

STATISTICAL ECO(-TOXICO)LOGY

IMPROVING THE UTILISATION OF DATA FOR
ENVIRONMENTAL RISK ASSESSMENT

by

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SUMMARY

Freshwaters are of immense importance for human well-being. Nevertheless, they are currently facing unprecedented levels of threat from habitat loss and degradation, over-exploitation, invasive species and pollution. To prevent risks from chemical substances, like agricultural pesticides, to the aquatic ecosystem, environmental risk assessment (ERA) is mandatory before approving the use of these substances. Concurrently, large-scale environmental monitoring is used for surveillance of the chemical pollution of rivers. This thesis examines statistical methods used in ERA, presents a national-scale compilation of chemical monitoring data, an analysis of driver of chemical pollution in streams, a combination with data ERA for a large-scale risk assessment and provides software tools to integrate different datasets used in ERA.

The thesis starts with a brief introduction to ERA, environmental monitoring, and statistical ecotoxicology and gives an overview of the objectives of the thesis. Chapter ?? addresses experimental setups and their statistical analyses using simulations. We show that current designs exhibit unacceptably low statistical power, that statistical methods chosen to fit the type of data provide higher power and that statistical practices in ERA need to be revised. In chapter ?? we compiled all available pesticide monitoring data from Germany. Hereby, we focused on small streams, similar to those considered in ERA and used threshold concentrations derived during ERA for risk assessment. This compilation resulted in the biggest dataset on pesticide exposure currently available for Germany. Using new statistical techniques, that explicitly take the limits of quantification into account, we demonstrate that 25% of small streams are exposed to risk from pesticides. Especially neonicotinoid pesticides are responsible for this risk. These risks are associated with agricultural uses and can be detected even at low levels of agricultural use. Moreover, our results indicated that current monitoring underestimates pesticide risks, because of sampling independent of precipitation events. Additionally, we provide a first large-scale study of annual pesticide exposure dynamics. Chapters ?? and ?? describe software solutions to simplify and accelerate the integration of different data sources from ERA, environmental monitoring and ecotoxicology.

Overall, this thesis contributes to the emerging discipline of statistical ecotoxicology and shows that pesticides pose a large-scale threat to small streams. Environmental monitoring can give a post-authorisation feedback to ERA. However, to protect freshwater ecosystems ERA and environmental monitoring need to be further refined and we provided software solutions to integrate data for this purpose.

THREATS TO FRESHWATER ECOSYSTEMS FROM CHEMICAL POLLUTION

Freshwater ecosystems, like streams, lakes and wetlands, amount to only 0.01% of the World's water and cover only 0.8% of Earth's surface (Dudgeon et al., 2006), yet they host an important component of global biodiversity. Freshwaters are a habitat for more than 125,000 species, which represents 10% of global biodiversity and 1/3 of all vertebrate species (Balian et al., 2007; Strayer and Dudgeon, 2010) and provide essential services for human well-being (Aylward et al., 2005). Small water bodies are of particular importance, because of their high abundance (Downing et al., 2012), the high biodiversity they host (Davies et al., 2008) and the ecosystem services they provide (Biggs et al., 2016).

The earth is currently experiencing a functional change driven by human activities which are so far-reaching, that a new geological epoch "Anthropocene" has been proposed (Steffen et al., 2011; Waters et al., 2016). Consequently, this is also associated with detrimental biotic changes: 65% of rivers are currently at threat (Vörösmarty et al., 2010), 21% of 27,516 assessed freshwater species are currently threatened with extinction (IUCN, 2016) and greatest biodiversity losses are observed in freshwater ecosystems (WWF, 2016). A multitude of stressors contribute to this deterioration of freshwater biodiversity, including habitat loss and degradation, overexploitation, invasive species and pollution (Collen et al., 2014; Dudgeon et al., 2006; Vörösmarty et al., 2010; WWF, 2016). Previous studies investigating water pollution have mainly focused on nutrient loading, acidification and pollution by organic loading (Schäfer et al., 2016). However, chemicals have become ubiquitous throughout humankind and are indispensable for society and economy. Currently, more than 100,000 chemicals are registered and in daily use (Schwarzenbach et al., 2010; Schwarzman and Wilson, 2009). Some of these chemicals are degraded quickly, while others rather accumulate in the environment (Fenner et al., 2013).

