on the aquatic community.

2. The status of water bodies according to the WFD, especially small streams, are unsatisfactory from a hydromorphological, biological and chemical point of view. The current protection of streams from pesticide exposure is too low. Risk mitigation measures do not fulfil their purpose, are not consistently implemented or monitored. The goal should be to design risk mitigation measures that significantly reduce pesticide exposure. Water bodies should also have better habitat conditions for potential recolonization with species that have disappeared.

8.2.2 Implications for the monitoring of pesticides

As identified in our results the current monitoring of surface waters by the WFD underestimates pesticide exposure at different scales. To achieve the good chemical and ecological status of surface waters, also small streams have to be monitored on a regular basis, as these make up a large part of the stream network (Downing, 2012), are ecologically relevant due to their ecosystem services (Ferreira et al., 2022) and have a spill-over effect on the larger rivers (Lorenz et al., 2017). It is of great importance to assess the full extent of stressors in order to initiate measures for improvement and draw conclusions for chemical risk management (Schäfer et al., 2011; Schäfer et al., 2013).

Our results, in addition to recent studies, have clearly shown that EDS are essential to correctly assess pesticide exposure in small streams (see publications 1, 2, 3 and 5). As the implementation of event-driven monitoring is usually labour-intensive and ultimately too costly and time-consuming, the sampling period should be shortened to a manageable level. This period should be chosen in such a way that it covers the maximum pesticide exposure in small streams as far as possible. This is where the results from publication 2 make a significant contribution. There we show that event-based monitoring could be limited to the period of the main pesticide application phase (approx. 2 months). Currently, this period is between May and June and can vary depending on weather conditions and the occurrence of pests. Nevertheless, this could limit the use of costly monitoring and provide a realistic picture of the pesticide exposure.

In order to enable a holistic assessment of pesticide contamination, it is also important to include the most important PPP active substances in the analyte spectrum. This should be as uniform as possible, so that effects of pesticides that are not measured are not overlooked (see publication 5). This would also make it easier to assess the dangers of mixtures at a later stage (see publication 4).

8.2.3 Implications for the environmental risk assessment and future pesticide use

Besides the underestimation of pesticide exposure by the governmental monitoring, we have identified numerous flaws in the current ERA of pesticides. Time delay in the authorisation process of pesticides (see publication 3) and insufficient protective thresholds (RAC) to assess the current vast pesticide exposure (see publication 5). Even though the current ERA requires exposure modelling to be repeated in the light of new scientific findings on toxicity (European Food Safety Authority (EFSA), 2013), it is not implemented in a timely manner and pesticide application rates are authorised on the basis of old findings. A solution to this problem is either to put more resources into the sector and more work to review exposure modelling, or to immediately restrict the use of substances when new scientific evidence suggests risks to aquatic communities.

The second major flaw, which involves the need for protective thresholds, could be solved by using the new field-based threshold AC_{field}. The AC_{field} reflects the actual exposure of invertebrate communities to pesticides in the field (see publication 3). This threshold is available for all insecticides and fungicides measured in the KgM-project for which invertebrates are the most sensitive organism group. In addition, an assessment can be made of the extent to which the RAC is protective. However, this threshold is currently only available for the assessment of pesticide effects to invertebrates. The assessment of effects on algae is lacking of a descriptive pesticide algae impairment indicator and link to in-stream pesticide concentrations. Additional research should be conducted in this field in order to holistically map the pesticide effects of herbicides on algae and macrophytes in the future.

A further major problem as described in chapter 8.1.1.2 is the widespread vast pesticide exposure. One way to reduce pesticide exposure would be to reduce pesticide application rates. This goal is also proposed in the European Green Deal (reduction of pesticide use by 50%) and NAP (reduction of pesticide use by 30%) (BMEL, 2013; European Commission, 2022). The aim of the European Green Deal (EGD) is to reduce pesticide application by 50% by 2030 and thus reduce the impact of pesticides on the environment and aquatic ecosystems (European Commission, 2022). But is this really the only way to ultimately protect aquatic communities? Addressing this question, we have also conducted a detailed analysis in publication 6. We lowered the measured concentration by different percentages (90%, 75%, 50%, 25% and 10%) and looked at how many RAC exceedances still occur. With a 50% reduction in concentration, as proposed by the EGD, there were still 39% of EDS and 59% of sampling sites with at least one RAC exceedance. The negative effects of widespread pesticide exposure can only be slightly (39% for EDS and 59% for sampling sites) reduced by halving the application rates. However, a reduction in pesticide use is a reasonable measure overall, as it also achieves further nature protection goals such as soil, pollinators and bird protection, but it cannot alone heal the current extensive damage to aquatic ecosystems.

