Ecological Risk Assessment of Organic Pollutants with Regard to their Direct and Indirect Effects on Stream-Dwelling Macroinvertebrates of Small Headwater Streams

Vom Fachbereich Biowissenschaften und Psychologie
der Technischen Universität Carolo-Wilhelmina
zu Braunschweig
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
(Dr.rer.nat.)
genehmigte
Dissertation

Kumulative Arbeit

von Peter Carsten von der Ohe
aus Bremen

1. Referent:	Prof. Dr. Georg Rüppell
2. Referent:	Prof. Dr. Gerrit Schüürmann
eingereicht am:	31.01.2005
mündliche Prüfung am:	20.05.2005

Braunschweig, 2005

Vorveröffentlichungen der Dissertation

Teilergebnisse aus dieser Arbeit wurden mit Genehmigung der Gemeinsamen Naturwissenschaftlichen Fakultät, vertreten durch den Mentor Prof. Dr. G. Rüppell, in folgenden Beiträgen vorab veröffentlicht:

Publikationen

Von der Ohe, P.C., Liess, M. 2004. Relative sensitivity distribution of aquatic invertebrates to organic and metal compounds. *Environmental Toxicology and Chemistry* **23**: 150-156.

Liess, M., Von der Ohe, P.C. 2005. Predicting effects of pesticides on invertebrate communities in streams. *Environmental Toxicology and Chemistry* **24**: 954-965.

Von der Ohe, P.C., Schriever, C.A., Ball, M., Holmes, C. & Liess, M. 2005. Agricultural intensity and upstream catchments quality – important determinates of the macroinvertebrate assemblage. - *in preparation*

Von der Ohe, P.C., Kühne, R., Ebert, R.-U., Altenburger, R., Liess M. & Schüürmann, G. 2005. Structural Alerts – A New Classification Model to Discriminate Excess Toxicity from Narcotic Effect Levels of Organic Compounds in the Acute Daphnid Assay. *Chemical Research in Toxicology* **18**: 536-555.

Tagungsbeiträge

SETAC Europe 12th annual meeting: “The relevance of recovery for the distribution of invertebrates in streams, impacted by pesticides.” P.C. von der Ohe, M. Liess. Vienna, 2002.

GdCh/SETAC GLB Jahrestagung: “Die Auswirkung temporärer Belastungen auf die Verbreitung sensitiver Arten.” P.C. von der Ohe, M. Liess. Braunschweig, 2002.

SETAC Europe 13th annual meeting: “Relative Sensitivity Distribution of aquatic invertebrates with respect to organic and metal compounds.” P.C. von der Ohe, M. Liess. Hamburg, 2003.

SETAC 24th annual meeting in North America. “Relative Sensitivity Distribution (RSD) of Aquatic Invertebrates – A Tool to Improve Risk Assessment in the Field.” P.C. von der Ohe, M. Liess. Austin, 2003.

SETAC Europe 14th annual meeting: “How many acute daphnid tests do we need in aquatic toxicology?” P.C. von der Ohe, R. Altenburger, R. Kühne, M. Liess und G. Schüürmann. Prag, 2004.

Acknowledgements

Although a PhD thesis is the work of a single person, many people are involved to finally succeed. I would like to especially acknowledge the following persons for their support:

Prof. Georg Rüppell for his great lectures - encouraging me in science, for his advice, helpful discussions on the manuscript and especially for being my mentor although he was already retired.

Dr. Matthias Liess, my supervisor, for the possibility to work at the UFZ, for endless inspiring discussions about research and becoming a friend in Leipzig.

Prof. Gerrit Schüürmann for being my supervisor lately, from whom I learned a lot about science and who kindly took over the second referee of my thesis.

My colleague Carola Schriever for preparing the “good morning coffee”, the nice atmosphere in our office and her technical sound help with Microsoft office problems.

Ralf Schäfer for helpful discussions concerning my General Introduction, for the reviewing of parts of my thesis and becoming a friend lately, bringing new wind into the group.

Dr. Rolf Altenburger for his afford in teaching me to write interesting papers.

Barry Pieters for distracting me from work for the length of a cigarette.

All my other colleagues of the group for the incredible nice atmosphere at the UFZ - making even work good fun and of course for proof reading parts of my thesis. Ralf Kühne and Ralf-Uwe Ebert for their support with many things concerning computers.

Stephanie Bopp and Anja Schreer for the Spanish courses we made, doing at least some other things besides science and for their help with the many little problems that arise when working at the UFZ.

Jacqueline Gerhard for all the nice little coffee breaks and especially for the final proof reading of my entire thesis.

Matthias Grote for nice parties, cultural events and helpful explanations in the dry subject of chemistry.

Vanessa Stauch for proof reading and correcting my English, especially the grammar.

My WG for standing my crazy working days and making me feel at home in our flat.

My friends Sven Steiner and Katrin Totschek for the relaxing weekends in Berlin.

My brother for endless telephone calls at night and his help in many things. My sister and her family for the good times we had together during my holidays.

Last but not least my parents for their love and care, for always being there for me and giving me the education to become the person I am.

Table of contents

Summary

Freshwater is more and more becoming one of the most valuable resources on earth, as it is essential to life but its availability is often limited. Therefore, its protection and conservation for future generations is a great challenge to mankind. To fulfill its functions as drinking water or as a habitat for aquatic life appropriately, a sufficient water quality should be guaranteed. At present however, around 100,000 chemicals are in widespread use and many of them are potential contaminants for our freshwater resources. In this context, pesticides form a special problem, as they are deliberately deployed into the environment in high quantities. Moreover, pesticides are a hazard to non-target organisms, as they are often persistent and become toxic at certain thresholds. Thus, there is a strong need to assess the risks that may originate from these compounds.

The aim of this work is to contribute to the ongoing development of appropriate risk assessment approaches. Therefore, both new exposure- and effect assessment tools are presented to address the toxic potential of organic pollutants and in particular their expected adverse effects on freshwater invertebrates. In **Chapter II**, a relative sensitivity ranking of aquatic invertebrate taxa regarding organic and metal pollutants was derived, considering the direct effects of toxicants. In **Chapter III**, this ranking together with life-cycle information of “wild”-species was used to derive the SPEcies A Risk (SPEAR) - index that enables detecting indirect effects on the composition of aquatic invertebrate communities in relation to toxic units, based on measured pesticide concentrations. In **Chapter IV**, the use of the SPEAR index was extrapolated to modeled pesticide impacts. In both field investigations, forested catchmentss were found to reduce the adverse effects of agricultural activities. In **Chapter V** finally, a discrimination scheme is presented that allows distinguishing between excess and narcotic toxic compounds. For the latter, the direct toxic effects on the model organism *Daphnia magna* could be predicted with reasonably accuracy, that would allow predicting Toxic Units from measured concentrations of organic pollutants. In combination with the findings of **Chapter III**, this furthermore allows for the prediction of likely threshold limits for indirect effects on macroinvertebrate communities in the field.

Chapter II: In ecological risk assessments, species sensitivity distributions are performed to address the effect of a single toxicant. However, invertebrate communities in their natural environment are usually exposed to mixtures of different toxicants. Hence, the respective sensitivity of each species for each toxicant should be known to predict potential changes in the species composition. For most species, however, no information about their sensitivity towards toxicants is available. To address this limitation, all available information of test species have been collected and separated into organic compounds and metal compounds. Then, each data was compared to the acute toxicity data of the reference species *Daphnia magna*, as a direct comparison was mostly not feasible for the majority of species and substances. Therefore, it was possible to assign a relative sensitivity for many aquatic invertebrate taxa and toxicants. In order to enable a profound ranking, similar species were grouped into higher taxa to calculate a relative sensitivity. Hence, the derived relative sensitivity distribution (RSD) enables the prediction of likely direct toxicant effects for many species and many toxicants.

Chapter III: The EU Uniform Principles for the assessment of pesticides require that no unacceptable impacts on the viability of exposed organisms occur under field conditions. The aim of this study was to find patterns in the species composition of 20 aquatic invertebrate communities that are related to the indirect effects of pesticides. To this end, measured pesticide concentrations were linked to the structure of respective invertebrate assemblages. To reduce the site-specific variation of community descriptors due to environmental factors other than pesticides, species were grouped according to their vulnerability to pesticides. Species were classified as SPEcies At Risk (SPEAR) and SPEcies not At Risk (SPE$_{\text{not}}$AR), due to (i) the relative sensitivity to organic toxicants (**Chapter II**), (ii) the generation time, (iii) the migration ability, and (iv) the presence of aquatic stages during time of maximum pesticide application in May. Results showed that pesticide concentrations of 1/10 to 1/100 of the acute 48h LC50 of *Daphnia magna* resulted in a short-term reduction of abundance and number of SPEAR. However, both descriptors are greatly increased when undisturbed stream sections are present in upstream reaches. The levels of biological impairment observed at sites with high pesticide contamination and good habitat quality in the upstream reaches were similar to those at sites with low pesticide contamination and poor habitat quality in the upstream reaches. These results suggest that the geographical unit of the risk assessment of streams should be extended to include the recovery potential of the landscape associated with undisturbed stream sections.

Chapter IV: The Water Framework Directive of the European Community aims to achieve a good biological quality of the aquatic ecosystems. In the present study, official data on invertebrate communities of 71 lowland streams, provided from local water boards, have been analyzed to investigate potential indirect effects, originating from agricultural activities. To this end, generic loads of pesticides were modeled as a general measure of agricultural intensity, reflecting the runoff potential that arises from crop growing according to the good agricultural practice. It could be shown, that among the considered community descriptors (SPEAR – **Chapter III**) some were negatively correlated to the modeled indicator of pesticide exposure, referred to as the Potential for Pesticide Runoff (PPR). Thereby, a reduction in the fraction of SPEAR abundance from about 50 % at the reference sites to below 5 % at the potentially highest impacted sites could be observed, indicating changes in the invertebrate community structure. Furthermore, for 7 sites with wastewater treatment plants in their upstream catchments, it could be demonstrated that their community descriptors showed a decreased biological quality, compared to similar classified streams without these point sources of pollution. In contrast, a significant increase in the number of SPEAR could be stated in the presence of relatively small undisturbed stream sections. Therefore, these sections account for higher species richness on a landscape level.

Chapter V: According to the upcoming European chemical policy it is likely that until 2012, around 30,000 chemical substances with annual production volumes of more than 1 ton will require data for their ecotoxicological evaluation, as they are likely hazards to the environment. Theoretical methods such as the presented quantitative or qualitative structure-activity relationships (QSARs) may form a particularly efficient component besides experimental *in vitro* methods and high-throughput techniques to predict the direct toxic effects of these chemicals. The advantages of this approach are that it is fast and cost-effective. In the present investigation, three classification models are derived, employing only simple structural features, based on the presence or absence of certain heteroatoms and their chemical functionality. These models allow for discriminating excess toxicity from narcotic effect levels in the acute *Daphnia magna* bioassay. For the latter, the toxicity can be estimated with reasonable accuracy from the given QSARs. Thus, respective compounds have a low priority for experimental testing. This would also enables the calculation of Toxic Units for the use in the indicator model of the SPEAR concept (**Chapter IV**), which allows for the estimation of likely ecological thresholds for respectively classified organic chemicals by simple structural features.

Zusammenfassung

Süßwasser wird zunehmend eine der wertvollsten Ressourcen auf unserem Planeten, da es für den Menschen zwar essentiell, seine Verfügbarkeit jedoch oft limitiert ist. Sein Schutz und seine Bewahrung für künftige Generationen stellen daher eine große Herausforderung für unsere Gesellschaft dar. Um seine Funktion als Trinkwasser und als Lebensraum für aquatische Organismen adäquat zu erfüllen, sollte immer eine ausreichende Wasserqualität garantiert werden. Gegenwärtig sind jedoch ungefähr 100.000 Chemikalien in vielfältigem Einsatz, die z. T. potentielle Gefahren für unsere Süßwasserressourcen darstellen. In diesem Zusammenhang sind insbesondere Pestizide zu nennen, da diese vorsätzlich und in hohen Mengen in die Umwelt ausgebracht werden. Dabei stellen viele Pestizide durch Ihre Persistenz sowie besondere Toxizität eine ernst zunehmende Gefahr für Nicht-Ziel-Organismen dar. Das Ziel dieser Arbeit ist es daher, die von den organischen Stoffen ausgehenden Risiken auf Süßwasser-Invertebraten abzuschätzen um zur Weiterentwicklung bestehender Risikobewertungen beizutragen. Zu diesem Zweck wurden sowohl neue Expositions- als auch Indikator-Modelle erstellt, um die zu erwartenden negativen Effekte zu beurteilen.

Kapitel II: In **Kapitel II** wurde für die Bewertung von Freiland-Gemeinschaften eine relative Rangfolge aquatischer Invertebraten-Taxa hinsichtlich deren Empfindlichkeit gegenüber organischen und metallischen Schadstoffen erstellt, um die direkten toxischen Effekte von Schadstoffen abzuschätzen. In der ökologischen Risikobewertung werden häufig Arten-Empfindlichkeits-Verteilungen von Laborarten verwendet, die auf einen einzelnen Schadstoff bezogen sind. Invertebraten-Gemeinschaften im Freiland sind jedoch normalerweise Gemischen von Schadstoffen ausgesetzt. Für eine adäquate Risikobewertung sollte daher möglichst die Empfindlichkeit jeder Art gegenüber allen Schadstoffen bekannt sein, um mögliche Änderungen in der Zusammensetzung der Gemeinschaft vorhersagen zu können. Für die meisten Arten sind jedoch keine Informationen über ihre Empfindlichkeit gegenüber dem Großteil der Schadstoffe verfügbar. Um dieser Limitation Rechnung zu tragen, wurden im Rahmen dieser Arbeit zunächst alle verfügbaren Toxizitäts-Informationen der üblichen Modellorganismen zusammen getragen, getrennt nach organischen und metallischen Schadstoffen. Der Standardtestorganismus *D. magna* wurde dabei als Referenz verwendet, da an ihm bereits eine Vielzahl von Schadstoffen getestet wurde. Um eine statistische Absicherung zu gewährleisten wurden die Ergebnisse von verwandten Arten auf höherer taxonomischer Ebene zusammengefaßt. Durch einen Vergleich der Sensitivität einer jeden getesteten Art oder Taxa mit der Sensitivität des Modellorganismus *Daphnia magna* war es möglich, eine relative Empfindlichkeitsverteilung aquatischer Invertebraten-Taxa (Relative Sensitivity Distribution - RSD) zu erstellen. Diese Methode ermöglichte es, eine große Zahl von aquatischen Invertebraten-Taxa hinsichtlich ihrer vermuteten Empfindlichkeit gegenüber organischen Schadstoffen einzuordnen und Grenzwerte für direkte toxische Effekte vorherzusagen.

Kapitel III: In diesem Kapitel wurde mit Hilfe der relativen Empfindlichkeitsverteilung aquatischer Invertebraten-Taxa (**Kapitel II**) und von zusätzlichen Informationen über den Lebenszyklus frei lebender Arten der Index der „Gefährdeten Arten“ (SPEcies At Risk - SPEAR) erstellt. Dieser ermöglichte es, indirekte Effekte von gemessenen Pflanzenschutzmittel-Konzentrationen zu detektieren. Die „EU Uniform Principles“ für die Bewertung von Pflanzenschutzmitteln (PSM) fordern nämlich, daß unter Freilandbedingungen keine unakzeptablen Auswirkungen auf die Lebensfähigkeit von Organismen auftreten dürfen. Das Ziel der vorliegenden Freilandstudie war es daher, Muster in der Zusammensetzung von 20 aquatischen Lebensgemeinschaften zu finden, die auf die Effekte von PSM zurückzuführen sind. Dazu wurden gemessene PSM-Konzentrationen mit den entsprechenden Ausprägungen der Invertebraten-Gemeinschaften korreliert. Um die lokale Variation der Gemeinschaftsparameter aufgrund anderer Umweltparameter als der Pflanzenschutzmittelbelastung zu verringern, wurden die Arten anhand ihrer Empfindlichkeit gegenüber diesen Schadstoffen gruppiert. Dazu wurden die Arten in „gefährdete Arten“ und in „nicht gefährdete Arten“ eingeteilt. Die Einteilung erfolgte anhand ihrer (i) Empfindlichkeit gegenüber organischen Schadstoffen, (ii) ihrer Generationszeit, (iii) ihrer Mobilität und (iv) dem Vorhandensein aquatischer Stadien während der maximalen Pflanzenschutzmittelanwendung im Mai. Die Ergebnisse zeigten, das PSM-Konzentrationen von 1/10 bis 1/100 der akuten letalen Konzentration gegenüber *Daphnia magna* zu einer kurzzeitigen Reduktion der Häufigkeit und Anzahl gefährdeter Arten führte. Diese beiden Gemeinschaftsparameter waren dagegen signifikant erhöht, wenn sich ungestörte Flußabschnitte im Oberlauf befanden. Die Beeinträchtigung der Gemeinschaft durch PSM an Stellen mit hoher Belastung und guter Habitatqualität im Oberlauf entsprach dabei der an Stellen mit geringer Belastung und schlechter Habitatqualität im Oberlauf. Die Ergebnisse untermauern die Forderung, die Geografische Komponente in die Risikobewertung von Fließgewässern mit einzubeziehen, um das Wiedererholungspotential der Landschaft, ausgehend von den ungestörten Flußabschnitten, zu berücksichtigen.

Kapitel IV: In diesem Kapitel wurde die Anwendbarkeit des SPEAR-Index (**Kapitel III**) auf modellierte landwirtschaftliche Einträge von PSM überprüft. Dabei wurde erneut festgestellt, daß bewaldete Stellen oberhalb des Eintragspunktes die ansonsten negativen Effekte landwirtschaftlicher Tätigkeit auf die Invertebraten-Lebensgemeinschaft zum Teil kompensieren. Die Wasserrahmenrichtlinie der Europäischen Gemeinschaft zielt darauf ab, eine gute biologische Qualität unserer aquatischen Ökosysteme zu erreichen. Die Ergebnisse der vorliegenden Arbeit deuten jedoch darauf hin, daß der Einfluß von PSM nicht ausreichend berücksichtigt wird. In der zweiten Studie der vorliegenden Arbeit wurden Freilanddaten der lokalen Wasserüberwachung von 71 Invertebraten-Gemeinschaften dazu verwendet, eventuelle negative Effekte der Landwirtschaft abzuschätzen. Zu diesem Zweck wurden generische Frachten eines Pflanzenschutzmittels als Maß der landwirtschaftlichen Intensität modelliert. Dieses so genannte „Runoff-Potential“ (Potential for Pesticide Runoff - PPR) besteht, wenn der Ackerbau nach der guten landwirtschaftlichen Praxis durchgeführt wird. Dabei konnte gezeigt werden, daß der verwendete Gemeinschaftsparameter (SPEAR) negativ mit dem modellierten PPR korreliert war. Ein Individuenanteil “gefährdeter Arten” von ungefähr 50 % an den Referenzstellen stand dabei einem Individuenanteil von unter 5 % an den potentiell höchstbelasteten Stellen gegenüber, was auf eine Veränderung in der Zusammensetzung der Lebens-Gemeinschaft hindeutet. Darüber hinaus konnte für sieben Stellen, in deren Oberlauf sich Kläranlagen befinden, gezeigt werden, daß die Struktur der Gemeinschaft im Vergleich zu ähnlich klassifizierten Stellen ohne punktuelle Belastungsquellen beeinträchtigt war. In diesem Zusammenhang führen die oben bereits erwähnten unbelasteten Flußabschnitte bereits zu einer signifikanten Zunahme der Zahl gefährdeter Arten und tragen demnach zu einer erhöhten Artenvielfalt auf Landschaftsebene bei. Daher ist es zu empfehlen, diesen Aspekt bei künftigen Risikobewertungen zu berücksichtigen.

Kapitel V: In **Kapitel V** wurde schließlich ein Klassifikations-Modell erstellt, mit dem es möglich ist, zwischen Basistoxizität und erhöhter Toxizität von Stoffen zu unterscheiden. Für erstere ließen sich dabei die direkten toxischen Effekte auf den Modelorganismus *Daphnia magna* mit ausreichender Genauigkeit vorhersagen. In Kombination mit den Ergebnissen aus **Kapitel III** wurde es dadurch möglich, Aussagen über Grenzwerte für die Gefährdung von Invertebraten-Lebensgemeinschaften aufgrund zu erwartender indirekter Effekte zu treffen. Gemäß der ausstehenden neuen Europäischen Chemikalien-Gesetzgebung ist damit zu rechnen, daß bis 2012 ungefähr 30.000 chemische Substanzen mit einer jährlichen Produktion von jeweils mehr als einer Tonne ökotoxikologisch bewertet werden müssen, da von ihnen im Zweifelsfall eine besondere Gefahr für die Umwelt ausgeht. Theoretische Methoden wie die vorgestellten quantitativen und qualitativen Struktur-Wirkungs-Beziehungen (engl. QSARs) könnten dabei neben experimentellen *in-vitro* Methoden und *hoher Durchsatz-Techniken* besonders effektive Komponenten darstellen, um direkte toxische Effekte von Chemikalien abzuschätzen. Die Vorteile der QSAR Methode sind dabei seine Kosten-Effizienz und Schnelligkeit. In der vorliegenden Arbeit wurden drei Klassifikations-Modelle erstellt, die lediglich einfache Strukturmerkmale zur Bewertung der Chemikalien verwenden, welche auf dem Vorhandensein bestimmter Heteroatome und deren chemischer Funktionalität basieren. Diese Modelle erlauben es, zwischen erhöhter und narkoseähnlicher Toxizität im akuten *Daphnia magna* Test zu unterscheiden. Für letztere läßt sich die narkoseartige Toxizität mit Hilfe von QSAR-Modellen ziemlich genau vorhersagen, was bedeutet, daß für diese Stoffe lediglich eine geringe Notwendigkeit einer experimentellen Überprüfung besteht.

Chapter I

General Introduction

The present thesis aims to provide a holistic ecological risk assessment approach on organic pollutants with regard to their adverse effects on aquatic invertebrates. To do so, four papers are presented, each covering distinct aspects of the risk assessment process. For the use of this thesis in a risk assessment approach, all four parts should be included. Therefore, some general ideas are initially introduced that are later employed in the concept of this work.

Protection goal: "the good ecological status"

Over the last 60 years, the application of risk assessment to protect human health has increased. It was only during the last 25 years that the ecological risk assessment has become more widely used (SOLOMON & SIBLY 2002). The novel aim of the latter is the estimation of risks that arise for natural communities of species, exposed to pollutants and other substances. More recently, the Water Framework Directive of the European Community (CEC 2000) aims to protect and enhance the status of the aquatic ecosystems. For the a priori description and the later monitoring of the present status of our streams, the assessment will be based on both chemical and ecological aspects, whereas the analysis of the ecological data is the most important component. According to the European Directive, there is a call for action in cases where a good ecological quality of streams cannot be stated. This means that for these streams, the respective governments must re-achieve a good ecological status until 2015 (CEC 2000). In this context, the monitoring of our streams is a good example as it has a long tradition in Germany. For the governmental water investigations regarding organic pollutants, the index of saprobic-scale (DIN 1990) was employed, which revealed strong ecological concerns regarding the water quality of many streams. The construction of new and the extension of existing wastewater treatment plants led to a good water quality (FAASCH 1997; FRIEDRICH 1998). With the new directive however, also other impacts such as structural degradation of streams (e.g. channelisation) or the pollution with toxic compounds (e.g. pesticides) will be considered in the assessment process (CEC 2000). Nevertheless, due to the findings of this thesis the author believes that the negative impacts of agricultural activities are not accordingly concerned at present. Hence, new indication tools are required to assess respective impacts.

Headwater streams as habitat

Running waters are one of the major aquatic ecosystems of the world (CAIRNS 1977). Usually, most stream reaches are characterized by many diverse microhabitats (FRISSELL et al. 1986), which result from physical factors (e.g. relief, substrate or woody debris) that generate an array of channel forms. Especially headwater streams play an important role in these continuous ecosystems, as they form the biggest part of the stream networks and moreover often consist of pristine sites. In Lower Saxony for example, the investigation area of the present studies, bigger streams of the 1st and 2nd order (rivers and tributaries) only amount to a length of 2,100 km and 27,000 km, respectively, whereas small headwater streams of the 3rd order amount to a length of 130,000 km (SELLHEIM 2000). Although usually small, headwater streams provide important ecological services, e.g. as breeding habitats for migrating fish (DAHL & HULLEN 1989). At the same time, they are more severely affectted by the surrounding land than almost any other aquatic habitat (BLANCHARD & LERCH 2000), especially in landscapes of intense agricultural activities. Nevertheless, streams with catchments of less than 10 km^2 (e.g. headwater streams) are not explicitly concerned in the Water Framework Directive (Annex II, 1.2.1 Rivers). For the achievement of a good ecological status of our larger rivers however, unimpaired tributaries and headwater streams may have an important contribution, as they can serve as source of colonists for impaired sections (FLANAGAN et al. 1979).

Invertebrates – important secondary producers

As consumers at intermediate trophic levels, macroinvertebrates that inhibit small headwater streams play an important role in the ecosystem functioning, as they have an influence on the nutrient cycles, primary productivity, decomposition and translocation of materials (WALLACE & WEBSTER 1996). For example, the application of an insecticide to an appalachian headwater stream eliminated 90 % of the insect biomass and therefore greatly reduced secondary production (MERRIT et al. 1984). This manipulation significantly reduced leaf litter breakdown and export of fine particulate organic matter (FPOM), compared with adjacent reference sites. It was demonstrated that macroinvertebrates accounted for 25 % of the annual leaf litter processing (CUFFNEY et al. 1990) and 56 % of the FPOM export (WALLACE et al. 1991). The exported FPOM may represent an important source of energy and nutrients to the downstream fauna, especially for those who are adapted to deposit- and filter-feeding (WALLACE & MERRIT 1980). The macroinvertebrates themselves in turn constitute an important prey for numerous fish (WALLACE & WEBSTER 1996).

Invertebrate communities usually consist of species that could be classified into different ecological groups, with regard to their feeding behaviour: *shredders*, that break down primarily large pieces of decomposing vascular plant tissues; *gatherer* (collectors), that feed primarily on fine particulate organic matter deposited in streams; *scrapers*, adapted to graze or scrape organic materials from mineral or organic substrates and finally *predators* that feed on animal prey (SCHWOERBEL 1999). Therefore, the macroinvertebrate assemblage of most streams is highly diverse, and many of the individual species may be redundant (LAWTON 1991) in the sense that ecosystem function can proceed if one species or another is absent (WALLACE et al. 1986). However, even normally rare species may have a critical role that becomes evident only after a major disturbance (WALLACE & GURTS 1986). Therefore, categorizing of any stream macroinvertebrate as a keystone species may be difficult (MILLS 1993), however macroinvertebrates as a group perform essential functions and are critical to the maintenance of stream functional integrity (ANGERMEIER & KARR 1994). The many roles performed by stream-dwelling macroinvertebrates underscore the importance of their conservation (WALLACE & WEBSTER 1996).

Direct and indirect effects

The term ecotoxicology has been defined as the study of the effects of anthropogenic toxicants on ecological systems (TRUHAUT 1977). This implies a focus on the impacts of toxicants on larger-scale phenomenon, such as ecosystem structure, e.g. species abundance and their diversity. However, in practice, ecotoxicologists focus mainly on the direct effects of toxicant exposure on a limited number of model test species (CLEMENTS & KIFFNEY 1994). Although there is most likely a connection between these two scales (CALOW 1994), the ability for extrapolating the individual level to toxicant effects at the ecosystem level is still limited (CHAPMAN 1995). This is probably due to the fact that toxicity assays are frequently conducted with a small body of test organisms, despite the significant differences in the sensitivity among, and the different composition of species in natural ecosystems. These communities consist of diverse populations of species interacting in complex ways both within and between populations. Therefore, the strength of these interactions depends on the way in which individuals and populations are affected indirectly through the direct toxicant effects on other species of an ecosystem with which they interact (PRESTON 2002).

The ecological definition describes indirect effects as interactions among independent species, particularly competition and predation. For example, an increase in the predation of one species may release another species from competition (Figure 1A). Often, interacting species have evolved together and thus may have become specialized to maximize niche exploitation and fitness. Live history traits such as generation time, clutch size and parental investment may influence competitive interactions among species. Given that within any trophic level each species may prey upon or be preyed upon by multiple other species, it is clear that indirect effects are frequent events that convey significant complexity to ecosystem structure (PRESTON 2002). Indirect effects carry a slightly different meaning in an ecotoxicological context. Toxicants, like species interactions, are capable of influencing species distribution and abundance. Frequently in ecotoxicology, only the direct effects of toxicity are considered, such as adverse effects on survival, growth or reproduction. In natural communities however, species may also be affected indirectly through their interactions with other species that are directly affected by toxicity (Figure 1B). Although a toxicant may directly affect species that are exposed and vulnerable to its mode of action, the subsequent restructuring of the community following a toxicant release is likely to be a function of the subsequent indirect effects of toxicity on species interactions (FLEEGER et al. 2003). For example, whereas slow-growing, stress-tolerant species may have had poor fitness relative to fast-growing species before a toxicant release, afterwards their resistance promotes a better relative fitness compared to the fast-growing species (PRESTON 2002). Models of toxicity based only upon the exposure of single species are not sufficient to account for such complex ecological phenomenon. In addition, it has been suggested that indirect effects have similar or greater influence on species abundance than direct effects (LAMPERT et al. 1989). Therefore, it appears that a better consideration of indirect effects in ecotoxicology would assist in the development of more relevant ecological risk assessments (PRESTON 2002).

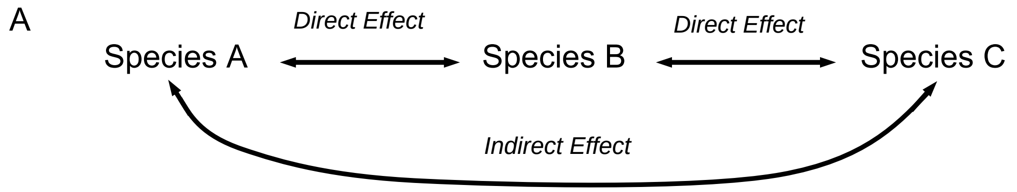


Figure 1: Conceptual models of indirect effects in (A) ecology and (B) ecotoxicology. Arrows indicate the direction of the respective effect. Indirect ecological effects may result from interactions among independent species (A) or from the effects of toxicants on such interactions (B). The figure was taken from PRESTON 2002.

Macroinvertebrates as bio-indicators

The distribution of macroinvertebrate species and their densities in agricultural headwater streams is influenced by many factors such as organic pollution (WHITEHURST 1991), habitat degradation (HILSENHOFF 1977, 1982; PLAFKIN et al. 1989) and pesticides (SCHULZ & LIESS 1999). As many invertebrate species have low dispersal abilities and constantly populate streams, macroinvertebrates may serve as valuable indicators of the degradation of streams, and as increasing demands are placed on our water resources, their value in the risk assessment and monitoring of these impacts will increase (WALLACE & WEBSTER 1996). So far, macroinvertebrates have successfully been used in numerous biological monitoring methods (ROSENBERG & RESH 1993), usually based on tolerance values for specific species according to their ability to inhabit streams differing in water quality (CHUTTER 1972; HILSENHOF 1987; LENAT 1993; PLAFKIN et al. 1989). Other approaches were based on empirical observations of the distribution of indicator species under certain environmental conditions, e.g. regarding the organic pollution or the acidity of streams (DIN 1990, BRAUKMANN 2000). According to NEWMAN (1995), the adverse effects of toxicants on the community structure can be quantified through various means, including measuring the species richness (number of species) or the species diversity (equatibility in species abundance among species), data that are routinely collected in ecological monitoring. Therefore, at present a lot of invertebrate samples are available and could be used in future applications of new assessment approaches. In this context, two different methods are usually employed to sample invertebrate communities. The first method is using a surber-sampler (SURBER 1932) that permits a quantitative statement about the abundance of invertebrate species as individuals/m^2. This method was employed for own investigations. The other semi-quantitative method is using a kitchen net to collect invertebrate species, whereas the species abundance is estimated in classes. This method was used for the governmental water investigations (DIN 1990). With the first method, about 90 % of all invertebrates are collected within the first two samples, whereas the latter method enables a more qualitative statement of the species diversity (SCHWOERBEL 1994). In both methods, difficult weather conditions during the sampling increase the data variability. These conditions include heavy rainfall events - resulting in an increased invertebrate drift or summer dehydration due to decreased rainfall in hot summers. Additionally, sampling results depend on the streambed structure and therefore sampling at different places may result in different invertebrate assemblages (e.g. SCHWOERBEL 1994).

One aim of the present study was to provide a new assessment tool to link the adverse effects of pesticides to the community structure of invertebrate communities. However, every index needs a conceptual basis for the stressor it wants to detect. In this case, the detection was based on the sensitivity of invertebrates to organic compounds. In this context, a fundamental consideration in toxicology is that different species possess different sensitivities to stress (SPRAGUE 1995). As the structure of natural communities is dependent upon the fitness of the individuals within the ecosystem, stressors (e.g. pesticides, organic pollution, and acidification) that differentially alter fitness among individuals act as novel selection factors that can potentially shift ecosystem structure to a different equilibrium. Hence, demonstrating the relative sensitivity of species assemblages to different toxicants may yield useful information regarding the response of communities in natural ecosystems to toxicant exposure (KOOIJMAN 1987). Recently, WOGRAM & LIESS (2002) demonstrated a rank ordering of 19 aquatic invertebrate orders, showing taxonomic differences in the sensitivity to organic and metal compounds. In fact, probabilistic modeling of species sensitivity distributions has been the most common method of comparing species sensitivities to toxicants (KOOIJMAN 1987). The advantage of this approach is that it is not assumed that one species is sufficiently sensitive or representative of a community of organisms. Furthermore, it could be predicted which populations within the communities will be most severely impacted. Subsequently, it is possible to deduce how species interactions will be altered after exposure (PRESTON 2002). The results of such an analysis have been demonstrated to yield estimates of toxicity thresholds comparable to mesocosm studies where assemblages of species were tested simultaneously (VERSTEEG et al. 1999).

Anthropogenic disturbances

Aquatic ecosystems are affected by various kinds of anthropogenic disturbances. WALLACE (1990) defined disturbances as “any event that results in a significant change (either positive or negative) in the community structure beyond that expected over the annual cycle”. BENDER et al. (1984) distinguished between pulse disturbances (characterized by limited duration with little effect on the surrounding watershed) and press disturbances (characterized by longer duration that often involve changes in the watershed or stream channel). Channelization and mining activities are examples of press disturbances, whereas most chemical stressors (e.g. pesticides) are examples of pulse disturbances (NIEMI et al. 1990). Channelization of streams has been shown to result in long-term decreases of invertebrate abundances and biomass, compared with unaltered reaches (EDWARDS et al. 1984). Other examples of anthropogenic disturbances are organic pollution, dredging of the streambed or removal of woody debris. The latter reduced the abundances of many invertebrates (ANGERMEIER & KARR 1984). Stream size, latitude, retention and local geomorphology may alter the rate of recovery from such disturbances (WALLACE 1990). Taking this into account, the present field studies were restricted to investigate the impact of pesticides on the macroinvertebrate communities in small headwater streams, located around Braunschweig. The example of pesticides should be described in more detail below.

Example pesticides

In the conventional agriculture of our days, pesticides are used to protect and raise the crop yield. Estimations show that pesticides could prevent up to 40 % of the harvest losses and therefore their use is generally accepted (REUS et al. 1999). In this context, the German Federal Office for Environmental Protection (UBA 2001) registered between 33,660 and 38,880 tons of active ingredients for the interior German use during the years 1993 to 2001. The UBA estimates that 1 to 2 % of the amount of pesticides applied enters streams via surface runoff (UBA 1997). Hence, pesticides form one of the biggest groups of chemicals that are deliberately released into the environment in large quantities. Thereby, pesticides can impact streams through various entry routes, such as drainage, spray drift, farm- and field-runoff (BACH et al. 2000). In this context, several authors emphasize the significance of rainfall-induced surface runoff as the most important non-point entry route of pesticides into streams (WAUCHOPE et al. 1978; AGASSIV et al. 1995; WILLIAMS et al. 1995). Compared to spray drift, runoff is the far more relevant pathway, as after heavy rainfall almost every field is susceptible to runoff, whereas spray-drift is more relevant in fruit orchards (BACH et al. 2000). Once arrived in other environmental compartments, pesticides affect also non-target organism, because of similar modes of toxic action. Therefore, the use of some of the most persistent pesticides has been restricted, for example lindane and atrazine. The latter has been detected in concentrations above 0.1 µg/L in ground water (FENT 1998).

Recovery from disturbance

Recovery constitutes the re-establishment of the community structure to within the range expected over the annual cycle prior to the initial disturbance (WALLACE 1990). In this context, the re-establishment of the community structure may be assessed on the basis of functional recovery (abundances, biomass) or taxonomic recovery (individual species). For example, after a seasonal insecticide treatment of a small headwater stream, the abundances of the aquatic insects were greatly reduced (MERRIT et al. 1984). Although the functional recovery occurred within two years after the disturbance (WALLACE et al. 1986), recovery in the taxonomic community structure required about five years (WALLACE 1990). Besides the problem of defining an appropriate endpoint to recover, the confirmation of the re-establishment of the community structure may still be difficult. Although ecological risk assessments are routinely executed on a landscape area (HALL et al. 1998), such assessments are usually conducted on the basis of actual data, and thus there may be no historical data that could deal as reference.

CAIRNS (1977) reported that, compared to other aquatic habitats, such as lakes and estuaries, streams have the highest resilience (rate to recover). Factors considered important for this resilience are (i) existence of nearby epicenters for reinvading organism (e.g. tributaries, calmer areas), (ii) the presence of organisms or other life stages not affected by the disturbance, (iii) aerial recolonization, (iv) high mobility of populations upstream and (v) the generation time of the organism constituting the community (CAIRNS & DICKSON 1977). This is in agreement with the findings of WALLACE et al. (1986), that low dispersal abilities and long life cycles in the studied stream prolonged the time of recovery. In this context, WILLIAMS & HYNES (1976) reported that drift (water-borne transport) is the most common means of transport for many stream invertebrates.

Use of QSAR in risk assessment

At present, about 100,000 chemicals are in widespread use worldwide that could, to some extent, be released into the environment (CEC 2000). Evaluating the likely hazards originating from all these potential pollutants is a challenge confronting national and international regulatory agencies like the U.S. Environmental Protection Agency (U.S. EPA), Environment Canada, and the European Union (U.S. EPA 1998; ENVIRONMENT CANADA 1997; CEC 2000). In order to ensure an efficient use of the available time and resources, a promising way forward would be to include low-cost screening methods that allow predicting the toxic potential of chemicals instead of unnecessary testing. In this context, theoretical methods such as quantitative or qualitative structure-activity relationships (QSARs) may form a particularly efficient component besides experimental in vitro methods and high-throughput techniques. Following a current paradigm in the field of QSAR research, identification of the mode of action allows the selection of respective models that are suitable for a mechanistically sound prediction of the toxic potency of chemical substances (LIPNICK 1986). The regulatory office of the U.S. EPA, the Toxic Substances Control Act (TSCA), employed QSARs as scientifically credible tools for predicting acute toxicity, when few or no empirical data are available (AUER et al. 1990). The development of reliable QSARs however requires empirical toxicity data of high quality. A recent study (BRADBURY et al. 2003) used databases especially created for the purpose of developing QSARs for estimating toxicity of organic chemicals to aquatic organism. These databases contain toxicity results for acute effects (e.g. the two fish species fathead minnow and the guppy), originated from single research facilities. They have resulted in the development of most QSARs in use today for predicting the acute toxic effects for the use in current risk assessment approaches (BRADBURY et al. 2003). In the present study, use was made of the extensive database existing for the acute daphnid assay, to derive respective QSAR for our model test organism *Daphnia magna*, taking its heterogeneous data sources into account.

Aims of this thesis

The aim of this work was to provide appropriate risk assessment tools to improve the hazard characterisation of organic compounds concerning their impact on aquatic invertebrates. Furthermore, these tools should contribute to the tiered legalization of organic chemicals and help to develop appropriate monitoring strategies in the framework of governmental water investigations with respect to the “real risk” of pesticides in streams.

Therefore, the following goals were addressed:

- A toxicological assessment of aquatic invertebrate species regarding differences in their physiological sensitivities towards organic and metal compounds **(Chapter II).**
- Analysis of the impact of pesticides compared to a variety of other environmental parameters on the community structure of invertebrate assemblages in small headwater streams **(Chapter III).**
- Derivation **(Chapter III)** and validation **(Chapter IV)** of a new community descriptor to address the impact of organic pollutants (e.g. pesticides) on the invertebrate communities of small headwater streams.
- Qualitative and quantitative analysis of the positive influence of forested stream-catchments on the distribution of invertebrates **(Chapter II)** and its validation **(Chapter IV).**
- The discrimination of excess toxicity from narcotic effect levels of organic compounds in the acute daphnid assay with reasonable accuracy, thus reducing the need for experimental testing for the legalization of newly assessed chemicals **(Chapter V).**
- To permit a prediction of the toxic potency of organic compounds towards *Daphnia magna* **(Chapter V)** for the hazard assessment of these compounds.

Concept

Risk assessment of organic compounds

To date, the most widely used method in ecological risk assessments is the hazard quotient (HQ) approach. Depending on the biological endpoint, the expected environmental concentration of a stressor is compared to an effect concentration. Hence, risk arises when the exposure concentration exceeds a certain effect threshold, or in other words, the HQ attains a value greater than 1. In this approach, the effect endpoint is based on the community structure of stream-dwelling macroinvertebrates, in order to additionally protect the more sensitive species of this important aquatic group. In order to enable a holistic risk assessment, the presented approach is based on a joint exposure and effect assessment. To this end, the effect assessment is based on the vulnerability of these species to organic compounds in combination with life history traits influencing the exposure to these pollutants. Hence, a new indicator is derived that enables a quantification of the impact of organic chemical stressors. The exposure assessment is based on measured and predicted concentrations of pesticides or other organic compounds. The conceptual model of these two parts of a risk assessment is shown in Figure 2 and described in more detail below.

Exposure assessment

In order to allow for an appropriate exposure assessment, twofold effort was given to estimate the toxic exposure of invertebrate communities to artificial organic pollutants (e.g. pesticides). In this context, exposure does not only involve the absolute concentrations of contaminants, but relative measures of toxicity based on two different reference endpoints (e.g. LC50 of the acute *Daphnia magna* assay). Firstly, measured concentrations were transformed into a relative toxicity (see below) in order to consider differences in the toxic potency of the diverse pesticides in use. The advantage of this approach is that other organic compounds could also be assessed, provided if respective life cycle traits are considered. Secondly, modeled impacts of agricultural activities were used to compare a sites potential to runoff, and hence possible exposure to pesticides.

In **Chapter III**, measured pesticide concentrations were used to compare the toxic exposure of 20 streams. Due to the prevailing crop grown at each site, different pesticides were used to protect the harvest. To compare the toxicity of these pesticides, the toxic unit approach was used (PETERSON 1994), based on the acute (48-h) LC50 of *Daphnia magna*. To this end, the measured concentration of a pesticide was divided by the respective LC50 of *D. magna*, taken from Literature (TOMLIN 2000). In **Chapter V** a classification model is presented, termed Structural Alerts, that enables classifying organic compounds to exert excess toxicity or narcotic effect levels. For the latter compounds, the acute toxicity of these compounds in the 48-h *Daphnia magna* assay could be predicted with reasonable accuracy. For the first compounds, the predicted baseline toxicity supplies at least the expected minimum toxicity. Hence, Toxic Units could be calculated allowing for a hazard assessment, e.g. for an accidental chemical spill, by using QSAR models.

Runoff induced short-term pesticide input has been identified as the main entry route into small headwater streams with agricultural catchments in the investigated landscape. As no measured pesticide concentrations are usually available and water samplings of short-term pesticides contaminations are difficult, time consuming and expensive, **Chapter IV** aims to assess a sites risk to be subject to field runoff. In order to enable a comparison between sites, a generic load based on the „simplified formula for indirect loading caused by runoff“ was calculated. The model used considers the amount of arable land in the buffer area, the slope, the soil-type and the carbon content prevailing at each site. Furthermore, precipitation between April and August and the corresponding plant interception for each single rain event were concerned to calculate loads of dissolved pesticides resulting from particular precipitation events. For the calculation of the Potential for Pesticide Runoff (PPR), the logarithm of the maximum load was taken due to the resulting large range of modeled loads.

Effect assessment

To indicate the adverse effects of organic pollutants (e.g. pesticides) on the community structure of invertebrate assemblages of small headwater streams, it was necessary to classify the respective taxa due to these stressors. In **Chapter II**, data on acute toxicity of organic compounds were used to rank macroinvertebrate taxa accordingly to their physiological sensitivity to organic compounds. To this end, available toxicity data on aquatic invertebrates were taken from literature, and quality checks were carried out in order to eliminate apparently odd data entries. Subsequently, each data was compared to acute toxicity data of the reference species *Daphnia magna*. This widely used model organism was taken as a reference, as for this species a great number of toxicants have been evaluated. Hence, relative sensitivity values could be calculated for many species and toxicants. For the calculation of the relative sensitivity of a species or taxa however, at least five different toxicants must be tested. Depending on the data availability, similar species were grouped to higher taxonomic groups in order to enable a statistically profound ranking. Theoretically, the resulting relative sensitivity distribution enables assessing the fraction of the species most likely affected by a toxicant. The strong point of this approach is that many field species can be classified due to many different pollutants.