Despite their potential negative effects on biota and their intentional release, pesticides have been neglected in the past by ecological studies investigating threats to freshwaters (Schäfer et al., 2016) and it is unknown how much they contribute to biodiversity loss (Persson et al., 2013; Rockström et al., 2009). However, recent studies indicate that pollution by pesticides may be a frequent threat to freshwaters and that risks might have been neglected in the past. Malaj et al. (2014) showed that almost half of the European water bodies are at risk from pesticides. In the United States, Stone et al. (2014) showed that 61% of assessed agricultural streams exceed aquatic-life benchmarks. On a global scale, Stehle and Schulz (2015) found that 52% of detected

insecticide concentrations ($n = 11,300$) exceeded risk thresholds. Small streams are particularly vulnerable to pesticide pollution because of their high contact with adjacent land and low water volume (Biggs et al., 2016). However, there is currently a lack of data on pesticide pollution of small streams (Lorenz et al., 2016).

As a reaction to the degradation of freshwaters, several legal frameworks have been established to safeguard and improve the quality of freshwater ecosystems. In the European Union (EU), the Water Framework Directive (WFD, European Union (2000)) regulates the protection of aquatic ecosystems and commits the member states to monitor chemical pollution and to achieve a 'good' status of all water bodies. Knowing of the toxicity of pesticides and their intentional release into the environment, also the introduction and use of new pesticides are highly regulated. Sophisticated environmental risk assessment procedures have been developed and are requested by the EU to ensure that the use of pesticides does not cause unacceptable effects to non-target organisms, soil, air and water (European Union, 2009).

ENVIRONMENTAL RISK ASSESSMENT

Environmental risk assessment (ERA) tries to estimate risks to animals, populations, communities or ecosystems. ERA investigates if a chemical can be used as intended without causing detrimental impacts to the environment. Moreover, ERA is used as a tool to support decision making under uncertainty (Newman, 2015). Environmental risk is defined as a combination of the severity and the probability of occurrence of a potential adverse effect on the environment (Suter, 2007). Therefore, ERA is based on two components: Effect- and exposure assessment. A combination of both is needed to characterise environmental risks.

Effect assessment characterises the strength of ecological effects using laboratory and semi-field experiments. It does so by establishing relationships between the concentration of a compound and the observed effects. In the EU a tiered approach with increasing complexity and realism has been established. Lower tier assessment is based on highly standardised single species laboratory experiments. If a low risk cannot be established in lower tiers, higher tier assessment refines the assessment by testing additional species, extended laboratory experiments or model ecosystem experiments and aims to reduce the uncertainty in the assessment (Brock et al., 2006; EFSA, 2013). To address the various uncertainties in effect assessment (e.g. experimental variation, variation between species, variation in environmental conditions etc.) the estimated toxicity values are divided by an assessment factor (AF) between 100 (lower tier assessment) and 2 (higher tier assessment) depending on data quality, which yields to a regulatory acceptable concentration (RAC) (Brock et al., 2006; EFSA, 2013).

Exposure assessment for freshwaters aims to characterise the probability of an adverse effect by deriving a predicted environmental concentration (PEC) in surface waters and sediments (Newman, 2015). This derivation is mainly based on modelling the fate of chemicals in the environment using computer simulations. In the European Union the FOCUS models are used to derive PECs (EFSA, 2013; FOCUS, 2001). For their calculations, these models need many compound specific input parameters like

the molecular weight, water solubility, partitioning coefficients and dissipation time. Additionally, information on the application regime and crop type is needed. FOCUS estimates the concentration within edge-of-field streams of 1 meter width (corresponding a catchment size of approx. 5 to 40 km², see Figure ??) and 30 cm depth (Erlacher and Wang, 2011). Nevertheless, recent research showed that FOCUS models fail to predict measured field concentrations of pesticides (Knäbel et al., 2014; Knäbel et al., 2012).

The final step in ERA is risk characterisation, putting together the information gained from effect and exposure assessment. Risk can be expressed in several ways, a simple quantitative way being the risk quotient approach: If the ratio PEC / RAC exceeds a value of one potential risks cannot be rebutted (EFSA, 2013; Solomon et al., 2000; Suter, 2007). Consequently, pesticides can be authorised only if the risk quotient is below one, indicating that harmful effects are unlikely to happen.

