STATISTICAL ECO(-TOXICO)LOGY

IMPROVING THE UTILIZATION OF DATA FOR ECOLOGICAL RISK ASSESSMENT

by

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1 INTRODUCTION AND OBJECTIVES

THREATS TO FRESHWATER ECOSYSTEMS FROM CHEMICAL POLLUTION

Freshwaters ecosystems, like streams, lakes and wetlands, make up only 0.01% of the World's water and cover only 0.8% of Earths 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 ⅓ 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 waterbodies 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).

Earth is currently experiencing a functional change driven by human activities which are so far-reaching, that a new geological epoche "Anthropocene" has been proposed (Waters et al., 2016). These changes are also associated with biotic changes: 65% of rivers are currently at threat (Vörösmarty et al., 2010) and freshwaters are experiencing the greatest losses of biodiversity (WWF, 2016). A multitude of stressors contribute to this deterioration of freshwater biodiversity including habitat loss and degradation, overexploitation, invasive species and pollution (Dudgeon et al., 2006; Vörösmarty et al., 2010; WWF, 2016). Studies investigating water pollution have mainly focused on nutrient loading, acidification and pollution by organic loading (Schäfer et al., 2016). Chemicals have become ubiquitous throughout humankind. Currently, more than 100,000 chemicals are registered and in daily use (Schwarzenbach et al., 2010; Schwarzman and Wilson, 2009). These substances will somewhen ultimately end in the environment.

Despite their potential negative effects for biota and humans 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 the contribute to biodiversity loss (Persson et al., 2013; Rockström et al., 2009). However, recent studies indicated that pollution by pesticides may be a frequent threat to freshwaters that might have been neglected by ecological studies in the past. Malaj et al., (2014) showed that almost half of 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 52.4% of detected insecticide concentrations (n = 11,300) exceeded risk thresholds. The high contact with adjacent land and low water volume of small streams make them particularly vulnerable to pesticide pollution (Biggs et al., 2016), however, there is currently a lack of data on pesticide pollution of small streams (Lorenz et al., 2016).

As 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 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 is highly regulated. Sophisticate environmental risk assessment procedures have been developed and are requested by the EU (European Union, 2009) to ensure that the use of pesticides does not cause unacceptable effects to non-target organism, soil, air and water.

ENVIRONMENTAL RISK ASSESSMENT

Environmental risk assessment (ERA) tries to estimate risks to animals, populations or ecosystems. It investigates if a chemical can be used as intended without a causing detrimental impacts to the environment. 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 to 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 effects using laboratory and semi-field experiments. It establishes relationships between the concentration of a compound and the observed effects. In the European Union a tiered approach with increasing complexity and realism. Lower tier assessment is based on highly standardised single species laboratory experiments, whereas higher tier assessment is refined by testing additional species, extended laboratory experiments or model ecosystem experiments. To address the various uncertainties in effect assessment (e.g. experimental variation, variation between species, variation in environmental conditions etc) the retrieved toxicity values are multiplied by an assessment factor between 0.01 (lower tier assessment) and 0.5

(higher tier assessment) depending on data quality, which yields to a regulatory acceptable concentration (RAC) (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). It is mainly based on modeling the fate of chemicals in the environment using computer simulations. In the European Union, the FOCUS models are used (EFSA, 2013; FOCUS, 2001). To calculate PECs 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 models the concentration within edge-of-field streams of 1 meter width and 30cm depth (Erlacher and Wang, 2011). Nevertheless, recent research showed that FOCUS models fail predict measured field concentrations of pesticides (Knäbel et al., 2014; Knäbel et al., 2012).

The final step in ERA is risk characterisation. It puts together the information gained from effect and exposure assessment. Risk can be expressed in several ways, a quantitative way being the risk quotient approach: A PEC / RAC ratio greater than one indicating potential risks (Amiard-Triquet, 2015; EFSA, 2013; Suter, 2007). Pesticides can be authorised only if the risk quotient is below one indicating that harmful effects are unlikely.

ENVIRONMENTAL MONITORING

Widespread anthropogenic activities and the induced environmental changes have resulted in concerns about the state of the environment and have lead to the development of environmental monitoring programs worldwide (Nichols and Williams, 2006). After authorization, pesticides applied on agricultural fields may enter aquatic ecosystems via diffuse sources like spray-drift, surface run-off or drainage (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 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 (European Union, 2013). Additionally, 14 substances (of which 8 are used as pesticides, including Neonicotinoids) that may pose a significant risk, have a insufficient data basis and are candidates for future priority substances are currently monitored until 2019 (European Union, 2015). Although national monitoring programs might monitor a broader spectrum of chemical substances, it is obvious that only a small fraction of the chemical space can be monitored.

Environmental monitoring produces humongous amounts of data containing information of realised pesticide concentrations in the field, which can be complementary to environmental risk assessment (Suter, 2007). If the risk assessment process captured all relevant sources of risk no concentration above the derived RAC should be observed in European rivers. Therefore, monitoring data could be used to provide feedback for ERA after approval (Knauer, 2016). However, it must be noted that there is a mismatch between streams assessed in ERA and streams monitored according the WFD: The WFD aims at monitoring medium size to large streams greater than 10 km^2 catchment size, whereas ERA assesses risks for streams corresponding to a catchment size of approximately 7 km^2 (corresponding to 1 meter width, see Figure **??** ref to small streams supplement). 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 modeling approaches.