The relative sensitivity ranking was simplified to classify species into sensitive or insensitive for its use as indicator in **Chapter III**. Moreover, information about the life history traits of respective species, such as a low generation time, a high migration ability, and presence of aquatic stages during exposure were considered to derive the SPEcies At Risk (SPEAR) Indicator (Figure 2). This indicator was then applied to the communities of the 20 investigated streams. A regression analysis was performed between the new community descriptor and the calculated toxic units that were based on the measured pesticide concentrations. In this context, undisturbed stream sections in the near upstream reaches were assumed to account for a better community structure. Hence, two equations are presented that consider the affiliation to one of these two different groups of streams.

In **Chapter IV**, the use of the indicator SPEAR was extrapolated to sampling data of governmental water investigations. As no measured pesticide concentrations were available, a potential of pesticide runoff was modeled. This enabled a regression analysis between the 66 invertebrate communities and the indicator PPR. Again, two regressions are presented that enable an assessment of streams due to expected pesticide impacts from agricultural activities, separate for streams with and without undisturbed stream sections in the upstream reaches.

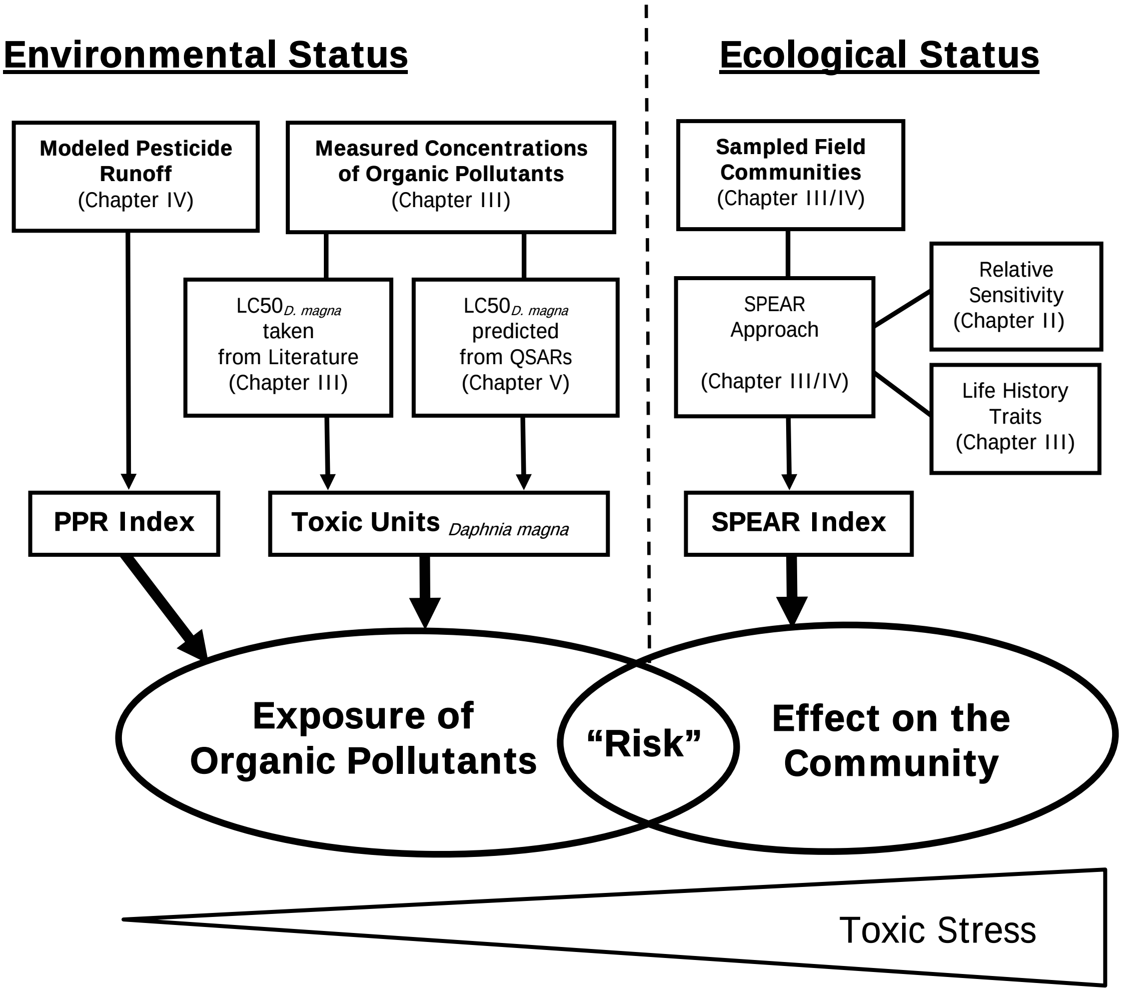


Figure 2: Conceptual model of the risk assessment approach of the present thesis. All methods and approaches shown in the concept that refer to a certain chapter are based on written papers. For details see text in Chapter I – Concept.

Discussion

Ecological risk assessment

In ecological risk assessments, endpoints and measures of effect can be defined at all levels of organization, ranging from the individual to the community level (SUTER et al. 1993). As this approach aims to provide an appropriate protection of the diversity of sensitive stream-dwelling invertebrates, the SPEAR approach is used. The author is not aware of any other indicator for pesticide impacts whose concept operates at the community level. In this context, a questionable issue is that of a functional redundancy (PRATT & CAIRNS 1996), which assumes that individuals and species are exchangeable, and loss of one species will be balanced by the gains in another species, provided that the latter occupies a similar functional niche within the ecosystem (WALKER 1991). Thus, ecosystems with high functional redundancies are expected to have an inherent capacity to adsorb disturbances while maintaining their functions. Nevertheless, the question remains weather we only want to maintain the function of our ecosystems or whether we also want to protect single species?

A major disadvantage of ecological risk assessment approaches is that they have not been widely calibrated against field observations (SOLOMON & SIBLEY 2002). The present risk assessment approach tries to capture this problem, providing both effect models and exposure models to assess the water qualities that are based on field investigation conducted at many streams. On the one hand, the given model allow for estimating the impact of a certain toxicant concentration or PPR on the structure of an exposed invertebrate community. On the other hand, the PPR-based regressions allow for assessing the water quality from invertebrate samples, collected for governmental water investigations. The present work employed these linear regression models, indicating significant negative effects of pesticides in a range far below the acute 48h LC50 of *Daphnia magna*. For example, pesticide concentrations of 1/10 to 1/100 of the LC50 resulted in a short-term reduction of abundance and number of SPEAR. This finding is somewhat confirmed by ROEX (2000) who found long-term effects of non-narcotic substances at concentrations that are 10 times lower than concentrations needed to cause acute (48-h) effects. The provided regression models could enable policy makers to decide about threshold limits, based on the fraction of SPEAR they would like to protect.

The use of relative sensitivity distributions

PRESTON (2002) concluded that for the improvement of current risk assessments, there are two possibilities: (i) a more detailed study of the ecology of the species of interest, to identify interspecies interactions which will be discussed in the next section or (ii) a risk assessment not for single species but rather for the ecosystems whole biota using probabilistic methods such as species sensitivity distributions. The species of a given community often exhibits a wide range of tolerance to a toxicant, with the consequence that a toxicant may exert lethal effects on some species, while it causes no observable effect on other species (FLEEGER et al. 2003). Hence, the utility of such species sensitivity distributions is dependent upon the number of species for which data are available, since if only limited data are available, sensitive species could be neglected (NEWMAN 2000). The more representative the assemblage is compared to natural communities, the more relevant the analysis is (PRESTON 2002). However, in most cases, only for one or few species of natural communities data on the toxicant of concern will be available (NOTENBOOM et al. 1995). VERSTEEG et al. (1999) examined species sensitivity distributions for 11 different toxicants, that all spanned at least two orders of magnitude difference in the sensitivity to surfactants and pesticides. The relative sensitivity distribution of Chapter II spanned two and a half orders of magnitude from the most sensitive species to the most insensitive taxa. Furthermore, the relative sensitivity of species, tested by VERSTEEG varied considerably among toxicants. In our approach however, only one relative sensitivity was calculated per species. Nevertheless, we did not aim to predict the direct effects of a single toxicant to a single species but the change in a natural community of species, exposed to mixtures of toxicants.

Indirect effects of pesticides

Indirect effects are a major consideration in ecology, and hence should also be used to improve current risk assessment approaches (PRESTON 2002). In this context, the most common indirect effect of toxicants seems to be a release of more tolerant species from the competition or predation of sensitive species, resulting in a shift in the ecosystem structure. HANSEN & GARTON (1982) found a reduction in the density of an insect community of a laboratory stream after exposure to the herbicide Diflubenzuron, causing indirect effects on the abundance of algae. The observed differences in the sensitivity among the insect species led to a reduced species diversity, as more sensitive species were eliminated. Another indirect effect of pesticides aims at predator-prey interactions that may be responsible for the bioaccumulation of toxicants through the food chains. Toxicants may therefore have adverse effects on predators that are not affected by the nominal concentrations they were exposed to, but through the accumulation of toxicants through ingestion of contaminated prey (PRESTON 2002). For example, SARMA et al. (1998) found a reduced reproduction of a predatory rotifer when fed to another rotifer that was exposed to methyl parathion. The observed adverse effects were only apparent after the first month of the experiment, indicating long-term effects of the toxicant. This result gives evidence that indirect effects can act already at toxic levels far below an acute LC50, measured in a single species experiment. Therefore, chronically exposed communities in agricultural areas should consist of more resistant species than communities at pristine sites. This could be shown with the results of the present studies, where the species were classified as sensitive to pesticides. Hence, an observed reduction in the fraction of these species implies a change in the community structure. Similar findings of NEUMANN & DUDGEON (2002) showed a significant effect of agricultural runoff on the benthic community. A review of the acute toxic and chronic effects of runoff identified pesticides as one of the major stressors on aquatic communities (COOPER 1993).

One aim of the present study was to use governmental water investigations to analyze the effects of pesticides on a landscape level, where no or only few information about field concentrations of pesticides was available. In this context, REUS et al. (1999) suggested that most models for the entry routes of indirect loads are too complex to be used as risk indicators and for the time being no validated models are available. They proposed the “simplified formula for indirect loadings caused by runoff”. Hence, the advantage of the model derived here was, that it needs only available landscape information to predict a runoff potential (PPR) that enables a comparison of different stream catchments. The disadvantage of the PPR is however, that there is no information about the actually use and the kind of pesticides applied. Nevertheless, this approach together with the SPEAR index enables a regression analysis that allows for identifying hot spots of high agricultural inputs. Considering the results of this study, this could help policy makers to minimize the adverse effects of agriculture through respective legislation.

SPEAR - an indicator of stream degradation

Besides chemical water samples, bio-indicators are commonly used to detect stressors in aquatic ecosystems. The disadvantage of the first is that the detection of short-term events and low concentrations is difficult and cost intensive (LIESS et al. 1996), whereas bio-indicators act as natural passive samplers (KLEE 1991). In the present study, the community structure of stream-dwelling macroinvertebrates was used to indicate in particular the adverse effects of pesticides. To this end, the above mentioned relative sensitivity distribution (RSD) was used in combination with life history traits influencing the vulnerability of invertebrate species to pesticides to derive the SPEAR Index. Here, the background of the theoretically ideas of the SPEAR indicator shall be discussed in more detail. Interestingly, NIEMI et al. (1990) reported that at the ordinal level, time to recovery of major invertebrate orders in field communities are ranked as follows: Diptera, Ephemeroptera, Trichoptera and

Plecoptera. This is exactly in agreement with the results of the relative sensitivity ranking of the RSD approach (Chapter II), indicating that the response of individuals to pesticides is partly dependent on differences in the physiological sensitivity of the species. In more temperate climates, like in Europe, univoltine life cycles are common (NIEMI et al. 1990). Therefore, they concluded that disturbance events, such as pesticide runoff can have greater impacts on recovery pattern if they occur during critical life stages. Hence, species with more than two generations per year were assigned as insensitive to pesticides in the SPEAR concept due to the high reproduction rates.

MINSHALL & PETERSEN, JR. (1985) suggested that even in variable stream environments, equilibrium conditions are found often and long enough to be of importance in the formation of stream community structure. As colonization proceeds, the contribution of the random probability element to community structure decreases as biotic interactions (e.g. predation, competition) increase. This view has certain similarities with the equilibrium model of MAC ARTHUR & WILSON (1963) for the colonization of oceanic islands. MINSHALL & PETERSEN, JR. (1985) stated further, that the number of species is allowed to fluctuate through time and space about some mean equilibrium value. A lower than the equilibrium number of species may be due to either stochastic events (e.g. runoff) or a life cycle adjustment such as emergence or pupation. Furthermore, SHAW & MINSHALL (1980) reported a 25 % greater number of benthic macroinvertebrates in Mink Creek during their summer samples compared to the respective winter samples. Hence, the restriction of the sampling period from April to August led to similar conditions of the community structures that enabled a comparison between different sites. As the most significant indicator is based on the relative fraction of SPEAR this approach could be applied to the data of the governmental water investigations, as it is independent from the sample method. A comparison of measured (Chapter III) and predicted (Chapter IV) pesticide inputs showed, that the approach enables to distinguish between impacted and pristine cites. Hence, it is recommended to include the SPEAR approach in the monitoring process of the European water investigations in order to assure a good ecological status of our aquatic ecosystems.

The use of Baseline-QSARs in the prediction of Toxic Units

The so-called baseline toxicity represents the expected minimum toxicity of organic compounds to a certain endpoint. In case of the present study, the chosen endpoint was the acute toxicity to *Daphnia magna*. This phenomenon, also known as narcosis, is based on the assumption that the pollutant is reversible soluble in the cell membranes of the organism, where its function as semi-permeable barrier is disturbed (LIPNICK 1986), leading to the death of the organism. In toxicology, this is a well-known phenomenon and abundant mode of toxic action in organic compounds (VAN LEEUWEN et al. 1992). According to a current paradigm in the field of quantitative structure/activity-relationship research, for organic compounds with a narcotic mode of action, the aquatic toxicity could be predicted with reasonable accuracy (LIPNICK 1989). In the present study (Chapter V), taking both data uncertainty (BRADBURY et al. 2003) and polar narcosis (VERHAAR et al. 1992) into account, the given QSAR models permit a high predictivity for compounds that exert narcotic effect level. Hence, the given QSAR models enable a reasonable prediction of LC50 values, compared to empirical data. Hence, in cases when few or no empirical data are available, QSARs enable the prediction of likely thresholds of hazard originating from organic pollutants.

Undisturbed stream sections – an important factor to recovery?

Compared to other aquatic habitats, such as lakes and estuaries, streams have the highest resilience or rate of recovery (CAIRNS 1977). Factors considered important for this resilience are (i) the existence of nearby epicenters for reinvading organism (e.g. tributaries, calmer areas) and (ii) the high mobility of the residents. In this context, MC LAY (1970) found that benthic invertebrates usually drift a mean daily distance of approximately 50m. Therefore, the existence of forested and undisturbed stream sections in the upstream reaches may be considered as such epicenters. The study of KREUTZWEISER & KINGSBURY (1982) also provided evidence that drift is the primary source of recovery from close undisturbed sections, whereas natural recolonization from aerial adults may become more important for distant stream reaches. GORE (1982) demonstrated that recolonization time of downstream populations depended on the distance to the upstream sources. Nevertheless, the results of the present investigation show only differences between streams with and without such epicenters of recovery.

At the one hand, NIEMI et al. (1990) reported examples of recovery times of more than three years where residual pollutants remained in the system and the system was isolated and recolonization was suppressed. On the other hand, the results of the investigation of 20 streams revealed that in-year recovery of the community structure to previous-year conditions takes place (Chapter III). Connell & Sousa (1983) concluded that more frequently disturbed communities have resident populations that colonize, grow rapidly, and recover between disturbances. Thus, the influence of undisturbed stream sections could not be completely revealed. Nevertheless, these results suggest that the geographical unit of the risk assessment of streams should be extended to include the recovery potential of the landscape associated with undisturbed stream sections.

References

Agassi, M., Letey, J., Farmer, W.J., Clark, P. 1995. Soil erosion contribution to pesticide transport by furrow irrigation. *Journal of Environmental Quality* **24**: 892-895.

Angermeier, P.L., Karr, J.R. 1984. Relationships between woody debris and fish habitat in a small warmwater stream. *Transactions of the American Fisheries Society* **113**: 716-726.

Angermeier P.L., Karr, J.R. 1994. Biological integrity versus biological diversity as policy directives. *BioScience* **44**: 690-697.

Auer, C.M., Nabholz, J.V., Baetcke, K.P. 1990. Mode of action and the assessment of chemical hazards in the presence of limited data: Use of structure activity relationships (SAR) under TSCA section 5. *Environmental Health Perspectives* **87**: 183–187.

Bach, M., Huber, A., Frede, H.-G., Mohaupt, V., Zullei-Seibert, N. 2000. Schätzung der Einträge von Pflanzenschutzmitteln aus der Landwirtschaft in die Oberflächengewässer Deutschlands. – Berichte des Umweltbundesamtes, Berlin.

Blanchard, P.E., Lerch, R.N. 2000. Watershed vulnerability to losses of agricultural chemicals: Interactions of chemistry, hydrology, and land-use. *Environmental Science and Technology* **34**: 3315-3322.

Bender, E.A., Case, T.J., Gilpin, M.E. 1984. Perturbation experiments in community ecology: theory and practice. *Ecology* **65**: 1-13.

Bradbury S.P., Russom C.L., Ankley G.T., Schultz, T.W., Walker J.D. 2003. Overview of data and conceptual approaches for derivation of quantitative structure-activity relationships for ecotoxicological effects of organic chemicals. *Environmental Toxicological and Chemistry* **22**: 1789-1798.

Braukmann, U. 2000. Hydrochemische und biologische Merkmale regionaler Bachtypen in Baden-Württemberg. Schriftenreihe der LfU Baden-Württemberg: Oberirdische Gewässer, Gewässerökologie 56, Karlsruhe.

Cairns, J. 1977. Quantification of biological integrity, Pages 171-185 in Balentine, R.K., Guarraia, L.J. (eds) The integrity of water. U.S. EPA, Office of Water & Hazardous Material, Washington, D.C.

Cairns, J., Dickson, K.L. 1977. Recovery of streams from spills of hazardous materials. Pages 24-42 in Cairns, J., Dickson, K.L., Hennricks, E.E. (eds) Recovery and Restoration of damaged ecosystems. University of Virginia Press, Charlottesville.

Calow, P. 1994. Ecotoxicology: What are we trying to protect? *Environmental Toxicology and Chemistry* **13**: 1049.

CEC. 2000. Directive of the European Parliament and of the Council: Establishing a framework for Community action in the field of water policy. 2000/60/EC, (23 October 2000). L 327/1, (22.12.2000). Official Journal.

Chapman, P.M. 1995. Extrapolating laboratory toxicity results to the field. *Environmental Toxicology and Chemistry* **17**: 99-108.

Cooper, C.M. 1993. Biological effects of agricultural derived surface-water pollutants on aquatic systems – a review. *Journal of Environmental Quality* **22**: 402-408.

Chutter, F.M. 1972. An empirical biotic index of the quality of water in South African streams and rivers. *Water Research* **6**: 19-30.

Clements, W.H., Kiffney, P.M. 1994. Assessing contaminant effects at higher levels of biological organization. *Environmental Toxicology and Chemistry* **13**: 357-359.

Connell, J.H., Sousa, W.P. 1983. On the evidence needed to judge ecological stability and persistence. *American Naturalist* **121**: 789-824.

Cuffney T.F., Wallace, J.B., Lugthart, G.J. 1990. Experimental evidence quantifying the role of benthic invertebrates in organic matter dynamics of headwater streams. *Freshwater Biology* **23**: 281-299.

Dahl, H.-J., Hullen, M. 1989. Studie über die Möglichkeiten zur Entwicklung eines naturnahen Fließgewässersystems in Niedersachsen (Fließgewässerschutzsystem Niedersachsen). – In: Naturschutz und Landschaftspflege in Niedersachsen. Niedersächsisches Landesverwaltungsamt, Fachbehörde für Naturschutz, Hannover, 18, 5–120.

DIN DIfNeV. 1990. Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung; Biologisch-ökologische Gewässeruntersuchung (Gruppe M); Bestimmung des Saprobienindex (M 2). Beuth Verlag, Berlin.

Edwards, C.J., Griswold, B.L., Tubb, R.A., Weber, E.C., Woods, L.W. 1984. Mitigating effects of artificial rifles and pools on the fauna of a channelized warmwater stream. *North American Journal of Fisheries Management* **4**: 194-203.

Environment Canada. 1997. Environmental Assessments of Priority Substances under the Canadian Environmental Protection Act. Guidance Manual Version 1.0. EPS/2/CC/3E. Chemicals Evaluation Division, Commercial Chemicals Evaluation Branch, Environment Canada. ISBN 0-662-25403-1.

Faasch, H. 1997. Gewässergütebericht - Ergänzungen 1997. Bezirksregierung Braunschweig

Fent, K . 1998. Ökotoxikologie. Thieme Verlag, Stuttgart.

Flannagan, J.F., Townsend, B.E., De March, B.G.E., Friesen, M.K., Leonhard, S.L. 1979. The effects of an experimental injection of methoxychlor on aquatic invertebrates: accumulation, standing crop, and drift. *Canadian Entomologist* **111**: 73-89.

Fleeger, J.W., Carman, K.R., Nisbet, R.M. 2003. Indirect effects of contaminants in aquatic ecosystems. The *Science of the Total Environment* **317**: 207-233.

Friedrich, G. 1998. Gewässerbewertung als Aufgabe beim Schutz der Fließgewässer. Deutsche Gesellschaft für Limnologie – Tagungsbericht 1997 Band **1**: 101-117.

Frissell C.A., Liss W.J., Warren C.E., Hurley M.D. 1986. A hierarchical framework for stream habitat classification: viewing streams in a watershed context. *Environmental Management* **10**: 199-214.

Hall, L.W., Scott, M.C., Killen, W.D. 1998. Ecological risk assessment of copper and cadmium in surface waters of Chesapeake Bay watershed. *Environmental Toxicology and Chemistry* **17**: 1172-1189.

Hansen, S.R., Garton, R.R. 1982. The effects of diflubenzuron on a complex laboratory stream community. *Archives of Environmental Contamination and Toxicology* **37**: 78-84.

Hilsenhoff, W.L. 1977. Use of arthropods to evaluate water quality of streams. Department of Natural Resources, Madison, WI, USA.

Hilsenhoff, W.L. 1982. Using a Biotic Index to Evaluate Water Quality in Streams. Department of Natural Resources, Madison, WI, USA.

Hilsenhoff, W.L. 1987. An improved index of organic stream pollution. *Great Lakes Entomology* **20**: 31-39.

Gore, J.A. 1982. Benthic invertebrate colonization: source distance effects on community composition. *Hydrobiologia* 94: 183-193.

Kooijman, S.A.L.M. 1987. A safety factor for LC50 values allowing for differences in sensitivity among species. *Water Research* **21**: 269-276.

Klee, O. 1991. Angewandte Hydrobiologie. Georg Thieme Verlag, Stuttgart.

Kreutzweiser, D.P., and Kingsbury, P.D. 1982. Recovery of stream benthos and its utilization by native fish following high dosage permethrin applications. Information Report FPM-X-59. Forest Pest Management Institute, Environment Canada.

Lampert, W., Fleckner, W., Pott, E., Schober, U., Storkey, K.U. 1989. Herbicide effects on planctonic systems of different complexity. *Hydrobiologia* **188/189**: 415-424.

Lawton, J. 1991. Are species useful? *Oikos* **62**: 3-4.

Lenat, D.R. 1993. A biotic index for the south-eastern United States: derivation and list of tolerance values, with criteria for assigning water quality ratings. *Journal of the North American Benthological Society* **12**: 279-290.

Liess, M., Schulz, R., Neumann, M. 1996. A method for monitoring pesticides bound to suspended particles in small streams. *Chemosphere* **32**: 1963-1969.

Lipnick, R.L. 1986. Charles Ernest Overton: Narcosis studies and a contribution to general pharmacology. *TIPS* **81**: 161-164.

Lipnick, R.L. 1989. Base-line toxicity predicted by quantitative structure-activity relationships as a probe for molecular mechanism of toxicity. In: Magee P. S., Henry D. R. and Block J. H., eds, Probing bioactive mechanisms, Vol 413. American Chemical Society, Washington, DC, pp. 366-389.

Mc Lay, C. 1970. A theory concerning the distance travelled by animals entering the drift of stream. *Journal of Fish. Res. Bd. Canada* **27**:359-370.

Merrit, R.W., Cummins, K.W., Burton, T.M. 1984. The role of aquatic insects in the processing and cycling of nutrients. Pages 134-163 in Resh, V.H., Rosenberg, D.M. (eds) The Ecology of aquatic Insects. Praeger, New York.

Mills L.S., Soule, M.E., Doak D.F. 1993. The keystone-species concept in ecology and conservation. *BioScience* **43**: 219-224.

Minshall, G.W., Pertersen Jr., R.C. 1985. Towards a theory of macroinvertebrate community structure in stream ecosystems. *Archiv für Hydrobiologie* **104**: 49-76.

Newman, M.C. 1995. Quantitative methods in aquatic ecotoxciology. Lewis Publishers, Boca Raton.

Newman, M.C., Ownby, D.R., Mezin, L.C.A., Powel, D.C., Christensen, T.R.L., Lerberg, S.B., Anderson, B. 2000. Applying species-sensitivity distributions in ecological risk assessment: Assumptions of distribution type and sufficient number of species. *Environmental Toxicology and Chemistry* **19**: 508-515.

Neumann, M., Dudgeon, D. 2002. The impact of agricultural runoff on stream benthos in Hong Kong, China. *Water Research* **36**: 3103-3109

Niemi, G.J., DeVore, P., Detenbeck, N., Taylor, D., Lima, A., Pastro, J. 1990. Overview of case studies on Recovery of Aquatic systems from disturbance. *Environmental Management* **14**: 571-587.

Notenboom, J., Vaal, M.A., Hoekstra, J.A. 1995. Using Comparative Ecotoxicology to develop quantitative Species Sensitivity Relationships (QSSR). *Environmental Science and Pollution Research* **2**: 242-243.

Peterson, D.R. 1994. Calculating the aquatic toxicity of hydrocarbon mixtures. *Chemosphere* **29**:2493-2506.

Plafkin, J.L., Barbour, M.T., Porter, K.D., Gross, S.K., Hughes, R.M. 1989. Rapid bioassessment protocols for use in streams and rivers: Benthic macroinvertebrates and fish. EPA 440-4-89-001. U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, D.C, USA.

Pratt, J.R., Cairns, Jr., J. 1996. Ecotoxicology and the redundancy problem: Understanding effects on community structure and function. Pages 347-370 in Newman M.C., Jagoe, C.H. (eds), Ecotoxicology: a hierarchical treatment. Lewis Publishers, Boca Raton.

Preston, B.L. 2002. Indirect effects in aquatic ecotoxicology: Implications for ecological risk assessment. *Environmental Management* **29**: 311-323.

Reus, J., Leendertse, P., Bockstaller, C., Fomsgaard, I., Gutsche, V., Lewis, K., Nilsson, C., Pussemier, L., Trevisan, M., Werf, H., van der Alfarroba, F., Blümel, S., Isart, J., McGrath, D., Sepplälä, T. 1999. Comparing Environmental Risk Indikators for Pesticides - Results of the European CAPER Project. Centre for Agriculture and Environment, Utrecht.

Rosenberg, D.M., Resh, V.H. (eds). 1993.Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapmann & Hall, New York.

Sarma, S.S.S., Nandini, S., Fernandez Araiza, M.A. 1998. Effect of methyl parathion-treated prey (Brachionus califlorus) on the population growth of he predator Asplanchna sieboldi (Rotifera). *Bulletin of Environmental Contamination and Toxicology* **61**: 135-142.

Shaw, D.W., Minshall, G.W. 1980. Colonization of an introduced substrate by stream macroinvertebrates. *Oikos* **28**: 256-261.

Schulz, R., Liess, M. 1999. A field study of the effects of agriculturally derived insecticide input on stream macroinvertebrate dynamics. *Aquatic Toxicology* **46**: 155-176.

Schwoerbel, J. 1994. Methode der Hydrobiologie – Süßwasserbiologie (4. Auflage). Gustav Fisher Verlag, Jena.

Schwoerbel, J. 1999. Einführung in die Limnologie - 8 ed. Fischer Verlag, Stuttgart, Jena, Lübeck, Ulm.

Solomon, K.R., Sibley, P. 2002. New concepts in ecological risk assessment: Where do we go from here? *Marine Pollution Bulletin* **44**: 279-285.

Sprague, J.B. 1995. Factors that modify toxicity. Pages 1012-1051 in Rand, G.M. (ed.) Fundamentals of aquatic toxicology, 2nd ed. Taylor and Francis, Washington, D.C.

Surber, E.W. 1937. Rainbow trout and bottom fauna production in one mile of stream-. *Trans. Amer. Fish. Soc.* **66**: 193-202.

Suter, G.W., Barnthouse, L.W., Bartell, S.M., Mill, T., Mackay, D., Patterson, S. 1993. Ecological Risk Assessment. Lewis Publishers, Boca Raton.

Tomlin, C.D.S. 2000. The pesticide manual, a world compendium. Crop Protection Publications, Farnham.

Sellheim, P. 2000. Mündliche Mitteilung, Niedersächsisches Landesamt für Ökologie, Hildesheim.

Truhaut, R. 1977. Ecotoxicology: Objectives, principles and perspectives. *Ecotoxicology and Environmental Safety* **1**: 151-173.

UBA. 1997. Daten zur Umwelt – der Zustand der Umwelt in Deutschland, Schmidt, Berlin, 252-257.

UBA. 2001. Wasserwirtschaft in Deutschland. Teil III – Emissionen in die Oberflächengewässer und Meere. Bonn.

U.S. EPA. 1998. Guidelines for Ecological Risk Assessment. EPA/630/R-95/002F

Van Leeuwen, C.J., Van der Zandt, P.T.J., Aldenberg, T., Verhaar, H.J.M. and Hermens, J.L.M. 1992. Application of QSARs, extrapolation and equilibrium partitioning in aquatic effects assessment. 1. Narcotic industrial pollutants. *Environmental Toxicology and Chemistry* **11**: 267–282.

Versteeg, D.J., Belanger, S.E., Carr, G.J. 1999. Understanding single species and model ecosystem sensitivity: a data based comparison. *Environmental Toxicology and Chemistry* **18**: 1329-1346.

Walker, B. 1991. Biodiversity and ecological redundancy. *Conservation Biology* **6**: 12-23.

Wallace, J.B., Merrit, R.B. 1980. Filter-feeding ecology of aquatic insects. *Annual review of Entomology* **25**: 103-132.

Wallace, J.B., Vogel, T.S., Cuffney T.F. 1986. Recovery of a headwater stream from an insecticide-induced community disturbance. *Journal of the North American Benthological Society* **5**: 115-126.

Wallace, J.B., Gurtz, M.E. 1986. Response of *Baetis* mayflies (Ephemeroptera) to catchment logging. *American Midland Naturalist* **115**: 25-41.

Wallace, J.B. 1990. Recovery of Lotic Macroinvertebrate Communities from Disturbance. *Environmental Management* **14**: 605-620.

Wallace, J.B., Cuffney T.F., Webster, J.R., Lugthart, G.J., Chung, K., Goldowitz, B.S. 1991. A five-year study of export of fine organic particles from headwater streams: effects of season, extreme discharge, and invertebrate manipulation. *Limnology and Oceanography* **36**: 670-682.

Wallace, J.B., Webster, J.R.1996. The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology* **41**: 115-139.

Wauchope, R.D. 1978. The pesticide content of surface water draining from agricultural fields - a review. *Journal of Environmental Quality* **7**: 459-472.

Whitehurst, I.T. 1991. The *Gammarus - Asellus* Ratio as an Index of Organic Pollution. *Water Research* **25**: 333-340.

Williams, D.D., Hynes, H.B.N. 1976. The recolonization mechanism of stream benthos. *Oikos* **27**: 265-272.

Williams, R.J., Brooke, D., Matthiesen, P., Mills, M., Turnbull, A., Harrison, R.M. 1995. Pesticide transport to surface waters within an agricultural catchment. *J. Inst. Wat. Envir. Man.* **9**: 72-81.

Wogram, J., Liess, M. 2001. Rank ordering of macroinvertebrate species sensitivity to toxic compounds by comparison with that of *Daphnia magna*. *Bulletin of Environmental Contamination and Toxicology* **67**: 360-367.

Chapter II

2004

Environmental Toxicology and Chemistry, Vol. 23

RELATIVE SENSITIVITY DISTRIBUTION OF AQUATIC INVERTEBRATES TO ORGANIC AND METAL COMPOUNDS

PETER CARSTEN VON DER OHE* and MATTHIAS LIESS

Department of Chemical Ecotoxicology, UFZ - Centre for Environmental Research
Leipzig-Halle GmbH, Permoserstrasse 15, 04318 Leipzig, Germany

ABSTRACT

In the field, a multitude of species can be exposed to numerous toxicants; thus, the sensitivity of individual species to particular toxicants must be known to predict effects and to analyze changes in species composition. For most species, no information about their toxicant sensitivity is available. To address this limitation, we have grouped the available information to assign sensitivities to aquatic invertebrate taxa relative to *Daphnia magna*. With respect to organic compounds, most taxa of the orders Anisoptera, Basommatophora, Coleoptera, Decapoda, Diptera, Ephemeroptera, Eulamellibranchiata, Heteroptera, Hirudinea, Isopoda, Oligochaeta, Prosobranchia, Trichoptera, Tricladida, and Zygoptera are less sensitive than *D. magna*. Some taxa of the Amphipoda, Plecoptera, and Cladocera (other than *D. magna*) are significantly more sensitive. For organic compounds, approximately 22 % of the investigated taxa were more sensitive than *D. magna*. Most taxa of the orders Amphipoda, Basommatophora, Diptera, Ephemeroptera, Eulamellibranchiata, Heteroptera, Isopoda, Oligochaeta, and Tricladida are significantly less sensitive than *D. magna* to metal compounds. The taxa belonging to the Crustacea, with the exception of the order Isopoda, are much more sensitive. For metal compounds, approximately 30 % of the investigated taxa were more sensitive than *D. magna*. Hence, *D. magna* is among the most sensitive taxa regarding both groups of toxicants. The sensitivities for several taxa are listed, and use of the relative sensitivity distribution to link toxicant effects in mesocosm studies and field investigations is discussed.

---

[*] To whom correspondence may be addressed: peter.vonderohe@ufz.de

INTRODUCTION

In the field, a multitude of species can be exposed to numerous toxicants. Therefore, to predict the effects of toxicants and to understand changes in species composition within communities, it is desirable to know how sensitive individual species are to specific toxicants. In most cases, no relevant information is available; sensitivities toward certain toxicants are known only for a limited number of species. Hence, efforts to predict the effects of toxicants are confronted by a major problem of data limitation [1]. Currently, two concepts are in use to deal with this lack of information.

First, a limited number of standard test organisms are used as representatives to predict toxicant effects on organisms at a similar trophic level. Because only a few standard test organisms are in use, it is possible to test their sensitivity toward a range of substances. In aquatic ecotoxicology, *Daphnia magna* is widely used as such a model organism. Nevertheless, the degree to which this species is representative of invertebrates in general has often been questioned [2].

Because of limitations associated with the use of standard test organisms, an alternative measure — namely, the species sensitivity distribution — has been employed in risk assessment. By testing a number of organisms, a distribution of sensitivity is obtained to assess the fraction of species affected in the environment. This refinement of the concept of representative test species enables a better assessment of environmental effects, because the sensitivity distribution allows the percentage of affected species in a community to be estimated. However, a major limitation of this approach is that extensive toxicological information is required for the great variety of species assemblages that exist in the field. In addition, such species sensitivity distributions refer to one substance only.

These limitations of current risk assessment were also recognized during the Higher-Tier Aquatic Risk Assessment for Pesticides (HARAP) workshop [3] and led to the following statement: ‘‘Perhaps the major area of uncertainty in preliminary effects assessments is the potential for differences in sensitivity between organisms tested, compared to those which occur in nature. For this reason, large uncertainty factors are often applied in a preliminary risk characterization’’ (page 8). As a possible solution to this problem, the HARAP workshop suggested comparing the sensitivity of other species with those of standard test organisms to classify the sensitivity of standard test organisms. Additional species data may also be used to develop probabilistic effects distributions, and the workshop recommended that probabilistic approaches be the subject of further discussion.

As one way to deal with the paucity of information described above, Wogram and Liess [4] suggested that aquatic invertebrate orders be ranked according to their sensitivity, with the sensitivity of *D. magna* being taken as a point of reference, because for this species, a great number of toxicants have been evaluated. This method enables the integration of various toxicants in the ranking of species, because a relative sensitivity compared to *D. magna* can be calculated for many species and toxicants. To ensure sufficient statistical power, related species are aggregated, and taxa are ranked separately with respect to organic compounds ($S_{organic}$) and to metal compounds (S_{metal}). This concept enables a great number of aquatic invertebrate species to be ranked according to their sensitivity within these two groups of toxicants. These particular groups were distinguished initially because of previous findings that indicated a differential sensitivity of various taxa to organics and metals. Within each

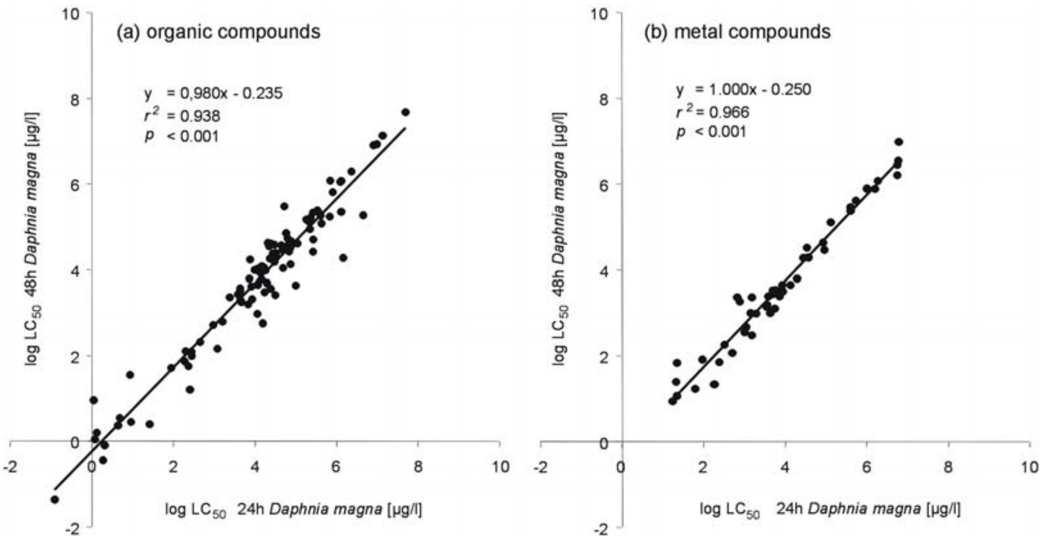


Fig. 1. The 48-h lethal concentration (LC50) of *Daphnia magna* plotted against the 24-h LC50 for (a) organic substances and (b) metal substances.

group, however, a large number of test results are available. The disadvantage of this concept lies in its reduced accuracy because of the aggregation of information. Nevertheless, this method has been successfully applied to predict the composition of the invertebrate community in streams subject to pesticide contamination [5].

The aim of the present study was to refine the concept of ranking taxa according to their sensitivity by identifying the taxa at the lowest taxonomic level possible according to the available data and selecting more comparable endpoints than those used in previous work [4]. Furthermore, by establishing sensitivity values for the taxonomic level of family and genus, the bandwidth of sensitivity within the different taxa can also be determined. The resulting information concerning relative sensitivity distributions is intended for use in linking the effect of toxicants in aquatic systems to the community responses.

MATERIALS AND METHODS

Database

The toxicity data were assembled in August 2002 from the online Aquatic Toxicity Information Retrieval (AQUIRE) database of the U.S. Environmental Protection Agency [6]. For the search in AQUIRE, the following orders were considered (taxonomic system [7]): Amphipoda, Basommatophora, Cladocera, Coleoptera, Decapoda, Diptera, Ephemeroptera, Heteroptera, Hirudinea, Isopoda, Eulamellibranchia, Megaloptera, Odonata, Oligochaeta, Plecoptera, Prosobranchia, Trichoptera, and Tricladida. The order Odonata was divided into the two suborders: Anisoptera and Zygoptera. The search was conducted at the genus level, including all genera listed by Illies [8] as being abundant in Central Europe. In contrast to Wogram and Liess [4], who considered sublethal endpoints, the endpoint considered in the present study was restricted to lethal concentration (LC50) values to increase comparability between datasets. Toxicity data were restricted to freshwater laboratory tests with an exposure duration of 24 and 48-h. Literature cited in AQUIRE that met the above criteria was included.

Calculation of relative sensitivity

Comparisons were made only between the test values that were obtained with the same substance and test duration. For this, the LC50 of *D. magna* was divided by the LC50 of each species obtained for each value i for organic and metal compounds.

$$S = \log \left(LC50_{Daphnia\ magna} / LC50_i \right) \qquad (1)$$

where S = relative sensitivity; LC50 *Daphnia magna* = experimental LC50 for *D. magna*; and LC50 i = experimental LC50 for a species i. The logarithm of this quotient was termed the relative sensitivity (S) with respect to the sensitivity of *D. magna* (Eqn. 1). A value of zero thus indicates a sensitivity equal to that of *D. magna*. For taxa more sensitive than *D. magna*, the S value is greater than zero; for less sensitive taxa, the value is less than zero. Because the S values are expressed logarithmically, a score of one means that the taxon in question is, on average, 10-fold more sensitive than *D. magna* to that particular substance.

When multiple test values were found for one combination of substance and species, these values were checked for inconsistencies. If values differed by more than a factor of 30 from the closest one in a group of at least three other references, the aberrant value was discarded to remove outliers from the dataset for both *D. magna* and the other species. This should ensure the quality of the unchecked raw data; thus, for the calculations of sensitivities, the arithmetic mean was chosen.

To increase the number of matching pairs of *D. magna* and the respective species, missing values of *D. magna* were extrapolated from 24-h to 48-h *D. magna* test results. The adjustment was performed when a particular toxicant and species combination did not match the test duration for the same toxicant and *D. magna.* Because the corresponding 24-h and 48-h values are highly correlated, the linear regression function between these values was used to extrapolate the 24-h values into 48-h values (Fig. 1) for both classes of compounds. As a next step, for all 48-h values (measured or extrapolated) of a certain substance, an arithmetic mean was taken. This mean was retransformed when a 24-h value for a respective species was available. For this, the relationship shown in Figure 1 was used. For each test value for a particular species and a particular substance, a relative sensitivity was calculated according to Equation 1. When multiple test values were found for one combination of species and substance, the arithmetic mean of the effect concentration values in each study was taken to derive a first-level S value to weight each study in a similar way. A secondary arithmetic mean was calculated by aggregating the results of different authors for one toxicant, which reflects the relative sensitivity of a species to this toxicant (second-level S value). A third arithmetic mean was calculated from all second-level S values of the respective species belonging to that taxon (third-level S value). This should avoid the overestimation of one substance during calculation of the relative sensitivity for each particular taxon. This relative sensitivity with respect to organic compounds is referred to as $S_{organic}$; relative sensitivity with respect to metal compounds are referred to as S_{metal}. These three levels of aggregation are represented in Table 1 for the number of test results, number of references, and number of different substances used to derive the S value for each taxa. According to this procedure, a higher taxonomic level always includes all the second-level

S values of the lower taxonomic level. The relative sensitivity (S) of a taxon was determined only when at least five different substances had been tested. When this criterion was met, an S value was assigned to the taxon regardless of its taxonomic level. Hence, according to the data available, every taxonomic level ranging from species to genus to family and to order can be assigned an S value. To define significant differences between the sensitivity of one taxa and that of *D. magna*, the one-sample Student's t test was performed (Table 1), with zero as the test value. Tests were only performed after the data had been shown to be normally distributed (Kolmogoroff-Smirnov test, $p \leq 0.05$). To represent graphically the distribution of the relative sensitivity of taxa (Fig. 2), data were plotted using a log-probability transformation, and linear regressions were performed with the aid of the Sigma Plot 4 graphics package (SPSS, Chicago, IL, USA). The sensitivities of each taxa are expressed as percentages, calculated from the formula 100 x *i*/(n + 1), where i is the rank of the taxon and n is the total number of data points in the set. Hence, the plotting positions calculated above are dependent on the number of data points. The log-normal model was used for characterizing toxicity distributions, because it has been recommended and is supported by the observations in other studies of species sensitivity distributions [1]. The percentiles have been derived graphically from Figure 2.