ENVIRONMENTAL MONITORING

Widespread anthropogenic activities and the induced environmental changes have resulted in concerns about the state of the environment and have led worldwide to the development of environmental monitoring programs (Nichols and Williams, 2006). After authorisation, pesticides applied on agricultural fields may enter aquatic ecosystems via diffuse sources like spray-drift, surface run-off or drainage (Carter, 2000; Liess et al., 1999; Schulz, 2004; Stehle et al., 2013). These entered pesticides may have ecological effects and worsen the chemical status, acting contrary to the goal of the WFD. For monitoring the progress towards the goal of a 'good' status and for assessment of the chemical status of surface waters the EU WFD established monitoring requirements for all European river basins (European Union, 2000). For chemical monitoring the WFD requires grab sampling and chemical analysis of 21 priority substances (of which 7 are pesticides) every third month and of 24 other pollutants (of which 12 are used as pesticides) every month and derived environmental quality standards (EQS) for these (European Union, 2013). Additionally, 14 substances (of which 8 are used as pesticides, including all Neonicotinoids) that may pose a significant risk, have currently an insufficient data basis and are candidates for future priority substances are currently monitored until 2019 (European Union, 2015). Nevertheless, monitoring programs on a national scale might consider a broader spectrum of chemical substances adapted to national requirements, e.g. for investigative monitoring. However, recent studies indicate that the current sampling and chemical analyses strategy greatly underestimates the pesticide exposure (Moschet et al., 2014; Stehle et al., 2013; Xing et al., 2013).

Environmental monitoring produces humongous amounts of data containing information on pesticide concentrations in the field on a large scale under many conditions. Moreover, data from long-term monitoring programs can be used to study hypotheses about spatial and temporal dynamics and interactions, that are not evident from short term and short scale studies (Gitzen, 2012) and provide insights for modelling approaches. Therefore, it can be complementary to ERA (Suter, 2007). If the environmental risk assessment process captured all relevant sources of risk, no concentrations

above the derived RAC should be observable in European rivers. Therefore, monitoring data could be used to provide feedback for ERA after approval (Knauer, 2016). However, monitoring under the WFD has its main focus on large water bodies $>10 \text{ km}^2$ catchment size (European Union, 2000), whereas ERA has its focus on small water bodies of approx. 5 to 40 km^2 catchment size (Figure ??, Brock et al. (2006) and European Union (2009)). At present little is known on pesticide concentrations in small streams comparable to those assessed in ERA (Biggs et al., 2016; Lorenz et al., 2016).

STATISTICAL ECOTOXICOLOGY

As outlined, environmental effect assessment is based on experimental approach and generates data on ecological effects. The produced datasets range from small univariate datasets (lower tier assessment) to medium-sized multivariate datasets (higher tier assessment). In order to extract usable information for assessment, these datasets are analysed using statistical techniques and therefore, statistics are crucial for effect assessment (Newman, 2012). Statistical ecotoxicology combines statistics with the specific needs and constraints of ecotoxicology. Ecotoxicologists deal generally with low replicated experiments, making statistical inference difficult (Van Der Hoeven, 1998). For example, a recent analysis of eleven mesocosm studies revealed that the sample sizes for these kind of experiments range between two and five (Szöcs et al., 2015). Statistical ecotoxicology aims to provide solutions to statistical challenges in ecotoxicology (Fox and Landis, 2016a), guidance on experimental designs (Johnson et al., 2015) and tools to integrate big data (Van den Brink et al., 2016). The ultimate goal is to improve the accuracy of ERA.

The relationships between the concentration of a compound and the observed effects are usually analysed using dose-response models, which can be used to derive an effective concentration (EC_x) for $x\%$ effect (Ritz, 2010). Nevertheless, such relationships cannot always be established from experimental data. For example, mesocosm experiments are conducted to characterise effects on whole biological communities. However, because of the multivariate response and potential indirect effects between species, there is no clear dose-response relationship and no models for this kind of data currently available. Recently, Green (2016) provided examples where fitting dose-response models is problematic. In such cases a no-observed-effect concentration (NOEC) usually derived to quantify the toxic potential.

The NOEC is the highest tested concentration that does not lead to significant deviation from the control response and therefore relies on null hypothesis significance testing (NHST). However, the use of NOEC as a toxicity measure in environmental effect assessment has been heavily criticised in the past (Chapman et al., 1996; Fox et al., 2012; Fox and Landis, 2016b; Jager, 2012; Laskowski, 1995; Warne and van Dam, 2008). One such critic is the low statistical power for NHST in common ecotoxicological experiments (Van Der Hoeven, 1998). *A priori* power calculations can provide useful guidance for choosing experimental designs (Johnson et al., 2015), but are rarely used by ecotoxicologists (Newman, 2008).