STATISTICAL ECOTOXICOLOGY

Environmental effect assessment generates data on ecological effects using experiments. The produced datasets range from small univariate datasets (lower tier assessment) to medium sized multivariate datasets (higher tier assessment). These datasets are analysed using statistical techniques in order to extract usable information for assessment and therefore, statistics are crucial for effect assessment (Newman, 2012). Statistical ecotoxicology combines statistics with the specific needs and constraints of ecotoxicology. It 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) to improve 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 for x% effect (EC_x) (Ritz, 2010). Nevertheless, such relationships cannot always be established from experimental data. For example, model ecosystem experiments are conducted to characterise effects on whole biological communities. However, because of multivariate responses and potential indirect effects, there is no clear dose-response relationship and no models for this kind of data available. There are also other examples were fitting dose-response models is problematic (Green, 2016). In such cases, there is usually a no-observed-effect concentration (NOEC) computed.

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 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 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 (Cortes-Ciriano, 2016; Murrell et al., 2015). Nevertheless, in order to improve these models to give sufficient prediction accuracy more data from experiments is needed (Kühne et al., 2013).

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 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, (ii) explore available monitoring data and (iii) provide tools to deal with big data. Figure 1.1 provides a conceptual overview on ERA and envi-

ronmental monitoring as outlined in the previous sections, as well as the parts of this thesis and its relations.
The thesis starts with a comparison of statistical methods to analyse ecotoxicological experiments in effect assessment (Chapter **??**). Specific questions addressed were:

- Are newer statistical methods more powerful than currently used methods for NHST?
- How much statistical power do current experimental designs in ecotoxicology exhibit?

Exposure assessment aims at predicting chemical concentrations in small streams. Chapter **??** focuses on measured large-scale environmental concentrations and the drivers thereof. Specific goals were:

- Compile all available monitoring data on pesticides in small streams in Germany
- Explore the relationship between agricultural land use and streams size and measured pesticide concentrations.
- Study annual dynamics of pesticide exposure, as well as the influence of precipitation on measured pesticide concentrations.
- Assess the current pollution in German streams and identify responsible pesticides.

The compilation of monitoring data from different data sources, lead to a big inhomogeneous amount of data that first needs to be harmonised. Chapter **??** (chemical data) and Chapter **??** (biological data) describe software solutions to simplify and accelerate the workflow of:

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

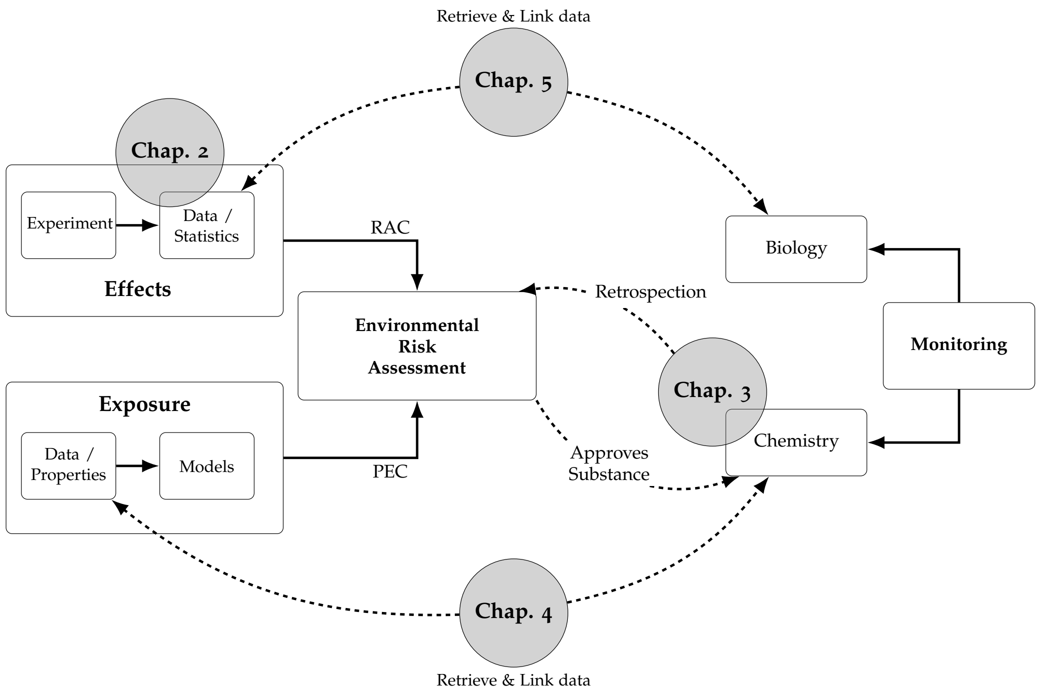

Figure 1.1: Conceptual overview on environmental risk assessment, environmental monitoring and the parts addressed by this thesis.

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