8.2.4 Implications for risk mitigation measures

Risk mitigation measures and in this context especially vegetated buffer strips (VBS) are an effective measure to reduce the inputs from pesticides by surface runoff (Cole et al., 2020; Reichenberger et al., 2007; Zhang et al., 2010). The current prescribed VBS width of 5 metres is not just too narrow to protect aquatic ecosystems from pesticide inputs via surface runoff, but is also not implemented in 26% of our investigated buffer strip transects (n=1,808) (see publication 6). We were able to derive a safe VBS width of 18 metres on each side to protect small stream communities. By implementing this protective VBS width no RAC or AC_{field} exceedances will occur at 95% of the streams.

A second problem of failing to achieve the good ecological status by the WFD is the lacking habitat quality of small streams. A VBS width of 18 metres on each side of the water body would not only drastically reduce the input of pesticides and nutrients and thus improve the chemical status of the water body, but this corridor also offers the possibility of providing the water body more space for the development of hydromorphological structures. Thus, within this corridor, the water body could meander more, different flow conditions could develop and riparian vegetation could establish. This would help to increase habitat diversity for the repopulation of damaged communities and would have other secondary benefits (see chapter 8.1.4) (Stutter et al., 2012). If this corridor were to remain uncultivated, the riparian vegetation could shade the watercourse, mitigating the increased solar radiation and subsequent effects on the aquatic community from rising water temperatures due to climate change (Brazier et al., 1973; Vought et al., 1995). Overall, this would additionally lead to improved areal water retention, which must be the main goal under future climate conditions (Stańczuk-Gałwiaczek et al., 2018). The expansion of an untreated riparian water corridor is one important measure to achieve the goals of the EGD, NAP, WFD and to adapt to the conditions of climate change.

9 Conclusion

The results from the publications of the dissertation and the KgM-project have contributed to a better understanding of the exposure to pesticides and its effects on the aquatic community. In addition, important insights and conclusions could be derived with regard to the ERA. This will help to identify discrepancies in the approval and evaluation of pesticides and to design measures to improve the overall condition of small streams. These results are based on a large-scale and representative survey that can contribute solving the most severe problems in surface water protection.

Altogether, the two main objectives that contribute to an improvement in the protection of small water bodies, (1) flaws in the current ERA and (2) reduction of vast pesticide exposure, can be achieved through a combination of measures. (1) The flaws in the ERA could be remedied by an updated evaluation of pesticides according to the current scientific knowledge standard and an improvement of the threshold value derivation. (2) A key to reduce the vast pesticide exposure in small streams and effects to aquatic communities could be tackled by implementing wider uncultivated buffer strips which reduce the input of pesticides and nutrients from agricultural fields into small streams and provide corridors for hydromorphological recovery to natural conditions, thereby improving the availability of habitats for aquatic organisms.

As conditions for small streams will continue to deteriorate in the future due to climate change and a growing world population enhancing food pressure, it is essential to take timely countermeasures and improve surface water protection (Herbst et al., 2019; Rose et al., 2023). We should invest more effort in protecting aquatic ecosystems as they are essential for our survival on this planet due to the provision of water as a drinking water resource and ecosystem services.