Table 1. Relative sensitivity ($S_{organic}$ and S_{metal}) of aquatic invertebrate taxa expressed as a logarithmic measure of sensitivity in comparison to that of *Daphnia magna*

order	family	genus	species	S organic	(su | re | te)	S metal	(su | re | te)
Annelida							
Hirudinea				- 0.60*	(18|18|18)		
	Erpobdellidae			- 0.41	(13|13|13)		
Oligochaeta				- 1.10***	(20|22|40)	- 0.80***	(35|37|92)
	Lumbriculidae			- 1.40*	(9|10|11)	- 0.51	(12|17|27)
	Tubificidae			- 0.93*	(6|7|15)	- 0.80***	(20|22|48)
Crustacea							
Amphipoda				0.16	(95|104|200)	- 0.30	(18|19|29)
			Gammarus fasciatus	0.19	(30|34|68)		
			Gammarus lacustis	0.32	(22|23|51)		
			Gammarus pseudolimnaeus	0.14	(11|13|25)		
			Gammarus pulex	0.04	(22|24|45)	- 0.03	(5|5|9)
Cladocera [1]				0.17***	(143|179|420)	0.48***	(70|91|280)
	Daphniidae [1]			0.20***	(136|172|413)	0.49***	(69|89|275)
		Ceriodaphnia		0.28*	(49|68|163)	0.62***	(17|25|77)
			Ceriodaphnia dubia	0.39***	(41|59|152)	0.64**	(10|16|62)
		Daphnia [1]		0.20*	(64|78|181)	0,50***	(34|43|145)
			Daphnia culcullata	0.08	(12|12|22)		
			Daphnia pulex	0.20***	(42|55|146)	0,38***	(18|27|112)
		Moina		- 0.22	(11|12|32)	0,40	(9|12|39)
			Moina macropoda	- 0.17	(10|11|31)	0,18	(5|8|13)
		Simocephalus		0.24	(12|14|37)	0,32*	(9|9|14)
			Simocephalus serrulatus	0.25	(8|8|8)		
Decapoda				- 0.08	(29|36|103)		
	Astacidae			- 0.57	(13|13|40)		
			Oronectes nais	- 0.41	(10|10|34)		
	Palaemonidae			0.26	(15|22|57)		
Isopoda				- 0.56*	(40|49|57)	- 1.22*	(7|10|24)
			Asellus aquaticus	- 0.17	(40|49|57)	- 1.55*	(5|8|14)
			Asellus brevicaudus	- 0.56	(12|17|17)		
Mollusca							
Basommatophora				- 1.23***	(89|127|171)	- 0.82***	(23|28|69)
	Lymnaeidae			- 0.64	(42|71|109)	- 0.93***	(12|17|27)

Table 1. *(continued)*

order	family	genus	species	S organic	(su | re | te)	S metal	(su | re | te)
			Lymnea acuminata	- 0.93	(11|15|26)		
			Lymnea stagnalis	- 0.62	(22|47|66)		
	Physidae			- 1.64***	(36|45|46)	- 0.35	(6|6|34)
			Physa acuta	- 1.88***	(25|34|34)		
	Planorbidae			- 1.94***	(11|12|18)		
Eulamellibranchia				- 2.09***	(21|24|41)	- 0.33	(7|8|13)
Prosobranchia				- 1.82*	(8|9|21)		
	Viviparidae			- 1.50*	(7|8|19)		
Insecta							
Anisoptera[2]				- 0.96	(12|12|16)		
	Libellulidae			- 1.53***	(8|8|12)		
		Orthetrum		- 1.75***	(7|7|11)		
Coleoptera				- 1.15***	(25|26|36)		
	Dytiscidae			- 0.81***	(10|11|15)		
	Haliplidae			- 1.83	(7|7|12)		
		Peltodytes		- 1.95	(6|6|11)		
	Hydrophilidae			- 0.89***	(7|7|8)		
Diptera				- 0.35***	(429|573|958)	- 1.57***	(35|41|81)
	Chironomidae			- 0.39***	(159|213|316)	- 1.53***	(28|32|69)
		Chironomus		- 0.33***	(110|141|202)	- 1.50***	(26|30|66)
			Chironomus tentans	- 0.19	(28|34|46)	- 1.36*	(6|8|19)
			Chironomus thummi	- 0.30	(36|46|63)	- 1.86**	(7|7|21)
		Tanytarsus		- 0.36	(28|43|56)		
			Tanytarsus dissimilis	- 0.34	(23|35|48)		
	Culicidae			- 0.30***	(259|349|631)	- 1.73**	(7|9|12)
		Aedes		- 0.29*	(114|137|224)		
			Aedes aegypti	- 0.14	(28|45|87)		
			Aedes cantans	- 0.18	(13|17|21)		
			Aedes punctor	- 0.34	(13|13|13)		
			Aedes vexans	- 0.07	(13|15|15)		
Ephemeroptera				- 0.30	(38|42|53)	- 1.61*	(5|8|14)
	Baetidae			- 0.25	(33|37|46)		
		Baetis		0.02	(7|9|11)		
		Cloeon		- 0.32	(26|28|35)		
Heteroptera				- 0.56***	(33|35|46)	- 1.63	(5|6|10)
	Corixidae			- 0.29	(18|20|28)		
		Corixa		- 0.31	(13|13|13)		
		Sigara		- 0.24	(5|7|15)		
	Notonectidae			- 0.86	(13|13|18)		
		Notonecta		- 0.82	(12|12|15)		
Plecoptera				0.38***	(15|15|16)		
	Nemouridae			0.25***	(13|13|13)		
			Nemoura cinerea	0.16*	(12|12|12)		
Trichoptera				- 0.06	(14|14|18)		
	Hydropsychidae			- 0.50	(10|10|14)		
		Hydropsyche		- 1.03*	(7|7|8)		
Zygoptera[2]				- 0.36	(22|22|29)		
	Coenagrionidae			- 0.24	(16|16|20)		
		Ischnura		- 0.32	(15|15|18)		
			Ischnura elegans	- 0.30	(12|12|12)		
	Lestidae			- 0.68	(6|6|9)		
Plathelminthes							
Turbellaria				- 0.43	(18|18|19)	- 1.25*	(6|7|15)
	Dugesiidae			- 0.47	(16|16|16)	- 0.91	(5|6|11)

Positive values indicate a higher sensitivity than *D. magna*; negative values indicate a lower sensitivity than *D. magna.* The number of substances (su), number of different references (re), and the number of tests (te) included in the analyses are given.

[1] other than *D. magna*

[2] suborder of Odonata

Database statistics

The database AQUIRE yielded toxicity data [6] meeting the requirements described above for a total of 2,941 different tests (2,269 for organic compounds and 672 for metal compounds) comprising 201 different substances (164 for organic compounds and 37 for metal compounds) and derived from 377 publications (271 for organic compounds and 106 for metal compounds). Substances considered within the group of organic compounds were distributed as follows: Insecticides, 25.6 %; herbicides, 9.8 %; fungicides, 4.3 %; and other, 60.3 %. Within the taxa, the number of substances for which comparable effect concentrations were available, both for the included species and also for *D. magna*, ranged from 5 (minimum requirement) to 104 (Diptera, organic compounds). The exposure durations in the experiments considered were distributed as follows: 49.7 % for 24-h and 50.3 % for 48-h. The number of different genera per order in this sample of toxicity data was between 1 (Isopoda, both classes of compounds) and 17 (Diptera, organic compounds). The sensitivity of the order Megaloptera to both substance groups was not calculated, because the data were insufficient.

RESULTS

General trends

The database used was sufficient to rank 92 taxa for organic compounds and 30 for metal compounds (Table 1), of which 35 and 21 taxa, respectively, exhibited significant differences in sensitivity compared to *D. magn*a. The distributions of the relative sensitivities to organic and metal compounds are shown in Figure 2. Insects show a wide range of sensitivities to organics; species within this taxa range from the most sensitive to the least sensitive. In contrast, they are consistently among the least sensitive taxa to metals. The taxa belonging to the Crustacea are similar to one another and to *D. magna* in terms of sensitivity to organics and metals. The one exception, Isopoda, is less sensitive than all other Cladocera to both organic and metal compounds. Molluscs are consistently among the species least sensitive to organics, but they show an average sensitivity to metals. An overview of the sensitivities to organics and metals is shown in Figure 3.

Organic substances

Most taxa of the orders Anisoptera, Basommatophora, Coleoptera, Decapoda, Diptera, Ephemeroptera, Eulamellibranchiata, Heteroptera, Hirudinea, Isopoda, Oligochaeta, Prosobranchia, Trichoptera, Tricladida, and Zygoptera show significantly less sensitivity than *D. magna* to organic compounds (Table 1). The order Eulamellibranchiata, with a $S_{organic}$ of –2.09, was the least sensitive. Some taxa in the Amphipoda, Plecoptera, and Cladocera (other than *D. magn*a) were significantly more sensitive. With a $S_{organic}$ of 0.39, the species *Ceriodaphnia dubia* was the most sensitive taxon of all. The most sensitive taxa all belong to Crustacea and Insecta, whereas three of the four least sensitive taxa are Mollusca. Regarding the sensitivity of aquatic invertebrates relative to *D. magn*a, the following characteristics of the distribution were calculated: For organic compounds, approximately 22 % of the investigated taxa were more sensitive than *D. magn*a. The 10th percentile intercepted at an $S_{organic}$ of 0.4, indicating that based on a normal distribution of sensitivities, the mostsensitive 10 % of taxa are at least 2.5-fold more sensitive than *D. magna* to organic substances (Fig. 2a).

Metal substances

Most taxa of the orders Amphipoda, Basommatophora, Diptera, Ephemeroptera, Eulamellibranchiata, Heteroptera, Isopoda, Oligochaeta, and Tricladida show significantly less sensitivity than *D. magna* to metal compounds (Table 1). The least sensitive taxon belongs to the order Diptera (the species *Chironomus thummi*, with an S_{metal} of –1.86). The species *Ceriodaphnia dubia* was again the most sensitive taxon, with a significant S_{metal} of 0.64. The 13 taxa belonging to the Crustacea, with the exception of the order Isopoda (two taxa), were the most sensitive, whereas the five least sensitive taxa are members of the Insecta. Regarding the sensitivity of aquatic invertebrates relative to *D. magna*, the following characteristics of the distribution were calculated: For metal compounds, approximately 30 % of the investigated taxa were more sensitive than *D. magna*, and the 10th percentile intercepted at an S_{metal} of 0.62, indicating that based on a normal distribution of sensitivities, the most sensitive 10 % of taxa are at least fourfold more sensitive than *D. magna* to metal substances (Fig. 2b).

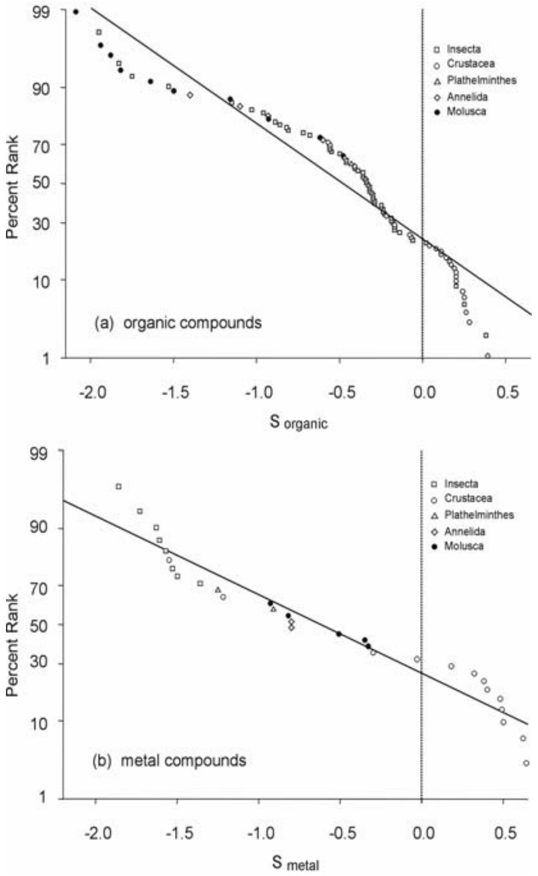


Fig. 2. Distribution of the relative sensitivity ($S_{organic}$ [a] and S_{metal} [b]) of aquatic invertebrate taxa expressed as a logarithmic proportion relative to the sensitivity of *Daphnia magna* (broken line). Positive values indicate a higher sensitivity than *D. magna*; negative values indicate a lower sensitivity than *D. magna*.

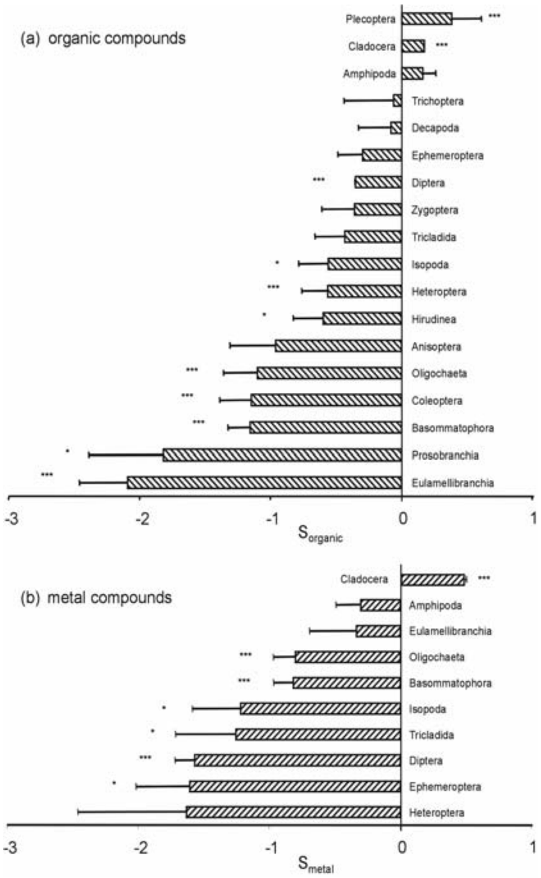


Fig. 3. Relative sensitivity ($S_{organic}$ [a] and S_{metal} [b]) of aquatic invertebrate taxa (order) expressed as a logarithmic proportion relative to the sensitivity of *Daphnia magna*. Positive values indicate a higher sensitivity than *D. magna;* negative values indicate a lower sensitivity than *D. magna* (* $p<0.05$; ** $p<0.01$, *** $p<0.001$, t-test).

DISCUSSION

Comparing sensitivity of taxa

During the course of the work on comparative toxicity sensitivity, several studies have revealed that some taxa are more sensitive than others. Nevertheless, these investigations were mostly restricted to closely related toxicants. When investigating the toxicity of several pyrethroids (Permethrin, Fenvalerate, Cypermethrin, and Deltamethrin), Leahey [9] found that compared with other invertebrates, the most sensitive organisms tested were consistently Ephemeroptera and Crustacea. Another study of several pyrethroids showed that Mollusca (particularly the bivalves) were generally less sensitive than Crustacea [10]. In addition, a review of the effect of the pyrethroid lamda-cyhalothrin on aquatic invertebrates showed a wide range of sensitivities, with the Crustacea and Insecta predictably being the more sensitive taxa [11]. Such predictability in the relative sensitivity of taxa was also found when taking into account a wider range of toxicants [4]. Hence, the present attempt to rank taxa has some precedents, but most of this work was concerned with narrowly circumscribed groups of substances.

Grouping organisms and toxicants

When comparing the sensitivity of invertebrates to toxicants, comparisons have usually been undertaken at the taxonomic level of species or genus [12,13]. To our knowledge, with the exception of Wogram and Liess [4], no comprehensive classifications at higher taxonomic levels (i.e., the order) have been performed. Given that more distantly related organisms resemble one another less in their physiological and morphological characteristics, a grouping at higher taxonomic levels reduces predictive accuracy and would be expected to produce a considerable variance of sensitivity. For example, Guerold [14] showed for several organism-based indices that as the taxonomic level at which the organisms were identified rose, the index values became progressively more erroneous. Nevertheless, it was decided to rank the sensitivities of taxa at higher taxonomic levels, because the paucity of data ruled out an assignment of sensitivities based on the species level. Therefore, when employing the sensitivity values presented in the present study, the interspecific sensitivity differences within a taxonomic group should be taken into account. However, the authors believe that many of the taxa chosen here are characterized by a specific sensitivity. The significant differences in sensitivity found between the various taxa justify this approach. Also, Hoekstra et al. [15], examining the literature regarding 26 chemicals, demonstrated that the variation of sensitivity between species within a class is usually less than the variation between classes. To minimize the problem associated with basing the present toxicity ranking on a taxonomic level higher than the species, an attempt was made to rank taxa at the lowest taxonomic level possible according to the availability of information. Unfortunately, to include as much information as possible for each taxon, this implies a reduction of distinguishable toxicant classes. It is therefore obvious that a trade-off must be reached between a refined differentiation of toxicant groups and a taxonomic differentiation of organisms. As a result, only organic and metal toxicants were differentiated in the present investigation. Future work should examine the possibility of further differentiating groups of toxicants.

Daphnia magna as reference

Daphnia magna is widely used in toxicological testing; thus, data are available regarding its sensitivity to many substances. For this reason, *D. magna* was chosen as a reference in the present study for toxicity in other species, because the taxa could not be compared directly as a result of the limited number of toxicants that have been tested for each taxon. *Daphnia magna* has been demonstrated to be representative for most insecticides [16], but the toxicity of certain other chemicals with very specific, receptor-mediated modes of action (e.g., neo-nicotinoids) may not be well represented [17]. Therefore, substances that have a taxon-specific mode of action should not be evaluated with the present database, because the actual, specific sensitivities of the taxa to these substances might deviate considerably from the general sensitivities found here.

Relative sensitivity distribution—a synthesis of existing concepts

At present, the toxicity of chemicals is evaluated by two methods. In the first method, a multitude of substances are tested against a few standard test organisms that are believed to be representative of a group of other organisms. This enables evaluation of several substances with a reasonable effort; the disadvantage of this approach is the restriction to a few standard test organisms. In the second method, several organisms are tested against one substance to obtain a sensitivity distribution for these species. This allows the percentage of affected species to be calculated for a given concentration; the disadvantage of this approach is that it is restricted to a few substances only. To evaluate the toxicity of chemicals, the described method of the relative sensitivity distribution is a synthesis of the two concepts currently in use: A great number of species (grouped into taxa) are ranked according to their sensitivity to a great number of substances (also grouped). Hence, the advantages of standard test organisms and species sensitivity distribution are combined to enable prediction of a great number of substances for a great number of species. The disadvantage of this method is a considerable decrease in predictability concerning the effect of a specific substance on a specific species. Keeping this in mind, we see an application of the concept as described below.

Prediction of effect

When comparing the sensitivity of *D. magna* with that of other species and taxa, it becomes clear that for both organic and metal compounds, *D. magna* is among the most sensitive of organisms. For organic compounds, approximately 22 % of the investigated taxa were more sensitive than *D. magna*, whereas the corresponding proportion for metal compounds was approximately 30 %. Hence, it is legitimate to state that in general, *D. magna* is a conservative predictor of toxicity for most taxa of interest. In principle, it is possible to predict the concentration at which a given toxicant will have a deleterious effect on a particular species if the acute LC50 for *D. magna* is known and reference is made to the relative sensitivity distribution in Figure 2. However, such a prediction is subject to major uncertainty and, hence, should not be attempted given the amount of data available at present. Two main reasons support this conclusion. First, if chronic endpoints were to be considered, it would be necessary to extrapolate these from the acute end-points employed here. Second, the toxicity of certain toxicants, which have very specific, receptor-mediated modes of action, may not be well represented by extrapolating the effect from *D. magna* to other taxa. Nevertheless, this approach has strong advantages when assessing the effect of toxicants on a multitude of species, such as those found in natural communities.

Interpretation of mesocosm and field data

The concept of relative sensitivity distribution presented here could be valuable when assessing the effect from exposure of an invertebrate community to single toxicants or to complex mixtures. It would be of use, for instance, when a comprehensive set of data from mesocosm studies or field investigations including species with unknown sensitivity are to be linked to the effect of a toxicant. The ratio between sensitive and less sensitive taxa abundance in a community can be used to infer the stress that such exposure will impose. This approach has been successfully applied to link pesticide contamination and invertebrate population response in agricultural streams [5]. Nevertheless, when assessing the effects of toxicants in communities, other potential contributory factors should be taken into account as well. For example, the effects of toxicants can be increased by natural stressors like low oxygen level [18,19] or increased ultraviolet-B radiation [20]. On the other hand, a decreased toxicant effect may result from recovery of species with a short generation time [21] and recolonization from undisturbed stream sections [22].

Acknowledgement—The authors would like to thank Martin Streloke, Jörn Wogram, Michael Kilemade, and two anonymous reviewers for their help with the present study.

REFERENCES

1. Posthuma L, Suter GW, Traas TP, eds. 2002. Species Sensitivity Distributions in Ecotoxicology. Lewis, Boca Raton, FL, USA.

2. Koivisto S. 1995. Is *Daphnia magna* an ecologically representative zooplankton species in toxicity tests? *Environ Pollut* **90**: 263–267.

3. Campbell PJ, Arnold DJS, Brock TCM, Grandy NJ, Heger W, Heimbach F, Maund SJ, Streloke M. 1999. Guidance Document on Higher-tier Aquatic Risk Assessment for Pesticides (HARAP). SETAC Europe, Brussels, Belgium.

4. Wogram J, Liess M. 2001. Rank ordering of macroinvertebrate species sensitivity to toxic compounds by comparison with that of *Daphnia magna. Bull Environ Contam Toxicol* **67**: 360–367.

5. Liess M, Schulz R, Berenzen N, Nanko-Drees J, Wogram J. 2001. Pflanzenschutzmittel-Belastung und Lebensgemeinschaften in Fliesgewässern mit landwirtschaftlich genutztem Umland. Texte 65/2001. UBA Texte, Berlin, Germany.

6. U.S. Environmental Protection Agency. 2002. AQUIRE (Aquatic toxicity information retrieval database). National Health and Environmental Effects Research Laboratory, Duluth, MN.

7. Schaefer M. 1992. Brohmer—Fauna von Deutschland, ein Bestimmungsbuch unserer heimischen Tierwelt. Quelle & Meyer Verlag, Heidelberg, Germany.

8. Illies J. 1978. Limnofauna Europaea, A Compilation of the European Freshwater Species with Emphasis on their Distribution and Ecology. G. Fischer, Jena, Germany.

9. Leahey JP. 1985. The Pyrethroid Insecticides. Taylor & Francis, London, UK.

10. Mian LS, Mulla MS. 1992. Effects of pyrethroid insecticides on nontarget invertebrates in aquatic ecosystems. *Journal of Agricultural Entomology* **9**: 73–98.

11. Maund SJ, Hamer MJ, Warinton JS, Kedwards TJ. 1998. Aquatic ecotoxicology of the pyrethroid insecticide l-cyhalothrin: Con-siderations for higher-tier aquatic risk assessment. *Pest Sci* **54**: 408–417.

12. Roshon RD, Mc Cann JH, Thompson DG, Stephenson GR. 1999. Effects of seven forestry management herbicides on Myriophyllum sibiricum, as compared with other nontarget aquatic organisms. *Can J For Res* **29**: 1158–1169.

13. Versteeg DJ, Belanger SE, Carr GJ. 1999. Understanding single-species and model ecosystem sensitivity: Data-based comparison. *Environ Toxicol Chem* **18**: 1329–1346.

14. Guerold F. 2000. Influence of taxonomic determination level on several community indices. *Water Res* **34**: 487–492.

15. Hoekstra JA, Vaal MA, Notenboom J, Slooff W. 1994. Variation in the sensitivity of aquatic species to toxicants. *Bull Environ Contam Toxicol* **53**: 98–105.

16. Brock TCM. 2000. Ecological risks of pesticides in freshwater ecosystems. Part 2: Insecticides. Alterra-Rapport 089. Alterra—Green World Research, Wageningen, The Netherlands.

17. European Commission. 2002. Guidance document on aquatic eco-toxicology in the frame of the Directive 91/414/EEC. Working Document of the European Commission 8075/VI/97 rev 9. European Commission, Brussels, Belgium.

18. Lowell RB, Culp JM. 1999. Cumulative effects of multiple effluent and low dissolved oxygen stressors on mayflies at cold temperatures. *Can J Fish Aquat Sci* **56**: 1624–1630.

19. Van der Geest HG, Soppe WJ, Greve GD, Kroon A, Kraak MHS. 2002. Combined effects of lowered oxygen and toxicants (copper and diazinon) on the mayfly *Ephoron virgo*. *Environ Toxicol Chem* **21**: 431–436.

20. Liess M, Champeau O, Riddle M, Schulz R, Duquesne S. 2001. Combined effects of ultraviolet-B radiation and food shortage on the sensitivity of the Antarctic amphipod *Paramoera walkeri* to copper. *Environ Toxicol Chem* **20**: 2088–2092.

21. Van den Brink PJ, Van Wijngaarden RPA, Lucassen WGH, Brock TCM, Leeuwangh P. 1996. Effects of the insecticide Dursbant 4E active ingredient chlorpyrifos in outdoor experimental ditches: II. Invertebrate community response and recovery. *Environ Toxicol Chem* **15**: 1143–1153.

22. Liess M, Schulz R. 1999. Linking insecticide contamination and population response in an agricultural stream. *Environ Toxicol Chem* **18**: 1948–1955.

Chapter III

2005

Environmental Toxicology and Chemistry, Vol. 24

ANALYZING EFFECTS OF PESTICIDES ON INVERTEBRATE COMMUNITIES IN STREAMS

MATTHIAS LIESS* and PETER CARSTEN VON DER OHE

Department of Chemical Ecotoxicology, UFZ - Centre for Environmental Research
Leipzig-Halle GmbH, Permoserstrasse 15, 04318 Leipzig, Germany

ABSTRACT

The aim of this investigation was to find patterns in aquatic invertebrate community composition that are related to the effects of pesticides. Investigations were carried out in 20 central European streams. To reduce the site-specific variation of community descriptors due to environmental factors other than pesticides, species were classified and grouped according to their vulnerability to pesticides. They were classified as SPEcies At Risk (SPEAR) and SPEcies not At Risk ($\text{SPE}_{\text{not}}\text{AR}$). Ecological traits used to define these groups were: (i) sensitivity to toxicants, (ii) generation time, (iii) migration ability, and (iv) presence of aquatic stages during time of maximum pesticide application. Results showed that pesticide concentrations of 1/10 to 1/100 of the acute 48-h LC50 of *Daphnia magna* resulted in a short-term reduction of abundance and number of SPEAR and a corresponding increase in $\text{SPE}_{\text{not}}\text{AR}$. Concentrations of 1/100 to 1/1000 of the acute 48-h LC50 of *Daphnia magna* correlated with a long-term change of community composition. However, number and abundance of SPEAR in disturbed stream sections are greatly increased when undisturbed stream sections are present in upstream reaches. This positive influence compensated for the negative effect of high concentrations of pesticides. The results emphasize the importance of considering ecological traits and re-colonization processes on the landscape level for ecotoxicological risk assessment.

---

* To whom correspondence may be addressed: matthias.liess@ufz.de

INTRODUCTION

The EU Uniform Principles for the assessment of pesticides require that if the preliminary risk characterization indicates potential concerns, registration cannot be granted unless it can be demonstrated that "... under field conditions no unacceptable impact on the viability of exposed organisms ..." occurs. To date, such assessments have been made by conducting higher-tier studies, which have included a range of laboratory and semi-field experiments. Therefore, it is still not clear to what extent pesticides change population dynamics and community structures in the field. Recently, some studies have quantified pesticide exposure, adverse effects on aquatic life and recovery of these invertebrate communities in the field. Mortality of six mayfly species in an Australian river was linked to endosulfan contamination due to runoff [1]. Other investigations also found a link between mortality of several invertebrate species and insecticide concentrations in streams [2,3]. Several invertebrate species that declined in abundance due to pesticides were found to recover within a year [2]. Nevertheless, most existing studies lack (i) sufficient numbers of investigations in various streams to evaluate the frequency of potentially harmful events in a specific region, (ii) evaluation of long-term effects on invertebrate communities, and (iii) quantification of the recovery of impacted communities due to re-colonization from undisturbed stream sections. The inclusion of habitat quality may put the risks resulting from contamination in context with other stressors.

According to these open questions, the aim of the present investigation was to find patterns in community composition that were related to the effect of pesticides. It is challenging in field investigations to reveal the importance of a specific environmental factor, because other environmental factors may mask possible effects. Therefore, to tackle this problem a new approach that aims at reducing variability in community characterization is presented.

METHODS

Study area

The study area is located around Braunschweig, Lower Saxony, Germany. The dominant land use is agricultural (field 61 %, forest 34 %, pasture 5 %; Fig. 1). The most common crops in the catchments are sugar beets, winter barley, and winter wheat. The investigation was carried out in an area where sites had a risk of runoff ranging from “very low” (level 0) to “high” (level 5), on a scale ranging from level 0 to level 6 (“very high”), defined for German agricultural areas [4].

Description of streams

Twenty sites, located on first-order streams, were selected to match the following requirements: all-year water flow; no dredging in the years before and during the investigation; no pollution from other than agricultural non-point sources; various pesticide loads, stemming from differences in the percentage of adjacent arable land. Nine streams were monitored for one year, six streams were monitored for two years, and five for three years (11 sites in 1998, 11 sites in 1999, and 14 sites in 2000). The streams investigated for three years spanned the entire range of measured toxicity. Data on streams that were investtigated for several years were pooled to avoid temporal pseudo-replication. Physical and chemical standard parameters were measured monthly in April (period before the application of insecticides), May, and June. Oxygen, pH and temperature were recorded with instruments made by WTW (Weilheim, Germany). Concentrations of nitrate, nitrite, ammonium and phosphate were determined in the field with colorimetric tests by Visicolor® (Macherey & Nagel, Germany). Detection limits were 5.0, 0.005, 0.025 and 0.25 mg/L, respectively. During April, May, and June 1998, the concentrations of suspended particles were estimated weekly at nine sites using sediment traps [5].

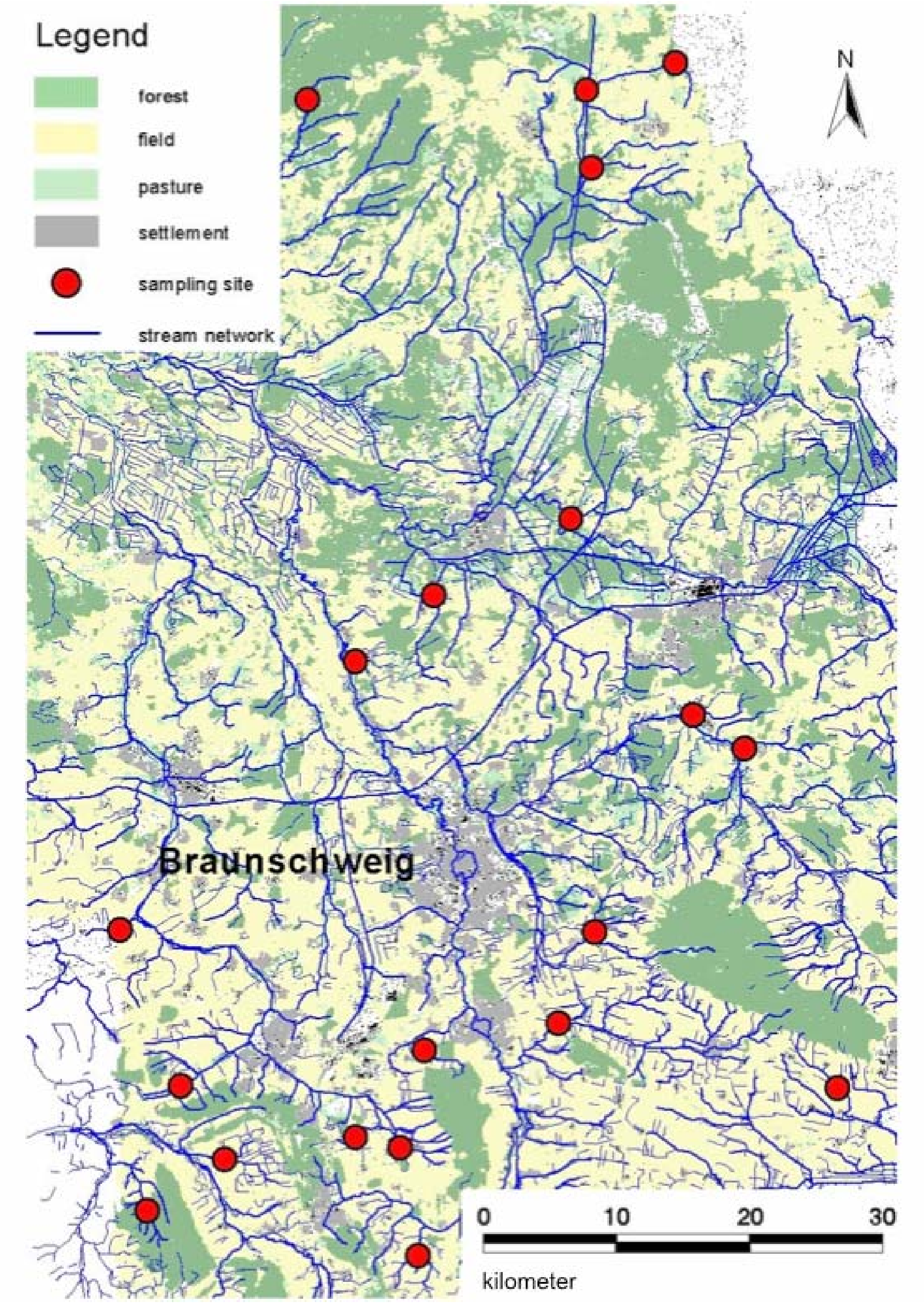


Fig. 1. Stream network, land use pattern and distribution of the sampling sites.

Stream width was measured at each location at mean water level. The biological oxygen demand was measured once during mid-March, as during this time oxygen depletion might occur due to field applications of liquid manure. However, application of liquid manure was not observed in any of the investigated catchments. We investigated in-stream structure and channel alteration of sites by visually estimating the percentage of various substrates and macrophytes in a 50 m reach within the stream on the base of the German classification system of morphological structure [6] (Table A1). Stream sections up- and downstream of the investigated sites that were bordered by forest or meadows at least 50 m wide were identified using GIS maps. The length of these stream sections and the distances from the investigated sites yielded estimates of the potential for in-stream re-colonization by invertebrates. No correlation between any of the investigated parameters with the measured toxicity was observed. Hence, the description of sites in Table A1 is an average of all sites.

Quantification of pesticides

The study sites did not receive point-source inputs of pesticide residues. Therefore, we assumed that pesticide peak concentrations in the streams we studied were due to runoff-induced inputs via nonpoint sources, and that these inputs were the dominant source of exposure to pesticides [7]. We used two event-controlled runoff sampling systems to characterize the exposures. One system was an automated active sampler that measured conductivity and water level in the stream continuously. Samples of 500 ml were obtained every 8 min for one hour (pooled) when runoff was indicated by a decline in conductivity of more than 10 % within 10 min, or if water level increased by ≥ 5 cm [8]. The samples were pre-filtered (0.2 µm) and pumped under high pressure (6 bar) on-site through a C18 column (Bakerbond, Baker; Hannover, Germany). The second system consisted of two passive samplers. Each of these was a 1-L bottle mounted in the stream. Rising water level triggered sampling, and the bottles then filled through a thin (5-mm dia) glass tube, 10 cm in length. The glass tubes of the samplers were positioned 5 and 10 cm above medium water level. The water samples collected by these devices were pre-filtered (0.2 µm) and processed by solid-phase extraction with C18 columns in the laboratory. Both sampling systems were checked every week from April to July, the period before and during the most toxic pesticides (insecticides) are used and detectable in this area [5,8].

Selection of analyzed pesticides was done on the base of use information provided by the local agricultural advisory board (Pflanzenschutzamt). Analyzed pesticides are listed in Table A1. Pesticide measurements were made with a GC/ECD (gas chromatograph NP 5990, Series II; Hewlett-Packard, Avondale, PA, USA). The values were confirmed with GC/MS (negative chemical ionization, a Varian 3400 gas chromatograph (Varian, Walnut Creek, CA, USA). The GC/MS was fitted with an HP 7673 autosampler, which was directly capillary-coupled to the quadruple mass spectrometer SSQ 700 (Finnigan; Bremen, Germany), with a quantification limit of 0.05 µg/L. The results obtained from the two sampling systems did not differ significantly, so the data were pooled by sampling method. Data on the measured pesticide concentrations are summarized in Table A1 and A3.

Derivation of the index of toxicity

To compare the toxicity of pesticides present during runoff events in the different streams, toxic units (TU) were calculated from the measured concentrations [9]. TU values for each compound were based on the acute (48-h) LC50 of *Daphnia magna* (equation 1, below; from [10]):

$$TU_{(D.\ magna)} = \log (C_i / LC50_i) \qquad (1)$$

where $TU_{(D.\ magna)}$ is the toxic unit of the pesticide "*i*" with the highest $TU_{(D.\ magna)}$ for one runoff event; C_i = the concentration (µg/L) of the respective pesticide "*i*", and $LC50_i$ = the corresponding LC50 (48-h) of *Daphnia magna* exposed to substance "*i*" (µg/L).

A TU value of –5 was assigned for the two sites where no pesticides were found. For additional calculations, the highest calculated $TU_{(D.\ magna)}$ based on the measurements of pesticides at one site was used. We also evaluated the possibility of (i) adding all pesticides detected in one runoff event, and (ii) adding all pesticides detected in one year. We used $TU_{(D.\ magna)}$ calculated with the highest $TU_{(D.\ magna)}$ for one substance, because this was the most basic assumption. This method also yielded slightly higher correlations between TU values and community endpoints, compared to correlation values computed with TU values calculated using methods (i) or (ii).

Macroinvertebrate sampling

Invertebrates were collected 3 times per year (April, May, June) from 1998 to 2000, with a Surber sampler (area: 0.062 m^2; 1 mm mesh). On each sampling date, four samples were taken randomly over a stream length of 50 m. Macroinvertebrates were sorted in white plastic tubs, preserved in 70 % EtOH and identified to species level except for dipterans, which were identified to family. Earlier investigations showed that the highest concentrations of the most toxic insecticides in the investigated area occurred during May and, to a lesser extent, in June [2,8]. Hence, our April surveys occurred before the main period of contamination and were regarded to represent possible long-term effects of pesticides from the previous year. Similarly, the May surveys occurred when pesticide levels were highest, so the May and June surveys represent possible short-term effects of pesticides from the same year.

Species at risk (SPEAR)

Site-specific combinations of environmental factors resulted in a unique composition of species at each site, masking the effect of individual environmental factors. In our investigation, this difficulty was reduced by grouping species according to their sensitivity to pesticides [11] and their life-cycle traits that are known to influence recovery from toxicant effects [12,13]. The sensitivity to organic toxicants was assigned on the basis of the classification of [14] and its revision by [11]. Species with a value of > –0.36 (median of sensitivity, from [11]) were regarded as sensitive. Three main life-cycle traits were used. These were generation time, migration ability and presence of sensitive aquatic stages during the time of maximum exposure to pesticides. Generation time ≥ 0.5/year was regarded as potentially sensitive due to a slow recovery potential [12,13,15]. If no data on generation time could be found for a species, we assigned it the value for its closest relative. Low migration ability was presumed to reflect reduced potential for recolonization. Species regarded as being not at risk due to their better ability to migrate included *Gammarus pulex, Limnephilus lunatus,* and *Anabolia nervosa* [16]. Species for which adults emerged before

May (before the time of maximum pesticide application) were regarded as insensitive, because exposure to aquatic stages would not occur. This categorization was made based on best professional judgment. The three traits were combined with the Boolean “AND” in our literature searches. Only if all three traits were present did we consider a species to have a potential sensitivity to pesticides; the species was then regarded as a species at risk of being affected by pesticides. All life-cycle traits used as SPEAR attributes were obtained from the literature [17-25]. All classifications followed the definitions listed in Table A2.

Community endpoints

The following endpoints for the invertebrate communities were calculated: Species number – the mean number of species found on the three sampling dates during one year. Diversity and evenness – according to Shannon-Wiener. $\mathrm{SPEAR}_{(\mathrm{number})}$ – the number of species at risk according to the definition given above. $\mathrm{SPEAR}_{(\mathrm{abundance})}$ – the abundance of species at risk calculated as the sum of the log abundance of each species. % $\mathrm{SPEAR}_{(\mathrm{abundance})}$ – the ratio of the abundance of species at risk compared to the abundance of all species. $\mathrm{SPE}_{\mathrm{not}}\mathrm{AR}_{(\mathrm{number})}$ – the number of species not at risk. And, $\mathrm{SPE}_{\mathrm{not}}\mathrm{AR}_{(\mathrm{abundance})}$ – the abundance of species not at risk. Temporal change of community endpoints was assessed from April to May, and from April to June, based on the concept of SPEAR.

Statistical analyses

For multivariate linear regressions, data normality was determined with the Kolmogoroff-Smirnoff test. Where significant deviation from the assumption of normality occurred, the data were transformed. Equality of variances was verified using Levene’s homogeneity-of-variance test. Environmental factors were not inter-correlated with $\mathrm{TU}_{(D.\ magna)}$ (linear regression, $p > 0.05$). Statistical procedures used were: stepwise entering of variables (criteria: probability of F to enter ≤ 0.05, probability of F to remove ≥ 0.10), adjusted r^2. Differences between groups of sites with different $\mathrm{TU}_{(D.\ magna)}$ were investigated using one-way ANOVA. Dunnett's multiple comparison test was used to detect significant differences among means. Differences between slopes of regression lines of sites with and without forested stream sections (covariate) were identified using ANCOVA. Multivariate linear regressions were carried out with SPSS® 11 for Macintosh® (Chicago, IL, USA). ANOVA and ANCOVA were carried out with Prism® for Macintosh® (GraphPad Software, San Diego California USA).

RESULTS

Environmental conditions

During the investigation period (1.8 years per site), pesticides were detected in 125 runoff events at 18 of the 20 sites. Runoff events with a toxicity > –2.5 occurred between May and July; most of the contaminated-runoff events occurred in May (57 %), followed by June (32 %) and July (11 %). In April, no runoff events with a toxic unit above –3 were detected. The four pesticides contributing the most to the Toxic Units ($\mathrm{TU}_{(D.magna)}$) were Parathion-ethyl, Azoxystrobin, Kresoxim-methyl, and Ethofumesat (Table A3). $\mathrm{TU}_{(D.magna)}$ varied between sites, but 40 % of the between-year variance among sites that were investigated more than 1 year was explained by the value measured in one year (linear regression of $TU_{(D.magna)}$ of $year_n$ with $year_{n+1}$; $r^2 = 0.40$, $p < 0.01$).

The morphological diversity of the streams was poor because stream beds contained a large percentage of fine substrates and the amount of plants or organic debris was slight in most cases. According to the German classification system for assessing stream morphology, the sites were classified as “strong and heavily modified” [6]. Based on the morphological and standard water quality parameters, the sites in the area of Braunschweig were typical of those for small lowland streams [26]. The physical and chemical characteristics of the streams are summarized in Table A1.

Correlating environmental parameters and community descriptors

The measure for toxic stress of pesticides $TU_{(D.\ magna)}$ best described the variance of community descriptors related to SPEAR (Table 4). In general, the number and abundance of SPEAR correlated negatively with $TU_{(D.\ magna)}$. In contrast, the average number and abundance of species not at risk ($SPE_{not}AR$) did not correlate with toxic stress. Other parameters contributing to the variability of SPEAR are length of forested stream sections, type of substrate, and coverage with submersed plants (Table 4).

The two subdivisions of the SPEAR parameter – the sensitivity of species to pesticides and life-cycle traits influencing the recovery potential (generation time, migration ability, and emergence time) – contributed about equally to the correlation of abundance of SPEAR with toxic stress. On average, 57 % ($p < 0.01$) of the explained variance was accounted for by species sensitivity, and 44 % ($p < 0.01$) of the explained variance was accounted for by life-cycle traits related to recovery potential.

Threshold of community response to toxic stress

To determine the $TU_{(D.\ magna)}$ at which a change in community structure became apparent, the most sensitive endpoint was used: ($SPEAR_{(abundance)}$-June), compared to the abundance of all species in June (Table 4). This ratio indicated a significant change in community structure compared to sites with a low $TU_{(D.\ magna)}$ (i.e., < –4), relative to sites in the range of –3 to –2 TU and higher (Fig. 2; ANOVA, Dunnett's multiple comparison test, $p < 0.01$). The total number and the total abundance of SPEAR in April showed a dependence on $TU_{(D.\ magna)}$ also, but to a lesser extent (ANOVA, Dunnett's multiple comparison test). Then, significant differences between sites with a TU < –4 compared to sites with a TU in the range of –3 to –2, –2 to –1, and –1 to 0 were indicated by *p* values of < 0.01, < 0.05, and < 0.01, respectively.

