Quantitative Structure-Activity Relationships for soil Ecotoxicity

Giesen, D.

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Chapter 7

General Discussion

Rising public awareness and increasing concerns regarding negative impacts of pollution with industrial organic compounds to the environment (Bone et al., 2010; Bone et al., 2011) and human health has prompted European Union (EU) authorities to enhance efforts in ecosystem protection and environmental risk assessment (ERA) (Gubbels-van Hal et al., 2005). ERA uses standardized methods and reproducible evaluation criteria to predict and address toxicity of toxicants to relevant ecological endpoints and ensure ecosystem structure and function and directly and indirectly human health (Brussaard et al., 1997; Cardinale et al., 2012; Hooper et al., 2012). In 2006 the European Commission implemented the legislation for Registration, Evaluation and Authorisation of Chemicals (REACH) (EU 2006). New and existing compounds, produced or traded within member states require thorough documentation and adequate risk assessment, making high quality biological endpoint datasets and optimized chemical description decisive factors (Hansson and Ruden, 2006; Heiss et al., 2006). To meet the goals of REACH within the period set, Intelligent Test Systems (ITS), e.g. *in silico* methods like Quantitative Structure-Activity Relationship (QSARs), as an alternative to animal tests form a crucial part of the evaluation processes. QSARs link toxicity of a series of compounds to their properties, for instance their lipophilicity (Hermens, 1995).

To be accepted under the REACH legislation, QSARs have to fulfil certain requirements for the quality assurance; they have to be well documented and scientifically valid, they have to meet defined and relevant endpoints and must be applicable in their domains. In the past quality assurance focused mainly on the reduction of variance deriving from standardized toxicity tests, as a control of the exact determination of the effect concentration to a test organism. These variations represent heterogeneous test-system-organism interactions, which should not be understood as a complication but be addressed as natural processes. Coincidental, the compound properties on which models are also relying rarely were optimized to meet compound interactions with a biological

matrix or with the surrounding test substrate. Physico-chemical parameters were accepted to be less variable and ready for use, though many of them were derived from models themselves (Piliszek et al., 2011; Sabljic et al., 1995; Wilczynska-Piliszek et al., 2012) but not experimentally validated and in some instances showed huge variations. This resulted in a discrepancy in the quality and efficiency assurance of ERA, between biological endpoints, e.g. 50% lethal concentration (LC_{50}) or 50 % reduction in reproduction (EC_{50}), and the chemical descriptors.

The studies presented in this thesis integrate biological and physico-chemical optimization for 1) the development of QSARs for two sets of compounds in different soils for the soil dwelling springtail *Folsomia candida* and validate estimated endpoint concentrations with advanced chemical analysis, 2) experimental determination of crucial physico-chemical parameters to optimize QSARs, and 3) applying newly developed biology-based alternative approaches implemented to assess the mode of action (MOA) of one set of compounds specifically to the chosen test organism. With this, the work presented in this thesis provides a basis for the application of QSARs for the prediction of the toxicity of chemicals to soil organisms within REACH.

Soil toxicity tests have batteries of soil dwelling test organism to chose from (Amorim et al., 2011; Dodd and Addison, 2010; Sverdrup et al., 2002b). In respect to ERA and REACH it is reasonable to perform necessary tests with the most sensitive species. Tests with *F. candida* have been shown to be reliable and highly reproducible with various test conditions (Fountain and Hopkin, 2001) and identified to be more susceptible to organic pollution than for instance earthworms (Droge et al., 2006). With the ISO11267 (ISO, 1999) test both sublethal endpoints, 10% (EC_{10}) or 50% (EC_{50}) reduction of reproduction and lethal endpoints can be assessed simultaneously. Furthermore, thorough phylogenetic (Timmermans et al., 2008; Timmermans et al., 2007) analysis prepared the foundation to establish transcriptome (mRNA)-based microarrays for physiological stress analysis (de Boer et al., 2010) and ecotoxicogenomic (Nota et al., 2011) analysis. These additional features opens the possibility to combine two ITS, namely QSARs based on standard toxicity tests and new age molecular bioassays. This unique position makes *F. candida* a key organism in soil ecotoxicological studies (Fountain and Hopkin, 2004, 2005). Quality of the estimated endpoints is

guaranteed by setting criteria to for the survival and reproduction in the control group and regular tests of the population performance with reference chemicals. The use of *F. candida* therefore fulfils the requirements for REACH.