Instead of conducting experiments, toxicity could be also predicted from molecular structures using quantitative structure-activity relationships (QSAR), which are usually calculated using machine-learning techniques (Breiman, 2001; Cortes-Ciriano, 2016; Murrell et al., 2015). Nevertheless, in order to improve and validate these models to give sufficient prediction accuracy more data from experiments is needed (Kühne et al., 2013). Indeed, a large amount of data is available that could be used for effect and exposure assessment. For example, the US EPA ECOTOX database (U.S. EPA, 2016), the Pesticides Properties Database (Lewis et al., 2016) and ETOX (Umweltbundesamt, 2016) provide toxicity data that could be used for effect assessment. Databases like Physprop (Howard and Meylan, 2016) and PubChem (Kim et al., 2016) provide chemical properties that are needed as input for exposure models. Monitoring data provides information on realised concentrations, could be used for validation of models and retrospective risk assessment. This "big data" can provide new information and opportunities for ERA (Dafforn et al., 2015). However, it needs to be harmonised, linked and easily accessible in order to be used effectively in ERA.

OBJECTIVES AND OUTLINE OF THE THESIS

The overall goal of this thesis was to contribute to the emerging field of statistical ecotoxicology, environmental risk assessment and environmental monitoring. The main objectives were (i) to scrutinise new methods in statistical ecotoxicology and effect assessment, (ii) explore risk dynamics using available monitoring data and (iii) provide tools to deal with and integrate big data in ERA. Figure 1.1 provides a conceptual overview on ERA and environmental monitoring as outlined in the previous sections, as well as the parts considered in this thesis and the relations between them.

The thesis starts with a comparison of statistical methods to analyse ecotoxicological experiments using NHST in effect assessment (Chapter ??). Specific questions addressed were:

- Are newer statistical methods, explicitly considering the type of analysed data, more powerful than currently used methods for NHST?
- How much statistical power do current experimental designs in ecotoxicology exhibit?

Risk assessment procedures in the European Union have the main focus on small water bodies adjacent to agricultural fields where plant protection products are applied. Therefore, chapter ?? focuses on measured environmental concentrations on a large spatial scale in small streams, the drivers thereof and comparison with RACs derived from ERA. Specific goals of this study were:

- Compile monitoring data on pesticides in small streams in Germany and check if the available data is suitable to inform ERA.
- Explore the relationship between agricultural land use and stream size and RAC exceedances.

- Scrutinise the annual dynamics of pesticide exposure, as well as the influence of precipitation on measured pesticide concentrations.
- We use RACs derived from ERA to assess the current pollution in German streams and identify pesticides exhibiting currently a risk to freshwaters.

The compilation of monitoring data from different data sources in Chapter ??, resulted in a big inhomogeneous amount of data. Moreover, Biologists, Chemists and ecotoxicologists face similar problems with the need to identify and harmonise their biological and chemical data. Chapters ?? (chemical data) and ?? (biological data) describe software solutions to simplify and accelerate the workflow of:

- validating and harmonising chemical and taxonomic data
- linking datasets from different databases
- retrieving properties and identifiers