The sensitivity of species and the life-cycle traits contributed about equally to the reduced proportion of SPEAR at toxic stress. The number of sensitive species and the number of species with a low recovery potential according their life-cycle traits were reduced substantially at $TU_{(D.\ magna)}$ from –3 and higher (ANOVA; Dunnett's multiple comparison test, $p < 0.01$).

Temporal changes in community structure

The abundance of SPEAR decreased from April until May at sites with values of $TU_{(D.\ magna)}$ exceeding (–2 to –1), compared to sites where $TU_{(D.\ magna)}$ values were below –4 (Fig. 3). Furthermore, an increase in abundance of $SPE_{not}AR$ occurred from April until June at sites where $TU_{(D.\ magna)}$ values were greater than (–3 to –2), compared to the sites where $TU_{(D.\ magna)}$, values were below –4 (Fig. 4).

Table 4. Coefficients of multiple determination (r^2) and standardized partial regression coefficients (beta; $p < 0.05^*$, $p < 0.01^{**}$, optimized model) for correlations between environmental parameters and community metrics for 20 sites in streams during April – June, 1998 – 2000 (1). All parameters not shown, but described in the Methods section, were minimally important in terms of explaining investigated community endpoints.

	r^2 (Sum)	df2	F	Tox. Unit (m)	Forest length (%)	Hard-substrate (%)	Sand (%)	Submerse plants (%)
Species number - Average	0.49	17	10.0	- 0.45*	+ 0.44*	-	-	-
Diversity - Average	0.28	18	8.2	-	+ 0.56	-	-	-
$SPEAR_{(number)}$ - Average	0.72	17	25.2	- 0.72**	+ 0.28*	-	-	-
$SPEAR_{(number)}$ - April	0.81	15	18.0	- 0.50**	+ 0.28*	+ 0.32*	-	+ 0.33**
$SPEAR_{(number)}$ - June	0.49	18	19.0	- 0.72**	-	-	-	-
$SPEAR_{(abund.)}$ - Average	0.60	18	29.5	- 0.79**	-	-	-	-
$SPEAR_{(abund.)}$ - April	0.69	16	15.2	- 0.53**	-	+ 0.43**	-	+ 0.30*
$SPEAR_{(abund.)}$ - June	0.53	18	22.6	- 0.75**	-	-	-	-
% $SPEAR_{(abund.)}$ - Average	0.62	18	31.6	- 0.80**	-	-	-	-
% $SPEAR_{(abund.)}$ - April	0.57	17	13.5	- 0.56**	+ 0.37*	-	-	-
% $SPEAR_{(abund.)}$ - June	0.60	18	29.1	- 0.79**	-	-	-	-
$SPEnotAR_{(abund.)}$ - Average	0.26	18	7.6	-	-	-	- 0.55**	-
$SPEnotAR_{(abund.)}$ - June/April (temporal change)	0.46	17	17.3	+ 0.70**	-	-	-	-

(1) Included in the table are parameters that account for at least 25% of the explained variance for the most relevant parameter, and adding at least 7.5 % of explained variance for all successive parameters

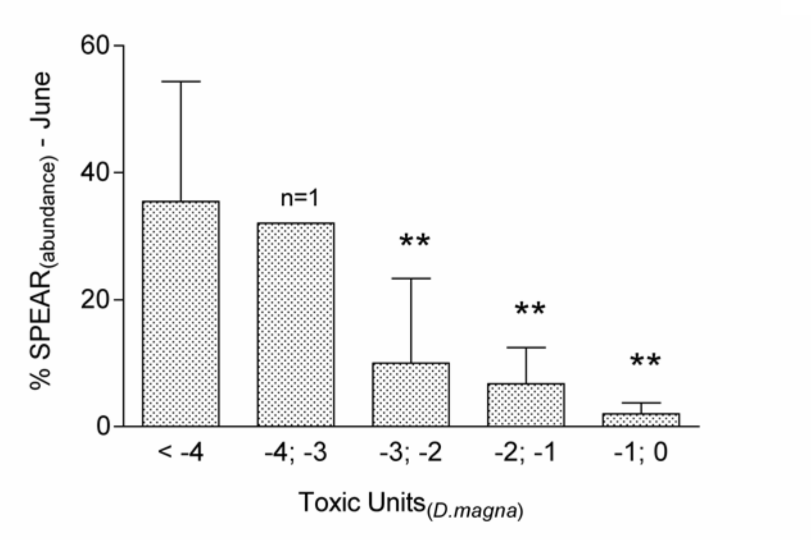


Fig. 2. Relation between Toxic $Units_{(D.\ magna)}$ and the benthic invertebrate community structure expressed as percentage of the abundance of species at risk in June (%$SPEAR_{(abundance)}$-June). Asterisks indicate significant differences from the sites with low $TU_{(D.\ magna)}$ (i.e., those below –4) (ANOVA, Dunnett's multiple comparison test, **p < 0.01). Error bars show SD's.

Contribution of uncontaminated stream sections to recovery

The presence of forested stream sections > 200 m in length and < 4000 m upstream of the investigated sites had a strong influence on the intercept and slope of the correlation between $\mathrm{TU}_{(D.\ magna)}$ and SPEAR in April. When forested stream sections were present, the numbers of SPEAR tended to be greater. At the same time, the reduction of SPEAR with increasing $\mathrm{TU}_{(D.\ magna)}$ was greater than at sites without forested stream sections (ANCOVA, $p < 0.05$). However, the positive influence of forested stream sections upstream of the investigated sites compensated the negative effect of high $\mathrm{TU}_{(D.\ magna)}$ on SPEAR. Indeed, sites with TU > –2 and forested stream sections contained a number and abundance of SPEAR similar to those at sites with TU < –3 without forested stream sections (Fig. 5). The described differences of sites with and without forested stream sections were apparent only in April – the time period before the highest toxic units have been measured. The differences of sites with and without forested stream sections were not detectable in June – the time period directly after the highest toxic units have been measured.

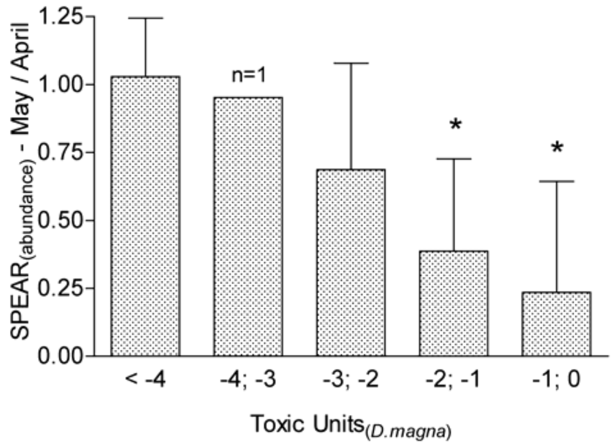


Fig. 3. Decrease of abundance in SPEAR, from April to May. Asterisks indicate significant differences from the sites with low $\mathrm{TU}_{(D.\ magna)}$ (i.e., those below –4) (ANOVA, Dunnett's multiple comparison test, $^{*}p < 0.05$). Error bars show SD’s.

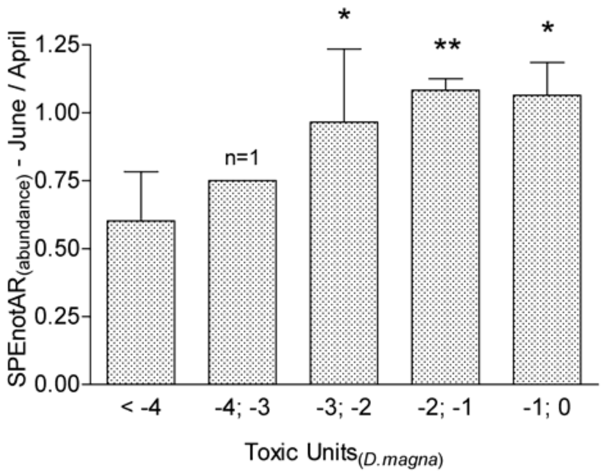


Fig. 4. Increase of abundance in $\mathrm{SPE_{not}AR}$, from April to June. Asterisks indicate significant differences from the sites with low $\mathrm{TU}_{(D.\ magna)}$ (i.e., below –4) (ANOVA, Dunnett's multiple comparison test, $^{*}p < 0.05$, $^{**}p < 0.01$). Error bars show SD’s.

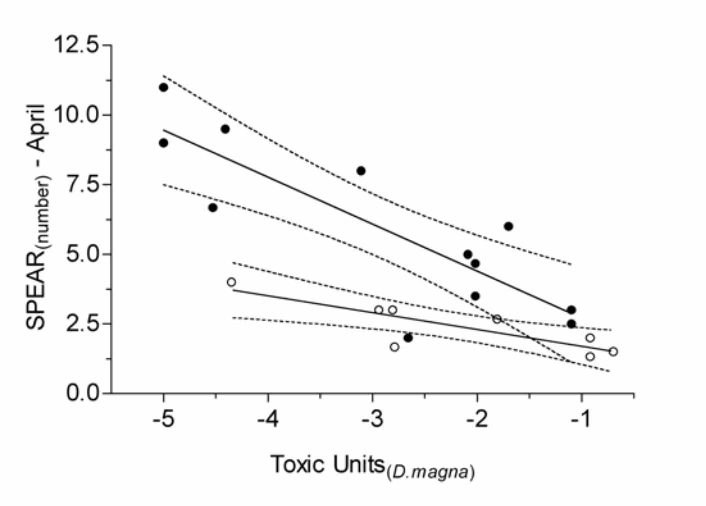


Fig. 5. Relation between Toxic Units$_{(D.\ magna)}$ and the number of species at risk in April (SPEAR$_{(\mathrm{number})}$-April). Sites are differentiated on the basis of the presence of forested stream sections closer than 4000 m upstream of the study site (filled circles; linear regression, $r^2 = 0.70$, $p < 0.01$) or absence of such sites (open circles; linear regression, $r^2 = 0.70$, $p < 0.05$). Confidence bands show the 95% confidence limit for the respective means. The slopes of the two regression lines differ (ANCOVA, $p < 0.05$).

DISCUSSION

Finding patterns in community composition

The aim of the present investigation was to find patterns in community composition that are related to the effect of agricultural activities. However, as is well known, it is very difficult to determine the importance of a specific environmental factor in field investigations that involve different sites. Each site has a unique combination of environmental factors and thus a unique composition of species. This situation obscures the effect of any particular environmental factor. In the present investigation, we reduced this problem by grouping species according to their sensitivity to pesticides [11] and life-cycle traits known to influence recovery from toxicant stress [12,13]. The approach of grouping species at risk (SPEAR) has the advantage of reducing the variability of the site-specific community characterization, and increasing the ability to detect the effect of pesticides on community composition at low toxic units (TU –2 to –3) - values that are equivalent to < 1/100 of the 48-h LC50 $_{(Daphnia\ magna)}$ (Table 4, Fig. 2). However, the SPEAR approach also has some disadvantages. For example, because species-level data are aggregated according to sensitivity and life-cycle traits related to recovery, the effect of a pesticide cannot be assigned to any particular species or taxon.

Temporal changes in community structure – reduction in SPEAR

Sites characterized by high TUs (between –1 and 0 based on the 48-h LC50 $_{(Daphnia\ magna)}$) showed a 75% reduction of SPEAR from April to May, when the highest concentrations of pesticides were measured (Fig. 3). Other investigations of streams in agricultural areas also reported that pesticides from surface runoff can cause acute mortality of benthic invertebrates when they reach the range of the 48-h LC50 $_{(Daphnia\ magna)}$: For example, mortality of the amphipod *Gammarus pulex* occurred at 26.8 µg/L Carbofuran, a level that is 2.5 times lower than the 48-h LC50 $_{(Daphnia\ magna)}$ [27]. Other examples include the amphipod *Gammarus pulex* and the caddisfly *Limnephilus lunatus*, which suffered mortality at

6.0 µg/L Parathion-ethyl (3.3 times above the 48-h LC50 $_{(Daphnia\ magna)}$) [2]. Similarly, the dipteran *Chironomus spp.* had greater mortality at 0.7 µg/L Azinphos-methyl (3.6 times below the 48-h LC50 $_{(Daphnia\ magna)}$) [28]. The present investigation also revealed a 60% reduction in SPEAR from April to May, when TUs were between –1 and –2. At that time, pesticide concentrations were about 10 times lower than the 48-h LC50 $_{(Daphnia\ magna)}$ (Fig. 3). This finding may be explained by the fact that long-term effects of non-narcotic substances can occur at concentrations that are 10 times lower than concentrations needed to cause acute (48-h) effects [29].

No indication was found in the present investigation that parameters other than pesticides (e.g., hydrodynamic stress, water quality parameters, etc.) might be responsible for the observed short-term reduction of sensitive species. In agricultural areas, hydrodynamic stress in streams due to increased current velocity and suspended particles during runoff events can occur frequently throughout the year [8]. Hence, this stressor is probably not responsible for the short-term reduction of individuals that occurred only during May. Furthermore, in another investigation involving year-long sampling, the only significant reduction in invertebrate taxonomic richness and abundance was found in May, when the highest pesticide concentrations were measured [2]. Concentrations of nitrite and ammonia high enough to cause toxicity also would not be restricted to May. Rather, maximum concentrations of these constituents should occur either near the beginning of the growing season (due to application of fertilizer or manure from animals), or during late summer (due to elevated temperature and low water levels) [30,31]. The emergence of SPEAR during May probably does not contribute much to the reduction of abundance at sites where TU levels are high, and abundance of SPEAR in stream benthic invertebrate communities at sites where TU levels were low did not change during May. Based on these considerations, the authors suggest that the short-term changes in SPEAR during May are best attributed to pesticides. If so, acute and chronic laboratory toxicity data for *D. magna* can provide an indication of the magnitude of concentrations at which pesticides cause a reduction in sensitive indigenous invertebrates.

Temporal changes in community structure – alteration of SPE$_{not}$AR

Sites characterized by low TUs (below –3, based on the 48-h LC50 $_{(D.\ magna)}$) showed a 60% reduction of SPE$_{not}$AR between April and June (Fig. 4). This reduction was not observed at sites where TU values were high (above the range of –3 and –2). This pattern could result from an indirect positive effect of pesticides on SPE$_{not}$AR due to negative effects of pesticides on sensitive species. Such negative effects might occur within the range of lethal effects (TU$_{(D.\ magna)}$ –2 to 0) and sublethal effects (TU$_{(D.\ magna)}$ –3 to –2) [32]. We did not demonstrate sublethal effects in this study. However, previous investigations support the idea that long-term effects occur with short-term exposure to concentrations that are more than 100 times lower than concentrations that can cause acute mortality. For example, development of the caddisfly *Limnephilus lunatus* was delayed in outdoor microcosms several months after a 1-h exposure to Fenvalerate at 1/1000 of the acute LC50 [33]. Similarly, an increase in mortality, a decrease in adult weight, and a delay in development of the caddisfly *L. lunatus* were observed in outdoor microcosms several months after a 1-h exposure to Fenvalerate at concentrations that were 1/100, 1/1000, and 1/10,000 of the acute LC50 [34].

Finally, mortality of Chironomidae and *Hyalella azteca* increased in littoral enclosures several weeks after short-term exposure to Es-Fenvalerate at a concentration that was 1/100 of the acute LC50 [35]. Such investigations show that low concentrations of pesticides ($TU_{(D.\ magna)}$ –3 to –2) may affect the taxonomic structure of benthic invertebrate communities, as indicated by the proportion of SPEAR in the current study.

Factors other than pesticides did not appear to be responsible for the observed long-term reduction of SPEAR. Hydrodynamic stress accompanying runoff, like other recurrent stressors, might favor species that have a high recovery potential due to a short generation time and good migration ability. So, the long-term reduction in SPEAR might be partly due to hydrodynamic stress. However, the affected taxa include those with a low recovery potential and species that are sensitive to toxicants, and no evidence suggests that the latter group is especially vulnerable to hydrodynamic stress. Thus, pesticides, rather than hydrodynamic stress, seem more likely to account for the observed reduction in sensitive species, even though they were present at low concentrations. The same logic applies to the rare events of dredging (not observed in the years before and during the investigation). Suspended particles also probably can be ruled out as causal factors. The results of experiments indicate that, compared to pesticides, suspended particles may not be very important to benthic invertebrates that dominate streams in agricultural areas. For example, the addition of suspended particles to experimental stream ecosystems in amounts up to 1.7 g/L did not alter the abundance or number of species of benthic invertebrates, or the rate of aquatic insect emergence, or the rate of leaf-litter decomposition [36]. The long-term survival of the caddisfly *L. lunatus* in outdoor test systems was not reduced by suspended particles (3 g/L), compared to controls [34,37]. The 3 g/L concentration of suspended particles in the cited investigations can be regarded as relevant to agricultural streams [8]. Finally, nitrite and ammonia probably cannot account for the change in SPEAR, because the concentrations of these constituents were lower than those that can be tolerated by most invertebrates. For example, *Gammarus pulex* is relatively sensitive to ammonia, nitrite and other types of organic pollution [38,39], but was abundant in our study, especially at sites with the highest pesticide concentrations. In contrast, *Asellus aquaticus* is more resistant than *Gammarus pulex* to hypoxia and to un-ionized ammonia [39-41], but is classified as sensitive to pesticides in the present investigation.

Based on these considerations, the observed changes in SPEAR may be attributed most parsimoniously to the effect of pesticides. Factors other than pesticides clearly can influence the benthic invertebrate community structure (and probably SPEAR), too, but we found no evidence that this occurred at the sites we studied. However, because the levels of contamination may have been insufficiently quantified, it remains uncertain at which concentration these changes occur.

Contribution of uncontaminated stream sections to recovery

In June, the correlation between community composition (SPEAR) and TUs was stronger than it was in April (Table 4). However, numbers of benthic invertebrate SPEAR in June were not affected significantly by the amount of forested stream sections. In contrast, the length of forested stream sections upstream of the investigated site did relate significantly to the number and proportion of SPEAR in April. The positive effect of upstream forested stream sections on SPEAR at the downstream sites was not due to lower concentrations of contaminants at the downstream sites, as the correlation between contamination at the investigated sites and length of forested stream sections was not significant. Their relatively large distance from the study sites (several km, for many of the forested stream sections) may explain the lack of a measurable influence on physical and chemical parameters measured at the sites. On the basis of this result, we suggest that the positive effect of forested stream sections on SPEAR at downstream sites can be attributed to in-stream re-colonization by invertebrates from the undisturbed stream sections where diversity is greater. This hypothesis is supported by the fact that forested stream sections had a positive effect on SPEAR only in April – 10 months after the time when pesticide concentrations were greatest. The positive effect of forested stream sections was not apparent in June, immediately after the period when pesticide concentrations were highest. Any water quality parameter that would have been ameliorated by forested stream sections should have exerted a positive influence in June, as well. In contrast, a positive effect of re-colonization from forested stream sections is likely to be greatest in April, when more time for re-colonization was available.

The hypothesis above is supported by the fact that in lotic habitats, passive dispersal of invertebrates by water currents or downstream drift can displace from 1 % to 2 % of benthic stream organisms. Drift is the most common means of transport for many stream invertebrates [42]. Various species of invertebrates emigrating from undisturbed stream sections can travel several km, by drifting, within a few months [43]. However, dispersal ability is species-dependent, and a more detailed evaluation would be needed to determine the particular requirements of species with very low dispersal abilities [44].

Cumulative risk

Including proximity and amount of uncontaminated stream sections in our habitat-quality assessments helped reveal the effects of pesticides. But they also allowed us to put risks due to contamination into context with other stressors. The levels of biological impairment observed at sites with high concentrations of pesticides and good habitat quality were similar to those at sites where pesticide concentrations were low but habitat quality was poor. For the streams we studied, habitat quality (indexed as undisturbed upstream sections) seemed about as important as toxicity, expressed as TU. Thus, landscape and land-use information may increase predictability in the assessment of risk due to pesticides. For streams, we suggest that the geographical unit of assessment should be extended to include the recovery potential of the landscape associated with undisturbed upstream sections.

Acknowledgement—This work was presented and discussed as an oral presentation at the EPiF workshop (Le Croisic, France, 21.10.03-24.10.03, organized by the EU and the SETAC-Europe). The authors are indebted to the participants, especially to Valery Forbes, Steve Maund, Martin Streloke, and Richard Sibly for valuable discussions. We are grateful to Norbert Berenzen, Holger Schulz, and Jörn Wogram for their invaluable contribution to the fieldwork. We thank anonymous reviewers for very helpful comments on the manuscript.

REFERENCES

1. Leonard AW, Hyne RV, Lim RP, Pablo F, Van den Brink PJ. 2000. Riverine endosulfan concentrations in the Namoi River, Australia: Link to cotton field runoff and macroinvertebrate population densities. *Environl Toxicol Chem* **19**: 1540-1551.

2. Liess M, Schulz R. 1999. Linking insecticide contamination and population response in an agricultural stream. *Environ Toxicol Chem* **18**: 1948-1955.

3. Schulz R, Thiere G, Dabrowski JM. 2002. A combined microcosm and field approach to evaluate the aquatic toxicity of azinphosmethyl to stream communities. *Environ Toxicol Chem* **21**: 2172–2178.

4. Bach M, Huber A, Frede H-G, Mohaupt V, Zullei-Seibert N. 2000. Schätzung der Einträge von Pflanzenschutzmitteln aus der Landwirtschaft in die Oberflächengewässer Deutschlands - Berichte. UBA, Berlin.

5. Liess M, Schulz R, Neumann M. 1996. A method for monitoring pesticides bound to suspended particles in small streams. *Chemosphere* **32**: 1963-1969.

6. LUA. 1999. Gewässerstrukturgüte in Nordrhein-Westfalen. Landesumweltamt Nordrhein-Westfalen, Essen.

7. Dabrowski JM, Schulz R. 2003. Predicted and measured levels of azinphosmethyl in the Lourens River, South Africa: Comparison of runoff and spray drift. *Environ Toxicol Chem* **22**: 494-500.

8. Liess M, Schulz R, Liess MH-D, Rother B, Kreuzig R. 1999. Determination of insecticide contamination in agricultural headwater streams. *Water Research* **33**: 239-247.

9. Peterson DR. 1994. Calculating the aquatic toxicity of hydrocarbon mixtures. *Chemosphere* **29**: 2493-2506.

10. Tomlin CDS. 2000. The pesticide manual, a world compendium. Crop Protection Publications, Farnham.

11. Von der Ohe P, Liess M. 2004. Relative sensitivity distribution (RSD) of aquatic invertebrates to organic and metal compounds. *Environ Toxicol Chem* **23**: 150-156.

12. Sherratt TN, Roberts G, Williams P, Whitfield M, Biggs J, Shillabeer N, Maund SJ. 1999. A life-history approach to predicting the recovery of aquatic invertebrate populations after exposure to xenobiotic chemicals. *Environl Toxicol Chem* **18**: 2512-2518.

13. Stark JD, Banks JE, Vargas R. 2004. How risky is risk assessment: The role that life history strategies play in susceptibility of species to stress. *Proc Nat Acad Sci* **101**: 732-736.

14. Wogram J, Liess M. 2001. Rank ordering of macroinvertebrate species sensitivity to toxic compounds by comparison with that of *Daphnia magna*. *Bull Environ Contam Toxicol* **67**: 360-367.

15. Van Urk G, Kerkum F, Van Leeuwen CJ. 1993. Insects and insecticides in the lower Rhine. *Water Research* **27**: 205-213.

16. Williams D, Williams NE. 1993. The upstream downstream movement paradox of lotic invertebrates - quantitative evidence from a Welsh mountain stream. *Freshwater Biol* **30**:199-218.

17. Reusch H. 1985. Zur Kenntnis der Köcherfliegenfauna des Niedersächsischen Tieflandes. Hannover.

18. Studemann D, Landolt P, Satori M, Hefti D, Tomka I. 1992. Ephemeroptera. Schweizerische Entomologische Gesellschaft.

19. Hynes HBN. 1977. A Key to the Adults and Nymphs of the British Stoneflies (Plecoptera) with notes on their Ecology and Distribution. Freshwater Biological Association., Scientific Publication 17; 90 S.; Ambleside, UK.

20. Heneghan PA. 2000. The Applied Stream Ecology class database. http://www.ent3orstedu/StreamEcology/database/streamlifehtm

21. Wallace ID, Wallace B, Philipson GN. 1990. A Key to the Case-bearing Caddis Larvae of Britain and Ireland. The Freshwater Biological Association, Cumbria, UK.

22. Tobias W, Tobias D. 1981. Trichoptera Germanica - Bestimmungstafeln für die deutschen Köcherfliegen. Senkenbergische Naturforschende Gesellschaft, Frankfurt am Main.

23. Glöer P, Meier-Brook C, Ostermann O. 1986. Süsswassermollusken - Ein Bestimmungsschlüssel für die Bundesrepublik Deutschland. Deutscher Jugendbund für Naturbeobachtung, Hamburg.

24. Nilsson A. 1996. Aquatic Insects of North Europe - A Taxonomic Handbook. Apollo Books Aps., DK - 5771 Stenstrup, Kirkeby Sand 19.

25. Klausnitzer B. 1984. Käfer im und am Wasser. A.Ziemsen Verlag, Wittenberg.

26. Faasch. 1997. Gewässergütebericht - Ergänzungen 1997. Bezirksregierung Braunschweig

27. Matthiesen P, Sheahan D, Harrison R, Kirby M, Rycroft R, Turnbull A, Volkner C, Williams R. 1995. Use of a *Gammarus pulex* bioassay to measure the effects of transient carbofuran runoff from farmland. *Ecotoxicol Environ Safety* **30**: 111-119.

28. Schulz R, Peall SKC, Hugo C, Krause V. 2001. Concentration, load and toxicity of spraydrift-borne Azinphos-methyl at the inlet and outlet of a constructed wetland. *Ecol Engin* **18**: 241-247.

29. Roex EWM, Van Gestel CAM, Van Wezel AP, Van Straalen NM. 2000. Ratios between acute aquatic toxicity and effects on population growth rates in relation to toxicant mode of action. *Environ Toxicol Chem* **19**: 685-693.

30. Kladivko EJ, Vanscoyoc GE, Monke EJ, Oates KM, Pask W. 1991. Pesticide and nutrient movement into subsurface tile drains on a silt loam soil in Indiana. *J Environ Qual* **20**: 264-271.

31. Chokmani K, Gallichand J. 1997. Use of indicators for assessing the diffused pollution potential on two agricultural watersheds. *Can Agric Engin* **39**: 113-122.

32. Fleeger JW, Carman KR, Nisbet RM. 2003. Indirect effects of contaminants in aquatic ecosystems. *Sci Total Environ* 317: 207-233.

33. Liess M, Schulz R. 1996. Chronic effects of short-term contamination with the pyrethroid insecticide fenvalerate on the caddisfly *Limnephilus lunatus*. *Hydrobiologia* **324**: 99-106.

34. Liess M. 2002. Population response to toxicants is altered by intraspecific interaction. *Environ Toxicol Chem* **21**: 138-142.

35. Lozano SJ, Ohalloran SL, Sargent KW, Brazner JC. 1992. Effects of Esfenvalerate on aquatic organisms in littoral enclosures. *Environ Toxicol Chem* **11**: 35-47.

36. Fairchild JF, Boyle T, English TR, Rabeni C. 1987. Effects of sediment and contaminated sediment on structural and functional components of experimental stream ecosystems. *Water Air Soil Pollut* **36**: 271-293.

37. Schulz R, Liess M. 2001. Runoff simulation with particle-bound fenvalerate in multispecies stream microcosms: importance of biological interactions. *Environ Toxicol Chem* **20**: 757-762.

38. Berenzen N, Schulz R, Liess M. 2001. Effects of chronic ammonium and nitrite contamination on the macroinvertebrate community in running water microcosms. *Water Research* **35**: 3478-3482.

39. Friedrich G. 1990. Eine Revision des Saprobiensystems. *Zeitschrift für Wasser- und Abwasserforschung* **23**: 141-152.

40. Maltby L. 1995. Sensitivity of the crustaceans *Gammarus pulex* (L) and *Asellus aquaticus* (L) to short-term exposure to hypoxia and unionized ammonia: Observations and possible mechanisms. *Water Research* **29**: 781-787.

41. Whitehurst IT. 1991. The *Gammarus* - *Asellus* Ratio As an Index of Organic Pollution. *Water Research* **25**: 333-340.

42. Bilton DT, Freeland JR, Okamura B. 2001. Dispersal in freshwater invertebrates. *Ann Rev Ecol Syst* **32**: 159-181.

43. Hatakeyama S, Yokoyama N. 1997. Correlation between overall pesticide effects monitored by shrimp mortality test and change in macrobenthic fauna in a river. *Ecotoxicol Environ Safety* **36**: 148-161.

44. Purse BV, Hopkins GW, Day KJ, Thompson DJ. 2003. Dispersal characteristics and management of a rare damselfly. *J Appl Ecol* **40**: 716-728.

Chapter IV

2004

Environmental Toxicology and Chemistry, in preparation

AGRICULTURAL INTENSITY AND UPSTREAM CATCHMENT QUALITY: DETERMINANTES OF AQUATIC INVERTEBRATE COMMUNITY STRUCTURE

Peter Carsten von der Ohe*, Carola Alexandra Schriever, Maria Ball,
Chris Holmes and Matthias Liess

Department of Chemical Ecotoxicology, UFZ - Centre for Environmental Research
Leipzig-Halle GmbH, Permoserstrasse 15, 04318 Leipzig, Germany

ABSTRACT

The aim of the present investigation was finding patterns in the structure of invertebrate communities in small headwater streams that could be linked to the potential effects of runoff related stress (e.g. pesticides, hydrodynamic stress). Investigations were carried out at 91 lowland streams that span a wide range of potential agricultural impacts. An indicator was modeled as a quantitative measure of the agricultural intensity, reflecting the potential stress that arises from runoff. To reduce the site-specific variation in the species composition, species were classified as species at risk (SPEAR) and species not at risk of being affected by pesticides according to the SPEAR concept. It could be shown that some of these specific community descriptors were negatively correlated to the indicator of runoff. Thereby, a reduction in the fraction of species at risk abundance from about 50 % at the reference sites to below 5 % at the potentially highest impacted sites could be observed, indicating changes in the community structure due to agricultural activities. Moreover, a reduced proportion of sensitive species was observed for streams receiving effluents from wastewater treatment plants. In contrast, a significant increase in the number of species at risk could be observed in the presence of uncontaminated stream sections. Therefore, the authors conclude that the SPEAR approach is suitable indicating the impairment of headwater streams due to agricultural activities on a landscape level.

---

* To whom correspondence may be addressed: peter.vonderohe@ufz.de

INTRODUCTION

Invertebrate communities in headwater streams are altered by natural disturbances, such as droughts or flooding (MINSHALL et al. 1983; MOLLES 1985) as well as by anthropogenic disturbances. The latter can cause long-term changes in the benthic communities: e.g. physical changes in the habitat (e.g. channelization) or stress due to organic pollution or toxicants. In natural communities, recovery from these disturbances would imply the reestablishment of the community structure to the status prior the initial disturbance (WALLACE 1990). However, in case of an annual insecticide treatment, recovery of the ecosystem functioning (e.g. shredder biomass and leaf litter processing) was observed after two years (WALLACE et al. 1986), whereas recovery in the taxonomic community structure required 5 years (HUTCHENS, JR. et al. 1998).

In this context, the impact of pesticides has been identified as an important agricultural stressor for invertebrate communities in the investigated area (LIESS & SCHULZ 1999). Other studies also found links between acute mortality of invertebrate species and the insecticide contamination of streams (LEONARD et al. 2000; SCHULZ et al 2002). A review of the acute toxic and chronic effects of runoff identified pesticides as one of the major stressors on aquatic communities (COOPER 1993). Moreover, runoff has been determined as the main entry route of short-term pesticide input into streams with agricultural catchments in the investigated landscape (LIESS et al. 1996; LIESS et al. 1999). In the present investigation, most streams are located in agricultural catchments and periodical treatments of similar amounts of pesticides were assumed (LIESS & VON DER OHE 2005). As a result, long-term effects of agricultural activities are expected and a runoff related indicator was modeled as a general measure of the agricultural intensity. In contrast, recent investigations identified forested stream sections upstream of sampling sites to be beneficial for the invertebrate communities (LIESS & VON DER OHE 2005) and are therefore considered as well.

An important aim of the Water Framework Directive of the European community (CEC 2000) is to protect and enhance the status of our aquatic ecosystems and to ensure a “good quality of streams”. According to the directive there is a call for action in cases where, e.g. a good biological quality couldn’t be stated. In this context, the authors believe that the possible negative impacts of agricultural activities are not concerned accordingly at present. Thus, the aim of the present study was to provide appropriate indication tools that enable a more realistic ecotoxicological risk assessment of headwater streams. To this end, the SPEcies At Risk concept (SPEAR - LIESS & VON DER OHE 2005) was used as an indicator of the biological quality of streams.

To focus on the impacts of agricultural activities, the streams have been selected due to the absence of other artificial pollutants. However, a subset of streams with wastewater treatment plants in the upstream reaches has been investigated separately. The invertebrate community may act as a convenient indicator to assess the biological quality of streams, as it is already being used by governmental water investigations. Examples include the organic pollution or the acidity of streams (DIN 1990; BRAUKMANN 2000). Therefore, a lot of invertebrate sampling data are available at present, which have been used in the present investigation.

METHODS

Study area

The study area is located around Braunschweig, Lower Saxony, Germany. The dominant land use in this area is agriculture (51 %), with sugar beets, winter barley and winter wheat as the most common crops, covering 2/3 of the arable land. The climatic conditions are temperate oceanic, with an average annual temperature of 8.8°C and an annual rainfall of approximately 620 mm, being distributed uniformly across the year (MÜLLER-WESTERMEIER 1996). The potential runoff for the investigated sites ranged from "very low" (level 0) to "high" (level 5) on a scale ranging from level 0 to level 6 "very high" - defined for German agricultural areas (BACH et al. 2000).

Description of the streams

The investigated streams were taken from two different sources. The local water board provided 71 sites, located at first-order streams, which were used as the training set to derive a regression model between a new community descriptor and the modeled indicator of runoff. Seven of the sites with wastewater treatment plants in their upstream catchments were classified as a subset of streams$_{\text{(+treatment plant)}}$. Another 20 streams of the test set have been taken from own previous investigations (LIESS & VON DER OHE 2005) and were used for the verification of the model. To derive the two data sets, the catchments of the streams were initially screened to match the following requirements: Other sources of pollution than agriculture and wastewater treatment plants, for example industry, as well as extensive urban or mining areas led to an exclusion from the data set as they are known to affect the invertebrate community (MALTBY 1995; ZELINKA & MARVAN 1961).

Morphological parameters of the streams, such as the substrate-composition (e.g. percentage of submersed plants) or the width of streams have been recorded once in October 2002, as no governmental data were available. Nonetheless, the authors assume that the recorded parameters still reflect the basic differences between the sampling sites. The streams concerned here flow throughout the year, with no dredging of the streambed occurring during the sampling periods. The width and depth of the streams were measured at the mean water level. The average current velocity was measured by taking the mean time for a drifting object to travel 10 m. The streambed structure and - cover at the sampling sites were quantified by estimating the percentage of various substrates and macrophytes within a 100 m reach of the sampling site. Other environmental parameters, such as nutrients, conductivity or pH have not been recorded. However, former investigations in this area (LIESS & VON DER OHE 2005; FAASCH 1997) did not state extreme values and are therefore implicitly expected to be of minor importance for the explanation of the calculated community descriptors (LIESS & VON DER OHE 2005). All environmental characteristics of the training set are summarised in Table A4.

Quantification of the agricultural intensity

In the present study, no measured impacts of agricultural activities could be used, as invertebrate data of governmental water investigations were used. Moreover, water sampling of short-term pesticide contamination is difficult, time consuming and expensive (LIESS et al. 1996; LIESS et al. 1999). Therefore, two different prediction models have been employed to address potential agricultural impacts. First, a runoff formula of the OECD was used to predict an indicator of runoff (OECD 2000), referred to as Potential for Pesticide Runoff (PPR). Additionally, this indicator should consider the amount of suspended particles and hydrodynamic stress, accompanying runoff events. Besides runoff, spray drift is also known as an input-pathway of pesticides into streams with agricultural catchments (DABROWSKI & SCHULZ 2003). Hence, predicted environmental concentrations of pesticides were also calculated, using the FOCUS Drift Calculator (EEC 2001).

Potential for Pesticide Runoff

This indicator has been used as a measure for runoff induced short-term pesticide input, because no water samples for the determination of real pesticide contaminations were available for this investigation. The calculations are based on the „simplified formula for indirect loading caused by runoff“ (OECD 2000), modified by SCHRIEVER et al. (2005). According to equation 1, a stream site can be characterized with respect to the influence of the near upstream environment on the level of potential runoff-induced losses of any pesticide from adjacent arable land. Irrespective of a specific pesticide, a generic dimensionless measure of the potential load originating from a single rainfall event is calculated as:

$$X = \frac{f(P)}{P} \cdot \sum_{i=1}^{n} A_i \cdot f(s_i) \cdot \left(1 - \frac{I_i}{100}\right) \cdot \frac{100}{1 + toc_i} \qquad (1)$$

where P is the precipitation depth [mm], f(P) is the volume of the resulting surface runoff (according to scenario 3) [mm] (OECD 2000), A_i is the size of arable land within a defined contributory area of the stream site [ha], the index i refers to different crops cultivated, s_i is the mean slope of arable land in the contributory area [%], $f(s_i)$ describes the influence of slope $\leq$ 20 % (OECD 2000), I_i is the plant interception at the time of the rainfall event [%] (LINDERS et al. 2000), and toc is the total organic carbon content in the soils of the considered arable land [%].

The generic dimensionless losses were log-transformed for the calculation of the PPR due to the large range of predicted values. Subsequently, the highest modeled value for all rainfall events between April and July within the year of the respective invertebrate samples was used as runoff indicator (eq 2).

$$PPR = \log (X_{max}) \qquad (2)$$

A threshold of –2 was set as lower boundary of the predicted PPR range, although some of the sites under-run this value. However, for these sites no significant differences in the specific community descriptors (SPEAR, as specified below) could be stated, when compared to the community descriptors of the six reference sites (Students t-test, $p > 0.05$). Therefore, streams with a PPR of –2 represent both reference sites as well as sites with a maximum generic load of 0.1 g within the contributory area, which were both assumed to be unimpaired. The upper boundary was not restricted due to sites that could show higher potentials as those in the data sets. Table A4 summarises the ranges of the modeled PPRs.

Spatial Estimation of Spray Drift

To also consider the sites´ potentials to be affected by spray drift, a predicted environmental concentration (PEC) of a generic compound was calculated. This approach uses the same regression fit as the FOCUS Drift Calculator (EEC 2001) that integrates across the width of the water body. It computes the maximum drift and resulting PEC based on crop type, application rate (100 g active ingredient/ha), and water body characteristics such as width (1 m) and depth (0.3 m).

A maximum PEC in a given wind direction will result from drift, originating from the crop directly adjacent to a water body. A PEC less than the maximum will occur if either (i) only a portion of the water body is potentially exposed to spray drift from that direction, or (ii) if the crop is not directly adjacent to the water body (assumption of 1 m minimum distance for arable crop). This applied method uses two ratios: the *affected ratio* estimates the portion of the water body that experiences spray drift from that direction; the *drift ratio* estimates the percentage of drift onto the water body in relation to the maximum. The final PEC is then calculated by multiplying the maximum PEC by the *affected ratio* and the *drift ratio.* Note that the *affected ratio* and the *drift ratio* may both be 1.0 if crop is directly adjacent to the water body and all potentially exposed points are actually exposed for a particular wind direction, yielding a PEC corresponding to the maximum value computed using the FOCUS drift calculator (EEC 2001).

Upstream catchment quality

Forested stream sections upstream sampling sites have recently been found to positively bias the invertebrate community of small headwater streams (LIESS & VON DER OHE 2005). To quantify this parameter, the land-use of the catchments were analysed using digital land-cover maps. All forested areas and adjacent meadows of at least 50 m lengths to both sides of the stream < 5000 m upstream of the sampling site were identified. Subsequently, the length of these sections and their distance from the investigated site provided estimates of the upstream catchments quality.

To investigate if and at which length such forested stream sections upstream act beneficial to the invertebrate community, the community descriptor $\mathrm{SPEAR}_{(\mathrm{number})}$ was used. For all sites belonging to the training set with a PPR value > 0.5, representing sites that are assumed to be impacted by agricultural activities, the $\mathrm{SPEAR}_{(\mathrm{number})}$ was correlated to the indicator of runoff. These sites span a similar PPR range but differ in the presence or absence of forested stream sections (Figure 3). Subsequently, all streams were ordered due to the length of forested stream sections. Starting with the stream containing the greatest length of these sections and continuing with streams that contain sections of declining length, these streams were classified into the subset of $\mathrm{streams}_{(+\mathrm{forest})}$. Consequently, the remaining sites form the subset of $\mathrm{streams}_{(-\mathrm{forest})}$. This results in two distinct linear regressions, as previously shown by LIESS & VON DER OHE (2005). The minimum length of a forested stream section, where the two regression lines of $\mathrm{streams}_{(+\mathrm{forest})}$ vs. PPR and $\mathrm{streams}_{(-\mathrm{forest})}$ vs. PPR, respectively, could be still significantly distinguished (ANCOVA, $p < 0.05$), was set as threshold for this parameter.

Hence, all streams were divided into two subsets: (i) $\mathrm{streams}_{(-\mathrm{forest})}$, without any forested stream sections and (ii) $\mathrm{streams}_{(+\mathrm{forest})}$, with these structures present in the upstream reaches. Subsequently, the training set was divided into 15 $\mathrm{streams}_{(-\mathrm{forest})}$ and

49 streams$_{(+forest)}$. Six of the latter sites could be referred to as reference sites without any sources of pollution. Accordingly, the test set was divided into 8 streams$_{(-forest)}$ and 12 streams$_{(+forest)}$, respectively.

To quantify a beneficial influence of the forested stream sections to the invertebrate community structure, the number of SPEAR and SPE$_{not}$AR at streams$_{(-forest)}$ and streams$_{(+forest)}$ were compared. To this end, all streams have been classified into three classes according to the existence of forested stream sections. Class 0 consisted of sites without any forested stream section and sites of Class < 500 m or Class < 1000 m that contained 200-500 m and 500-1000 m of forested stream sections, respectively. For this analysis, an important requirement was that the mean PPR for each class did not differ significantly (ANOVA, $p > 0.05$), which holds also for the mean width of each class (ANOVA, $p > 0.05$).

Invertebrate sampling

Data on invertebrate surveys of the training set were taken from local governmental water investigations, whereas data of the test set were taken from LIESS & VON DER OHE (2005). For the first set, at least three sampling surveys per site during the whole investigation period from 1986 to 1999 were required for the analysis. Furthermore, only sampling dates from April to August were considered, due to expected similar monthly community descriptors (as described below). Invertebrates were collected according to the Deutsche Industrie Norm DIN 38410-instructions for saprobic-scale (DIN 1990). Samples were taken randomly in a stream segment of approximately 100 m. Invertebrates were preserved in 70 % ethanol and identified to species level with the exception of dipterans identified to the family level. The abundance has been valuated in classes: from one animal in class 1 to very abundant in class 7. Former investigations showed that the highest pesticide concentrations in the investigated area were observed during May and June (LIESS et al. 1996; 1999). Consequently, surveys in April should reflect the situation before the main period of application in this area and were considered to represent possible long-term effects of agriculture from the previous years. Surveys from May to August should reflect the situation during and after the main application of insecticides and therefore represent possible short-term effects of pesticides from the same year.

Community descriptors

A number of different indices were calculated to assess the unique structure of the invertebrate communities. Besides the commonly used endpoints species number (SCHWOERBEL 1994), total abundance, diversity and evenness (according to Shannon-Wiener), the index for saprobic-scale (DIN 1990) was calculated to verify its use for the detection of potentially impaired sites. Furthermore, a number of specific endpoints have been employed to address potential impacts of agricultural activities. The respective classification scheme of species at risk (SPEAR) was taken from LIESS & VON DER OHE (2005). There, the species were classified due to physiological differences in the physiological sensitivity of species (VON DER OHE & LIESS 2004) and additional life history information (e.g. generation time). As a result, the species were regarded to be at risk or not to be at risk of being affected by pesticides: (i) species with a value > -0.36 (median of sensitivity, from VON DER OHE & LIESS (2004)) were regarded as sensitive; (ii) a generation time ≥ 0.5/year was regarded as potentially sensitive due to a slow recovery potential;

(iii) low migration ability was presumed to reflect reduced potential for recolonization (species regarded as being not at risk due to their better ability to migrate included *Gammarus pulex* and *Anabolia nervosa*); (iv) species of which adults emerged before April (before the time of maximum pesticide application) were regarded as insensitive, because exposure to aquatic stages would not occur. Only if all four traits indicate sensitive, a species was considered as being potentially at risk. This index was especially derived with regard to organic pollutants, such as pesticides, impacted in the season of late April to June.