QSAR optimization

Validation and optimization of QSARs also need new insight on the test substrate composition and the applied chemical descriptors. Soil as one of the most heterogeneous ecosystems known to man consists of inorganic particles, soil organic matter, dissolved organic matter and ions in the pore water (Levine et al., 1996). To evaluate the dynamics and behaviour of organic toxicants in soils, a clear description of the solid and aqueous compartments is crucial. Sorption of hydrophobic organic compounds towards the inorganic particles or organic matter and the dissolved organic fraction has been described on several occasions for soils and sediments (Haigh, 1996). From the sediments, the general accepted pore water theory was adopted for soil ecotoxicological studies, focusing on equilibrium establishing itself between sorbed fraction → bioavailable concentration in the porewater (interstitial water in soils) → bioaccumulation (Van Gestel and Ma, 1988; Van Gestel and Van Dis, 1988). Examples show that the paradigm of this three phase model, generally, can be applied to soil science as well (Sverdrup et al., 2002a; Van Gestel and Ma, 1988). However, origin and physico-chemical properties of components differ between sediments and soils. They only appear similar in some aspects, for instance they contain particulate and aqueous fractions. Yet, structure and function of soils are results of long-term weathering effects, e.g. extreme temperature changes compared to water-buffered sediments, and large scale biological activity and interactions, for instance podzolization (mainly restricted to the sandy soils and coniferous trees). Specific to sediments on the other hand is the pattern of the horizontal stratification of microbial processes and reduced redox-potential and oxygen depletion zones. The most obvious physical difference yet is the fact that soils are not water saturated like sediments. As this is the toxic fraction carrying compartment in both systems a good description, qualitatively and quantitatively, is absolutely mandatory, especially when multiple soils are compared. The different water volumes of the individual soils need to be included when toxic concentrations,

corresponding to toxic endpoints, are expressed as molar concentrations are used in QSARs. This is different from sediments, which do not require any further correction. In the past deviations in the regression models between soils were explained by referring at the different composition of the soil organic matter together with the used non test soil specific organic carbon – water partition coefficient ($logK_{oc}$), as measure for soil sorption. One way to circumvent less optimal chemical descriptors like $logK_{oc}$ and assess differences in the interstitial water is the direct measurement with passive samplers. These measured values then can be calculated to comparable concentrations and directly used in QSARs. To conclude, when developing QSARs for soil, it is not possible to directly rely on methods that have been shown to be valid for sediments.

In hindsight, the use of the n-octanol-water partition coefficients as basic parameters to correlate with toxicity or to classify compounds is only understandable under the perspective of the evolution of QSARs (Hermens, 1995; Könemann, 1981; Van Gestel and Ma, 1988). However, its further application without clearly discussing differences in the structure and composition with membrane lipids is unfortunate. Octanol consists of a single aliphatic chain of eight carbon atoms, with a terminal polar hydroxyl group. Lipids contain two chains of more than twelve carbons engaged in both saturated and unsaturated bonds, plus glycerin as central structure and a complex polar group containing e.g. phosphates or sulphates. At the supramolecule level, octanol behaves like a bulk solvent, whereas lipids form double layer vesicles or micelle spheres. Baring these discrepancies of the solvent structure in mind it becomes obvious that the structure-activity is not only determined by the properties of the compound but also the structure of the interacting matrix. This means that QSARs using $logK_{ow}$ are accepted although they do not match the assumed biological endpoint structure. Furthermore a classification system to distinguish groups of compounds and their mode of actions, just for their good correlation, entirely neglects the effective interaction with matrix and biological and biochemical facts of a test system. Modes of action have to be reviewed within biological context and their potential sides of action in an organism, which can change within the animal kingdom. Other partition coefficients regarding proteins or large biopolymers, such as chitin from arthropods exoskeleton or spider webs, which are crucial for the fitness of a species, have not been regarded as

indicators of chemical mode of action. Our understanding of toxicity and sensitivity of life traits would benefit substantially from such descriptors.