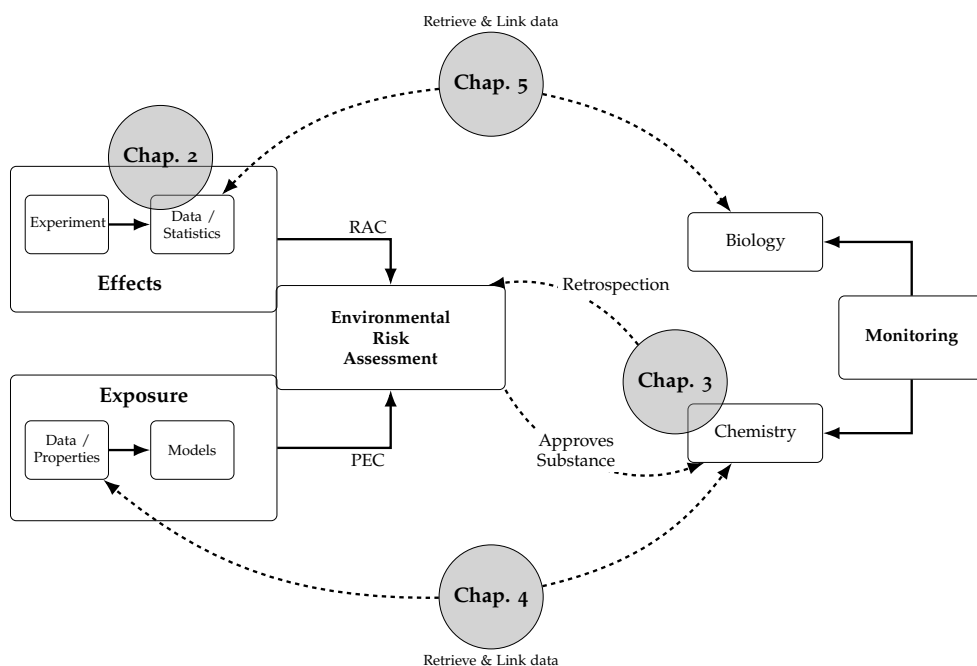


Figure 1.1: Conceptual overview on environmental risk assessment, environmental monitoring and the parts addressed by this thesis.

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TOPICS IN STATISTICAL ECOTOXICOLOGY

The simulation study performed in chapter ?? clearly showed that common experimental designs in ecotoxicology exhibit unacceptably low statistical power (Szöcs and Schäfer, 2016; Van Der Hoeven, 1998). This underpins the criticism accumulated over the last 30 years towards the usage of NOEC as an endpoint for ERA (Fox and Landis, 2016). Nevertheless, the NOEC is still one of the standard endpoints of mesocosm experiments in higher tier risk assessment (EFSA, 2013). Therefore, further advances in the statistical evaluation of mesocosm experiments is needed.

Recently, *a posteriori* calculations of statistical power have been proposed to counteract these limitations and aid the interpretation treatment-related effects in model ecosystems (Brock et al., 2015). The "minimum detectable difference" (MDD) estimates the difference between two means that must exist in order to produce a statistically significant result ($p < 0.05$ (Gelman and Stern, 2006)) and could be used to interpret NOEC. However, *a posteriori* calculations have been shown to have logical flaws when used for interpretation of non-significant results (Hoenig and Heisey, 2001; Nakagawa and Foster, 2004). However, conducting and reporting of *a priori* power calculations, as performed in chapter ??, might provide researchers important information to optimise their study designs, ensuring that their experimental designs have appropriate power and can lead to interpretable results (Johnson et al., 2015).

Moreover, similar simulations could not only be used to study factorial but also regression designs. Indeed, simulations could be used to determine the optimal experimental design for dose-response models and EC_x determination, balancing precision and usage of resources. Regression designs are generally more powerful and provide more information than factorial designs (Cottingham et al., 2005). In mesocosm experiments, such designs, assigning the replicates to more tested concentrations, might also provide additional insights. Currently, statistical tools to analyse a community dose-response relationship are not well explored and no equivalent $EC_{x,mesocosm}$ can be derived. One possibility could be to fit separate dose-response models to each species, leading to a EC_x for each species in a mesocosm study. Subsequently, these EC_x values could be combined and summarised using Species Sensitivity Distributions (Posthuma et al., 2002), providing a single measure for the community response, e.g. a hazardous concentration ($HC_{x,mesocosm}$) for x % of species affected in mesocosms (Maltby et al., 2005). Another possibility would be to use a logistic type of ordination (van den Brink et al., 2003). Reduced-Rank vector generalised linear models (RR-VGLM) could be used to fit such type of models (Yee, 2015; Yee and Hastie, 2003), but they have not been applied in ecotoxicology yet.