Hence, some specific endpoints for the invertebrate communities were calculated from the SPEAR classification. The number of species at risk, $\text{SPEAR}_{(\text{number})}$, addresses the number of potentially endangered species. For comparison, we calculated the number of species not at risk, $\text{SPE}_{\text{not}}\text{AR}_{(\text{number})}$. The abundance of species at risk, $\text{SPEAR}_{(\text{abundance})}$, calculated as the sum of the single abundances of all species at risk, concerns the total amount of animals at risk in the respective communities. Furthermore, $\%\text{SPEAR}_{(\text{abundance})}$, calculated as the ratio of the total abundance of the species at risk compared to the total abundance of all species, was verified for its use to reveal a threshold at which the community structure of invertebrate communities are significantly impaired. Moreover, the indicator $\%\text{SPEAR}_{(\text{abundance})}$ was used to investigate monthly changes in the community structure and to reveal potential indirect effects of wastewater treatment plants.

%SPEAR$_{(abundance)}$ – a new indicator

To assess the “quality of streams” concerning agricultural impacts quantitative, the most robust endpoint $\%\text{SPEAR}_{(\text{abundance})}$ was correlated to the PPR. Due to observed differences between the community descriptors of $\text{streams}_{(\text{-forest})}$ and $\text{streams}_{(\text{+forest})}$, the community descriptors have been correlated to the PPR, separately for each subset. The endpoint $\%\text{SPEAR}_{(\text{abundance})}$ is a relative measure of the fraction of sensitive species abundance and hence almost independent from the sampling method. Therefore it was possible to include the invertebrate data of the test set, which were also correlated to the PPR. The respectively derived regression model allows for calculating the quality of any invertebrate community compared to the prevailing agricultural intensity (modeled as the PPR) for a landscape level assessment. Moreover, the community descriptors of the test set were additionally correlated to the $\text{Toxic Units}_{(\textit{Daphnia magna})}$ that were calculated as the measure of agricultural intensity of our former investigation. This enables an assessment of measured concentrations of pollutants, where an $\text{LC50}_{(\textit{Daphnia magna})}$ is available.

All samples of the training set have been previously verified to eliminate apparently odd sampling surveys (e.g. due to difficult weather conditions or summer dehydration of sites, which have not been reported). In this context, the findings of our former investigation empirically revealed that the index values of $\%\text{SPEAR}_{(\text{abundance})}$ for all samplings of one site do not differ by more than 12 % from the average (not shown). Thus, for the calculation of a mean community descriptor, a minimum of at least 3 sampling surveys between April and August have been required. Subsequently, the average of the $\%\text{SPEAR}_{(\text{abundance})}$ values for all samplings of one site was taken. Those samplings, whose index values exert the threshold of 12 % deviation from the average, were excluded from the data set. It has to be noted that for some of the sites only two samplings remained for the analysis.

Statistical analysis

To evaluate the dependency of the community responses to environmental parameters, multivariate linear regressions were performed. The normal distribution of data was tested with the Kolmogoroff-Smirnoff test. Equality of variances was verified using White's homogeneity-of-variance test. Statistical procedures used were: stepwise entering of variables (criteria: probability of F to enter ≤ 0.05, probability of F to remove ≥ 0.10), resulting in an adjusted coefficient of determination r^2. The beta values of the multiple regressions are given in Table 2, representing the explanation power of the single parameter. Differences between slopes of regression lines (e.g. to reveal differences in $\text{streams}_{(\text{-forest})}$ and $\text{streams}_{(\text{+forest})}$ as well as monthly changes in the community structure) were investigated using the analysis of covariance (ANCOVA). Differences between classes of sites (e.g. classified due to the PPR, the width of streams at the sampling sites or the length of forested stream sections) were investigated by means of one-way analysis of variance (ANOVA). Scheffé`s F-Test was applied to detect significant differences among means. Multivariate linear regressions, ANOVA and ANCOVA were carried out with SPSS® (Chicago, IL, USA).

RESULTS

Environmental conditions

Due to the lack of measured agricultural impacts (pesticides, nutrients or hydrodynamic stress), only relative differences between sites could be modeled, assuming farming according to the good agricultural practice. The modeled PPR for the training set ranged from –2 to +2 for $\text{streams}_{(\text{+forest})}$, and from +0 to +2 for $\text{streams}_{(\text{-forest})}$. To compare those ranges to measured pesticide concentrations, the PPRs were also calculated for the 20 streams of the test set where this exposure information was available. This resulted in a respective Potential for Pesticide range from –2 to +1 (not shown). The respective pesticide peak concentrations at each site, expressed as $\text{Toxic Units}_{(\textit{Daphnia magna})}$, still corresponded to concentrations below the LC50 of the standard test organism *Daphnia magna*. Therefore, it could be concluded that the PPR range of the training set also corresponds to a sublethal toxicity range. Likewise, the PPR reflects potential differences in the amount of suspended particles and hydrodynamic stress of the respective sites, whereas these parameters could not be further quantified.

The predicted environmental concentrations from the spray drift calculations ranged from 0 µg/L for unpolluted sites to 0.64 µg/L for the most impacted site. Both indicators of agricultural intensity were partly interrelated (linear regression, $r^2 = 0.41$, $p < 0.01$), indicating a similar toxicity classification. Nevertheless, according to the multiple regressions performed on the community descriptors and the environmental parameters, the predicted environmental concentrations were found to be of minor importance and are therefore not further considered in the present investigation.

The morphological diversity of the streams was poor as most streambeds contained a large percentage of fine substrates and the amount of plants or organic debris was slight (Table A4). Based on these morphological parameters, the streams of the training set were typical for small lowland streams. Regarding the standard water quality parameters (e.g. organic pollution, nutrients), no toxic concentrations are expected (FAASCH 1997).

Correlating community descriptors with environmental parameters

Site-specific combinations of environmental factors result in unique compositions of species, masking the effects of individual environmental factors. Nevertheless, PPR described best the observed variability in the community structures of the training set, when using the species at risk community descriptors (Table 2). For both subsets, the number as well as the abundance of species at risk (SPEAR) were negatively correlated with the PPR, whereas the average number and abundance of $SPE_{not}AR$ was not correlated with this parameter. The existence of sand contributed additionally to the explanation of $SPEAR_{(number)}$ for $streams_{(-forest)}$, whereas the length of forested stream sections added to the explanation of $streams_{(+forest)}$. The latter environmental parameter was partially interrelated with the PPR (linear regression, $r^2 = 0.43$, $p < 0.01$), indicating that streams containing longer forested stream sections less tend to runoff.

The commonly used descriptor "species number" only depends on the width of streams and the percentage of sand present for streams lacking forested stream sections upstream. In contrast, for $streams_{(+forest)}$, the upstream catchments quality was found to be most important for the species number (Table 2). The same holds for the diversity, which solely depended on the width of the streams and the upstream catchments quality. Note that the total abundance was not correlated to any of the measured environmental parameters, whereas it was higher for $streams_{(+forest)}$ (not shown).

Threshold of community response to the Potential for Pesticide Runoff

For both subsets, the $\%SPEAR_{(abundance)}$ was found to be the most sensitive endpoint with respect to the PPR (Table 2). Therefore, it was used as an indicator system to determine the level at which a change in the community structure was apparent. To this end, streams were classified due to PPR Classes. Figure 1 showed the first significant reduction in the community structure of $streams_{(+forest)}$ at Class –1 to 0, compared to six reference sites (Class < –2). For the classes 0 to 1 and >1 this effect was even more pronounced. The same holds for $streams_{(-forest)}$, for which both classes showed a significant reduction of the $\%SPEAR_{(abundance)}$. Class 0 to 1 and Class > 1 differed significantly for both subsets, whereas the only significant difference between the two subsets was found for Class > 1. In this context, the subset of seven $streams_{(+treatment\ plant)}$ was also classified into PPR classes and compared to similar classified $streams_{(+forest)}$ (Figure 1). This revealed significantly lower mean community descriptors for both Classes, Class –1 to 0 and Class 0 to 1, respectively (ANOVA, $p < 0.05$).

Application of the indicator % $SPEAR_{(abundance)}$

For both own investigations, the most sensitive endpoint $\%SPEAR_{(abundance)}$ was closely correlated to the used indicator of agricultural intensity, namely the PPR and the Toxic $Units_{(Daphnia\ magna)}$, respectively (LIESS & VON DER OHE 2005). In the following, linear regressions are provided for future assessments of small headwater streams, using the $\%SPEAR_{(abundance)}$ as a new indicator for agricultural impacts. To this end, streams of the training- and the test set were pooled, separately for $streams_{(-forest)}$ and $streams_{(+forest)}$. The authors believe that the resulting regressions better reflect the reality, because the data are more uniformly distributed over the whole PPR range.

Table 2. Adjusted coefficients of multiple determination (r^2) and standardized partial regression coefficients (beta; $p < 0.05$*, $p < 0.01$**, optimized model) for correlations between environmental parameters and community metrics at 64 streams during April – August, 1986-1999 (1), listed separately for subset of 49 $\text{streams}_{(+\text{forest})}$ (+) and the subset of 15 $\text{streams}_{(+\text{forest})}$ (–). All parameters not shown, but described in the Methods section, were minimally important for the explanation of the investigated community endpoints.

Community Descriptor	subset	r^2	df	F	PPR	Uncontaminated Stream Sections [m]	Width [%]	Sand [%]
Species number - Average	+	0.38	47	30.4	-	+ 0.63**	-	-
	–	0.44	12	6.4	-	-	+ 0.49*	+0.46
Diversity - Average	+	0.28	47	19.8	-	+ 0.54**	-	-
	–	0.32	13	7.6	-	-	+ 0.61**	-
$\text{SPEAR}_{(\text{number})}$ - Average	+	0.68	46	51.3	- 0.37**	+ 0.54**	-	-
	–	0.78	12	20.7	- 0.77**	-	-	+0.30*
$\text{SPEAR}_{(\text{abundance})}$ - Average	+	0.61	46	38.1	- 0.46**	+ 0.41**	-	-
	–	0.73	13	38.8	- 0.87**	-	-	-
$\%\text{SPEAR}_{(\text{abundance})}$ - Average	+	0.72	47	126.1	- 0.85**	-	-	-
	–	0.71	13	34.9	- 0.85**	-	-	-
$\text{SPE}_{\text{not}}\text{AR}_{(\text{number})}$ - Average	+	-	-	-	-	-	-	-
	–	0.21	13	4.8	-	-	+ 0.52*	-

(1) Included in the table are parameters that account for at least 20% of the explained variance for the most relevant parameter, and adding at least 7.5% of explained variance for all successive parameters.

For all $\text{streams}_{(+\text{forest})}$ (Figure 2a), linear regression yields

$$\%\text{SPEAR}_{(\text{abundance})} = -0.094\ (\pm 0.008)\ \text{PPR} + 0.324\ (\pm 0.010) \qquad (3)$$

where $n = 61$, $r^2 = 0.71$, SE = 0.07, $F_{1,59} = 148.51$. For all $\text{streams}_{(-\text{forest})}$ (Figure 2d), the linear regression yields

$$\%\text{SPEAR}_{(\text{abundance})} = -0.084\ (\pm 0.015)\ \text{PPR} + 0.117\ (\pm 0.016) \qquad (4)$$

where $n = 23$, $r^2 = 0.58$, SE = 0.05, $F_{1,21} = 31.88$. The slope of regression 4 is similar to the first one (0.084 vs. 0.094, respectively), while the intercept is significantly lower (ANCOVA, $p < 0.05$). The fractions of species at risk are about 20 % lower at streams without forested stream sections (0.117 vs. 0.324, respectively). As can be seen from figure 2d, the scatter of data for $\text{streams}_{(-\text{forest})}$ is somewhat larger, which is also reflected in the lower calibration r^2 of the regression statistics.

For future applications, a risk assessment based on measured pesticide concentrations should also be enabled. Therefore, respective regressions were additionally given, based on the community descriptors and $\text{Toxic Units}_{(\textit{Daphnia magna})}$ of the test set. To calculate the $\text{Toxic Units}_{(\text{Daphnia magna})}$, the measured pesticide concentrations have to be divided by the LC50 of *Daphnia magna*. For the $\text{streams}_{(+\text{forest})}$ of the test set, linear regression yields

$$\%\text{SPEAR}_{(\text{abundance})} = -0.080\ (\pm 0.013)\ \text{Toxic Units}_{(\textit{Daphnia magna})} + 0.132\ (\pm 0.018) \qquad (5)$$

where $n = 12$, $r^2 = 0.78$, SE = 0.05, $F_{1,10} = 128.51$.

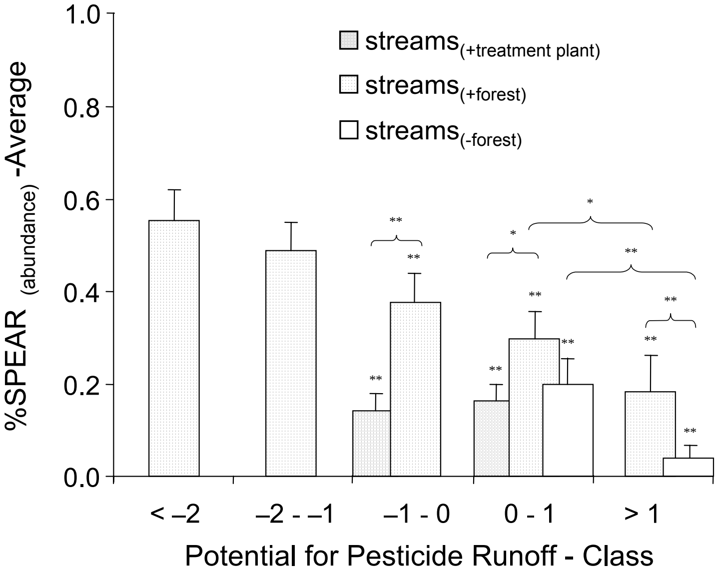


Fig. 1. Relation between predicted agricultural intensity, expressed as Potential for Pesticide Runoff classes and the benthic invertebrate community structure expressed as percentage of the abundance of species at risk (%SPEAR$_{(abundance)}$-Average) for three subsets: 44 streams$_{(+forest)}$, 15 streams$_{(-forest)}$ and 7 streams$_{(+treatment\ plant)}$ - compared to six reference sites (Class < -2). Asterisks show significant difference from the reference sites or differences between classes (ANOVA, Scheffé`s *F* test, * $p < 0.05$, ** $p < 0.01$). Error bars show the respective SD`s.

For the streams$_{(-forest)}$ of the test set, linear regression yields

$$\%SPEAR_{(abundance)} = -0.060\ (\pm 0.011)\ Toxic\ Units_{(Daphnia\ magna)} + 0.003\ (\pm 0.014) \qquad (6)$$

where $n = 8$, $r^2 = 0.67$, SE = 0.09, $F_{1,6} = 48.51$. Here, the slope of regression 5 is still similar to regression 6 (0.080 vs. 0.060, respectively), while the intercept is significantly lower (ANCOVA, $p < 0.05$). The fractions of SPEAR are about 13 % lower at streams without forested stream sections (0.132 vs. 0.003, respectively).

The two subdivisions of the index %SPEAR$_{(abundance)}$, namely the physiological sensitivity of species and the life cycle traits influencing the recovery potential, were both correlated to the indicator of agricultural intensity. For streams$_{(+forest)}$, 65 % ($p < 0.01$) of the explained variance was accounted for by the species sensitivity (Fig. 2b), and 46 % ($p < 0.01$) of the explained variance was accounted for by life-cycle traits (Fig. 2c). For streams$_{(-forest)}$, the species sensitivity accounted for 53 % ($p < 0.01$) of the explained variance (Fig. 2e), whereas this could not be stated for the life cycle traits alone (Fig. 2f). It has to be noted that the values of the two subdivisions were interrelated, indicating that species with a high recovery potential due to life cycle traits also tend to be insensitive to organic pollution.

In this context, potential effects of treatment plants were also investigated in more detail. In Figure 2a it could be seen that the %SPEAR$_{(abundance)}$ of the seven streams$_{(+treatment\ plant)}$ were significantly reduced, compared to streams$_{(+forest)}$ with similar PPR range. To find a possible explanation for this, the index was also calculated for the two subdivisions of this index. The largest deviation of the index values for streams$_{(+treatment\ plant)}$ is observed for the subdivision of the sensitivity index (Fig. 2b), whereas this was less pronounced for the subdivision of the life cycle traits index (Fig. 2c). To verify the impact of organic pollution in this context, the index of saprobic-scale was calculated for all streams of the training set. Nevertheless, no significant differences were found for streams$_{(+\text{treatment plant})}$ compared to similar classified streams$_{(+\text{forest})}$ (ANOVA, $p > 0.05$, not shown). Generally it can be stated that the commonly used index of saprobic-scale was not correlated to any of the environmental parameter.

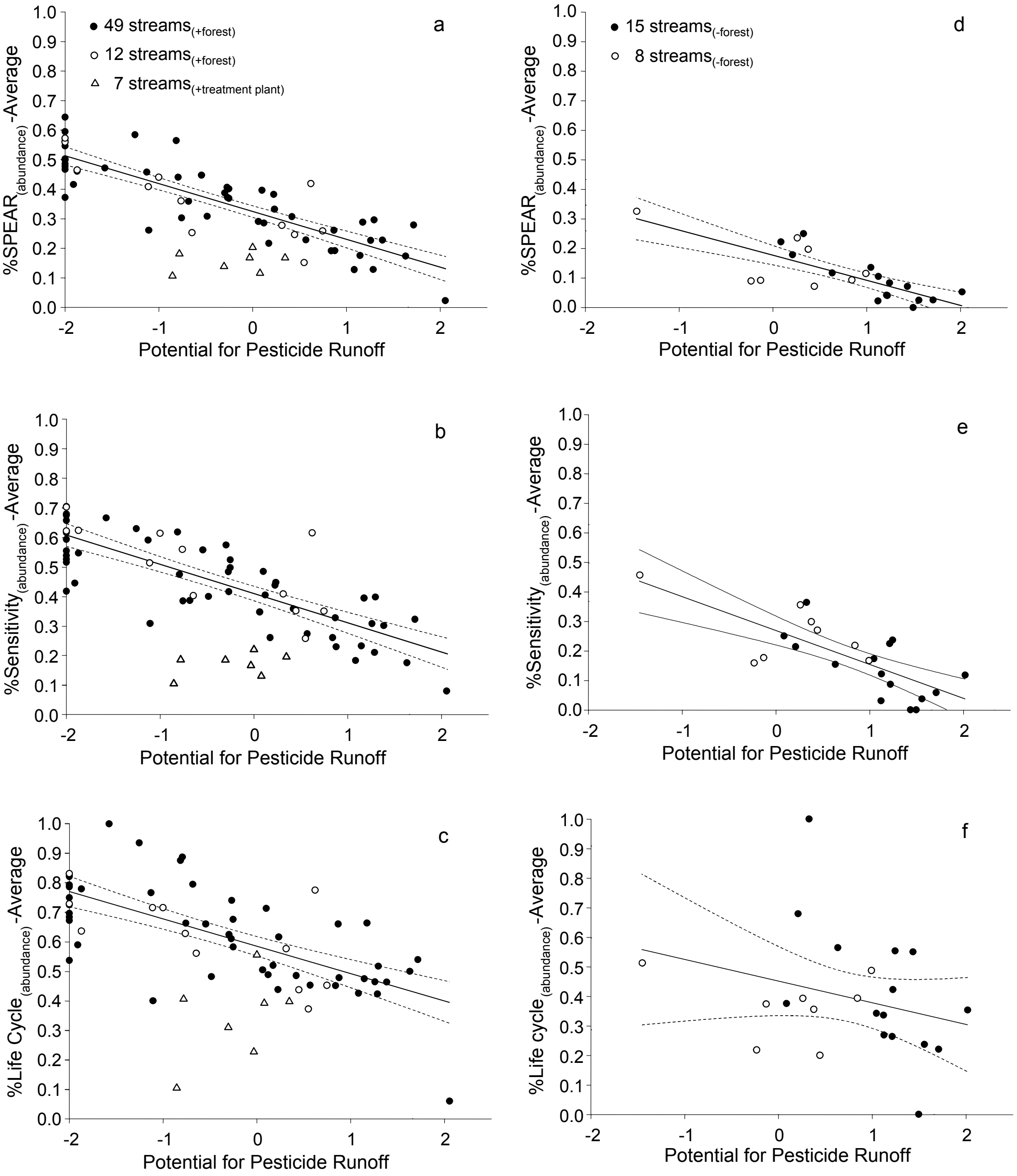


Fig. 2. Relation between the Potential for Pesticide Runoff and specific community descriptors, based on the SPEAR concept (for details see text). Plots a-c represent streams$_{(+\text{forest})}$, with uncontaminated stream sections closer than 4000 m upstream of the study site present: 49 streams of the training set (open circles); 12 streams of the test set (filled circles) and 7 streams$_{(+\text{treatment plant})}$ (open tringles). Plots d-f represent streams$_{(-\text{forest})}$ without such sections: 15 streams of the training set (open circles) and 8 streams of the test set (filled circles). The dotted lines show the 95 % confidence limit for the respective mean.

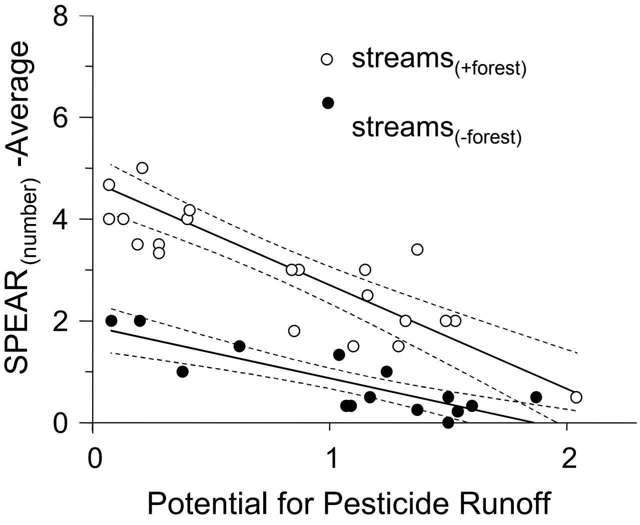


Fig. 3. Relation between the Potential for Pesticide Runoff and the $\text{SPEAR}_{(\text{number})}$-Average. Sites are differentiated on the basis of the presence of uncontaminated stream sections closer than 4000 m upstream of the study site (22 $\text{streams}_{(+\text{forest})}$ = open circles, linear regression, r^2 = 0.72, $p \leq 0.01$) or absence of such sections (15 $\text{streams}_{(-\text{forest})}$ = filled circles, linear regression, r^2 = 0.68, $p \leq 0.01$). The dotted lines show the 95 % confidence limit for the respective mean. The slopes of the two regression lines are different (ANCOVA, $p \leq 0.05$).

Temporal changes in community structure

To reveal temporal changes in the community structures, again data for both the training set and the test set were pooled. For $\text{streams}_{(+\text{forest})}$, a monthly correlation of $\%\text{SPEAR}_{(\text{abundance})}$ to the PPR was found as early as April. The same holds for the correlations from May to August, although the number and composition of streams considered for the regressions were different each time. A first significant reduction of $\%\text{SPEAR}_{(\text{abundance})}$ compared to the regression line in April was found in May. This regression line was significantly steeper than the one in June, which was still reduced compared to the one in April. The regression line in July and August however were already similar to the one in April.

For $\text{Streams}_{(-\text{forest})}$, a significant correlation between $\%\text{SPEAR}_{(\text{abundance})}$ and the PPR was found for all months, although the number and composition of sites considered were different each time. A significant reduction of $\%\text{SPEAR}_{(\text{abundance})}$ compared to the regression line in April was found in May as well as in June. In contrast to $\text{streams}_{(+\text{forest})}$, the regression line in July was significantly steeper than the one in April, but was at the same time not different from the ones in May and June. The regression line in August was again similar to the one in April.

Contribution of the upstream catchments quality

The area for the quantitative definition of the upstream catchments quality was finally set to a continual stream-section > 150 m in length, with adjacent forested areas or meadows to both banks that are located < 4000 m upstream of the investigated site. Figure 3 illustrates that two groups of streams could be distinguished when classified accordingly to the existence or absence of these sections. The slopes of the two regression lines were significantly different (ANCOVA, $p < 0.05$), indicating that the number of species at risk depended on the affiliation

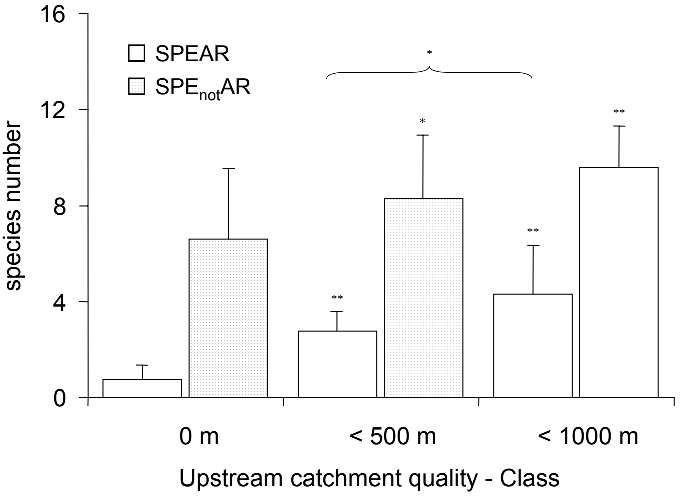


Fig. 4. Relation between the upstream catchments quality class, classified due to the length of undisturbed stream sections in m and the number of species at risk (SPEAR$_{(number)}$) and number of species not at risk (SPE$_{not}$AR$_{(number)}$): 12 sites without any recovery potential (Class 0 m); 7 sites with up to 500 m (Class < 500 m) and 5 sites with more than 500 m and up to 1000 m (Class < 1000 m) length of uncontaminated stream sections. The mean PPR and the mean width of streams of each class are not significantly different (ANOVA, $p > 0.05$). Asterisks show significant differences from Class 0 or differences between groups (ANOVA, Scheffé`s F test, * $p < 0.05$, ** $p < 0.01$). Error bars show the respective SD`s.

to one of the two subsets. Thereby, streams$_{(+forest)}$ show significantly more species at risk, when compared to streams$_{(-forest)}$, within a similar PPR range. Nevertheless, for all sites with a PPR > 1, a clear reduction of the SPEAR$_{(number)}$ could be observed for both subsets. Less impacted streams$_{(+forest)}$ with PPR < 0.5 consist of 4.4 species at risk on average vs. 1.7 species at streams$_{(-forest)}$ (ANOVA, $p < 0.01$). High impacted streams$_{(+forest)}$ with a PPR > 1 show an average of 2 species at risk vs. 0.5 species at streams$_{(-forest)}$ (ANOVA, $p < 0.01$). Hence, potentially high impacted streams$_{(+forest)}$ have a similar SPEAR$_{(number)}$ to potentially low impacted streams$_{(-forest)}$. A comparison with the community descriptors of the test set showed an overall lower number of species identified in the training set.

One aim was to quantify the positive influence of forested stream sections for the number of species at risk and species not at risk. To this end, sites have been pooled to classes concerning this parameter, while requiring was that the classes did not differ in the mean width of streams and the mean PPR. Figure 4 illustrates that for Class < 500 m as well as for Class < 1000 m a significantly increased average number of species at risk and average number of species not at risk could be observed. Thereby, the number of species at risk increased from 0.7 species for class 0 over 2.8 at Class < 500 m to 4.3 species at Class < 1000 m. Considering the increase in the number of species not at risk, 200-500 m uncontaminated stream sections account for additional 3.7 species and 500-1000 m uncontaminated stream sections for additional 6.5 species.

DISCUSSION

%SPEAR$_{(abundance)}$ as indicator

So far, field investigations about invertebrates and agricultural activities were performed at one or few streams only (Matthiesen et al. 1995). To reveal lasting changes in the community structure in these cases, long history data on invertebrate communities are necessary to show the initial community structure. In particular, investigations of one or two streams lack of sufficient data-ranges to reveal quantitative changes concerning a certain environmental parameter of interest. In this context, the present analysis of the commonly used indicators species number on the one hand and the more specific index of saprobic scale (DIN 1990) on the other hand revealed that these indices were not appropriate to indicate adverse effects originating from agricultural activities. In our previous investigation, close correlations between measured pesticide concentrations and invertebrate community descriptors could be observed, when using the SPEAR concept (LIESS & VON DER OHE 2005). The strong point of the present investigation is the number of investigated streams and therefore the wide range of expected agricultural intensities. On the minor site, no measured pesticide concentrations could be considered. Nevertheless, the Potential for Pesticide Runoff should enable a relative comparison between sites on a landscape level, using multivariate analysis of environmental parameters. A former study of reference sites in the UK (WALLY & FONTAMA 1998) successfully applied a similar approach to the RIVPACS data set (WRIGHT et al. 1993), focusing the adverse effects of agriculture activities.

The EU Uniform Principles for the assessment of pesticides require that if the preliminary risk characterization indicates potential concerns, registration cannot be granted unless it can be demonstrated that "... under field conditions no unacceptable impact on the viability of exposed organisms ..." occurs. However, the given regressions clearly state a correlation between the community descriptors and the respective indicator of agricultural impacts. To which extent such changes should be tolerated cannot be answered here but needs further discussion. Hence, the given regression lines enable a risk assessment on a landscape level, for both the modeled PPR and the Toxic Units$_{(\mathit{Daphnia\ magna})}$, when using the SPEAR classification in LIESS & VON DER OHE (2005). The only exception made here was the classification of *Limnephilus lunatus* that now was regarded to be at risk. In our previous investigation, streams$_{(+\mathrm{forest})}$ and streams$_{(-\mathrm{forest})}$ were not investigated separately and hence the importance of *Limnephilus lunatus* for an indicator system was disguised. The separate analysis of streams$_{(-\mathrm{forest})}$ and streams$_{(+\mathrm{forest})}$ considered the differences in the habitat for the invertebrate communities of these streams.

To investigate potential reasons for the impairment of sites in more detail, the two subdivisions of the index %SPEAR$_{(\mathrm{abundance})}$, namely the physiological sensitivity and the life cycle traits, were used. Thereby, the first subdivision proved to be more important to the explanation of the community structure and hence for the compiled indicator. This result indicates toxic stress to be mainly responsible for the altered communities. Moreover, the results confirm the importance of combined approaches in the risk assessment on the population level, considering both physiological and life-history characteristics (SCHULZ & LIESS 2001).

Changes in community structure related to the agricultural intensity

In using the indicator system %SPEAR$_{(\mathrm{abundance})}$ in regression models, a significant reduction in the community structure was found to be correlated with the indicator of agricultural activities for both subsets of streams (eqs. 3-6). A significant negative correlation could be observed as early as April, although these samples should rather reflect the status prior the application of pesticides. Hence, the communities in April must have been influenced by the conditions of the prior year. In another field study (BROCK et al. 2000), long-term effects of pulse exposure to pesticides could be shown. ROEX (2000) found that long-term effects of toxicants can occur at concentrations 10 times lower than concentrations needed to cause acute (48-h) effects. Other investigations of streams in agricultural areas also reported that pesticides from surface runoff could cause acute mortality of benthic invertebrates when they reach the range of the 48-h LC50 $_{(\textit{Daphnia magna})}$ (MATTHIESEN et al. 1995; SCHULZ et al. 2001). IDE (1967) studied the response of aquatic macroinvertebrates to large-scale forest spraying with DDT and found that recovery times varied for various taxa. Some plecopteran taxa did not recover for 2 years, some ephemeroptera required three years and some caddiesflies required four years. Note that these taxa were also regarded at risk of being effected by pesticides by our current approach. Furthermore, it was concluded that the large-scale contamination apparently inhibited the recolonization abilities of some taxa that were in vulnerable life cycle stages at the time of application. Those with a short life cycle (e.g. Chironomidae), recovered within a few weeks, whereas a few mayflies and plecopterans apparently survived the spraying in the egg stage. They emerged within a year after the spraying and served as early colonists. Note further that these considerations were also carried out for the SPEAR concept. These findings together with the results of the present study gave evidence of chronic effects and therefore long-term changes of the community structure that could be attributed to pesticides. In respect to the runoff-related increase of suspensions the results of experimentally based investigations indicate a relatively minor importance of the adverse effects on invertebrates compared to pesticides (FAIRCHILD et al. 1987). Hydrodynamic stress accompanying runoff as well as other recurrent stressors might favor species that have a high recovery potential due to a short generation time and good migration ability. Therefore, long-term reduction in SPEAR might be partly present due to this stressor. However, many taxa of SPEAR are those with a low recovery potential and species that are sensitive to toxicants, but not particularly vulnerable to hydrodynamic stress.

Moreover, it could be shown that the total number of species recorded at the sampling sites did not depend on the PPR (Table 2) whereas this was observed for the number of species at risk (Fig. 3). Therefore, a change in the species composition could be assumed to explain this instance. The species at risk probably have been replaced by other species being not at risk. This result agreed with the findings of other studies, outlined by BROCK et al. (2000), that insensitive species benefit from reduced competition to more sensitive species. For a landscape assessment, an overall change in the species diversity would be expected for areas with a predominantly agricultural land-use and high Potentials for Pesticide Runoff (> 1) or high Toxic Units (> –2).

Temporal changes in the community structure

When analysing the time dependency (in terms of monthly changes) of the indicator %SPEAR$_{\text{(abundance)}}$, similar community descriptor values could be observed for the months of April to August. This is in agreement with the investigation of HUTCHENS JR. et al. (1998), who concluded that in humid-temperate climates with less pronounced differences in precipitation there also emerge less pronounced differences in the macroinvertebrate abundances. Furthermore, HUTCHENS JR. et al. (1998) reported only about small differences in abundance throughout the whole investigation period of five years. This finding justifies our approach of using monitoring data sampled over a long investigation period.

Nevertheless, acute effects for streams$_{(+forest)}$ could be stated for May and June as the months of the main application period of pesticides in this area. This is in agreement with another investigation where the only significant reduction in invertebrate taxonomic richness and abundance was found in May when the highest pesticide concentrations were measured (LIESS & SCHULZ 1999). Nevertheless, invertebrate populations affected by toxic stress have been observed to recover (SCHULZ et al. 2002; SHERRATT et al. 1999), in particular when they remain connected to pristine sites (SHELDON et al. 2002). The linear regression lines for July as well as for August were again similar to the one in April, indicating within-year recovery of the community structure after acute effects. CONNELL & SOUSA (1983) concluded that more frequently disturbed communities have resident populations that colonize, grow rapidly and recover between disturbances.

For streams$_{(-forest)}$ the acute effects were even more pronounced as a reduction of %SPEAR$_{(abundance)}$ could be still observed in July. A linear regression of %SPEAR$_{(abundance)}$ again equal to the one in April was only observed in August, one month later than for streams$_{(+forest)}$. This results give evidence that the within-year recovery takes longer in streams$_{(-forest)}$ than in streams with forested stream sections present.

Evidently, other agricultural stressors could have been responsible for the observed acute effects. In agricultural areas, hydrodynamic stress arising from increased current velocity and suspended particles can occur during runoff events, but this is observed frequently throughout the year (LIESS et al. 1999). Hence, these stressors are probably not responsible for the short-term reduction of %SPEAR$_{(abundance)}$ that mainly occurred during May and June. In addition, concentrations of nitrite and ammonia being high enough to cause toxicity would also not be restricted to May, but even more to the beginning of the growing season (due to the application of fertilizer or manure from animals), or during late summer (due to elevated temperature and low water levels: KLADIVKO et al. 1991; CHOKMANI & GALLICHAND 1997).

Positive influence of upstream catchments quality

The present investigation revealed that the mean number of species at risk was correlated to the PPR for both subsets, whereas these numbers were significantly higher at streams$_{(+forest)}$. This finding is remarkable, as both subsets span a comparable PPR range. Therefore, the positive effect of undisturbed stream sections does not necessarily involve a reduced contamination within the investigated catchments, although the interrelation of these two parameters first indicated this. The increase in the species number probably could be attributed to in-stream colonisation by invertebrates from undisturbed stream sections in the upper reaches of the stream that do not receive agricultural impacts from adjacent areas. The same pattern was observed in (HATAKEYAMA & YOKOYAMA 1997) where the invertebrate community in the lower reaches was exposed to aerial insecticide spraying whereas the upper reaches remained uncontaminated. It was concluded that the recovery of the benthic community was brought over through recruitment from the benthic community in a distance of 5 km upstream. Another studies reported rapid colonization by drift of invertebrates from upstream sources (e.g. GORE 1982). FLANAGAN et al. (1997) found rapid recovery of fauna in a large river following methoxychlor application, being linked to undisturbed upstream areas and tributaries, which serve as sources of colonists. In this context, ANGERMEIR & KARR (1984) observed a reduction in the abundances of many invertebrates after short-term removal of woody debris. These snag habitats are important substrates of high secondary production for macroinvertebrates (BENKE et al. 1984) and are related to forested watersheds that serve as a source of in-stream colonisation. However, all presented results indicate an increased amount and number of species at risk for streams$_{(+forest)}$ compared to streams$_{(-forest)}$ that is related to the existence of forested stream sections. Note further that for streams$_{(+forest)}$ the total number of species was positively correlated with the length of uncontaminated stream sections. In contrast, for streams$_{(-forest)}$ the total number of species solely depend on the width at the sampling site that was in agreement with a recent study, where this parameter was the most important factor (PROBST et al. 2005).

To quantify the beneficial "amount" attributed to the upstream catchments quality, three classes of sites with comparable average PPR and widths of streams have been compared. It could be shown that both SPEAR$_{(number)}$ and SPE$_{not}$AR$_{(number)}$ are significantly increased, compared to the class of streams$_{(-forest)}$. Sites with up to 500 m undisturbed areas upstream exhibit about four times as many species at risk and additionally an increased number of species not at risk. Therefore, it could be concluded that relatively small undisturbed areas at every headwater streams would increase in the species richness on a landscape level.

Other sources of pollution

Industrial and municipal wastewaters have been identified as non-agricultural stressors of invertebrate communities (MALTBY 1995; ZELINKA & MARVAN 1961). This finding was somewhat verified with the results of the analysis of seven streams$_{(+treatment\ plant)}$ located in the effluent of wastewater treatment plants. With regard to the new indicator %SPEAR$_{(abundance)}$, the community structures were significantly affected. This result indicates adverse effects of wastewater treatment plants whereas the source being responsible for this could not be revealed. Potential reasons include an increased organic pollution with respective oxygen consumption, pollution with toxic nitrogen compounds, pesticides or other artificial pollutants (e.g. hormonal effective compounds). However, the possibility that the communities were affected by the increased oxygen consumption due to organic pollution at these sites was enervated through the fact that the community index of saprobic-scale was not significantly reduced. The investigation of the two subdivisions of the community descriptor %SPEAR$_{(abundance)}$ revealed a stronger influence on the sensitivity index than on the life cycle traits. This indicated toxic organic compounds to be responsible for the reduction in %SPEAR$_{(abundance)}$ of streams$_{(+treatment\ plant)}$.

However, the exclusion of all sites located at streams with these point sources led to a reduced variance in the community response of the training set. Due to this restriction, the water quality standard parameters at the remaining sites would not be expected to have any deleterious effects on the invertebrate communities (FAASCH 1997).

CONCLUSIONS

The results of this investigation give strong evidence that agricultural activities cause long-term effects on invertebrate communities of small headwater streams, although the level of impact at which these changes occur has been insufficiently quantified. Nonetheless, the Potential for Pesticide Runoff yields a convenient way of modelling potential hot spots that arise on a landscape level. In this context, the SPEAR concept proved to be a useful tool in the risk assessment of streams, indicating an important aspect of the biological quality of headwater streams. Furthermore, the importance of life-history characters could be shown and should be considered in future applications.

Moreover, positive effects of forested stream sections could be stated positively biasing the adverse effects of agricultural impacts, as it accounted for a higher number and abundance of species at risk. Therefore, landscape information should be considered in future risk assessments to increase the predictability of the “real risk” of pesticides. Moreover, the adverse effects observed for wastewater treatment plants suggest further investigations.

Acknowledgements—We are grateful to Helga Faasch for her invaluable fieldwork.

REFERENCES

Angermeier, P.L., Karr, J.R. (1984). „Relationships between woody debris and fish habitat in a small warmwater stream.” *Transactions of the American Fisheries Society* **113**: 716-726.

Bach, M., A. Huber, et al. (2000). Schätzung der Einträge von Pflanzenschutzmitteln aus der Landwirtschaft in die Oberflächengewässer Deutschlands - Berichte. Umweltforschungsplan des Bundesumweltministeriums für Umwelt, Naturschutz und Reaktorsicherheit - Gewässerschutz - Forschungsbericht 295 24 034 - UBA-FB 99-114. Umweltbundesamt. Berlin, UBA. 3/00: 273.

Benke A.C., T.C. Vanarsdall, D.M. Gillespie et al. (1984). “Invertebrate productivity in a sub-tropical blackwater river – the importance of habitat and life history.” *Ecological monographs* **54**: 25-63.

Braukmann, U. (2000). "Hydrochemische und biologische Merkmale regionaler Bachtypen in Baden-Württemberg." Schriftenreihe der LFU Baden Württemberg "Oberirdische Gewässer, Gewässer-ökologie 56".

Brock, T. C. M., R. P. A. van Wijngaarden, et al. (2000). Ecological risks of pesticides in freshwater ecosystems. Part 2: Insecticides, Alterra, Green World Research.

CEC (2000). "Directive of the European Parliament and of the Council: Establishing a framework for Community action in the field of water policy. 2000/60/EC, (23 October 2000). L 327/1, (22.12.2000)." Official Journal.

Chokmani, K. and J. Gallichand (1997). "Use of indicators for assessing the diffused pollution potential on two agricultural watersheds." *Canadian Agricultural Engineering* **39**: 113-122.

Connell, J.H., Sousa, W.P. 1983. “On the evidence needed to judge ecological stability and persistence.” *American Naturalist* **121**: 789-824.

Cooper, C.M. (1993). Biological effects of agricultural derived surface-water pollutants on aquatic systems – a review. *Journal of Environmental Quality* **22**: 402-408.

Dabrowski, J. M. and R. Schulz (2003). "Predicted and measured levels of azinphosmethyl in the Lourens River, South Africa: Comparison of runoff and spray drift." *Environmental Toxicology and Chemistry* **22**: 494-500.

DIN, D. I. f. N. e. V. (1990). Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlamm-untersuchung; Biologisch-ökologische Gewässeruntersuchung (Gruppe M); Bestimmung des Saprobienindex (M2). Beuth Verlag, Berlin.

EEC (2001). FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC**:** 245 pp.

Faasch (1997). Gewässergütebericht - Ergänzungen 1997, Bezirksregierung Braunschweig.

Fairchild, J.F., T. Boyle et al. (1987). "Effects of sediment and contaminated sediment on structural and functional components of experimental stream ecosystems." *Water, Air and Soil Pollution* **36**: 271-293.

Flanagan, D.C., L.D. Norton and I. Shainberg, (1997). "Effect of water chemistry and soil amendments on a silt loam soil - Part 1: Infiltration and runoff." *Transactions of the American Society of Agricultural Engineers* **40**: 1549-1554.

Gore, J.A. (1982). Benthic invertebrate colonization: source distance effects on community composition. *Hydrobiologia* **94**: 183-193.

Hatakeyama, S. and N. Yokoyama (1997). "Correlation between overall pesticide effects monitored by shrimp mortality test and change in macrobenthic fauna in a river." *Ecotoxicology and Environmental Safety* **36**: 148-161.

Hutchens, J.J, K. Chung and J.B. Wallace. (1998). "Temporal variability of stream macroinvertebrate abundance and biomass following pesticide disturbance." *Journal of the North American benthological Society* **17**: 518-534 DEC 1998

Ide, F.P. (1967). "Effects of Forest Spraying with DDT on Aquatic Insects of Salmon Streams in New Brunswick." *Journal of the Research Board of Canada* **24**: 769-805.

Kladivko, E. J., G. E. Vanscoyoc, et al. (1991). "Pesticide and nutrient movement into subsurface tile drains on a silt loam soil in Indiana." *Journal of Environmental Quality* **20**: 264-271.

Leonard, A. W., R. V. Hyne, et al. (2000). "Riverine endosulfan concentrations in the Namoi River, Australia: Link to cotton field runoff and macroinvertebrate population densities." *Environmental Toxicology and Chemistry* **19**: 1540-1551.

Liess, M., R. Schulz, et al. (1996). "A method for monitoring pesticides bound to suspended particles in small streams." *Chemosphere* **32**: 1963-1969.

Liess, M. and R. Schulz (1999). "Linking insecticide contamination and population response in an agricultural stream." *Environmental Toxicology and Chemistry* **18**: 1948-1955.

Liess, M., R. Schulz, et al. (1999). "Determination of insecticide contamination in agricultural headwater streams." *Water Research* **33**: 239-247.

Liess, M. and P. C. von der Ohe (2005). "Analyzing effects of pesticides on invertebrate communities in streams." *Environmental Toxicology and Chemistry* **24**: 954-965.