Concluding the optimisation process of QSARs for soil toxicity reveals a fairly worrying picture. The wrong adaptation of the sediment based pore water hypothesis for varying soil interstitial water volume, though the general concepts still is correct, shows how undervalued the description of the environmental matrix is. Focusing solely on chemical interactions, simply does not explain toxicity though significant relations were and will be found. The same is true for the application of both physico-chemical descriptors, $\log K_{oc}$ and $\log K_{ow}$. Sorption to organic carbon does not solely explain differences in interstitial water concentration when soils are compared. The use of the logKow for the estimation of bioaccumulation or toxicity classification, and QSARs based on it should be cautiously reviewed in future studies that include polar compounds. Structure-activity relationships for ERA should also include the structure-activity of biological and environmental matrices. Only in combination the results would be scientifically correct.

Biology based compound categorisation

Although the classification system by Verhaar et al. (1992 and 1996) has been challenged already with the application of artificial membrane – water partition coefficients, explaining quantitative differences between non-polar and polar compounds, the question remains if differences in the mode of action still occur as have been reported for, e.g. algae tests (Aruoja et al., 2011). Interaction of the toxicant with the biological matrix still might occur in different steps and paces, leading to different physiological pathways and biochemical reactions (Bradbury, 1995; McKim et al., 1987). Toxicity is after all the biological response towards a chemical stressor and the assessment of differences over all steps in the interaction pathway necessary to be able to fully understand and evaluate the risk of a specific compound. Gene-response can give an insight into the reaction pathway initiated in the organism after accumulation of a toxicant or mixtures and compensates for the limited amount of information obtainable by toxicity tests, which cannot provide any mechanistic biological information (van Straalen and Feder, 2012). Information on modes of action, however, is not only interesting to fundamentally understand toxicity to organisms. It can assist in a new

classification system, which is needed as the traditional one has been proven to be insufficient. Biological categorisation based on gene responses can also help in extrapolating toxic effects through taxa in the animal kingdom, once specific key genes have been identified. Yet several obstacles have to be addressed.

Biological finger printing is the result of statistical comparison of the transcriptome of exposed and control animals including over 5000 genes potentially up-or down regulated. It is therefore not as robust as a one parameter definition by a single chemical descriptor. Similarities in the gene expression to analogous chemical structures are therefore also depending on the interpretation of the scientist and risk assessors. More research with a wide range of chemicals and a better understanding of the total genome are needed to reduce this limitation. Natural genetic variation between populations might also alter the genetic response pattern. International consensus therefore is vital for the technique, standardizing which populations to use and how to avoid the risk of genetic erosion. Further obstacles derive from the need of standardized toxicity test still to be performed to be able to calculate effective concentrations before gene-response can be assessed. Until now, biological techniques cannot work on their own. The need for toxicity tests raises also the question if the toxicity is caused by effects on the adult or if it is caused by the different physiology of immobile eggs in case of *F. candida*. Anyway, for bioassays 25-day old animals are exposed for 2 days to corresponding EC_{50} concentrations. Sensitivity in the molecular response can also derive from inherent soil physico-chemical properties (de Boer et al., 2011). Higher clay content forces animals to stay on the soil surface which may limit the contact with the toxicants. Other physical differences include water content and pH (de Boer et al., 2010). Soil organic matter might include compounds that itself are toxic or do not allow for growth of beneficial or disease-carrying microbial communities. Nevertheless, with advanced testing of more compounds and further standardisation of increasingly sophisticated bio-mathematical tools, bioassays will become an intrinsic part of ecotoxicological testing, compound classification and risk assessment.

Similar to the optimization of QSARs and the environmental matrix, biological aspects (Kooijman et al., 2007) to determine the mode of action are less valued than chemical approaches. This is concerning at least, as

actually only the reaction of and in the organism ultimately determines by what mechanism a toxicant causing toxic effects. Higher variability might be a blessing in disguise, as it might in the future enable taxa-specific risk assessment and extrapolation through ecosystems.

To conclude, this thesis brought new insights on the development and use of QSARs for soil organisms. It for instance confirmed the use of SPME as a tool for reliably assessing interstitial water concentrations of organic chemicals in soil. It also provided insight into the development with time of pore water concentrations, which showed to be quite different depending on the properties of the chemicals. The use of liposome-water partitioning, for the first time applied to soil organisms, showed promising. The results of this study showed that it is possible to arrive at a unified QSAR for different chemicals and different soils. The results of this study also showed that we need more biology-based mechanistic understanding on the way soil organisms interact with chemicals in soil.