In a similar vein, community ecology is currently experiencing a shift towards a new class of multivariate methods, incorporating statistical models for abundances across many taxa simultaneously (ter Braak and Šmilauer, 2015; Warton et al., 2015a; Warton et al., 2015b; Warton et al., 2012). However, these methods have not been applied frequently and their applicability to ecotoxicological data is currently unclear (Szöcs et al., 2015). All these models have in common, that the choice of statistical model is primarily based on the grounds of data properties. In chapter ?? we showed, that using statistical models that fit the type of data analysed, can provide higher statistical power. Simultaneously, Ives (2015) published a study reaching contradictory conclusions (*"For testing the significance of regression coefficients, go ahead and log-transform count data"*). It must be noted, that the simulation designs differed significantly between both studies: We used a low-replicated factorials design, whereas Ives (2015) simulated a well-replicated regression designs with two predictors. We both found that the negative-binomial GLMs were surprisingly prone to Type I errors, although the assumptions of this model closely matched the data. Nevertheless, as we show in chapter ??, the parametric bootstrap might provide a solution to this problem, but is computationally intensive and not widely used. The parametric bootstrap is akin to Bayesian methods (Gelman et al., 2014), which might provide an alternative inference method for inference. The main point, leading Ives (2015) to his conclusions, was that GLM showed undesirable Type I errors in case of correlated predictors, a case not commonly encountered in ecotoxicology and not studied in chapter ?. Recently, the current state-of-the-art was discussed by Warton et al. (2016): i) choose the statistical model based on the grounds of data properties; ii) fix Type I errors using parametric bootstrap or resampling; iii) take mean-variance relationship into account, which is in line with the findings of chapter ?. However, there are still open questions regarding the use of GLMs for count data (see e.g. raised by Prof. John Maindonald, <http://uni-ko-ls.de/fb>). To diagnose issues such as overdispersion and excess of zeros in count data models new tools like the recently developed *"Rootograms"* (Kleiber and Zeileis, 2016) provide useful additions.

In chapter ?? we applied new statistical modelling techniques that explicitly consider the limit of quantification. The currently most often used methods to deal with such censored data is to omit or substitute non-detects. Censoring is very common when dealing with chemical and ecological datasets, but is rarely taken into account (Fox et al., 2015). Recent examples from ecotoxicology and environmental chemistry show that omission (Hansen et al., 2015), randomization (Goulson, 2015) or substitution by a fixed value (Helsel, 2010; Helsel, 2006) can lead to biased results. Hansen et al. (2015) used a Tobit regression (Tobin, 1958) that takes the amount of censored data into account, assuming a (log-) normal distribution of concentrations. In chapter ?? we used a slightly different approach, using a zero adjusted gamma model (ZAGA). We modelled measured concentrations as two separate processes, generating i) zero values and ii) non-zero values assuming a gamma distribution of concentrations. In ecological statistics this type models is also known as *hurdle* models (Martin et al., 2005). The log-normal Tobit model has no probability mass at zero, whereas ZAGA model has a probability at zero. Generally, the difference between Tobit and two-part models are small (Min and Agresti, 2002) and the same holds for differences between the log-normal and Gamma distribution.

Indeed, a Tobit-like model could be also fitted assuming a Gamma distribution (Sigrist and Stahel, 2010).

Grab sampling likely underestimates chemical concentrations because of short term peak concentrations (Stehle et al., 2013; Xing et al., 2013). Although this leads to an increased variation in chemical measurements, we still can learn from the process generating values above LOQ, even if the absolute value is subject to error. This is also highlighted by the results of chapter ??, with estimated coefficients for the absolute concentration showing much larger uncertainty than coefficients for the probability of exceeding LOQ (Figure ??). Currently, models explicitly taking the censored nature of chemical monitoring data are not well explored and seldom applied. Further research on those is needed and might provide useful information for analysing monitoring data, assessing the chemical status and trends thereof.

LEVERAGING MONITORING DATA FOR ENVIRONMENTAL RISK ASSESSMENT

In chapter ?? we compiled and analysed monitoring data leading to the currently most extensive dataset on pesticide exposure available for Germany. We demonstrated, that within the current monitoring scheme small streams below 10 km² are underrepresented (Figure ??, top). Given their importance, we must admit that we currently do not have much knowledge about these and their threats (Biggs et al., 2016; Lorenz et al., 2016). To fill these gaps, monitoring networks need to be adapted to give a better representation of small streams.

We provide results in chapter ?? only for small streams, however, data on lentic systems is even more scarce. Although, more the 95% of German standing waters are lentic small waterbodies a recent meta-analysis revealed that only 5% of studies investigating pesticides in freshwaters were performed on lentic small water bodies (Lorenz et al., 2016). The query to the federal states in chapter ?? included also lotic systems. However, the returned data revealed that there are currently no such monitoring data available (Brinke et al., 2016). This highlights the urgent need to adapt monitoring schemes to also include small standing waters.