Linders, J., H. Mensink, et al. (2000). "Foliar interception and retention values after pesticide application. A proposal for standardized values for environmental risk assessment." *Pure and Applied Chemistry* **72**: 2199-2218.

Maltby, L. (1995). "Sensitivity of the crustaceans Gammarus pulex (L) and *Asellus aquaticus* (L) to short-term exposure to hypoxia and unionized ammonia: Observations and possible mechanisms." *Water Research* **29**: 781-787.

Matthiesen, P., D. Sheahan, et al. (1995). "Use of a *Gammarus pulex* bioassay to measure the effects of transient carbofuran runoff from farmland." *Ecotoxicology and Environmental Safety* **30**: 111-119.

Minshall, G.W., R.C. Petersen et al (1983). "Interbiome comparison of stream ecosystem dynamics." *Ecological monographs* **53**: 1-25.

Molles, M.C. (1985). "Recovery of a stream invertebrate community from a flash-flood in Tesuquee creek, New Mexico." *Southwestern Naturalist* **30**: 279-287

Müller-Westermeier, G. (1996). Klimadaten von Deutschland, Zeitraum 1961-1990. Offenbach am Main, Deutscher Wetterdienst.

OECD (2000). Report of the OECD pesticide aquatic risk indicators expert group. Braunschweig, Germany, OECD.

Probst, M., N. Berenzen, et al. (2005). "Linking land use variables and invertebrate taxon richness in small and medium-sized agricultural streams on a landscape level." *Ecotoxicology and Environmental Safety* **60**: 140-146.

Roex, E. W. M., C. A. M. Van Gestel, et al. (2000). "Ratios between acute aquatic toxicity and effects on population growth rates in relation to toxicant mode of action." *Environmental Toxicology and Chemistry* **19**: 685-693.

Schulz, R. and M. Liess (2001). "Runoff simulation with particle-bound fenvalerate in multispecies stream microcosms: importance of biological interactions." *Environmental Toxicology and Chemistry* **20**: 757-762.

Schulz, R., S. K. C. Peall, et al. (2001). "Concentration, load and toxicity of spraydrift-borne Azinphos-methyl at the inlet and outlet of a constructed wetland." *Ecological Engineering* **18**: 241-247.

Schulz, R., G. Thiere, et al. (2002). "A combined microcosm and field approach to evaluate the aquatic toxicity of azinphosmethyl to stream communities." *Environmental Toxicology and Chemistry* **21**: 2172–2178.

Schwoerbel, J. (1994). Methode der Hydrobiologie – Süßwasserbiologie (4. Auflage). Gustav Fisher Verlag, Jena.

Sheldon, F., A. J. Boulton, et al. (2002). "Conservation value of variable connectivity: aquatic invertebrate assemblages of channel and floodplain habitats of a central Australian arid-zone river, Cooper Creek." *Biological Conversation* **103**: 13-31.

Sherratt, T. N., G. Roberts, et al. (1999). "A life-history approach to predicting the recovery of aquatic invertebrate populations after exposure to xenobiotic chemicals." *Environmental Toxicology and Chemistry* **18**: 2512-2518.

Schriever, C.A., P.C. von der Ohe and M. Liess. (2005). „An indicator of the potential aquatic exposure caused by pesticide runoff.” *Environmental Toxicology and Chemistry* - submitted

Von der Ohe, P. and M. Liess (2004). "Relative Sensitivity Distribution (RSD) of Aquatic Invertebrates to Organic and Metal Compounds." *Environmental Toxicology and Chemistry* **23**: 150-156.

Wallace, J.B. (1990). Recovery of Lotic Macroinvertebrate Communities from Disturbance. *Environmental Management* **14**: 605-620.

Wallace, J.B., Vogel, T.S., Cuffney T.F. (1986). Recovery of a headwater stream from an insecticide-induced community disturbance. *Journal of the North American Benthological Society* **5**: 115-126.

Wally, W. F. and V. N. Fontama (1998). "Neural Network predictors of average score per taxon and number of families at unpolluted sites in Great Britain." *Water Research* **32**: 613-622.

Wright, J. F., M. T. Furse, et al. (1993). "RIVPACS - a technique for evaluating the biological quality of rivers in U.K." *European Water Control* **3**: 15-25.

Zelinka, M. and P. Marvan (1961). "Zur Präzisierung der biologischen Klassifikation der Reinheit fließender Gewässer." *Archiv für Hydrobiologie* **57**: 389-407.

Chapter V

2004

Chemical Research in Toxicology, accepted

STRUCTURAL ALERTS – A NEW CLASSIFICATION MODEL TO DISCRIMINATE EXCESS TOXICITY FROM NARCOTIC EFFECT LEVELS OF ORGANIC COMPOUNDS IN THE ACUTE DAPHNID ASSAY

PETER CARSTEN VON DER OHE, RALPH KÜHNE, RALF-UWE EBERT, ROLF ALTENBURGER,
MATTHIAS LIESS, GERRIT SCHÜÜRMANN*

Department of Chemical Ecotoxicology, UFZ - Centre for Environmental Research
Leipzig-Halle GmbH, Permoserstrasse 15, 04318 Leipzig, Germany

ABSTRACT

Quantitative and qualitative structure-activity relationships (QSARs) have a great potential to support the risk assessment of chemicals, provided there are tools available that allow evaluation of the suitability of QSARs for the compounds of interest. In this context, a pragmatic approach is to discriminate excess toxicity from narcotic effect levels, because the latter can be estimated from QSARs and thus have a low priority for experimental testing. To develop a respective scheme for the acute daphnid toxicity as one of the primary ecotoxicological endpoints, 1067 acute toxicity data for 380 chemicals involving the daphnid species *Daphnia magna* were taken from the on-line literature, and quality checks such as water solubility were employed to eliminate apparently odd data entries. For 36 known narcotics with LC50 values referring to *Daphnia magna*, a reference baseline QSAR is derived. Compounds with LC50 values above a certain threshold defined relative to their predicted baseline toxicity are classified as exerting excess toxicity. Three simple discrimination schemes are presented that enable the identification of excess toxicity from structural alerts based on the presence or absence of certain heteroatoms and their chemical functionality. Moreover, a two-step classification approach is introduced that enables a prioritisation of organic compounds with respect to their need for experimental testing. The discussion includes reaction mechanisms that may explain the association of structural alerts with excess toxicity, a comparison with predictions derived from MOA-based classification schemes, and a statistical analysis of the discrimination performance in terms of detailed contingency table statistics.

---

* To whom correspondence may be addressed: gerrit.schuurmann@ufz.de

INTRODUCTION

According to the upcoming European chemical policy (*1*), it is likely that until 2012, approximately 30,000 chemical substances with production volumes of more than one ton per year will require data for their toxicological and ecotoxicological evaluation. At present, however, we are far from having a complete picture with respect to the chemicals in use (*2*). It was estimated recently that for less than 1 % of the chemicals in commerce in the United States, experimental results for their aquatic toxicity are available (*3*).

In order to enable an efficient use of the available testing resources, a promising way forward would be to include low-cost screening methods that allow the identification of those chemicals where according to present knowledge, substantial toxicity for the endpoints of interest is to be expected. In this context, theoretical methods such as quantitative or qualitative structure-activity relationships (QSARs) may form a particularly efficient component besides experimental *in vitro* methods and high-throughput techniques. Recent QSAR research includes a variety of endpoints such as skin sensitisation (*4*), androgen receptor binding (*5*), aryl-hydrocarbon receptor binding (*6*), mixture toxicity prediction (*7*, *8*), acute aquatic toxicity (*9*) and rat chronic toxicity (*10*). Besides models to predict effect levels there are also classification schemes that relate modes of toxic action to structural features (*11*, *12*) or to property profiles calculated from molecular structure (*13-15*).

For the regulation of industrial chemicals, the US American and European legislation still differ significantly as regards the use of QSARs. Whilst the U.S. Environmental Protection Agency (EPA) as well as the Interagency Testing Committee (ITC) of the Toxic Substances Control Act (TSCA) use QSARs to estimate the toxicity of existing and new chemicals, the current European regulation is based on experimental data, employing QSARs only for specific and limited purposes such as data evaluation or the provision of additional evidence for conducting long-term tests (*16, 17*). However, it is likely that under the new REACH (Registration, Evaluation and Authorization of Chemicals) system to be introduced in Europe (*1*), more efforts will be given to include non-animal methods such as *in vitro* techniques and QSAR models in the regulatory decision making process, provided these have been validated appropriately.

Among the few QSAR packages with history in US regulatory programs, ECOSAR (*18*) and ASTER (*12*, *19*) are mainly based on linear regression equations relating toxicity to the octanol/water partition coefficient, K_{ow} (*3*). For a given chemical, the selection of the appropriate QSAR proceeds with ECOSAR by chemical class, which may be problematic for multifunctional compounds as well as for compounds where the log K_{ow} is not a good descriptor for the effect level of interest (*3*). ASTER first allocates the chemical to a likely mode of toxic action through analysis of substructural features. If narcotic-type toxicity or oxidative uncoupling was predicted as mode of action, the associated effect level is then predicted by accordingly selected QSARs, again employing log K_{ow} as only molecular descriptor.

Among the commercial packages that are used by the U.S. EPA is TOPKAT (*20*), which employs both classification schemes and multiple linear regression relationships to predict toxicity from molecular structure. According to a recent independent evaluation of the prediction power of TOPKAT for chronic oral rat toxicity, however, TOPKAT could not yield predictions for 108 (34 %) out of 315 organic compounds (*10*). Among the remaining 208 chemicals, the predicted lowest observed adverse effect level (LOAEL) was within a factor of 2, 5, and 10 of the experimental value for 43 %, 66 % and 80 % of the test set, respectively (*10*). For a different test set of 313 compounds, TOPKAT could not yield LOAEL predictions for 84 chemicals (27 %), and for the remainder the prediction error was similar to the one achieved with the other test set (*10*).

A recent comparative analysis of the performance of six QSAR packages to estimate the acute fish toxicity showed some variation between the different methods, also as regards the application range (*3*). Although the test set of 130 compounds consisted mainly of simple organics with low functionality, with more than 60 % being nonpolar narcotics, the overall performances of the six QSAR packages were only low to moderate: The mean absolute prediction error of the LC50 ranged from 0.9 to 1.3 log units, and the squared correlation coefficient (r^2) from 0.154 to 0.279. The proportion of chemicals with prediction errors above a factor of 10 was between 32 % and 45 %, and prediction errors above factors of 100 and 1000 were observed for test set proportions between 13 % and 22 %, and between 5 % and 9 %, respectively (*3*).

Here, the performance of TOPKAT was particularly interesting: Only 37% of the compounds were in the so-called optimum prediction space, and for these compounds the model performance was significantly better than when considering all formally applicable chemicals. With regard to the latter, there were also substantial differences observed: Only two of the six packages (including ECOSAR) could handle all compounds of the test set, whilst the ASTER methodology could be applied to only 92 of the 130 compounds.

As regards mode of action (MOA) classification schemes in the area of aquatic toxicology (*11, 12, 22*), we are not aware of a corresponding comparative study about the statistical performance with a test set of compounds that had not been included in the training sets. However, some experience has been reported with individual packages. In a recent evaluation of ASTER, MOA-based QSAR selection and subsequent application lead to an r^2 of 0.90 for a validation set of 97 chemicals (*21*), which is somewhat surprising when considering the much inferior statistics reported in the above-mentioned comparative study (*3*). Note further that with a descriptor-based classification of chemicals according to prevalent MOAs in fish, systematic errors were reported for epoxides, fluorinated hydrocarbons, thiols and α,β -unsaturated carbonyl compounds (*14*).

According to REACH (*1*), the acute daphnid toxicity will become the primary trigger for the aquatic toxicity evaluation in the European legislation. So far, there is no independent analysis available about the performance of QSAR models for this endpoint, and there is also no classification scheme available that allocates chemical structures to MOAs prevalent in the daphnid bioassay. In the present investigation, three classification models (CMs) are introduced that allow discrimination between compounds exerting narcotic effect levels and those exerting excess toxicity in the acute 48-h daphnid test, employing only simple structural features as molecular descriptors. To this end, 1067 LC50 (lethal concentration 50 %) values have been collected from the on-line literature (*23*) and critically evaluated, resulting in a final set of 300 organic compounds.

For the *a priori* identification of the narcotic effect range, the excess toxicity (T_e) concept (*24, 25*) is employed in connection with a reference baseline QSAR developed for a subset of 36 compounds that had already been classified as narcotic (*26*). Considering factors of 10 to account for both data uncertainty and the difference between nonpolar and polar narcotics when referring to the octanol/water partition coefficient (K_{ow}) as hydrophobicity parameter (*11*), LC50 values within a factor of 100 from baseline toxicity are classified as belonging to the narcotic effect range, and the remainder as indicating significant excess toxicity. For the three CMs, the concordance (overall agreement between experimental and predicted toxicity categories) ranges from 0.63 to 0.92, with predictivities (for each category the ratio of the numbers of correctly predicted over predicted compounds) up to 0.98.

Moreover, three existing classification models (*11*, *12*, *22*) that make use of substructural features to predict MOAs of organic compounds in the acute fish toxicity test are evaluated for their suitability to separate excess toxicity from the narcotic effect range. The results show that more than 60 % of the chemicals with reactive or specific MOAs in the fish test have acute daphnid toxicity values in the narcotic effect range. It suggests that for setting test priorities in tiered chemical hazard evaluation schemes, T_e-based CMs are superior to MOA-based CMs.

METHODS

From the US EPA database AQUIRE (*23*), 1067 acute toxicity values (48-h LC50, lethal concentration 50 %) for the cladoceran *Daphnia magna* were collected for a total of 380 compounds. The query was conducted for the endpoint mortality as recorded in AQUIRE. Note, however, that some studies use mortality (LC50) and immobilization (EC50, effective concentration 50 %) as identical endpoints in the context of daphnid toxicity, as is for example reported in the toxicity analysis of parathion (*27*) that is also included in the presently selected AQUIRE data set.

When multiple test values were found for one substance, these values were checked for consistency. If values differed by more than a factor of 30 from the closest one in a group of at least three other references, the aberrant value was discarded so as to remove outliers from the data set. Of all remaining values for a given substance, the arithmetic mean was taken as the valid experimental value.

Training set. From the initial set of 1067 LC50 data, 77 values were excluded as outliers as described above, which led to a set of 349 organic chemicals with at least one LC50 value per substance. Subsequently, 49 chemicals were excluded because their LC50 values exceeded the predicted water solubility (*28*), or because they contained metal atoms or were inorganic, leading to the final set of 300 organic compounds that cover a log K_{ow} (octanol/water partition coefficient) range from –2 to 8. All log K_{ow} were predicted using the KOWWIN software (*28*).

As regards the chemical domain, the data set includes hydrocarbons; aliphatic alcohols; phenols; ethers and esters; anilines; amines; nitriles; nitroaromatics; amides and carbamates; urea and thiourea derivatives; isothiocyanates; thioles; phosphorothionate and phosphate esters; halogenated derivatives.

For 36 compounds of the data set, the prevalent mode of toxic action had been reported as narcosis (*26*). Consequently, these 36 compounds were used to derive a baseline QSAR for the acute toxicity towards *Daphnia magna*. The remaining 264 compounds formed the training set for the derivation of classification models to discriminate between narcotic effect levels and excess toxicity.

Excess toxicity. The ratio between the QSAR-predicted baseline toxicity and the experimental toxicity was evaluated in terms of the excess toxicity T_e (*24*, *25*):

$$T_e = \frac{\text{LC50 (baseline)}}{\text{LC50 (exp.)}} \tag{1}$$

Polar narcotic compounds are known to be on the average ten times more toxic than non-polar narcotics (*11*), which corresponds to a log T_e of one. Moreover, data uncertainty is estimated to cover one order of magnitude, keeping in mind that the LC50 values collected in AQUIRE come from different laboratories. Therefore, it was decided that the narcotic toxicity range should comprise compounds whose toxicity values are within a factor of 100 of the baseline toxicity. A log T_e greater than two, corresponding to toxicity 100 times above baseline toxicity, was defined to indicate excess toxicity. In this way, excess toxicity is most likely associated with a reactive or specific mode of action, whilst it is not unlikely to find some non-narcotic chemicals with T_e values below 100 and even below 10, which is indeed the case and will be discussed in more detail below. Following this approach, all 264 compounds of the training set were classified as narcotic-level or excess toxic, which served as experimental categories for the subsequent classification modelling.

Derivation of classification models. Visual inspection of the chemical structures of the compounds exerting excess toxicity, followed by cross-checking the structural patterns of narcotic effect level compounds, led to the empirical identification of structural alerts as indicators of excess toxicity in terms of the presence or absence of certain heteroatoms and chemical functionalities. This approach was applied on two levels of complexity: Firstly, only the presence or absence of certain heteroatoms was analysed in comparison with log T_e. Secondly, substructural units encoding specific functional groups were identified as indicators of excess toxicity. To this end, all compounds were allocated to major chemical classes, keeping in mind that a unique classification is not feasible for multifunctional compounds. The distribution of chemical classes and subclasses with regard to the observed T_e values resulted in initial hypotheses about functional groups that might discriminate, as good as possible, between narcosis-level and excess toxicity. Detailed visual analyses of the chemical structures in relation to their log T_e values finally led to the derivation of three classification models (CMs), which are comparatively analysed for their overall prediction performance (concordance) as well as for their sensitivity (recognition power) and predictivity (prediction power) using contingency table statistics (see below).

For comparative purposes, the following three existing MOA-based classification schemes (CMs) were included in the analysis: a 4-MOA CM (*11*), a 7-MOA CM (*12*), and a 2-MOA CM (*22*). For convenience, all classification rules have been implemented in our ChemProp software system (*29*), allowing automated classification runs according to all six schemes.

Contingency table statistics. The performance of the different classification schemes was evaluated in terms of detailed contingency table statistics as outlined elsewhere (*15*), considering the two categories narcotic effect range and excess toxicity. Here, the concordance is calculated as the sum of all compounds that are correctly predicted to belong to one of the two categories (n_{cpred}), divided by the total number of compounds (N). It represents the proportion of compounds where the predicted and the experimental classification agree:

$$\text{concordance} = \frac{1}{N}\sum_{i=1}^{2} n_{\mathrm{cpred}} \tag{2}$$

To evaluate the category-specific performances, the following two parameters have been employed (*15*). For a given category, the sensitivity

$$\text{sensitivity} = \frac{n_{\mathrm{cpred}}}{n_{\mathrm{exp}}} \tag{3}$$

is calculated as the number of compounds correctly predicted to belong to this category (n_{cpred}), divided by the number of compounds that actually belong to this category (n_{exp}). As such, the sensitivity may also be referred to as the recognition power of the classification model (*15*). Correspondingly, the predictivity

$$\text{predictivity} = \frac{n_{\mathrm{cpred}}}{n_{\mathrm{pred}}} \tag{4}$$

is defined as the number of compounds correctly predicted to belong to one category (n_{cpred}), divided by the total number of compounds predicted to belong to this class (n_{pred}).

QSAR modelling. For subsets of narcotic effect level compounds, linear regression of log LC50 on calculated log K_{ow} was performed and compared with baseline toxicity QSARs. The statistical tests and graphs have been carried out with SPSS (*30*) and the SigmaPlot graphics package (*31*).

RESULTS

Baseline toxicity. For the 36 compounds reported as narcotics (26), linear regression of log LC50 on log K_{ow} yields

$$\text{log LC50 [mol/L]} = -0.857\ (\pm\ 0.049)\ \log K_{\mathrm{ow}} - 1.281\ (\pm\ 0.125) \tag{5}$$

where n = 36, r^2 = 0.90, SE = 0.44, $F_{1,34}$ = 311. Eq. 5 thus represents a baseline QSAR for the acute toxicity towards *Daphnia magna*, and was used to evaluate the excess toxicity in terms of T_{e} values according to Eq. 1. Interestingly, the subset of 33 compounds classified as polar narcotics according to the 4-MOA scheme trained on acute fish toxicity (11) yields

$$\log LC50\ [mol/L] = -0.802\ (\pm\ 0.085)\ \log K_{ow} - 2.206\ (\pm\ 0.244) \qquad (6)$$

for the acute daphnid toxicity ($n = 33$, $r^2 = 0.74$, $SE = 0.45$, $F_{1,31} = 89.9$), with a quite similar slope (–0.802 vs. –0.857) and an intercept one log unit lower (–2.206 vs. –1.281) as compared to Eq. 5 that represents daphnid baseline narcosis. For the derivation of Eq. 6, all anilines were left out, because there is evidence that anilines show substantial excess toxicity towards daphnids despite their polar narcotic effect level in fish (32).

A similar result is achieved when applying the 7-MOA scheme that was also derived from acute fish toxicity data (12), and the respective data distributions are shown in the left and right part of Figure 1, respectively. It follows that in contrast to findings with polar narcosis towards fish (33), a convergence of the log LC50 vs. log K_{ow} relationships for baseline and polar narcosis with increasing log K_{ow} is not observed for the acute daphnid toxicity according to the presently available AQUIRE data. Note also that the log K_{ow} -based regression relationships for nonpolar and polar narcosis towards the fish species *Pimephales promelas* (slope: –0.85 vs. –0.73; intercept: –1.39 vs. –2.16) as recommended by the European Union (17) intersect each other only at the high log K_{ow} of 6.42.

Discrimination between narcotic effect levels and excess toxicity. For the training set of 264 compounds, baseline daphnid toxicity was predicted through Eq. 5, and their actual experimental LC50 values were classified through Eq. 1 as narcotic effect level or excess toxic. Here, log $T_e > 2$ indicated excess toxicity, whilst LC50 values with log $T_e \leq 2$ were classified as belonging to the narcotic effect range (see section Materials and Methods). In this way, 78 of the 264 compounds were classified as exerting excess toxicity, and the remaining 186 compounds as yielding narcotic effect levels. The associated LC50 data cover more than nine orders of magnitude, ranging from 8.04×10^{-10} mol/L (deltamethrin) to 0.34 mol/L (triethylene glycol). Note further that for the subset of 78 compounds exerting excess toxicity, the LC50 variation is seven orders of magnitude. In Table A5, all 300 compounds are listed together with their mean experimental daphnid toxicities in terms of log LC50, calculated log K_{ow} values and the logarithmic excess toxicities (log Te). In addition, the toxicity category (narcotic effect range vs. excess toxicity) predicted by CM1, CM2 and CM3 as well as by the three literature schemes 4-MOA CM (11), 7-MOA CM (12) and 2-MOA CM (22) are summarized and will be explained in more detail below.

Classification model 1 (CM1). According to Könemann (*34*), baseline toxicity is expected for simple organic compounds such as aliphatic and aromatic hydrocarbons, aliphatic alcohols, aldehydes and ketones, and halogenated derivatives. A generalisation of this approach leads to the following simple condition for a chemical to exert a narcotic-level toxicity: The compound may contain any combination of C, H, O and halogen, excluding α,β-unsaturated carbonyl groups as electrophilic functionalities. As will be shown below, this simple approach is particularly powerful in predicting chemicals as exerting narcotic effect levels.

According to CM1, all compounds containing heteroatoms other than O and halogen as well as all α,β-unsaturated carbonyl derivatives would be classified as excess toxic in the 48-h *Daphnia magna* test. Interestingly, this prediction of excess toxicity is much less reliable a

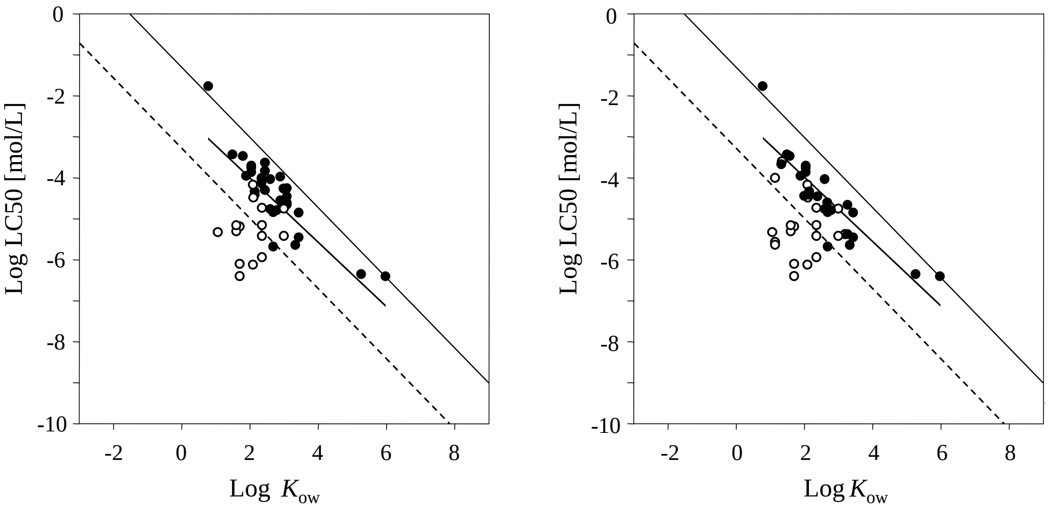


Figure 1. Log LC50 [mol/L] vs log K_{ow} for 33 polar narcotics (filled circles) and 15 anilines (open circles), classified by the 4-MOA CM (left plot) and for 25 polar narcotics (filled circles) and 20 anilines (open circles), classified by the 7-MOA CM (right plot). The regression lines for the polar narcotics read log LC50 [mol/L] = -0.795 log K_{ow} - 0.831 (left plot) and log LC50 [mol/L] = -0.795 log K_{ow} - 0.831 (right plot). In both plots, the regression line of Eq. 5 representing baseline toxicity (solid line) and the threshold of excess toxicity (dashed line) are included for comparison.

compared to the complementary prediction of narcotic effect levels, because CM1 tends to predict too many compounds as excess toxic. Despite this disadvantage, the predictive mode of CM1 for narcotic effect levels appears to be highly reliable, suggesting that in a tiered approach, CM1 could serve as a first-tier component for the predictive identification of compounds that are highly probable to exert only narcotic effect levels (see below). A more detailed evaluation of the statistical performance of CM1 is given below.

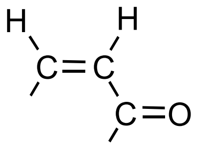
Classification models 2 and 3 (CM2 and CM3) - Structural Alerts (SAs). CM2 and CM3 are based on structural alerts as indicators of excess toxicity. CM2 is based on nine SAs (SA1 – SA9) each of which is defined through a single substructural unit (see below), forming the primary rules to identify excess toxic chemicals. The statistical selection criterion was that for each correspondingly defined SA, at least three compounds with T_e values above 100 are present in the data set.

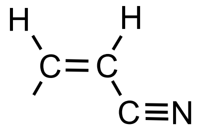
Inspection of the remaining classification errors led to the identification of further eight substructural units, each of which belonged to one or two compounds exerting excess toxicity (of course, without counterexamples). Because these structural features form variants of the primary rules of CM2, they were considered as additional tentative alerts, and led to the definition of a correspondingly augmented classification scheme 3, CM3, consisting of nine partially extended structural alerts (SA1 – SA2, SA3*, SA4, SA5* – SA7*, SA8 – SA9; see below).

In the respective list of SAs given below, open valences indicate attachment to any of the elements C, H, N, O, P, S, F, Cl, Br, or I. For CM2, only the first substructural unit of each SA is relevant (e.g. in case of SA3: 3.1: Phosphorothionates with different heteroatoms), and

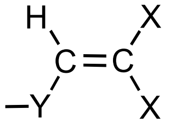
CM3, the additional tentative rules – as far as present – are marked through a star (e.g. in case of SA3: 3.2*: Thiophosphonates).

SA1. α,β-unsaturated carbonyl and nitrile compounds



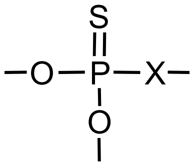


SA2. Carbon-carbon double bond activated by two halogens (X = halogen; Y = C, O or N).



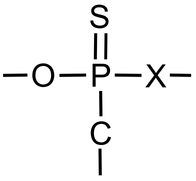
SA3. Organophosphorus compounds

3.1 Phosphorothionates with different heteroatoms (X = O or S).



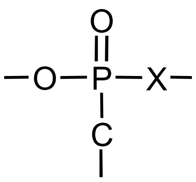
$-\mathrm{O}-\overset{\mathrm{S}}{\overset{\|}{\underset{\underset{\underset{|}{\mathrm{O}}}{|}}{\mathrm{P}}}}-\mathrm{X}-$

3.2* Thiophosphonates (X = O or S).



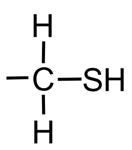
$-\mathrm{O}-\overset{\mathrm{S}}{\overset{\|}{\underset{\underset{\underset{|}{\mathrm{C}}}{|}}{\mathrm{P}}}}-\mathrm{X}-$

3.3* Phosphonates (X = O or S).



$-\mathrm{O}-\overset{\mathrm{O}}{\overset{\|}{\underset{\underset{\underset{|}{\mathrm{C}}}{|}}{\mathrm{P}}}}-\mathrm{X}-$

SA4. Aliphatic thioles



$-\overset{\mathrm{H}}{\overset{|}{\underset{\underset{\mathrm{H}}{|}}{\mathrm{C}}}}-\mathrm{SH}$

SA5.Isothiocyanates and thiocyanates

5.1 Isothiocyanates.

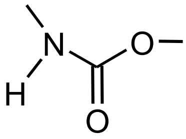
$$-N{=}C{=}S$$

5.2[*] Thiocyanates.

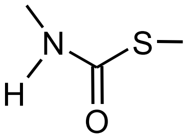
$$-S-C{\equiv}N$$

SA6. Carbamates

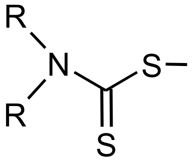
6.1 Simple carbamates.



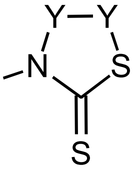
6.2[*] Thiocarbamates.



6.3[*] Dithiocarbamates (R = H or CH_3).

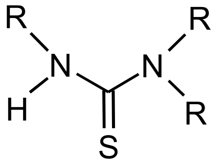


6.4[*] Rhodanin derivatives (Y = C, O, N or S).

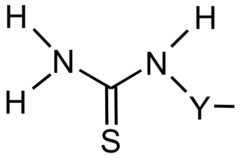


SA7. Thiourea derivatives

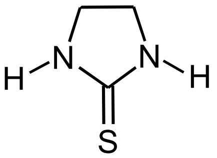
7.1 with at least two hydrogen atoms and a maximum of two methyl groups attached to the two nitrogens (R = CH_3).



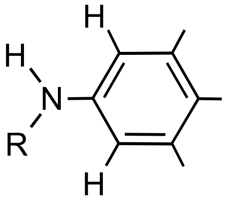
7.2[*] with one aliphatic chain attached to nitrogen (Y ≠ H, CH_3).



7.3[*] Cyclic ethylene-thiourea derivatives.



SA8. Primary or secondary anilines without ortho substituents (R = H, CH_3 or C_2H_5).



SA9. Imid derivatives with different heteroatoms (Y= C, O or N; Z = O or N).

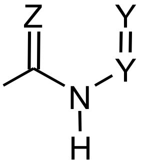


Table 2. Classification Models used for Discriminating between Narcotic Effect Levels and Excess Toxicity.

	Predicted category	
Models	Excess Toxicity	Narcotic Effect Level
CM1	All organic chemicals other than narcosis.	Organic chemicals that contain any combination of C, H, O and halogens, not showing SA 1.
CM2	Organic chemicals containing SA1-SA9	All other organic chemicals
CM3	Organic chemicals containing SA1-SA2, SA3*, SA4, SA5*-SA7*, SA8, SA9	All other organic chemicals
4-MOA CM (*11*)	Organic chemicals classified as (3) reactive chemicals and (4) specifically acting chemicals	Organic chemicals classified as (1) inert chemicals and (2) less inert chemicals
7-MOA CM (*12*)	Organic chemicals classified as (4) oxidative phosphorylation uncouplers, (5) electrophiles and proelectrophiles, (6) AChE inhibitors, and (7) CNS seizure agents	Organic chemicals classified as (1) base-line narcotics, (2) polar narcotics, and (3) ester narcotics
2-MOA CM (*22*)	Organic chemicals classified as (1) electrophiles or (2) proelectrophiles	All other organic chemicals

In Table 2, the relevant structural rules of CM1, CM2 and CM3 are summarized. Moreover, Table 2 contains the rules used to apply the MOA-based schemes 4-MOA CM (*11*), 7-MOA CM (*12*) and 2-MOA CM (*22*) for distinguishing between narcotic effect levels and excess toxicity as defined in the present context. To this end, for each MOA all narcotic modes of action (e.g. "inert chemicals" and "less inert chemicals" in the case of the 4-MOA CM) were allocated to the narcotic toxicity range, and all reactive or specific MOAs (e.g. electrophilic and proelectrophilic toxicity in the case of the 2-MOA CM) were allocated to the excess toxicity. It should be noted that the original derivation of these three CMs did not consider the presently employed T_e criterion. As a consequence, the statistical performances as presented below do not evaluate their validity in terms of MOA classifications, but only their suitability to discriminate between the narcotic effect range and excess toxicity.

Coming back to the prediction results of these six CMs for the present set of 300 compounds, the information summarized in Table A5 is illustrated with two examples: For DDT (compound #1 in Table A5), CM1, CM2, CM3 and the 2-MOA CM agree in their prediction of a narcotic toxicity range, whilst the 4-MOA CM classifies DDT as specifically acting compound, and the 7-MOA CM as CNS seizure agent. Note, however, that in the acute daphnid test, the log T_e of DDT is 0.79 and thus well in the range expected already from narcotics, despite the well-known fact that DDT has the potential to affect specifically the central nervous system.

With carbaryl (compound #18 in Table A5), the 4-MOA CM does not offer structural rules to predict any MOA, whilst the 7-MOA CM classifies this compound as exerting nonpolar narcosis, which agrees with the respective implicit prediction when applying the 2-MOA CM (because carbaryl does not contain any of the electrophilic and proelectrophilic structural features as defined in this CM). According to CM1, CM2 and CM3, carbaryl is classified as excess toxic in agreement with the experimental log T_e value of 4.04 towards *Daphnia magna.* With CM1, the relevant structural criterion is that carbaryl contains nitrogen (in form of –NH–), whilst the relevant structural alert of CM2 and CM3 is the carbamate functionality (SA5, –O–CO–NH–). Note that in both cases, fish-trained MOA predictions (if applicable) would suggest toxic effect levels in ranges significantly different from what is actually found in the daphnid bioassay.

Sterical Influence. Figure 2 contains examples of chemicals classified as thiourea derivatives by SA7. These compounds show similar molecular structures but differ in their log T_e. With increasingly bulky substitution at the thiourea nitrogen, the excess toxicity is lowered: The parent substance thiourea showed the highest toxicity with a log T_e of 3.55, whereas 1,3-diethylthiourea with two ethyl side chains was not excess toxic.

Classification Performances. In Table 3, the performance of each CM is evaluated in terms of contingency tables. The last column shows the number of compounds that could actually be classified by the given CM. Taking CM2 as an example, 205 of the 264 compounds were predicted to exert a narcotic-level toxicity, of which 179 actually show LC50 values in the narcotic range as defined through log $T_e \leq 2$, and 26 compounds have LC50 values exceeding that toxicity range. Moreover, with CM2 seven of the 186 narcotic-level compounds are (wrongly) predicted to exert excess toxicity, and 52 of the 78 compounds with experimental excess toxicities are correctly recognized.

Besides the newly introduced CM1, CM2 and CM3, the 2-MOA CM (a scheme to identify electrophilic and proelectrophilic structures as indicators of excess toxicity) and the 7-MOA CM are able to provide classifications for all 264 compounds of the training set. The latter, however, yields conflicting MOA allocations for 15 of the compounds (cf. Table A5). With the simpler 4-MOA CM, only 139 substances (52 %) can be classified (which means that structural rules are missing for 125 compounds), and here one compound is allocated to two different MOAs.

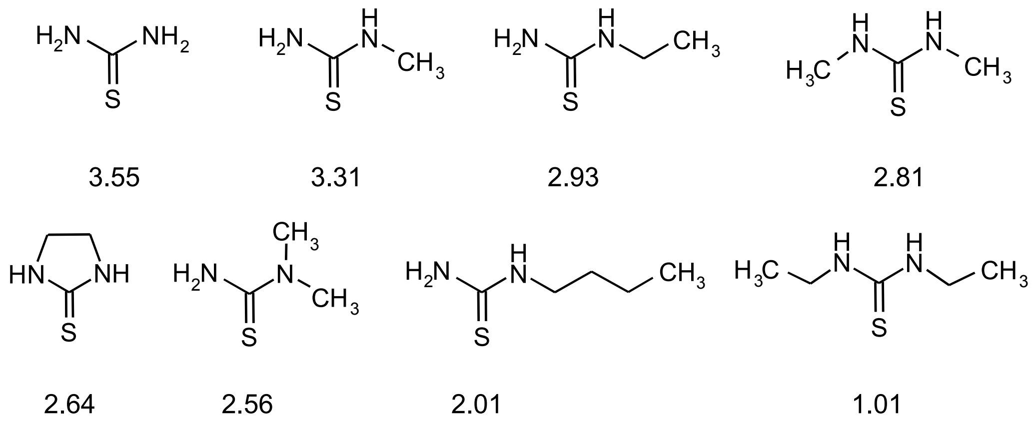


Figure 2. Thiourea derivatives that contain similar molecular structures, ranked according to their excess toxicity (log T_e).

The associated performance statistics in terms of the concordance (Eq. 2), sensitivity (Eq. 3) and predictivity (Eq. 4) are summarized in Table 4. Taking CM1 as example, a high predictivity for narcotic-level toxicity (0.978) contrasts with a relatively low respective sensitivity (0.478). The former indicates that compounds predicted to exert a narcotic-level LC50 have a high probability that their acute daphnid toxicity is actually in the narcotic range. However, with the present training set 52 % of the narcotic-level compounds are not recognized as such when applying CM1. By contrast, the CM1 recognition power for compounds exerting excess toxicity is very high (sensitivity 0.974), whilst its predictivity for such compounds is low (0.439). As a consequence, the overall agreement between predicted and actual toxicity category is only moderate for CM1 (concordance = 0.625).

CM2 provides a significant overall improvement as compared to CM1, except that the latter is particularly strong in predicting narcotic effect levels towards daphnids, which could be very helpful in identifying those compounds where narcotic effect level QSARs for the acute daphnid toxicity can be applied. The greatest concordance is achieved with CM3, keeping in mind that here several rules have been applied that at this stage are only tentative.

All three MOA-based CMs taken from the literature (*11, 12, 22*) show only moderate concordances in the present context of discriminating narcotic effect levels from excess toxicity. Interestingly, their prediction power for excess toxicity is particularly poor (0.35 – 0.43). Note further that when applying the 2-MOA CM structural alerts for electrophilic and proelectrophilic substructures, only 16.7 % of the compounds exerting excess toxicity in the *Daphnia magna* test are actually recognized.

QSAR modelling of narcotic-level toxicity. Figure 3 shows the data distributions of log LC50 vs. log K_{ow} for different subsets of compounds that are classified as exerting narcotic-level toxicities towards *Daphnia magna*. In the top left part of the figure, the respective plot with the subset of 36 compounds classified previously as narcotics (*26*) is shown, and the associated regression line of the baseline QSAR (Eq. 5) is included for comparison also in all other plots of the figure.

For the subset of 91 compounds predicted to exert narcotic-level toxicities according to CM1, the respective data distribution in the top right of Figure 3 shows that most of the LC50 values are slightly below the baseline regression line. Consequently, the respective regression equation CM1 narcotic-level toxicity:

$$\log LC50\ [mol/L] = -0.801\ (\pm 0.038) \log Kow - 2.139\ (\pm 0.144) \qquad (7)$$

(n = 91, r2 = 0.83, SE = 0.66, F1,89 = 448) has a similar slope as compared to Eq. 5 (–0.801 vs. –0.857), but an intercept that is almost one log unit lower than the one of the baseline QSAR (–2.126 vs. –1.281). The latter has two causes: Firstly, narcotic-level compounds according to CM1 include, by definition, substances with excess toxicities (Te) up to 100 to account for both data uncertainty and effect level differences between nonpolar and polar narcosis. Secondly, the 36 known baseline narcotics had – by intention – been excluded from the training set used for deriving CM1 (as well as CM2 and CM3), and so do not belong to the subset of 91 compounds used for the calibration of Eq. 7. Note also that Eq. 7 is very similar to Eq. 6. However, when applying the fish-trained 4-MOA scheme (11)

Table 3. Two Dimensional 2 × 2 Contingency Table of Six Classification Models.

	Category type			
	Predicted Category	Experimental Category		
Models		Narcotic Effect Level	Excess Toxicity	Total
CM1	Narcotic effect level	89	2	1
	Excess toxic	97	76	73
	Total	186	78	64
CM2	Narcotic effect level	179	26	05
	Excess toxic	7	52	9
	Total	186	78	64
CM3	Narcotic effect level	179	14	93
	Excess toxic	7	64	1
	Total	186	78	64
4-MOA CM	Narcotic effect level	59	11	0
	Excess toxic	44	25	9
	Total	103	36	39
7-MOA CM	Narcotic effect level	125	45	70
	Excess toxic	61	33	4
	Total	186	78	64
2-MOA CM	Narcotic effect level	169	65	34
	Excess toxic	17	13	0
	Total	186	78	64

to identify polar narcotics and leaving out anilines for the reasons mentioned above, 2/3 of the compounds with polar narcotic effect levels towards daphnids are actually overlooked. By construction, Eq. 7 yields an LC50 estimate of compounds in the narcosis toxicity range, whilst application of Eq. 5 provides the minimum daphnid toxicity expected from baseline narcosis. Note further that Eq. 5 is in fact similar to a previously published baseline QSAR based on 17 narcotics (26):

$$\log \mathrm{LC50}\ [\mathrm{mol/L}] \ = \ -0.95 \log \mathrm{Kow} \ - 1.19 \qquad (8)$$

where n = 17, r2 = 0.99, SE = 0.21. Because of the low CM1 sensitivity for narcotic-level daphnid toxicity (cf. Tables 3 and 4), 97 compounds with experimental LC50 values in the log Te range up to 2 are not included in Eq. 7. With CM2 and CM3, the respective sensitivity is significantly higher (0.962), with slightly reduced predictivities (0.873 and 0.927) as compared to CM1 (0.978).

Table 4. Contingency Table Statistics of Six Classification Models [a]

Model	Concordance	Category	Statistical Evaluation	
			Sensitivity	Predictivity
with anilines				
CM1	0.625	Narcotic effect level	0.478	0.978
		Excess Toxicity	0.974	0.439
CM2	0.875	Narcotic effect level	0.962	0.873
		Excess Toxicity	0.666	0.881
CM3	0.920	Narcotic effect level	0.962	0.927
		Excess Toxicity	0.820	0.901
4-MOA CM	0.604	Narcotic effect level	0.573	0.843
		Excess Toxicity	0.694	0.362
7-MOA CM	0.598	Narcotic effect level	0.672	0.735
		Excess Toxicity	0.423	0.351
2-MOA CM	0.689	Narcotic effect level	0.909	0.722
		Excess Toxicity	0.167	0.433
without anilines				
4-MOA CM	0.642	Narcotic effect level	0.563	0.964
		Excess Toxicity	0.926	0.373
7-MOA CM	0.616	Narcotic effect level	0.655	0.784
		Excess Toxicity	0.508	0.351
2-MOA CM	0.715	Narcotic effect level	0.904	0.755
		Excess Toxicity	0.200	0.433

For the subset of 193 compounds predicted as exerting narcotic-level toxicities according to CM3, linear regression of log LC50 on log K_{ow} yields:

$$\log \mathrm{LC50}\ [\mathrm{mol/L}] = -0.748\ (\pm\ 0.030) \log K_{ow} - 2.393\ (\pm\ 0.101) \quad (9)$$

where n = 193, r2 = 0.76, SE = 0.76, $F_{1,191}$ = 614. Again, the slope of the regression is similar to the baseline QSAR of Eq. 5, while the intercept is still lower as compared to Eqs. 5 and 7. As can be seen from the plot in the bottom left of Figure 3, the scatter of the data is somewhat larger due to the greater number of misclassified excess toxic chemicals, which is also reflected in the lower calibration r^2 and greater standard deviation of the regression statistics.

For comparison, all compounds predicted to exert excess toxicity according to CM3 are shown in the bottom right of Figure 3. Except for seven misclassified compounds (cf. Tables 1 and 3), all other substances have T_e values above 100, corresponding to graphical locations in the lower left triangle of the log LC50 vs. log K_{ow} plot.