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10 Declaration

Eidesstattliche Erklärung

Ich, Carl Philipp Vormeier,
erkläre hiermit, dass diese Dissertation und die darin dargelegten Inhalte die eigenen sind und selbstständig, als Ergebnis der eigenen originären Forschung, generiert wurden.
Hiermit erkläre ich an Eides statt
1. Diese Arbeit wurde vollständig oder größtenteils in der Phase als Doktorand dieser Fakultät und Universität angefertigt;

2. Sofern irgendein Bestandteil dieser Dissertation zuvor für einen akademischen Abschluss oder eine andere Qualifikation an dieser oder einer anderen Institution verwendet wurde, wurde dies klar angezeigt;

3. Wenn immer andere eigene- oder Veröffentlichungen Dritter herangezogen wurden, wurden diese klar benannt;

4. Wenn aus anderen eigenen- oder Veröffentlichungen Dritter zitiert wurde, wurde stets die Quelle hierfür angegeben. Diese Dissertation ist vollständig meine eigene Arbeit, mit der Ausnahme solcher Zitate;

5. Alle wesentlichen Quellen von Unterstützung wurden benannt;

6. Wenn immer ein Teil dieser Dissertation auf der Zusammenarbeit mit anderen basiert, wurde von mir klar gekennzeichnet, was von anderen und was von mir selbst erarbeitet wurde;

7. Ein Teil oder Teile dieser Arbeit wurden zuvor veröffentlicht und zwar in:
   - Water Research;
   - Science of the Total Environment

31.08.2023

11 Contributions to publications

1 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. In: *Water Research.*

15% - Investigation, data curation, formal analysis, writing (review & editing)

2 **Vormeier, P.**; Liebmann, L.; Link, M.; Schäfer, R.B.; Schneeweiss, A.; Schreiner, V.C.; Weisner, O.; Liess, M. (2023): Temporal scales of pesticide exposure and risks in German small streams. In: *Science of the Total Environment*.

60% - Sampling, conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing (original draft)

3 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. In: *Water Research.*

20% - Investigation, data curation, formal analysis, visualisation, writing (review & editing)

4 Weisner, O.; Frische, T.; Liebmann, L.; Reemtsma, T.; Roß-Nickoll, M.; Schäfer, R.B.; Schäffer, A.; Scholz-Starke, B.; **Vormeier, P.**; Knillmann, S.; Liess, M. (2021): Risk from pesticide mixtures - The gap between risk assessment and reality. In: *Science of the Total Environment.*

10% - Investigation, data curation, writing (review & editing)

5 Weisner, O.; Arle, J.; Liebmann, L.; Link, M.; Schäfer, R.B.; Schneeweiss, A.; Schreiner, V.C.; **Vormeier, P.**; Liess, M. (2021): Three reasons why the Water Framework Directive (WFD) fails to identify pesticide risks. In: *Water Research*.

10% - Investigation, data curation, writing (review & editing)

6 **Vormeier, P.**; Liebmann, L.; Weisner, O.; Liess, M. (2023): Width of vegetated buffer strips to protect aquatic life from pesticide effects. In: *Water Research*.

60% - Sampling, conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing (original draft)

12 List of additional publications during the dissertation

2023 Liess, M.; Böhme, A.; Gröning, J.; Liebmann, L.; Lück, M.; Reemtsma, T.; Römerscheid, M.; Schade, U.; Schwarz, B.; **Vormeier, P.**; Weisner, O. (2023): Belastung von kleinen Gewässern in der Agrarlandschaft mit Pflanzenschutzmittel-Rückständen – TV1 Datenanalyse zur Pilotstudie Kleingewässermonitoring 2018/2019. In: UBA Texte | 63/2023

2023 Schreiner, V.C.; Liebmann, L.; Feckler, A.; Liess, M.; Link, M.; Schneeweiss, A.; Truchy, A.; von Tümpling, W.; **Vormeier, P.**; Weisner, O.; Schäfer, R.B.; Bundschuh, M. (2023): Standard vs. Natural: Assessing the impact of environmental variables on organic matter decomposition in streams using three substrates. In: Environmental toxicology and chemistry. DOI: 10.1002/etc.5577.