Our results revealed that chemical monitoring schemes within Germany differed largely in terms of spatio-temporal coverage and compound spectra between federal states. Similarly, Malaj et al. (2014) showed big differences between European countries. Overall, a homogenisation and standardisation of chemical monitoring programs would enhance the comparability and the possibility for a large-scale assessment.

We found that the signal from agricultural pesticides can be detected down to a small percentage of agriculture within the catchment (Figure ??). Thus, we can conclude that if there is agriculture with a catchment, it is very likely that pesticides will be applied, enter the streams and are detected. This has implications for selection of reference sites for environmental monitoring, that need to have no agricultural influence. We studied only the influence of agricultural non-point sources, however, point-sources

like wastewater treatment plants can also contribute to pesticide pollution of streams (Bunzel et al., 2014).

We were able to detect a small, but distinct increase of risks after precipitation events. This is in line with findings that pesticides mainly enter surface waters via edge-of-field runoff (Schulz, 2001). Moreover, our results suggest that absolute measured concentrations are subject to a high error due to the sampling process and adds evidence that current monitoring schemes, largely unconnected from precipitation events, underestimate pesticide risks (Stehle et al., 2013; Xing et al., 2013). Automatic event-driven samplers in small streams could provide knowledge on pesticide risk dynamics that are currently unknown.

Monitoring data can provide an opportunity to inform ERA after authorization and could possibly trigger a refinement of the assessment (Knauer, 2016). However, current monitoring mainly addresses streams bigger than those considered in ERA. Our results indicated that small streams are frequently exposed to high risks from pesticides. Moreover, to provide a suitable feedback for ERA small agricultural streams must be integrated into environmental monitoring schemes. As the measurements within the current monitoring schemes provide an underestimation, all exceedances of risk thresholds represent an unacceptable risk and indicate that current ERA might miss potential risks and further enhancements of the current authorisation procedure are needed.

Risk thresholds in chapter ?? were especially exceeded for the organophosphate Chlorpyrifos and neonicotinoid substances. This adds to the existing evidence that this particular class of insecticides poses currently a high threat to freshwaters and stricter regulations are warranted (Goulson, 2013; Morrissey et al., 2015). The high number of exceedances shows that ERA for these substances was not accurate enough and lead to risks for the environment. Recent studies investigating large-scale pesticide risks did not consider neonicotinoid insecticides (Malaj et al., 2014; Stehle and Schulz, 2015) and therefore likely underestimated the risks to freshwaters. However, this also shows that the analysed spectrum is an important driver of detected compounds (Malaj et al., 2014; Schreiner et al., 2016) and must be taken into account when evaluating monitoring data for risk assessment. The WFD currently considers on a few, relatively well-known substances (European Union, 2013) and a status assessment based on EQS likely missed the actual chemical pollution (Moschet et al., 2014). Compared to the data presented in chapter ?? the WFD considers currently only 4% of the pesticides (19 out of 478 pesticides). Recently, neonicotinoid substances have been incorporated in the watch list of substances for Union-wide monitoring (European Union, 2015), so that more information on the environmental fate of compound group will be available. Monitoring data provide also valuable information for the prioritisation of emerging pollutants and future monitoring with the WFD (Brack et al., 2017) and the compiled data we compiled could be a valuable input for such a prioritisation.

Monitoring under the WFD is also performed for biological components of freshwaters and a combination with pesticide exposure data might provide valuable insights into large-scale field effects of chemical substances (Schipper et al., 2014). Currently, chemical and biological monitoring are not synchronised. On a continental scale, Malaj et al. (2014) was able to compile ecological status data only for 5% of sites with chemical

measurements. For the dataset presented in chapter ?? we found a spatial match with biological monitoring for 60% of sites (Brinke et al., 2016). However, as biological data in Germany is sampled at lower frequencies (often less than once per year) a spatio-temporal match would result in much less accordance. Synchronising these samplings in a future monitoring would possibly enable to assess large-scale post-authorization field effects of chemical substances.