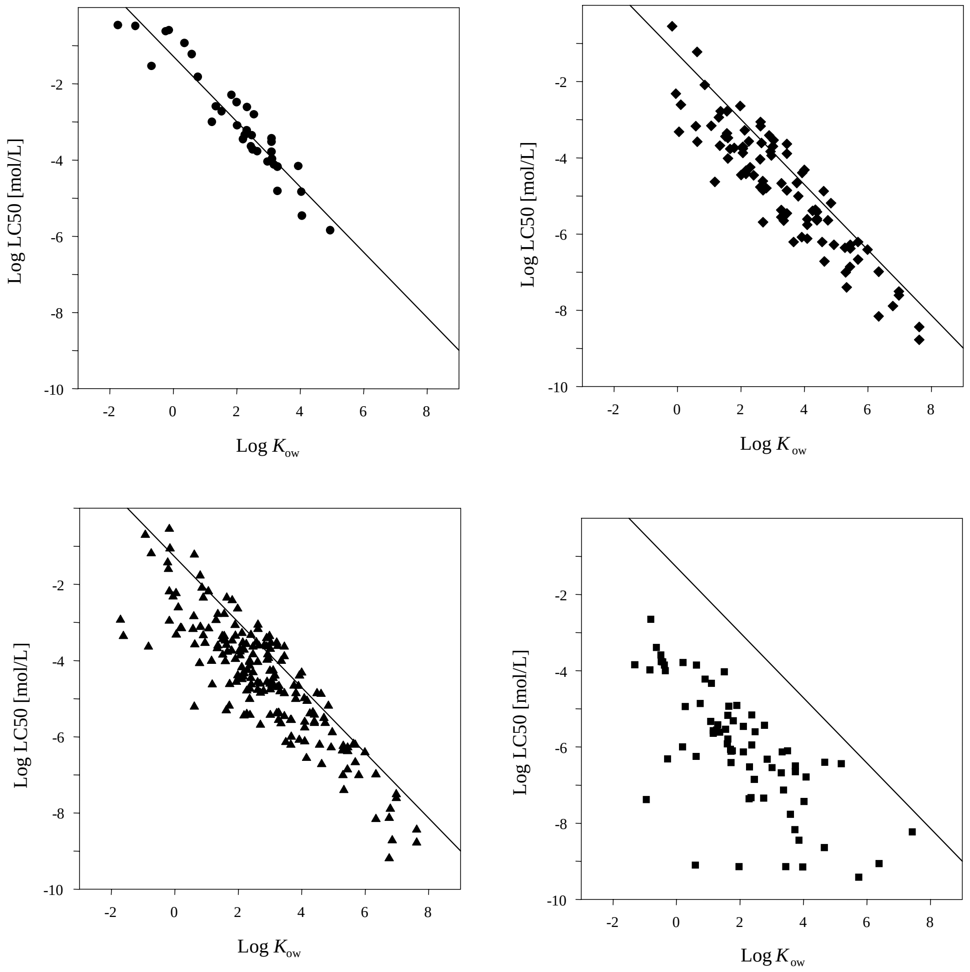


Figure 3. Log LC50 (mol/L) vs. log K_{ow} for 36 known nonpolar narcotics (circles, top left), 91 CM1-narcotic-level compounds (diamonds, top right), 193 CM3-narcotic-level compounds (triangles, bottom left), and 71 chemicals predicted to exert excess toxicity according to CM3 (squares, bottom right). In all four plots, the regression line of Eq. 5 representing baseline toxicity is included for comparison.

Validation. Because the structural alerts have been derived through visual inspection of the chemical structures and T_e values of all compounds of the training set, internal validation procedures such as cross-validation do not apply. Moreover, for the training set all suitable *Daphnia magna* LC50 entries from the AQUIRE database had been included, such that the collection of an additional compound set with respective toxicity values does not appear to be feasible at this point in time.

In order to still perform some kind of validation, all AQUIRE entries referring to other species of the daphniidae family were collected and subjected to the same selection and quality criteria (only uniquely defined organic chemicals without metals, mean values without apparent outliers, water solubility cut-off) as with *Daphnia magna* (Table 5). In this way, an initial validation set of 74 compounds with LC50 values covering six different daphnid species (*Daphnia pulex*: 36 values; *Ceriodaphnia dubia*: 23 values; *Daphnia carinata:* 5 values; *Moina macrocopa:* 4 values; *Daphnia laevis*: 3 values; *Daphnia pulicaria*: 3 values) was constructed (validation set 1). However, 47 of the respective compounds belong also to the *Daphnia magna* training set, leaving a final validation set of 27 compounds (validation set 2).

Only the latter offers an external validation of the presently derived CMs, whilst the former allows one to evaluate the possibility of extrapolating the T_e-based classification concept to other daphnid species.

In the upper part of Table 6, the contingency table statistics for the extrapolation across species are summarized using validation set 1 (74 compounds). The statistical performances are similar to the ones achieved with the *Daphnia magna* training set (Table 4), indicating that the Te criterion applied to discriminate narcotic effect levels from excess toxicity can well be extrapolated across different daphnid species. Because none of the CM3-specific tentative rules were applicable, the validation results of CM2 and CM3 are identical. As regards the 27 compounds outside the *Daphnia magna* training set (validation set 2), the CM1 prediction power for narcotic-level toxicities is similar to the corresponding training result, whilst the CM1 validation performance for the excess toxicity is inferior. With CM2, the validation statistics are overall of similar quality as the training statistics.

DISCUSSION

The intention of this work was to derive structural rules as a tool for a first-tier risk assessment that allows for discrimination of compounds with narcotic effect levels from those that are likely to exert excess toxicity in the acute daphnid test. Baseline toxicity QSARs allow one to estimate, with reasonable accuracy, the aquatic toxicity of narcotics. It follows that such compounds would have little priority for experimental testing. Thus, the ability to identify – directly from chemical structure – compounds that are likely to be toxic only in the narcotic range would offer a possibility to reduce the need for experimental testing, and thus provide an attractive component of a tiered chemical hazard classification scheme.

Table 6. External validation of CM1, CM2 and CM3 using LC50 values of Six Daphnid Species other than *Daphnia magna*.[a]

			Statistical Evaluation	
Model	Concordance	Category	Sensitivity	Predictivity
Validation set 1 (74 compounds)				
CM1	0.662	Narcotic effect level	0.574	0.940
		Excess Toxicity	0.900	0.439
CM2/CM3	0.932	Narcotic effect level	0.981	0.930
		Excess Toxicity	0.800	0.941
Validation set 2 (27 compounds)				
CM1	0.481	Narcotic effect level	0.435	0.909
		Excess Toxicity	0.750	0.188
CM2/CM3	0.963	Narcotic effect level	1.000	0.958
		Excess Toxicity	0.750	1.000

[a] Validation set 1 covers the following six daphnid species (and associated number of compounds): *Daphnia pulex* (36), *Ceriodaphnia dubia* (23), *Daphnia carinata* (5), *Moina macrocopa* (4), *Daphnia laevis* (3), *Daphnia pulicaria* (3). However, only 27 of the 74 compounds do not belong to the *Daphnia magna* training set, and form validation set 2.

Excess-toxic and narcotic-level chemicals. In the presently analysed data set, 36 of the 78 compounds with log T_e values above 2 are pesticides, and nine of the ten compounds with a log T_e above 4 are also pesticides. At the same time, 30 pesticides have log T_e values below 2, and 17 even below 1. It demonstrates that the knowledge about whether or not a given compound is a pesticide would not be a reliable predictor of excess toxicity, although highly toxic chemicals have a high probability of being used as pesticides.

Among the 91 compounds with T_e values in the range 1-10 are 23 hydrocarbons, 11 phenols, one aniline, 17 nitroaromatics, six urea and thiourea derivatives, three esters, and three organophosphorus compounds. Note that in a previous MOA classification study, the fish toxicity of nitroaromatics was allocated to a reactive mode of action with a likely enhanced toxicity (*14*), whilst with algae both narcotic-type and excess toxicity were observed and related to electronic structure characteristics of the compounds (*35*). In the T_e range 10-100 are four hydrocarbons, 12 phenols, seven anilines, three urea and thiourea compounds, and three isothiocyanates. Monofunctional compounds with T_e values above 100 include 11 anilines, but only one phenol. This distribution of chemical classes across effect levels shows that a classification scheme based on simple compound classes would not perform well in discriminating between narcotic-level and excess toxicity towards daphnids.

Similarly, the focus on previously published lists of electrophilic structural features (*12*, *22*) would not yield a reliable identification of excess-toxic chemicals with respect to daphnids. On the one hand, well-known electrophiles such as the Michael-type acceptors acrylamid (#10), acrolein (#98) and acrylonitrile (#102) as well as the S_NAr-sensitive 2,4-dinitro-1-chlorobenzene (#70) have T_e values above 100. On the other hand, however, a variety of electrophiles classified according to the fish-trained 7-MOA scheme (*12*) or according to the 2-MOA scheme (*22*) is associated with the T_e range 1-100, and a still substantial number of electrophiles has T_e values in the range 1-10: When applying the 7-MOA scheme, 15 electrophiles have T_e values below 10, and 13 electrophiles have T_e values in the range 10-100. With the 2-MOA scheme, 17 electrophiles have T_e values below 100, and seven of them below 10. These findings show that existing schemes to identify electrophiles do not provide a good discrimination between excess-toxic and narcotic-level compounds.

Allylamine (#101) is an example of a total of 20 compounds with log T_e values in the range 1.5-2.0 that could be considered as grey zone between narcotic-level and excess toxicity. With mammals, allylamine is highly toxic to the heart, which is traced back to a metabolic activation by monoamine oxidase to yield acrolein (*36*). However, direct daphnid exposure to acrolein results in a much higher toxicity with a log T_e value of 4.55. One possible reason for this discrepancy could be that the strong basicity of allylamine makes this compound significantly less bioavailable for the aqueous exposure pathway (through formation of the ammonium form prevalent under neutral pH conditions). In any case, allylamine belongs to the narcotic range as defined in this study, and is correctly classified through CM2, CM3 as well as through the 2-MOA scheme, whilst CM1 and the 7-MOA scheme predict this compound to be excess toxic, and the 4-MOA scheme does not offer a classification due to missing structural features. Interestingly, three of the seven compounds misclassified by CM2 and CM3 as excess toxic (cf. Table 3) have log T_e values in this grey zone range of 1.5-2.0. It should be noted, however, that there are also classes of electrophiles with only little representation in the presently analysed set of 300 compounds. In the AQUIRE database, only two epoxides (#26 and #68) were found that both have daphnid LC50 values in the narcotic level (with log T_e values of 1.08 and 1.37, respectively). Another example is given by the S_N2 reactants epichlorohydrin (#97) and 2-chloroethanol (#100), where again the daphnid toxicity is in the narcotic range for both compounds (log T_e 1.76 and 1.24, respectively). It follows that a more comprehensive analysis of such underrepresented electrophilic structures cannot be undertaken at this stage, but requires additional test data, as is similarly the case for existing fish-trained MOA classification schemes. A further aspect of interest in future studies may be, how the presently selected T_e value of 100 as cutoff between the narcotic range (including both data uncertainty and the systematic difference between nonpolar and polar narcosis as outlined above) and excess toxicity would perform as compared to alternative approaches.

Chemical reactivity and metabolic potential of structural alerts. The structural alerts (SAs) of CM1 (SA1- SA2), CM2 (SA1- SA9) and CM3 (SA1 – SA2, SA3*, SA4, SA5* – SA7*, SA8 – SA9) are used as indicators for the potential of chemical structures to exert excess toxicity in the acute daphnid toxicity test. Whilst these SAs have been identified empirically through visual inspection of the chemical structures and T_e values of all training set compounds, mechanistic reasoning provides possible explanations for the correlation between the presence of individual SAs and the observed enhanced toxicity of the chemicals.

In Table 7, possible biotransformation reactions associated with individual SAs are listed that could explain why compounds containing these structural features are more toxic than narcotic-level chemicals. The underlying reaction mechanisms are based on standard considerations about the reactivity associated with functional groups as discussed in organic chemistry textbooks. Some also include known metabolic processes such as the mechanism of acetylcholine esterase inhibition through certain classes of insecticides. Note further that the reaction products listed in Table 7 may well undergo subsequent biotransformations, which are not considered further.

The chemical reactivity associated with SA1 is illustrated for the case of α,β-unsaturated carbonyl compounds. Due to the electronegative carbonyl oxygen, the β carbon is electron deficient and thus susceptible for an attack by endogeneous nucleophiles (Nu–H). The resultant conjugated 1,4-addition leads to an enol, which is likely to be tautomerized to the final carbonyl compound (reaction 1). A corresponding attack can also take place at the (somewhat less) electrophilic α carbon, yielding an allyl alcohol as 1,2-adduct (reaction 2). Respective transformations may also occur for α,β-unsaturated nitrile compounds, which form a related class of xenobiotic electrophiles.

SA2 has been mainly found in pyrethroids that block the gate of the sodium channel protein and therefore affect the balance of nerve membranes (37). Apart from this toxicological route, it may also be hypothesized that due to the strong electron-attracting effect of halogen substituents in geminal alkene halogenides, 2-step elimination via the vinyl cation as intermediate may lead to alkynes, which are substantially more reactive towards addition of endogeneous nucleophiles (reaction 3) than their alkene counterparts. Organic phosphorothionates containing SA3 are known as potent acetylcholine esterase (AChE) inhibitors (38, 39). Initial monooxygenase-mediated oxidation yields the bioactive phosphate, which is sufficiently reactive to attack the nucleophilic hydroxyl oxygen of the AChE serine group, resulting in a phosphorylated and thus deactivated AChE. In addition, an SN2 attack of endogeneous nucleophiles (Nu–H) at alkoxy ester functions of correspondingly substituted phosphorothionates may take place, leading to alkylated derivatives (Nu–R; reaction 5 in Table 7).

Aliphatic thiols (SA4) are more acidic than corresponding alcohols by around seven orders of magnitude, and the thiolate anions are among the strongest nucleophiles. Thus, a possible reaction path could be started by an initial deprotonation that yields the thiolate anion, which in turn is sufficiently reactive to dealkylate alkoxy functionalities such as the methoxy group of tyrosine (CH3O–Tyr), one of the aromatic amino acid side chains (reaction 6). Here, a further toxicologically relevant sideway is the reaction of the thiolate anion with intracellular oxygen, leading to the formation of superoxoide anion and subsequently further reactive oxygen species. Another possible route is the stepwise oxidation via monooxygenases until the formation of sulfonic acid derivatives. Here, the intermediate sulfinic acid (R–SO–OH) should be quite reactive due to the electron-deficient character of the sulfur atom, and may thus interfere with endogeneous nucleophiles (reaction 7). Note that as regards the acute toxicity towards fish, thiols have been classified as baseline narcotics (12, 14), which contrasts with the clear excess toxicity in the acute daphnid test according to the present data set. Isothiocyanates and thiocyanates (SA5) are electrophilic at the central sp2 and sp3 carbon, respectively, enabling the addition of endogeneous nucleophiles, possibly followed by hydro-lysis (reactions 8 and 9). Carbamates (SA6) are another class of insecticides known as AChE inhibitors (38). In contrast to phosphorothionates, however, an oxidative activation step is not needed, and attack of the carbonyl carbon at the serine OH group yields the carbamylated enzyme (reaction 10). Thiourea derivatives (SA7) also contain an electrophilic carbonyl carbon atom that may attack electron-rich sites of biological macromolecules (Nu–H), leading to respective adducts that may further undergo desamination (reaction 11). Here, the tautomeric form, an isothiourea compound, would yield the same reaction product.

Aromatic amines (SA8) form a special case as regards their acute toxicity towards daphnids, although the mechanistic origin has not been disclosed so far. Whilst anilines act as polar narcotics towards fish under acute exposure regimes, certain substitution patterns of the aromatically bound amino group lead to a considerably enhanced toxicity towards Daphnia magna and other waterfleas (32).

Interestingly, prolonged exposure of rainbow trout (*Onchorhyncus mykiss*) and medaka (*Oryzia latipes*) towards aniline and 4-chloroaniline results in metabolic conversion and effects different from polar narcosis (40, 41), which indicates that the mode of action of aromatic amines in fish depends also on the duration of exposure. In line with previous findings that ortho substitution reduces the aniline excess toxicity towards daphnids (32), SA8 is confined to derivatives without ortho substituents. Possible metabolic transformations include the formation of the highly toxic hydroxylamine, which may attack endogeneous nucleophiles through an addition-elimination reaction (reaction 12).

Table 7. Reaction Mechanisms associated with the Nine Structural Alerts.

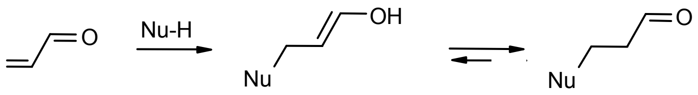
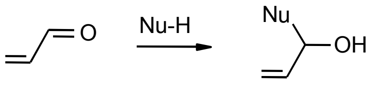
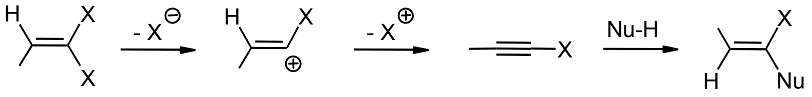
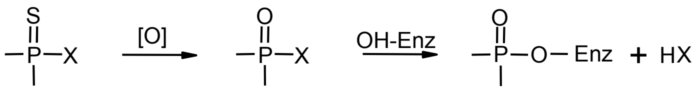
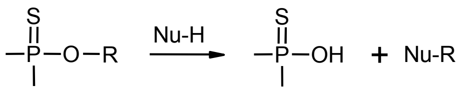
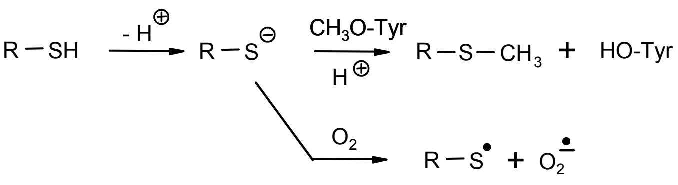
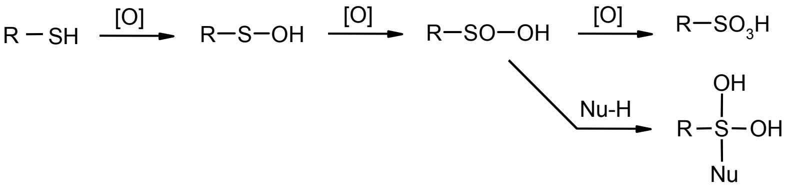
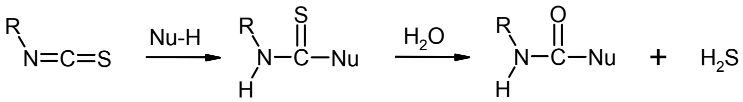
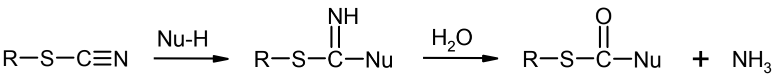
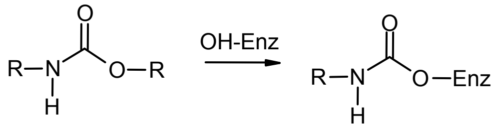
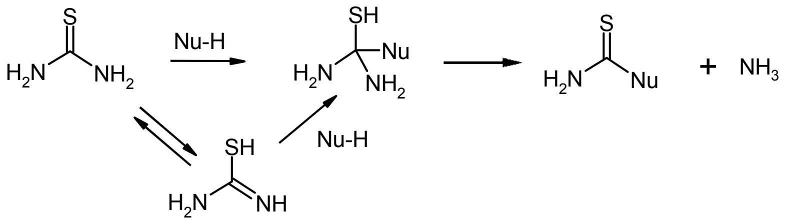
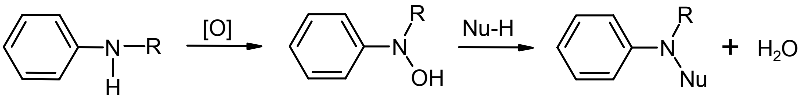
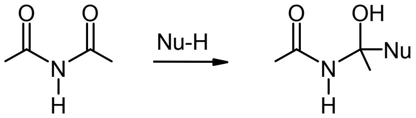
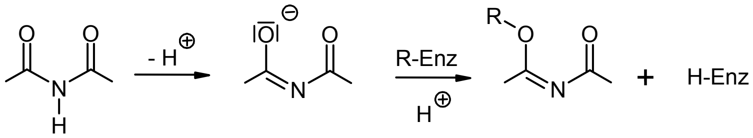
Structural Alert	Reaction Mechanism	Reaction No.
SA1		(1)
		(2)
SA2		(3)
SA3	 $-\overset{S}{\overset{\parallel}{P}}-X \xrightarrow{[O]} -\overset{O}{\overset{\parallel}{P}}-X \xrightarrow{OH\text{-}Enz} -\overset{O}{\overset{\parallel}{P}}-O-Enz + HX$	(4)
	 $-\overset{S}{\overset{\parallel}{P}}-O-R \xrightarrow{Nu\text{-}H} -\overset{S}{\overset{\parallel}{P}}-OH + Nu\text{-}R$	(5)
SA4	 $R-SH \xrightarrow{-H^{\oplus}} R-S^{\ominus} \xrightarrow[H^{\oplus}]{CH_3O\text{-}Tyr} R-S-CH_3 + HO\text{-}Tyr$; $R-S^{\ominus} \xrightarrow{O_2} R-S^{\bullet} + O_2^{\bullet -}$	(6)
	 $R-SH \xrightarrow{[O]} R-S-OH \xrightarrow{[O]} R-SO-OH \xrightarrow{[O]} R-SO_3H$; $R-SO-OH \xrightarrow{Nu\text{-}H} R-\underset{Nu}{\overset{OH}{S}}-OH$	(7)
SA5		(8)
	 $R-S-C\equiv N \xrightarrow{Nu\text{-}H} R-S-\overset{NH}{\overset{\parallel}{C}}-Nu \xrightarrow{H_2O} R-S-\overset{O}{\overset{\parallel}{C}}-Nu + NH_3$	(9)
SA6		(10)

Table A5. (continued)

Structural Alert	Reaction Mechanism	Reaction No.
SA7		(11)
SA8		(12)
SA9		(13)
		(14)

Imides (SA9) are derivatives of carbonyl compounds. Although the carbonyl carbon atoms are somewhat reduced in their electrophilic reactivity as compared to the ones in aldehydes, ketones and esters, the imide could still form adducts with electron-rich sites of biological macromolecules (Nu–H; reaction 13). Because imides are relatively acidic, another possible metabolic route could be given by an initial deprotonation, followed by a dealkylation of enzymes (R–Enz), membrane proteins or DNA side chains through the strong nucleophile formed intermediately (reaction 14).

As mentioned above, the transformation reactions summarized in Table 7 provide possible pathways that may explain the enhanced toxicity of compounds containing the relevant structural features. In some cases such as with the thiols and imides, however, the proposed reactions are based only on principal reactivity considerations, without experimental biochemical or toxicological evidence except that such chemical structures are apparently associated with enhanced acute toxicities towards daphnids.

Statistical performance of CM1, CM2 and CM3. Among our newly introduced classification schemes, CM1 is particularly strong in identifying excess toxicity (recognition power) as well as in predicting narcotic effect levels (prediction power). It follows that for a given compound, the CM1 prediction of a narcotic effect level in the acute daphnid test has a high probability to hold true. At the same time, the CM1 criterion for excess toxicity is likely to be less reliable, keeping in mind that a substantial portion of the respectively classified compounds may in fact turn out to exert effect levels in the range of narcosis.

The overall best-performing scheme is CM3, which however contains eight structural rules that at this stage are only tentative. These tentative rules require additional experimental data to prove or disprove their statistical significance. It follows that for the time being, CM3 should not be used alone, but only in the context of further information. Nonetheless, most of the tentative rules can be traced back to distinct biotransformation reactions as outlined above, and thus can be considered as mechanistic hypotheses about the association of certain structural features with the occurrence of excess toxicity.

CM2 is confined to statistically significant rules, and shows an overall performance between CM1 and CM3. Whilst the CM2 prediction power with respect to narcotic effect levels is lower than the one of CM1 (0.873 vs. 0.978, Table 4), the corresponding recognition power is much better than with CM1 (0.962 vs. 0.478). In absolute numbers, with CM2 only seven of the 186 compounds with narcotic effect levels were overlooked to belong to this class.

As regards excess toxicity, the CM2 prediction power is again twice as good as with CM1 (0.881 vs. 0.439), but at the same time the respective recognition power is significantly inferior to the CM1 result (0.666 vs. 0.974). The results suggest that a properly combined use of CM1 (optimised to predict narcotic effect levels) and CM2 or CM3 (optimised to predict excess toxicity) is the current method of choice to screen organic compounds for their priority to undergo an experimental test of their acute daphnid toxicity.

Combined two-step CM approach. Using the specific strengths of the classification models CM1 and CM2/CM3, a combined two-step approach can be applied that yields high prediction rates for both compounds with narcotic effect levels and compounds exerting excess toxicity. In a first step, CM1 is used to predict narcotic effect levels as well as excess toxicity. At this stage, only the category of narcotic effect levels is used, for which CM1 is relatively conservative and was shown to have a high prediction power. The correspondingly identified compounds have a high probability of not exceeding the narcotic range in the acute daphnid test, and thus have a low priority for experimental testing. The latter results from the fact that baseline narcosis can be relatively well predicted through K_{ow}-based QSARs.

Secondly, CM2 (or CM3) is applied to the prediction of both narcotic range compounds and compounds exerting excess toxicity. In general, there will be a subset of compounds that should exert a narcotic effect level according to CM2 (or CM3), but at the same time should be excess toxic according to CM1. This subset is allocated an intermediate priority for experimental testing.

Finally, the remaining subset where both CM1 and CM2 (or CM3) agree consists of compounds with the highest probability of exerting excess toxicity. For these compounds, reasonable QSAR-based predictions are generally not available (except possibly for structurally related groups based on specific knowledge), and as a consequence the priority for undertaking an experimental test to determine the acute daphnid toxicity is high.

The approach is visualized in Figure 4, where the three corresponding subgroups are ordered according to their predicted priority for experimental testing. With this two-step procedure, 91 compounds are predicted (by both CM1 and CM2) to exert narcosis-level toxicity, of which 89 actually belong to this class (predictivity = 0.978; see respective CM1 entry in Table 4). These 91 compounds would be allocated lowest test priority when applying the classification in a predictive mode.

For 59 compounds, both CM1 and CM2 agree in predicting excess toxicity in the sense of $T_e > 100$, of which 52 compounds are actually excess toxic (predictivity = 0.881; see respective CM2 entry in Table 4). This subset of 59 compounds would thus be allocated with the highest test priority, because their toxicity is expected to exceed the baseline toxicity at least by a factor of 100 and is generally difficult to be predicted quantitatively from existing QSAR models.

For the remainder of 114 compounds, CM1 and CM2 yield conflicting prediction results. This is mainly caused by the low predictivity of CM1 for excess toxic compounds (0.439, see Table 4). As noted above, CM1 is biased towards a high predictivity for the narcotic-level toxicity of organic compounds, and in this respect is superior to CM2 and CM3 (cf. Tables 3 and 4). 90 of the 114 compounds have LC50 values in the narcosis range in agreement with the respective CM2 prediction, while 24 compounds are in fact excess toxic (as predicted by CM1). Within the two-step approach as outlined in Figure 4, this intermediate class would thus be allocated an intermediate priority for experimental testing.

Statistical performance of MOA-based CMs. For the present training set of 264 compounds, the 4-MOA CM (*11*) could be applied to only half of the compounds. It indicates that the applicability domain of this classification scheme is somewhat restricted. Moreover, the overall concordance is only moderate (0.604, Table 4) and in fact inferior to all other CMs except the 7-MOA CM (0.598), the latter of which refers, however, to a much greater number of compounds (264 vs. 139, cf. Table 3).

In view of the above-mentioned systematic difference between the daphnid and fish toxicity of anilines, one might suspect that the only moderate performance of the fish-trained CMs could be driven mainly by misclassifications for this compound class. However, leaving out the 22 anilines results in only moderately improved overall concordances of 0.642 (4-MOA CM), 0.616 (7-MOA CM) and 0.715 (2-MOA CM), with still very low predictivities for excess toxicity (0.373, 0.358, 0.211). Only for the 4-MOA CM, the sensitivity for excess toxicity as well as the predictivity for narcotic effect levels are now much better than before (0.926 vs. 0.694 and 0.964 vs. 0.843, respectively; cf. Table 4 for the statistics with inclusion of anilines), whilst the predictivity for excess toxicity and the sensitivity for narcotic-level toxicity are essentially unchanged (0.563 vs. 0.573 and 0.373 vs. 0.362, respectively). Note further than when restricting the chemical domain to the subset of 123 compounds (without anilines) that can be classified by the 4-MOA scheme, the overall concordances of CM2 and CM3 increase from 0.875 (Table 4) to 0.911 and from 0.920 (Table 4) to 0.943, respectively.

Another group with an apparently systematic difference in the toxic level between daphnids and fish are thiols. All three compounds (#24, #99, #224) are clearly excess toxic towards daphnids with T_e values above 100, in contrast to their classification as narcotics according to the fish-based 7-MOA scheme (*12*) and a separate MOA classification study (*14*). Note, however, that the 4-MOA scheme has no classification rule for this functional group, and that according to the 2-MOA scheme all three thiols would have been classified as excess toxic.

A further interesting case is given by the only two aldehydes present in the data set under investigation. Acetaldehyde (#23) is even less toxic than according to baseline narcosis ($\log T_e$ -0.59), suggesting that the metabolic oxidation via aldehyde dehydrogenase to acetic acid acts as a quite efficient detoxification pathway. Although salicylaldehyde (#49) shows a moderately elevated toxicity ($\log T_e$ 1.44), its daphnid LC50 is still in the narcotic range, and both aldehydes are classified accordingly by CM1, CM2 and CM3 (cf. Table A5). By contrast, application of the 4-MOA scheme would classify both compounds as excess toxic, whilst according to both the 7-MOA scheme and the 2-MOA scheme only acetaldehyde would be predicted to be excess toxic. Here, the 7-MOA scheme would allocate salicylaldehyde to the group of polar narcotics (cf. Table A5).

The generally only moderate statistical performance of the MOA-based CMs for predicting the daphnid toxicity level has probably two causes: Firstly, reactive and specific MOAs need not result in significant excess toxicities. This means, however, that MOA-based classification schemes are not necessarily suited to provide good testing priorities in the context of tiered chemical hazard evaluation schemes that include QSAR predictions for narcotic effect levels as potential or preliminary alternative to experimental results.

Secondly, two of the three classification schemes were derived using acute fish toxicity data (actually LC50 towards guppy, *Poecilia reticulata*, for the 4-MOA CM, and 96h- LC50 data towards fathead minnow, *Pimephales promelas*, for the 7-MOA CM). It suggests that except for simple narcotics, the scope for extrapolating knowledge about prevalent MOAs across trophic levels appears to be limited.

The 2-MOA CM (*22*) was developed to identify electrophilic and proelectrophilic substructures as indicators for the likely occurrence of excess toxicity of the respective compounds towards fish and other aquatic organisms, and has a strong basis on chemical reaction mechanisms. In the present context of acute daphnid toxicity, however, both its recognition and prediction power for excess toxic chemicals are surprisingly poor (0.167 and 0.433; Table 4). Among the 30 compounds predicted to show excess toxicity, only 13 actually belong to this class, and 65 of the 78 chemicals with experimental excess toxicities would be (implicitly) classified as narcotic effect level compounds (Table 3).

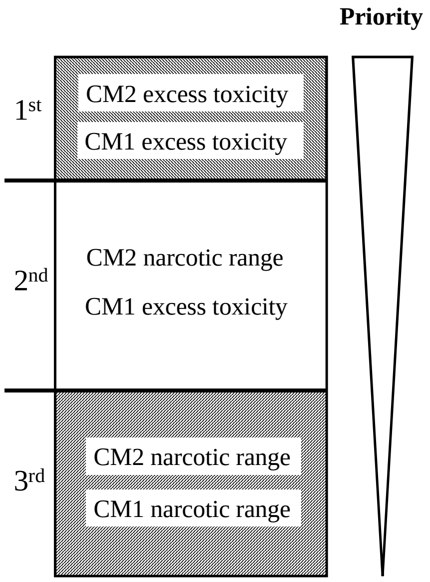


Figure 4. Two-step classification approach combining CM1 and CM2. Compounds predicted to exert narcotic effect levels according to CM1 are likely to have corresponding LC50 values, and thus have a low priority for experimental testing. By contrast, compounds predicted by both CM1 and CM2 to exert excess toxicity have a high priority for experimental testing, because their effect level can not be estimated reasonably well from QSARs. Compounds with conflicting predictions according to CM1 and CM2 are allocated an intermediate priority for experimental testing. Note that in this two-step scheme, CM2 may also be replaced by CM3.

These results suggest that the applicability domain of the 2-MOA CM in terms of actually covered electrophilic and proelectrophilic substructures were too restricted for the presently analysed set of 264 compounds. When comparing the 2-MOA CM with the presently derived CM2 and CM3, the former contains structural alerts identical or similar to SA1, SA4 and SA5.

Excess toxicity and specific modes of toxic action. For the acute daphnid toxicity, the currently developed classification schemes allow discrimination of chemicals exerting excess toxicity from compounds with narcotic effect levels. The respective distinction is based on a T_e criterion of 100: Only LC50 values with a $T_e > 100$ are classified as excess toxic, in order to account for both data uncertainties and systematic effect level differences between nonpolar and polar narcosis when employing the K_{ow} scale of hydrophobicity. As a consequence, specifically acting compounds with T_e values below 100 would also be classified as narcotic-level, in line with the goal to sort out those compounds where the acute daphnid toxicity is within two orders of magnitude from baseline narcosis, the latter of which can be predicted pretty well from respective QSARs.

In the daphnid test, oxidative uncouplers are one group of specifically acting compounds with T_e values below 100. Inspection of Table A5 reveals that according to the 7-MOA CM (*12*), the following nine compounds (eight phenols and one aniline) are classified as oxidative uncouplers: 2,4-dinitrophenol (#2), 2,3,4,6-tetrachlorophenol (#9), pentachlorophenol (#42), 2-(1-methylpropyl)-4,6-dinitrophenol (#45), 2,4,6-trinitrophenol (#46), dinitro-*o*-cresol (#162), 2,3,4,5-tetrachloroaniline (#184), 2,3,5,6-tetrachlorophenol (#193), and 2,4,6-trinitro-1,3-benzenediol (#236). Moreover, 2-methoxy-tetrachlorophenol (#216) and 2,3,4,5-tetrachlorophenol (#241) are predicted to exert different specific MOAs including oxidative uncoupling. Interestingly, all of these mostly phenolic uncouplers have T_e values below 100 including 2,4-dinitrophenol and pentachlorophenol as well-known reference compounds for this MOA, and thus belong to the narcotic toxicity range.

For the five uncouplers pentachlorophenol, 2,4,6-trinitrophenol, 2,3,5,6-tetrachlorophenol, 2,3,4,5-tetrachlorophenol (that would exert both polar narcosis and oxidative uncoupling according to the 7-MOA scheme) and 2,4,6-trinitro-1,3-benzenediol, the T_e values are even below 10 and thus in the effect level range of baseline and polar narcotics. It demonstrates that the T_e criterion is clearly not strict in terms of distinguishing between narcosis and specific or reactive modes of action, but straightforward in its focus on the effect level as compared to baseline toxicity.

For the subset of 11 oxidative uncouplers, the mean log T_e is 1.08 with a standard deviation of 0.57, indicating that their toxicity is on the average a factor of 10 greater than baseline toxicity. Moreover, regression of log LC50 on log K_{ow} yields

$$\log \text{LC50 [mol/L]} = -0.879\ (\pm\ 0.149) \log K_{ow} - 2.287\ (\pm\ 0.505) \qquad (10)$$

($n = 11$, $r^2 = 0.77$, SE = 0.60, $F_{1,9} = 34.8$). As compared to the baseline QSAR (Eq. 5), the intercept is lowered by one log unit (–2.287 vs. –1.281), and the slope is similar (–0.879 vs. –0.857). The moderate statistics reflect the somewhat greater scatter within the range of two log units above baseline toxicity. It follows that Eq. 10 can be used as reasonable estimate for the acute daphnid toxicity of (phenolic) oxidative uncouplers, with expected LC50 values within two log units distant from baseline narcosis.

Interestingly, the log K_{ow}-based regression relationship for oxidative uncoupling in the fish *Pimephales promelas* (*12*) has a quite different slope (–0.67) and intercept (–2.95) as compared to Eq. 10 that refers to daphnids, and is in fact almost parallel to the corresponding fish polar narcosis QSAR (*12, 33*) with a slope of –0.65 and an intercept of –2.29. The latter indicates that with this fish species, LC50 predictions for oxidative uncouplers would be consistently ca. 0.65 log units below the ones according to polar narcosis.

Nonetheless, the mean log T_e of all 12 oxidative uncouplers of the Duluth database (*12*) is only 0.82 with a standard deviation of 0.46, and thus even slightly closer to fish baseline narcosis as was found for the comparison of uncouplers and narcotics in the daphnid toxicity test. It follows that even when employing a T_e value of 10 as criterion to distinguish excess toxicity from the narcotic range, most of these uncouplers would have been classified as belonging to the group of narcotic-type chemicals. Moreover, in the ciliate assay with *Tetrahymena pyriformis* (*15*), 19 oxidative uncouplers have a mean log T_e of 1.29 with a standard deviation of 0.43. From these findings with three different aquatic organisms it may be concluded that oxidative uncouplers appear to exert an only moderate excess toxicity towards aquatic organisms that is in fact still in the range expected for the combined group of nonpolar and polar narcotics.

CONCLUSIONS

In the context of tiered chemical hazard assessment schemes, QSARs enable prediction of narcotic effect levels of organic compounds with reasonable accuracy, thus reducing the need for experimental testing. To this end, however, tools are required that allow identification of compounds likely to exert effect levels in the narcotic range. With the presently introduced T_e-based classification schemes, discrimination between narcotic-level and excess toxic compounds referring to the acute daphnid test is feasible, employing only information about the chemical structure of the substances. As such, this approach is well suited for the priority setting of organic compounds as regards their need for experimental testing. Moreover, extrapolation to other invertebrate species and other taxonomic groups would allow to extend the scope of QSAR-supported risk assessment schemes to be extended to a broader range of endpoints. The inferior performance of MOA-based classification schemes reflects the fact that toxic effects based on reactive and specific MOAs do not necessarily exceed the narcotic effect range, indicating that the knowledge about prevalent MOAs is in general not sufficient to predict the likely effect level.

Acknowledgements—P.C. von der Ohe would like to thank Matthias Grote for helpful discussions.

REFERENCES

(1) White paper, COM (2003) Directive of the European Parliament and of the Council: amending council Directive 67/548/EEC in order to adapt it to Regulation (EC) of the European Parliament and of the Council concerning the registration, evaluation, authorisation and restriction of chemicals. 2003/0256/COD, 2003/0257/COD, (29 October 2003).

(2) Betts, K. S. (1998) Chemical industry pressured to test high-production volume chemicals. *Environ. Sci. Technol.* **32***,* 251A.

(3) Moore, D. R. J., Breton, R. L., MacDonald, D. B. (2003) A comparison of model performance for six quantitative structure-activity relationship packages that predict acute toxicity to fish. *Environ. Toxicol. Chem.* **22**, 1799-1809.

(4) Estrada, E., Patlewicz, G., Chamberlain, M., Basketter, D., Larbey, D. (2003) Computer-aided knowledge generation for understanding skin sensitization mechanisms: The TOPS-MODE approach. *Chem. Res. Toxicol.* **16**, 1226-1235.

(5) Fang, H., Tong, W., Branham, W. S., Moland, C. L., Dial, S. L., Hong, H., Xie, Q., Perkins, R., Owens, W., Sheehan, D. M. (2003) Study of 202 natural, synthetic, and environmental chemicals for binding to the androgen receptor. *Chem. Res. Toxicol.* **16**, 1338-1358.

(6) Arulmohhiraja, S., Morita, M. (2004) Structure-activity relationships for the toxicity of poly-chlorinated dibenzofurans: Approach through density functional theory-based descriptors. *Chem. Res. Toxicol.* **17**, 348-356.

(7) Altenburger, R., Nendza, M., Schüürmann, G. (2003) Mixture toxicity and its modeling by quantitative structure-activity relationships. *Environ. Toxicol. Chem.* **22**, 1900-1915.

(8) Lin, Z., Yin, K., Shi, P., Wang, L., Yu, H. (2003) Development of QSARs for predicting the joint effects between cyanogenic toxicants and aldehydes. *Chem. Res. Toxicol.* **16**, 1365-1371.

(9) Serra, J. R., Jurs, P. C., Kaiser, K. L. E. (2001) Linear regression and computational neural network prediction of *Tetrahymena pyriformis* acute toxicity for aromatic compounds from molecular structure. *Chem. Res. Toxicol.* **14**, 1535-1545.

(10) Aptula, A.O., Kühne, R., Ebert, R.-U., Cronin, M. T. D., Netzeva, T. I., Schüürmann, G. (2003) Modeling discrimination between antibacterial and non-antibacterial activity based on 3D molecular descriptors. *QSAR Comb. Sci.* **22**, 113-128.

(11) Verhaar, H. J. M., van Leeuwen C. J. and Hermens J. L. M. (1992) Classifying environmental pollutants. 1: Structure-activity relationships for prediction of aquatic toxicity. *Chemosphere* **25***,* 471–491.

(12) Russom, C. L., Bradbury, S. P., Broderius, S. J., Hammermeister, D. E. and Drummond, R. A. (1997) Predicting modes of toxicity action from chemical structure: Acute toxicity in the fathead minnow (*Pimephales Promelas*). *Environ. Toxicol. Chem.* **16**, 948–967.

(13) Venkatapathy, R., Moudgal, C. J., Bruce, R. M. (2004) Assessment of the oral rat chronic lowest observed adverse effect level model in TOPKAT, a QSAR software package for toxicity prediction. *J. Chem. Inf. Comput. Sci.* **44**, 1623-1629.

(14) Boxall, A. B. A., Watts, C. D., Dearden, J. C., Bresnen, G. M., Scoffin, R. (1997) Classification of environmental pollutants into general mode of toxic action classes, based on molecular descriptors. In: Chen, F., Schüürmann, G. (eds.) QSAR in Environmental Sciences VII.. SETAC Press, Pensacola, FL, USA, pp. 263-275.

(15) Schüürmann, G., Aptula, A. O., Kühne, R., Ebert R.-U. (2003) Stepwise discrimination between four modes of toxic action of phenols in the *Tetrahymena pyriformis* assay*. Chem. Res. Toxicol.* **16***,* 974–987.

(16) Comber, M. H. I., Walker, J. D., Watts, C., Hermens, J. (2003) Quantitative structure-activity relationships for predicting potential ecological hazard of organic chemicals for use in regulatory risk assessment. *Environ. Toxicol. Chem.* **22**, 1822-1828.

(17) Russom, C. L., Breton, R. L., Walker, J. D., Bradbury, S. P. (2003) An overview of the use of quantitative structure-activity relationships for ranking and prioritizing large chemical inventories for environmental risk assessment. *Environ. Toxicol. Chem.* **22**, 1810-1821.

(18) U.S. Environmental Protection Agency (1994) ECOSAR: A computer program for estimating the ecotoxicity of industrial chemicals based on structure-activity relationships. EPA-748-R-002. Office of Pollution Prevention and Toxics, National Center for Environmental Publications and Information, Cincinnati, OH, USA.

(19) Russom, C.L., Anderson, E.B., Greenwood, B.E., Pilli, A. (1991) ASTER: An integration of the AQUIRE database and the QSAR system for use in ecological risk assessments. *Sci. Total Environ.* **109/110**, 667-670.

(20) Gombar, V. K, Enslein, K. (1995) Use of predictive toxicology in the design of new chemicals. ACS Symposium Series, Vol. 589, pp. 236-249.

(21) Bradbury, S.P., Russom, C.L., Ankley, G.T., Schultz, T.W., Walker, J.D. (2003) Overview of data and conceptual approaches for derivation of quantitative structure-activity relationships for ecotoxicological effects of organic chemicals. *Environ. Toxicol. Chem.* **22**, 1789-1798.

(22) Hermens, J. L. M. (1990) Electrophiles and acute toxicity to fish. *Environ. Health Perspect.* **87**, 219–225.

(23) U.S. Environmental Protection Agency (2002) AQUIRE (Aquatic toxicity information retrieval database). National Health and Environmental Effects Research Laboratory, Duluth, MN.

(24) Lipnick, R. L., Watson, K. R., Strausz, A. K . (1987) A QSAR study of the acute toxicity of some industrial organic chemicals to goldfish. Narcosis, electrophile and proelectrophile mechanisms. *Xenobiotica* **17**, 1011–1025.

(25) Lipnick, R. L. (1989) Base-line toxicity predicted by quantitative structure-activity relationships as a probe for molecular mechanism of toxicity. In: Magee P. S., Henry D. R. and Block J. H., eds, Probing bioactive mechanisms, Vol 413. American Chemical Society, Washington, DC, pp. 366–389.

(26) Van Leeuwen, C. J., Van der Zandt, P. T. J., Aldenberg, T., Verhaar, H. J. M. and Hermens, J. L. M. (1992) Application of QSARs, extrapolation and equilibrium partitioning in aquatic effects assessment. 1. Narcotic industrial pollutants. *Environ. Toxicol. Chem.* **11**, 267–282.