2022 Liebmann, L., **Vormeier, P.**, Weisner, O., Liess, M. (2022): Balancing effort and benefit – How taxonomic and quantitative resolution influence the pesticide indicator system $SPEAR_{pesticides}$. Science of the Total Environment 848, art. 157642. https://doi.org/10.1016/j.scitotenv.2022.157642

2022 Liess, M., Liebmann, L., Lück, M., **Vormeier, P.**, Weisner, O., Foit, K., Knillmann, S., Schäfer, R.B., Schulze, T., Krauss, M., Brack, W., Reemtsma, T., Halbach, K., Link, M., Schreiner, V.C., Schneeweiss, A,, Möder, M., Weitere, M., Kaske, O., von Tümpling, W., Gunold, R., Ulrich, N., Paschke, A., Schüürmann, G., Schmitt-Jansen, M., Küster, E., Borchardt, D. (2022): Umsetzung des Nationalen Aktionsplans zur nachhaltigen Anwendung von Pflanzenschutzmitteln (NAP) – Pilotstudie zur Ermittlung der Belastung von Kleingewässern in der Agrarlandschaft mit Pflanzenschutzmittel-Rückständen. In: UBA Texte | 07/2022

2020 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.

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14 Curriculum vitae

Carl Philipp Vormeier

Date of birth:	May 9th 1993
Place of birth:	Berlin
Nationality:	Germany

Career

Since 07/2022	Scientist German Environment Agency, Dessau-Roßlau, Department Inland waters
07/2021 – 07/2022	Scientist Helmholtz-Centre for Environmental Research Leipzig - UFZ, Department System-Ecotoxicology - Prof. Dr. Matthias Liess

Education

Since 04/2018	PhD study Helmholtz-Centre for Environmental Research Leipzig - UFZ, Department System-Ecotoxicology - Prof. Dr. Matthias Liess „The Kleingewässermonitoring (KgM) – A Monitoring of German Small Streams and Its Implications for the Environmental Risk Assessment of Pesticides“
07/2015 – 01/2018	M.Sc. Environmental hydrology Freie Universität Berlin Thesis: „Comparison of satellite-derived chlorophyll-a and turbidity measurements with probe data Lake Rummelsburg, Berlin“
10/2012 – 07/2015	B.Sc. Physical geography Freie Universität Berlin Thesis: „Land use change classification of the Pune region in India using Landsat data, India“
09/2005 – 07/2012	Abitur / High School Diploma Lilienthal Gymnasium Berlin

August 31, 2023

15 Annex - Supporting information of publications

15.1 Small streams - Large concentrations? Pesticide monitoring in small agricultural streams in Germany during dry weather and rainfall – Supporting Information

The amount and/or format of the Supporting Information is not suitable to be presented here. Please see the homepage related to the publication (https://www.sciencedirect.com/science/article/abs/pii/S0043135421007314) to view this supporting information.

15.2 Temporal scales of pesticide exposure and risks in German small streams. In: Science of the Total Environment – Supporting Information

The amount and/or format of the Supporting Information is not suitable to be presented here. Please see the homepage related to the publication (https://doi.org/10.1016/j.scitotenv.2023.162105) to view this supporting information.

15.3 Pesticides are the dominant stressors for vulnerable insects in lowland streams – Supporting Information

The amount and/or format of the Supporting Information is not suitable to be presented here. Please see the homepage related to the publication (https://www.sciencedirect.com/science/article/abs/pii/S0043135421004607) to view this supporting information.

15.4 Risk from pesticide mixtures - The gap between risk assessment and reality – Supporting Information

The amount and/or format of the Supporting Information is not suitable to be presented here. Please see the homepage related to the publication (https://www.sciencedirect.com/science/article/abs/pii/S0048969721040894) to view this supporting information.

15.5 Three reasons why the Water Framework Directive (WFD) fails to identify pesticide risks – Supporting Information

The amount and/or format of the Supporting Information is not suitable to be presented here. Please see the homepage related to the publication (https://www.sciencedirect.com/science/article/pii/S0043135421010423) to view this supporting information.

15.6 Width of vegetated buffer strips to protect aquatic life from pesticide effects – Supporting Information

The amount and/or format of the Supporting Information is not suitable to be presented here. Please see the homepage related to the publication (https://doi.org/10.1016/j.watres.2023.119627) to view this supporting information.

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