CHALLENGES UTILISING 'BIG DATA' IN ENVIRONMENTAL RISK ASSESSMENT

Effect assessment and environmental monitoring produce huge amounts of data. However, the profoundness of environmental risk assessment is often determined by the available data (Van den Brink et al., 2016). Useful data for ERA is currently spread over several largely unconnected databases. E.g. ecotoxicity data is spread over database maintained by the U.S. EPA (ECOTOX, U.S. EPA (2016)), the University of Hertfordshire (PPDB, Lewis et al. (2016)), the German Environment Agency (ETOX, Umweltbundesamt (2016)) and others. Chemical information is similarly spread over several databases, like PubChem (Kim et al., 2016) or Chemspider (Pence and Williams, 2010). Additional complications arise because these databases use different identifiers for chemical substances. The U.S. EPA (U.S. EPA, 2016) uses solely the CAS-Number for identification, whereas other databases uses SMILES (Weininger, 1990) or InChI and InChIKeys (Heller et al., 2015). Integrating these databases is currently a challenge in ERA, which is complicated by ambiguous identifiers, e.g. should different salts be considered separately for aquatic risk assessment? Projects like the NORMAN EMPDAT database (Brack et al., 2012) or the STOFF-IDENT (Huckele and Track 2013, <http://uni-ko-ld.de/fc>) are first attempts for such an integration. Integration monitoring data and risk assessment data is a mandatory requirement for landscape level ecotoxicology and risk assessment (Focks, 2014) and needed for an improved model development and validation (Brock et al., 2006; Knäbel et al., 2012). Chapter ?? is an example for such an integration, but represents only a preliminary assessment and spatial-temporal risk dynamics should be further investigated.

The webchem package, presented in chapter ??, can foster such an integration. However, data must also be accessible in order to be retrievable by webchem. Unfortunately, major parts of data produced for environmental risk assessment are not available (Daforn et al., 2015; Schäfer et al., 2013). Recently, it has been demonstrated that data from the European Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) database can be used to improve the characterisation of ecotoxicity in life cycle assessment (LCA) (Müller et al., 2016). Although this database hosts humongous amounts of data used for risk assessment, it is currently not available in a convenient way. Indeed, a systematic data collection contravenes the legal usage of the REACH database (<http://uni-ko-ld.de/fd>). This may be also the reason, why the quality of chemical property data submitted this database is currently unknown (Müller et al., 2016; Stieger et al., 2014). Webchem currently can retrieve data from 11

data sources. However many other data sources are available and implementation of more than 15 other data sources in the pipeline will be implemented in the future (<http://uni-ko-lid.de/fi>) and collaborators are welcome.

The software tools described in chapters ?? and ?? assist researchers handling and cleaning their data. Aggregating taxonomic data to a higher taxonomic level is a common task when analysing data from mesocosm experiments or from field sampling. Taxize facilitates the retrieval of taxonomic classification, which is the basis also for more sophisticated aggregation methods (Cuffney et al., 2007). Today, taxize has been used in more than thirty scientific publications. Recent applications of the webchem package, have been demonstrated by Münch and Galizia (2016) and Ranke (2016): Münch and Galizia (2016) compiled a database for odorant responses of *Drosophila melanogaster* and webchem "likely saved [him] hundreds of working hours". Ranke (2016) is using webchem to compile and store chemical information for further analyses. The analyses performed in chapter ?? needed to integrate monitoring, chemical and risk assessment data which would have been difficult without the webchem package. These examples show that researchers have been missing such tools in the past. If they can reduce their time spend on data retrieval and handling, they could focus more on the quintessence of their research.

CONCLUSIONS

In the near future, big amounts of data will be available for environmental risk assessment. Integration and analysis of these data are forthcoming challenges in ecotoxicology (Dafforn et al., 2015; Van den Brink et al., 2016). This thesis provides insights into statistical analyses and experimental designs for a future effect assessment. Statistical ecotoxicology is just at the beginning and many problems are pending to be solved.

Environmental monitoring data can provide an important feedback to improve environmental risk assessment. The integration of both in this thesis showed that in Germany highly toxic insecticides pose a major threat to freshwaters. However, we also demonstrated current problems and further improvements of those are needed to safeguard freshwaters from chemicals. It needs to be re-evaluated if the current use of neonicotinoid insecticides is within the safe operating space to provide long-term health of ecosystems upon which humanity depends (Rockström et al., 2009).

Big data and modern statistical tools are a means of improving the accuracy and reducing the uncertainty of environmental risk assessment (Van den Brink et al., 2016). The software described in this thesis contributes its part to these improvements. Nevertheless, science and politics need to develop a culture of openness to promote the safety of our environment.

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