(27) Sosak-Swiderska, B., Tyrawska, D., Dzido, D. (1998) *Daphnia magna* ecotoxicity test with parathion. *Chemosphere* **37***,* 2989–3000.

(28) U.S. Environmental Protection Agency (2003) *EPI Suite for Windows*, v3.11 National Health and Environmental Effects Research Laboratory, Duluth, MN.

(29) Schüürmann, G., Kühne, R., Kleint, F., Ebert, R.-U., Rothenbacher, C., Herth, P. (1997) A software system for automatic chemical property estimation from molecular structure. In: Chen, F., Schüürmann, G. (eds.) QSAR in Environmental Sciences VII. SETAC Press, Pensacola, FL, USA, pp. 93–114.

(30) *SPSS for Windows*, Release 7.5.1 (1996) SPSS Inc., Chicago, IL.

(31) *Sigma Plot for Windows*, Release 4.0 (1997) SPSS Inc., Chicago, IL.

(32) Ramos, E.U., Vaal, M. A., Hermens, J. L. M. (2001) Interspecies sensitivity in the aquatic toxicity of aromatic amines. *Environ. Toxicol. Pharmacol.* **11,** 149-158.

(33) Veith, G.D. and Broderius, S.J. (1987) Structure-toxicity relationships for industrial chemicals causing type (II) narcosis syndrome. In: Kaiser, K.L.E., ed., QSAR in Environmental Toxicology – II. D. Reidel Publishing Company, Dordrecht, NL, pp. 385-391.

(34) Könemann, H. (1981) Quantitative structure-activity relationships in fish toxicity studies Part 1: Relationship for 50 industrial pollutants. *Toxicology* **19**, 209–221.

(35) Schmitt, H., Altenburger, R., Jastorff, B., Schüürmann, G. (2000) Quantitative structure-activity analysis of the algae toxicity of nitroaromatic compounds. *Chem. Res. Toxicol.* **13**, 441-450.

(36) Timbrell, J. (2000) Principles of Biochemical Toxicology, Third Edition, Taylor & Francis, London, UK, p.86.

(37) Krieger, R. (2001) Handbook of pesticide toxicology – Volume II, Second Edition, Academic Press, San Diego, CA, USA.

(38) Tomlin, C.D.S. (2000) *The pesticide manual, a world compendium*. Crop Protection 23 Publications, Farnham.

(39) Schüürmann, G. (1992) Ecotoxicology and structure-activity studies of organophosphorus compounds. In: Fujita, T. and Draber, W. (eds.) Rational Approaches to Structure, Activity and Ecotoxicology of Agrochemicals. CRC Press, Boca Raton (Florida, USA), pp. 485-541.

(40) Dady, J. M., Bradbury, S. P., Hoffman, A. D., Voit, M. M., Olson, D. L. (1991) Hepatic microsomal N-hydroxylation of aniline and 4-chloroaniline by rainbow trout (*Onchorhyncus mykiss*). *Xenobiotica* **29**, 1605-1620.

(41) Bradbury, S. P., Dady, J. M., Fitzsimmons, P. N., Voit, M. M., Hammermeister, D. E., Erickson, R. J. (1993) Toxicokinetics and metabolism of aniline and 4-chloroaniline in medaka (*Oryzias latipes*). *Toxicol. Appl. Pharmacol.* **118**, 205-214.

Table A1. Mean ± SD, minimum, and maximum of environmental parameters at 20 sites in streams during April-July, 1998-2000.

Parameter (units)	Mean	± SD	Min.	Max.
Physical				
Width (m) [a]	1.30	± 0.44	0.50	2.50
Depth (m) [a]	0.16	± 0.10	0.04	0.60
Current (m/s) [a]	0.17	± 0.09	0.02	0.50
Temperature (°C) [a]	13.3	± 3.0	3.5	19.5
Suspended particles (ml/week) [b]	161	± 69	77	294
Catchment area (km^2) [c]	12.6	± 4.9	6.0	20.4
Gradient of streams (%)	4.4	± 1.9	2.1	8.1
Forest, length upstream (m) [c]	730	± 800	0	3300
Forest, distance upstream (m) [c]	2640	± 2300	0	5300 [e]
Streambed substrate				
Cobble (%) [a]	2	± 7	0	30
Gravel (%) [a]	5	± 10	0	40
Sand (%) [a]	24	± 37	0	100
Silt (%) [a]	55	± 46	0	100
Streambed cover				
Allochton leaves (%) [a]	20	± 28	0	100
Submersed plants (%) [a]	8	± 11	0	50
Emerged plants (%) [a]	5	± 9	0	65
Filamentous algae (%) [a]	1	± 4	0	25
Water quality - standard				
Oxygen (mg/L) [a]	10.2	± 2.2	3.4	13.8
pH [a]	7.9	± 0.34	6.8	8.6
Nitrate (mg/L) [a]	3.4	± 9.2	0.5	47.5
Nitrite (mg/L) [a]	0.15	± 0.13	0.01	0.80
Ammonium (mg /L) [a]	0.07	± 0.21	0.00	1.75
Ortho-phosphate (mg/L) [a]	0.19	± 0.13	0.00	0.60
Insecticides				
beta-Cyfluthrin (μg/L) [a]	nd			
Cypermethrin (μg/L) [a]	nd			
Es-Fenvalerate (μg/L) [a]	nd			
Lambda-Cyhalothrin (μg/L) [a]	nd			
Lindane (μg/L) [a]	0.25	± 0.07	nd	0.3
Parathion-ethyl (μg/L) [a]	0.24	± 0.12	nd	0.5
Pirimicarb (μg/L) [a]	nd			
Fungicides				
Kresoxim-methyl (μg/L) [d]	0.41	± 0.49	nd	2.9
Epoxiconazol (μg/L) [d]	0.40	± 0.69	nd	5.6
Azoxystrobin (μg/L) [d]	0.46	± 1.31	nd	11.1
Propiconazol (μg/L) [d]	0.60	± 0.34	nd	0.8
Fenpropimorph (μg/L) [d]	0.20	± 0.14	nd	0.4
Tebuconazol (μg/L) [d]	0.56	± 1.74	nd	9.1
Herbicides				
Bifenox (μg/L) [d]	0.27	± 0.14	nd	0.5
Chloridazon (μg/L) [d]	5.63	± 12.12	nd	33
Ethofumesat (μg/L) [d]	8.66	± 23.35	nd	129
Isoproturon (μg/L) [d]	0.62	± 0.67	nd	2.6
Metamitron (μg/L) [d]	1.18	± 2.08	nd	9.3
Metribuzin (μg/L) [d]	0.27	± 0.33	nd	1.2
Pendimethalin (μg/L) [d]	0.40	± 0.00	nd	0.4
Prosulfocarb (μg/L) [d]	0.35	± 0.39	nd	1.0
$TU_{(D.\ magna)}$ [d]	-2.52	± 1.46	-5.00	-0.70

nd = not detected

[a] measured monthly

[b] measured at nine sites

[c] measured once.

[d] measured event controlled

[e] distances to forested stream sections greater than 6000m were not taken into account.

Table A2. Classification of invertebrate species at risk of being affected by pesticides (SPEAR). E = month of emergence (- not applicable), G = generations per year, S = sensitivity according to [11], SPEAR = species at risk (1), $SPE_{not}AR$ = species not at risk (0).

major taxa	species	E [m]	R [a]	S	SPEAR	major taxa	species	E [m]	R [a]	S	SPEAR
Annelida						**Heteroptera**					
Hirudinea						Corixidae		-	1	-0.29	1
Erpobdellidae		-	0.25	-0.41	0		*Corixa punctata*	3	1	-0.31	0
Glossiphoniidae		-	0.25	-0.60	0		*Sigara sp.*	-	1	-0.24	1
Hirudidae		-	0.25	-0.60	0	Gerridae		2	0.25	-0.56	0
Piscicolidae		-	0.25	-0.60	0	Hydrometridae	*Hydrometra stagnorum*	6	1	-0.56	0
Oligochaeta						Naucoridae	*Ilyocoris cimicoides*	-	1	-0.56	0
Lumbricidae		-	0.25	-1.10	0	Nepidae		-	1	-0.56	0
Lumbriculidae		-	0.25	-1.40	0	Notonectidae		-	1	-0.82	0
Naididae		-	0.25	-1.10	0	Pleidae		-	1	-0.56	0
Tubificidae		-	0.25	-0.93	0	Veliidae	*Velia caprai*	6	1	-0.56	0
Crustacea						**Megaloptera**					
Amphipoda						Sialidae		-	2	-	1
Corophiidae		-	0.25	+0.17	0	**Plecoptera**					
Gammaridae [a]		-	0.75	+0.04	0	Capniidae	*Capnia bifrons*	2	1	+0.38	0
Cladocera						Chloroperlidae		5	1	+0.38	1
Daphniidae	*Daphnia sp.*	-	0.25	+0.20	0		*Isoptena serricornis*	2	1	+0.38	0
Decapoda							*Siphonoperla sp.*	2	1	+0.38	0
Astacidae	*Orconectes limosus*	-	2	-0.57	0	Leuctridae		4-9	1	+0.38	1
Atyidae	*Atyaephyra desmaresti*	-	1	-0.08	1		*Leuctra hipopus*	3	1	+0.38	0
Grapsidae	*Eriocheir sinensis*	-	1	-0.08	1		*Leuctra niveola*	2	1	+0.38	0
Isopoda							*Leuctra proma*	2	1	+0.38	0
Asellidae	*Asellus aquaticus*	-	0.75	-0.17	1	Nemouridae		4	1	+0.25	1
	Proasellus coxalis	-	0.75	-0.56	0		*Nemoura sp.*	4	1	+0.20	1
Insecta						Perlodidae		5	1-2	+0.38	1
Coleoptera							*Perlodes microcephalus*	2	2	+0.38	0
Donaciidae		5	1	-1.15	0	Taeniopterygidae		1-3	1	+0.38	0
Dryopidae		-	1	-1.15	0		*Brachyptera risi*	3	1	+0.38	0

Table A2. *(continued)*

major taxa	species	E [m]	R [a]	S	SPEAR	major taxa	species	E [m]	R [a]	S	SPEAR
Dytiscidae		6-7	0.5-2	-0.81	0	**Trichoptera**					
	Agabus sp.	3	1-2	-0.81	0	Beraeidae		5	1	-0.06	1
Elmidae		8	1-2	-1.15	0	Brachycentridae	*Brachycentrus subnubilus*	3	1	-0.06	0
Gyrinidae		3-6	1	-1.15	0	Ecnomidae	*Ecnomus tenellus*	7	1	-0.06	1
Haliplidae		4-5	1-2	-1.83	0	Glossosomatidae	*Agapetus fuscipes*	5	0.5	-0.06	1
	Brychius elevatus	3	2	-1.83	0	Goeridae		5	1	-0.06	1
Helodidae		8-9	1	-1.15	0	Hydropsychidae		4-8	1	-1.03	0
Hydraenidae		3-6	1	-1.15	0	Hydroptilidae		4-8	0.5-1	-0.06	1
Hydrophilidae		3-7	1	-0.89	0		*Hydroptila vectis*	2	0.5-1	-0.06	0
Noteridae		4-7	1	-1.15	0	Lepidostomatidae		8	1	-0.06	1
Scirtidae	*Scirtes sp.*	8	1	-1.15	0	Leptoceridae		5-8	0.5-1	-0.06	1
Diptera						Limnephilidae		4-10	1	-0.06	1
Ceratopogonidae		-	0.25	-0.35	0		*Anabolia nervosa* [a]	7	1	-0.06	0
Chaoboridae		-	0.26	-0.35	0		*Limnephilus lunatus* [a]	4	1	-0.06	0
Chironomidae		3	0.25	-0.39	0		*Micropterna sp.*	3-5	1	-0.06	0
Culicidae		-	0.25	-0.29	0	Molannidae	*Molanna angustata*	8	1	-0.06	1
Dixidae		-	0.25	-0.35	1	Odontoceridae	*Odontocerum albicorne*	5	1	-0.06	1
Dolichopodidae	*Poecilobothrus sp.*	-	1	-0.35	1	Philopotamidae	*Wormaldia occipitalis*	6	1	-0.06	1
Limoniidae		-	1-2	-0.35	1	Phryganeidae		4-6	1	-0.06	1
Muscidae	*Limnophora sp.*	-	1	-0.35	1		*Oligostomis reticulata*	3	1	-0.06	0
Psychodidae		-	0.25	-0.35	0		*Oligotricha striata*	3	1	-0.06	0
Ptychopteridae	*Ptychoptera sp.*	5	1	-0.35	1	Polycentropodidae		5-8	1-2	-0.06	1
Rhagionidae		-	1	-0.35	1	Psychomyiidae		5-6	1	-0.06	1
Simuliidae		-	0.25	-0.35	0	Rhyacophilidae		4-8	1-2	-0.06	1
	Simulium sp.	-	0.25	-0.46	0	Sericostomatidae		4-7	1-3	-0.06	1
Stratiomyiidae	*Strictotarsus sp.*	-	1	-0.35	1	Uenoidae		5	1	-0.06	1
Syrphidae		3	1	-0.35	0	Mollusca					
Tabanidae		-	1	-0.35	1	**Basommatophora**					
Tanypodinae		-	0.25	-0.35	0	Acroloxidae		-	0.25	-1.16	0
Tipulidae		-	1	-0.35	1	Ancylidae	*Ancylus fluviatilis*	3	0.25	-1.16	0

Table A2. *(continued)*

major taxa	species	E [m]	R [a]	S	SPEAR	major taxa	species	E [m]	R [a]	S	SPEAR
Ephemeroptera						Lymnaeidae		-	0.25	-0.48	0
Baetidae		2-5	0.5-1	+0.02	0		*Lymnaea stagnalis*	-	0.25	-0.68	0
	Baetis rhodani	2	0.25	+0.02	0	Physidae		-	0.25	-1.64	0
	Centroptilum luteolum	5	0.25	-0.25	0		*Physa acuta*	-	0.25	-1.88	0
	Cloeon dipterum	4	0.5	-0.32	1	Planorbidae		-	0.25	-2.20	0
	Procloeon sp.	5	1	-0.25	1	**Eulamellibranchia**					
Caenidae		5-6	0.5-1	-0.30	1	Dreissenidae		-	0.25	-2.50	0
Ephemerellidae		4-6	1	-0.30	1	Sphaeriidae		-	0.25	-2.50	0
Ephemeridae		5-7	2-3	-0.30	1	Unionidae		-	0.25	-2.50	0
Heptageniidae		4-7	1	-0.30	1	**Prosobranchia**					
	Heptagenia sulphurea	3	0.5-1	-0.30	0	Bithyniidae		-	0.25	-1.82	0
Leptophlebiidae		4-5	1	-0.30	1	Hydrobiidae		-	0.25	-1.82	0
Siphlonuridae		5	1	-0.30	1	Valvatidae		-	0.25	-1.82	0
Odonata						Viviparidae	*Viviparus contectus*	5	0.25	-1.50	0
Aeshnidae		6	1-3	-0.96	0	Plathelminthes					
	Aeshna cyanea	6	1	-0.96	0	**Turbellaria**					
Calopterygidae		5	2	-0.36	1	Dendrocoelidae	*Dendrocoelum lacteum*	-	0.25	-0.43	0
Coenagrionidae		4-6	1-2	-0.24	1	Dugesiidae		-	0.25	-0.47	0
Cordulegasteridae	*Cordulegaster boltoni*	5	2	-0.96	0	Planariidae		-	0.25	-0.43	0
Corduliidae		5	1-2	-0.96	0						
Gomphidae		5	1-2	-0.96	0						
Lestidae		5	1	-0.68	0						
Libellulidae		5	1-2	-1.53	0						
Platycnemididae	Platycnemis pennipes	5	1	-0.96	0						

[a] not at risk due to migration ability

Table A3. Short-term peak concentrations of pesticides in streams during runoff events. Only concentrations with a toxicity of $TU_{(D. magna)}$ > –2.5 are shown.

Site	Date	Compound	LC_{50} *D. magna*	Conc, µg/L)	TU *(D. magna)*
1	03.05.98	Parathion-Ethyl	2.5	0.5	-0.70
1	02.06.98	Parathion-Ethyl	2.5	0.3	-0.92
1	14.07.98	Parathion-Ethyl	2.5	0.3	-0.92
2	03.05.98	Parathion-Ethyl	2.5	0.3	-0.92
3	24.05.00	Parathion-Ethyl	2.5	0.3	-0.92
4	03.05.98	Parathion-Ethyl	2.5	0.2	-1.10
1	23.05.00	Parathion-Ethyl	2.5	0.2	-1.10
5	19.07.98	Parathion-Ethyl	2.5	0.2	-1.10
2	03.05.98	Parathion-Ethyl	2.5	0.2	-1.10
5	12.05.99	Azoxystrobin	259	11.1	-1.37
1	30.05.98	Parathion-Ethyl	2.5	0.1	-1.40
6	24.05.00	Parathion-Ethyl	2.5	0.05	-1.70
7	12.05.99	Kresoxim-methyl	168	2.9	-1.81
7	12.05.99	Azoxystrobin	259	3.8	-1.83
8	15.06.00	Azoxystrobin	259	3.8	-2.02
9	14.06.99	Ethofumesat	13500	129	-2.02
10	15.06.00	Azoxystrobin	259	2.1	-2.09
7	05.07.99	Azoxystrobin	259	2.0	-2.11
2	12.05.99	Kresoxim-methyl	168	1.0	-2.27
7	03.06.98	Azoxystrobin	259	1.3	-2.30
1	30.05.98	Kresoxim-methyl	168	0.9	-2.32
2	02.06.98	Kresoxim-methyl	168	0.9	-2.32
2	27.05.98	Kresoxim-methyl	168	0.8	-2.37
7	03.06.98	Kresoxim-methyl	168	0.7	-2.42
7	14.06.98	Kresoxim-methyl	168	0.7	-2.42
7	27.05.98	Kresoxim-methyl	168	0.6	-2.49
1	23.05.98	Kresoxim-methyl	168	0.6	-2.49
2	24.06.98	Kresoxim-methyl	168	0.6	-2.49

Table A4. Mean, standard deviation (SD), minimum (Min.), and maximum (Max.) of environmental parameters for 50 streams$_{(+forest)}$ (+) and for 15 streams$_{(-forest)}$ (–), recorded in October 2002.

Environmental Parameter	group	Mean	±	SD	Min.	Max.
Physical						
Width [m]	+	1.81	±	0.60	0.65	3.00
	–	1.08	±	0.45	0.50	1.90
Depth [m]	+	0.34	±	0.22	0.05	0.90
	–	0.22	±	0.16	0.05	0.70
Current [m/s]	+	0.29	±	0.20	0.01	0.83
	–	0.30	±	0.13	0.03	0.80
Length of recovery potential [m] [a]	+	2050	±	2200	200	6000
	–	0	±	0	0	0
Distance to recovery potential [m]	+	360	±	750	0	3300
	–	> 4000	±	0	-	> 4000
Streambed substrate						
Stones [%]	+	10	±	18	0	70
	–	5		9	0	35
Gravel [%]	+	13	±	17	0	60
	–	12	±	16	0	55
Sand [%]	+	60	±	32	2	100
	–	45	±	34	0	100
Silt [%]	+	15	±	22	0	40
	–	16	±	21	0	75
Streambed cover						
Leaves [%]	+	20	±	27	0	90
	–	5	±	8	0	30
Debris [%]	+	9	±	12	0	50
	–	4	±	4	0	15
Submersed plants [%]	+	7	±	10	0	35
	-	10	±	14	0	50
Emerged plants [%]	+	4	±	6	0	25
	-	18	±	24	0	80
Filamentous algae [%]	+	4	±	12	0	61
	–	2	±	4	0	15
Modelled agricultural intensity						
Potential for Pesticide Runoff	+	-0.48	±	0.26	-2.00	2.05
	–	0.99	±	0.56	0.09	2.02
Predicted environmental concentration [mg/L]	+	222	±	210	0	642
	–	378	±	169	14	640

[a] distances to forested stream sections greater than 6000m were not taken into account.

Table A5. Compounds with 48-h Daphnia Toxicity in Terms of Log LC_{50}, Log K_{ow}, Log T_e and the Prediction Results of Six Classification Models.[a]

No.	CAS	Name	log LC_{50} [mol/L]	log K_{ow}	log T_e	CM1	CM2	CM3	4-MOA CM	7-MOA CM	2-MOA CM
Training Set											
1	50293	DDT	-7.89	6.79	0.79	0	0	0	4	7	0
2	51285	2,4-dinitrophenol	-4.62	1.73	1.86	1	0	0	3	4	0
3	52686	trichlorofon	-6.31	-0.28	5.27	1	0	3.2	n.a.	5/6	0
4	55389	fenthion	-6.79	4.08	2.01	1	3.1	3.1	4	6	0
5	55630	nitroglycerine	-3.85	1.51	1.28	1	0	0	n.a.	1	0
6	56382	parathion	-8.17	3.73	3.70	1	3.1	3.1	4	6	0
7	58140	pyrimethamine	-4.63	2.41	1.29	1	0	0	n.a.	1	0
8	58899	lindane	-5.39	4.26	0.46	0	0	0	1	7	0
9	58902	2,3,4,6-tetrachlorophenol	-6.12	4.09	1.34	0	0	0	n.a.	4	0
10	59063	ethopabate	-3.07	1.90	0.17	1	0	0	3	3	0
11	59507	4-chloro-3-methylphenol	-4.85	2.70	1.26	0	0	0	2	2	0
12	60515	dimethoate	-4.94	0.28	3.42	1	3.1	3.1	4	6	0
13	60571	dieldrin	-6.28	5.45	0.33	0	0	0	3	5/7	1
14	62533	aniline	-5.33	1.08	3.13	1	8.1	8.1	2	2	0
15	62555	thioacetamide	-3.64	-0.83	3.07	1	0	0	n.a.	1	0
16	62566	thiourea	-3.84	-1.31	3.68	1	7.1	7.1	n.a.	1	0
17	62737	dichlorvos	-9.10	0.60	7.30	1	2.1	2.1	n.a.	6	1
18	63252	carbaryl	-7.33	2.35	4.04	1	6.1	6.1	n.a.	1	0
19	68122	N,N-dimethylformamide	-0.70	-0.93	0.22	1	0	0	n.a.	5	0
20	72208	endrin	-6.38	5.45	0.43	0	0	0	3	5/7	1
21	74839	methyl bromide	-4.63	1.18	2.34	0	0	0	1	1	0
22	75058	acetonitrile	-1.06	-0.15	-0.10	1	0	0	n.a.	1	0
23	75070	acetaldehyde	-0.55	-0.17	-0.59	0	0	0	3	5	1
24	75081	ethyl mercaptan	-5.56	1.27	3.19	1	4.1	4.1	n.a.	1	1
25	75150	carbon disulfide	-4.56	1.94	1.62	1	0	0	n.a.	1	0
26	75218	ethylene oxide	-2.32	-0.05	1.08	0	0	0	3	5	1
27	75252	bromoform	-3.74	1.79	0.92	0	0	0	1	1	0
28	75354	1,1-dichloroethene	-3.28	2.12	0.18	0	0	0	1	1	0
29	77474	hexachlorocyclopentadiene	-6.72	4.63	1.47	0	0	0	1/3	7	0
30	78591	isophorone	-3.06	2.62	-0.47	1	1.1	1.1	3	5	1
31	78999	1,1-dichloropropane	-3.57	2.25	0.36	0	0	0	1	1	0
32	79061	acrylamide	-2.65	-0.81	2.06	1	1.1	1.1	n.a.	5	1
33	79094	propionic acid	-3.17	0.58	1.39	0	0	0	n.a.	1	0

Table A5. *(continued)*

No.	CAS	Name	log LC_{50} [mol/L]	log K_{ow}	log T_e	CM1	CM2	CM3	4-MOA CM	7-MOA CM	2-MOA CM
34	83410	1,2-dimethyl-3-nitrobenzene	-4.56	2.91	0.78	1	0	0	2	1	0
35	83421	2-chloro-6-nitrotoluene	-4.61	3.00	0.76	1	0	0	2	1	0
36	84662	diethyl phthalate	-3.61	2.65	0.06	0	0	0	3	3	0
37	84742	dibutyl phthalate	-4.88	4.61	-0.36	0	0	0	3	3	0
38	85018	phenanthrene	-5.36	4.35	0.35	0	0	0	1	1	0
39	85687	butyl benzyl phthalate	-5.19	4.84	-0.24	0	0	0	3	3	0
40	86306	N-nitrosodiphenylamine	-4.40	3.16	0.42	1	0	0	3	5	0
41	86748	carbazole	-4.70	3.23	0.65	1	0	0	n.a.	1	0
42	87865	pentachlorophenol	-5.64	4.74	0.30	0	0	0	n.a.	4	0
43	88722	1-methyl-2-nitrobenzene	-4.14	2.36	0.84	1	0	0	2	1	0
44	88733	1-chloro-2-nitrobenzene	-3.64	2.46	0.25	1	0	0	2	1	1
45	88857	2-(1-methylpropyl)-4,6 -dinitrophenol	-6.00	3.67	1.57	1	0	0	3	4	0
46	88891	2,4,6-trinitrophenol	-3.43	1.54	0.83	1	0	0	3	4	0
47	89598	4-chloro-2-nitrotoluene	-4.27	3.00	0.41	1	0	0	2	1	0
48	89612	1,4-dichloro-2-nitrobenzene	-4.26	3.10	0.32	1	0	0	2	1	1
49	90028	salicylaldehyde	-4.45	2.01	1.44	0	0	0	3	2	0
50	90040	o-aminoanisole	-4.01	1.16	1.74	1	0	0	n.a.	2	0
51	90051	2-methoxyphenol	-3.68	1.34	1.25	0	0	0	n.a.	2	0
52	90131	1-chloronaphthalene	-5.01	3.81	0.46	0	0	0	n.a.	1	0
53	90437	2-phenylphenol	-5.38	3.28	1.29	0	0	0	n.a.	2	0
54	91225	quinoline	-3.53	2.14	0.41	1	0	0	n.a.	5	0
55	91645	coumarin	-4.03	1.51	1.46	0	0	0	n.a.	5	0
56	91941	3,3'-dichlorobenzidine	-5.38	3.21	1.35	1	0	0	n.a.	2	0
57	92524	biphenyl	-4.66	3.76	0.15	0	0	0	1	1	0
58	92693	4-phenylphenol	-4.67	3.28	0.58	0	0	0	n.a.	2	0
59	94757	2,4-dichlorophenoxyacetic acid	-3.17	2.62	-0.35	0	0	0	n.a.	5	0
60	95158	benzo[b]thiophene	-3.36	2.99	-0.49	1	0	0	n.a.	1	0
61	95487	o-cresol	-3.87	2.06	0.82	0	0	0	2	2	0
62	95512	2-chloroaniline	-5.19	1.72	2.44	1	0	0	2	2	0
63	95534	ortho-toluidine	-5.31	1.62	2.64	1	0	0	2	2	0
64	95578	2-chlorophenol	-4.34	2.16	1.21	0	0	0	2	2	0
65	95761	3,4-dichlorobenzenamine	-5.95	2.37	2.64	1	8.1	8.1	2	2	0
66	95829	2,5-dichloroaniline	-4.74	2.37	1.43	1	0	0	2	2	0
67	95954	2,4,5-trichlorophenol	-4.86	3.45	0.63	0	0	0	2	2	0

Table A5. *(continued)*

No.	CAS	Name	log LC_{50} [mol/L]	log K_{ow}	log T_e	CM1	CM2	CM3	4-MOA CM	7-MOA CM	2-MOA CM
68	96093	1,2-epoxyethylbenzene	-4.02	1.59	1.37	0	0	0	3	5	1
69	96457	ethylene thiourea	-3.59	-0.49	2.73	1	0	7.3	n.a.	1	0
70	97007	1-chloro-2,4-dinitrobenzene	-5.40	2.27	2.18	1	0	0	3	5	1
71	97745	bis(dimethylthiocarbamyl)sulfide	-4.86	0.75	2.93	1	0	6.3	n.a.	1	0
72	97778	bis(diethylthiocarbamoyl)disulfide	-5.56	3.67	1.13	1	0	0	3	5	0
73	98828	cumene	-3.64	3.45	-0.60	0	0	0	1	1	0
74	98953	nitrobenzene	-3.48	1.81	0.65	1	0	0	2	1	0
75	99081	1-methyl-3-nitrobenzene	-4.04	2.36	0.74	1	0	0	2	1	0
76	99514	1,2-dimethyl-4-nitrobenzene	-3.98	2.91	0.20	1	0	0	2	1	0
77	99650	1,3-dinitrobenzene	-3.59	1.63	0.91	1	0	0	3	5	0
78	99876	cymene	-4.32	4.00	-0.39	0	0	0	1	1	0
79	99990	4-methylnitrobenzene	-4.01	2.36	0.71	1	0	0	2	1	0
80	100005	4-chloronitrobenzene	-4.31	2.46	0.92	1	0	0	2	1	1
81	100027	4-nitrophenol	-3.96	1.91	1.04	1	0	0	2	2	0
82	100414	ethyl benzene	-3.54	3.03	-0.34	0	0	0	1	1	0
83	100425	styrene	-3.41	2.89	-0.35	0	0	0	1	5	0
84	100618	N-methylaniline	-5.79	1.62	3.12	1	8.1	8.1	n.a.	1	0
85	102089	diphenylthiourea	-3.53	3.21	-0.50	1	0	0	n.a.	1	0
86	103695	ethylaniline	-5.46	2.11	2.37	1	8.1	8.1	n.a.	1	0
87	103720	isothiocyanatobenzene	-6.13	3.33	2.01	1	5.1	5.1	n.a.	5	0
88	103855	phenylthiourea	-3.54	0.95	1.44	1	0	0	n.a.	1	0
89	104949	4-methoxybenzenamine	-5.57	1.16	3.30	1	8.1	8.1	n.a.	2	0
90	105373	ethyl propionate	-2.78	1.36	0.34	0	0	0	n.a.	3	0
91	105555	1,3-diethylthiourea	-2.84	0.60	1.04	1	0	0	n.a.	1	0
92	105679	2,4-dimethylphenol	-4.77	2.61	1.25	0	0	0	2	2	0
93	106412	4-bromophenol	-4.46	2.40	1.13	0	0	0	n.a.	2	0
94	106445	p-cresol	-3.71	2.06	0.66	0	0	0	2	2	0
95	106478	4-chloroaniline	-6.41	1.72	3.65	1	8.1	8.1	2	2	0
96	106489	4-chlorophenol	-4.42	2.16	1.28	0	0	0	2	2	0
97	106898	epichlorohydrin	-3.58	0.63	1.76	0	0	0	3	5	1
98	107028	acrolein	-6.00	0.19	4.55	1	1.1	1.1	3	5	1
99	107039	1-propanethiol	-6.10	1.76	3.31	1	4.1	4.1	n.a.	1	1
100	107073	2-chloroethanol	-2.61	0.11	1.24	0	0	0	n.a.	5	0
101	107119	allylamine	-3.15	0.21	1.69	1	0	0	n.a.	1	0
102	107131	acrylonitrile	-3.78	0.21	2.32	1	1.1	1.1	3	5	1
103	107153	ethylenediamine	-3.36	-1.62	3.46	1	0	0	n.a.	1	0

Table A5. *(continued)*

No.	CAS	Name	log LC_{50} [mol/L]	log K_{ow}	log T_e	CM1	CM2	CM3	4-MOA CM	7-MOA CM	2-MOA CM
104	107926	n-butyric acid	-3.16	1.07	0.96	0	0	0	n.a.	1	0
105	108189	bis(isopropyl)amine	-2.35	1.64	-0.33	1	0	0	1	1	0
106	108394	m-cresol	-3.76	2.06	0.71	0	0	0	2	2	0
107	108429	3-chloroaniline	-6.11	1.72	3.35	1	8.1	8.1	2	2	0
108	108441	m-toluidine	-5.17	1.62	2.50	1	8.1	8.1	2	2	0
109	108850	bromocyclohexane	-3.89	3.45	-0.35	0	0	0	1	1	0
110	108952	phenol	-3.44	1.51	0.87	0	0	0	2	2	0
111	109466	dibutylthiourea	-3.52	2.57	0.04	1	0	0	n.a.	1	0
112	109524	pentanoic acid	-3.36	1.56	0.74	0	0	0	n.a.	1	0
113	109897	diethylamine	-3.12	0.81	1.14	1	0	0	1	1	0
114	110021	thiophene	-2.42	1.81	-0.41	1	0	0	n.a.	1	0
115	110838	cyclohexene	-3.94	2.96	0.12	0	0	0	1	1	0
116	110861	pyridine	-1.77	0.80	-0.19	1	0	0	2	2	0
117	111422	2,2'-iminobisethanol	-2.93	-1.71	3.11	1	0	0	n.a.	1	0
118	111444	2,2'-dichlorodiethyl ether	-2.78	1.56	0.16	0	0	0	3	5	1
119	111911	propoxur	-2.94	1.30	0.54	0	0	0	3	5	0
120	114261	2-(1-methylethoxy)phenol, methyl carbamate	-4.91	1.90	2.02	1	6.1	6.1	n.a.	6	0
121	115297	endosulfan	-6.14	3.50	1.86	1	0	0	3	7	0
122	115311	isobornyl thiocyanatoacetate	-6.50	3.75	2.01	1	0	5.2	3	5	1
123	115866	phosphoric acid	-5.51	4.70	0.20	1	0	0	n.a.	6	0
124	116063	aldicarb	-5.61	1.36	3.16	1	6.1	6.1	n.a.	6	0
125	118967	2,4,6-trinitrotoluene	-4.39	1.99	1.40	1	0	0	3	5	0
126	119653	isoquinoline	-3.71	2.14	0.60	1	0	0	n.a.	5	0
127	120832	2,4-dichlorophenol	-4.80	2.80	1.12	0	0	0	2	2	0
128	120934	ethyleneurea	-1.19	-0.74	0.54	1	0	0	n.a.	1	0
129	121142	2,4-dinitrotoluene	-3.72	2.18	0.57	1	0	0	3	5	0
130	121299	pyrethrine II	-7.40	5.33	1.55	0	0	0	4	5/7	1
131	121733	3-nitrochlorobenzene	-3.84	2.46	0.45	1	0	0	2	1	0
132	121755	malathion	-7.36	2.29	4.11	1	3.1	3.1	4	5/6	0
133	121879	2-chloro-4-nitroaniline	-4.49	2.12	1.39	1	0	0	2	2	0
134	122145	fenitrothion	-6.68	3.30	2.57	1	3.1	3.1	4	6	0
135	122349	simazine	-3.33	2.40	-0.01	1	0	0	n.a.	1	0
136	122667	1,2-diphenylhydrazine	-4.65	3.06	0.75	1	0	0	3	5	0
137	123546	2,4-pentanedione	-3.32	0.05	1.99	0	0	0	n.a.	1	0
138	124403	dimethylamine	-2.96	-0.17	1.82	1	0	0	1	1	0

Table A5. *(continued)*

No.	CAS	Name	log LC_{50} [mol/L]	log K_{ow}	log T_e	CM1	CM2	CM3	4-MOA CM	7-MOA CM	2-MOA CM
139	126738	tributyl phosphate	-4.86	3.82	0.31	1	0	0	n.a.	6	0
140	131113	dimethyl phthalate	-3.77	1.66	1.07	0	0	0	3	3	0
141	132650	dibenzothiophene	-5.06	4.17	0.20	1	0	0	n.a.	5	0
142	135193	2-naphthol	-4.61	2.69	1.02	0	0	0	n.a.	2	0
143	137268	thiram	-6.06	1.70	3.32	1	0	6.3	3	5	0
144	140669	4-tert-octylphenol	-6.36	5.28	0.55	0	0	0	2	2	0
145	141786	ethyl acetate	-2.09	0.86	0.07	0	0	0	n.a.	3	0
146	141902	thiouracil	-4.22	0.90	2.17	1	9.1	9.1	n.a.	1	0
147	142961	butyl ether	-3.70	3.01	-0.16	0	0	0	1	1	0
148	148016	dinitolmide	-3.14	0.19	1.69	1	0	0	3	5	0
149	149315	2-methyl-1,3-pentanediol	-1.22	0.62	-0.59	0	0	0	1	1	0
150	150196	3-methoxyphenol	-3.48	1.59	0.84	0	0	0	n.a.	2	0
151	156605	trans-1,2-dichloroethylene	-2.64	1.98	-0.33	0	0	0	1	1	0
152	206440	fluoranthene	-6.28	4.93	0.78	0	0	0	1	1	0
153	260946	acridine	-4.81	3.32	0.68	1	0	0	n.a.	5	0
154	298000	methyl parathion	-7.34	2.75	3.70	1	3.1	3.1	4	6	0
155	298022	phorate	-7.13	3.37	2.96	1	3.1	3.1	4	6	0
156	311455	diethyl p-nitrophenyl phosphate	-9.14	1.97	6.17	1	0	0	n.a.	6	0
157	333415	diazinon	-8.45	3.86	3.86	1	3.1	3.1	4	6	0
158	470906	chlorfenvinfos	-6.56	4.15	1.72	1	0	0	n.a.	5/6	0
159	503877	2-thioxo-4-imidazolinone	-3.77	-0.43	2.86	1	0	7.3	n.a.	1	0
160	532558	benzoyl isothiocyanate	-4.93	1.65	2.24	1	5.1	5.1	n.a.	5	1
161	534134	N,N'-dimethylthiourea	-3.85	-0.38	2.90	1	7.1	7.1	n.a.	1	0
162	534521	dinitro-o-cresol	-4.79	2.27	1.57	1	0	0	3	4	0
163	536903	3-methoxybenzeneamine	-5.64	1.16	3.37	1	8.1	8.1	n.a.	2	0
164	542756	1,3-dichloropropene	-4.25	2.29	1.01	0	0	0	3	5	0
165	542858	isothiocyanatoethane	-5.31	1.79	2.49	1	5.1	5.1	n.a.	5	1
166	554007	2,4-dichloroaniline	-5.43	2.37	2.12	1	0	0	2	2	0
167	556616	isothiocyanatomethane	-5.42	1.30	3.02	1	5.1	5.1	n.a.	5	1
168	576261	2,6-dimethylphenol	-4.04	2.61	0.52	0	0	0	2	2	0
169	578541	2-ethylbenzenamine	-4.18	2.11	1.09	1	0	0	2	2	0
170	589162	4-ethylaniline	-6.13	2.11	3.04	1	8.1	8.1	2	2	0
171	592825	1-isothiocyanatobutane	-5.43	2.77	1.77	1	5.1	5.1	n.a.	5	1
172	598163	tribromoethene	-4.33	2.18	1.18	0	0	0	1	1	0
173	598527	methylthiourea	-3.98	-0.84	3.42	1	7.1	7.1	n.a.	1	0
174	602017	2,3-dinitrotoluene	-5.44	2.18	2.29	1	0	0	3	5	0

Table A5. *(continued)*

No.	CAS	Name	log LC_{50} [mol/L]	log K_{ow}	log T_e	CM1	CM2	CM3	4-MOA CM	7-MOA CM	2-MOA CM
175	609198	3,4,5-trichlorophenol	-5.46	3.45	1.23	0	0	0	2	2	0
176	611063	2,4-dichloro-1-nitrobenzene	-4.66	3.10	0.72	1	0	0	2	1	1
177	618622	1,3-dichloro-5-nitrobenzene	-4.46	3.10	0.52	1	0	0	2	1	0
178	622786	benzylisothiocyanate	-6.54	3.01	2.68	1	5.1	5.1	n.a.	5	1
179	625536	ethylthiourea	-4.00	-0.35	3.01	1	0	7.2	n.a.	1	0
180	626437	3,5-dichloroaniline	-5.16	2.37	1.85	1	8.1	8.1	2	2	0
181	630206	1,1,1,2-tetrachloroethane	-3.84	2.93	0.05	0	0	0	1	1	0
182	632224	1,1,3,3-tetramethylurea	-1.60	-0.20	0.49	1	0	0	n.a.	1	0
183	634673	2,3,4-trichloroaniline	-5.43	3.01	1.57	1	0	0	2	2	0
184	634833	2,3,4,5-tetrachloroaniline	-5.56	3.65	1.15	1	0	0	n.a.	4	0
185	636306	2,4,5-trichloroaniline	-4.76	3.01	0.90	1	0	0	2	2	0
186	680319	hexamethyl phosphoramide	-1.43	-0.22	0.34	1	0	0	n.a.	1	0
187	693210	diethylene glycol dinitrate	-3.34	0.90	1.29	1	0	0	n.a.	1	0
188	732116	phosmet	-5.60	2.48	2.19	1	3.1	3.1	4	6	0
189	759944	dipropylcarbamothioic acid, S-ethyl ester	-4.61	3.02	0.74	1	0	0	n.a.	1	0
190	786196	carbophenothion	-6.44	5.19	0.71	1	3.1	3.1	4	6	0
191	825445	benzo[b]thiophene S,S-dioxide	-4.07	0.78	2.12	1	0	0	n.a.	5	0
192	877430	2,6-dimethylquinoline	-3.62	3.24	-0.44	1	0	0	n.a.	1/5	0
193	935955	2,3,5,6-tetrachlorophenol	-5.61	4.09	0.82	0	0	0	n.a.	4	0
194	944229	fonofos	-7.43	4.02	2.71	1	0	3.3	n.a.	1	0
195	1014706	simetryn	-3.63	2.90	-0.14	1	0	0	n.a.	1	0
196	1016053	dibenzothiophene-5,5-dioxide	-4.57	2.61	1.05	1	0	0	n.a.	1	0
197	1024573	heptachlor epoxide	-6.21	4.56	1.02	0	0	0	3	5/7	1
198	1516321	butylthiourea	-3.85	0.63	2.03	1	0	7.2	n.a.	1	0
199	1563662	carbofuran	-6.52	2.30	3.27	1	6.1	6.1	n.a.	6	0
200	1570645	4-chloro-o-cresol	-5.69	2.70	2.10	0	0	0	2	2	0
201	1570656	2,4-dichloro-6-methylphenol	-5.65	3.35	1.50	0	0	0	2	2	0
202	1582098	trifluralin	-6.24	5.31	0.41	1	0	0	3	5	0
203	1825214	pentachloroanisole	-7.01	5.30	1.19	0	0	0	n.a.	5	0
204	1836777	chlornitrofen	-5.88	4.96	0.34	1	0	0	3	1	0
205	1897456	chlorothalonil	-6.21	3.66	1.79	1	0	0	3	1	0
206	1912249	atrazine	-3.60	2.82	-0.10	1	0	0	n.a.	1	0
207	1918021	picloram	-3.61	1.36	1.16	1	0	0	3	2	0
208	1982474	chloroxuron	-4.99	4.08	0.22	1	0	0	n.a.	1	0
209	2008584	2,6-dichlorobenzamide	-2.35	0.90	0.29	1	0	0	3	1	0

Table A5. *(continued)*

No.	CAS	Name	log LC$_{50}$ [mol/L]	log K_{ow}	log T_e	CM1	CM2	CM3	4-MOA CM	7-MOA CM	2-MOA CM
210	2051607	2-chlorobiphenyl	-5.42	4.40	0.37	0	0	0	n.a.	1	0
211	2051618	3-chlorobiphenyl	-5.64	4.40	0.59	0	0	0	n.a.	1	0
212	2051629	4-chloro-1,1'-biphenyl	-5.60	4.40	0.55	0	0	0	n.a.	1	0
213	2257092	(2-isothiocyanatoethyl)benzene	-6.10	3.50	1.82	1	5.1	5.1	n.a.	5	1
214	2437798	2,4,2',4'-tetrachlorobiphenyl	-6.99	6.34	0.27	0	0	0	n.a.	1	0
215	2489772	trimethylthiourea	-2.19	-0.17	1.05	1	0	0	n.a.	1	0
216	2539175	2-methoxytetrachlorophenol	-6.08	3.92	1.44	0	0	0	n.a.	4/5	0
217	2556425	tetrapropylthioperoxydicarbonic-diamide	-6.19	5.63	0.08	1	0	0	3	5	0
218	2668248	2-methoxy-4,5,6-trichlorophenol	-5.37	3.27	1.29	0	0	0	n.a.	2/5	0
219	2741062	1-phenyl-3-ethyl thiourea	-3.35	1.91	0.43	1	0	0	n.a.	1	0
220	2764729	diquat	-5.01	2.36	1.71	1	0	0	n.a.	1/5	0
221	2782914	tetramethyl thiourea	-2.23	0.04	0.92	1	0	0	n.a.	1	0
222	2921882	clorpyrifos	-8.64	4.66	3.37	1	3.1	3.1	4	6	0
223	3209221	1,2-dichloro-3-nitrobenzene	-4.62	3.10	0.69	1	0	0	2	1	1
224	3483123	dithiothreitol	-3.76	-0.48	2.89	1	4.1	4.1	n.a.	1	1
225	3547044	DDE	-6.86	5.44	0.91	0	0	0	n.a.	5	0
226	3689245	TEDP	-9.15	3.98	4.45	1	3.1	3.1	4	1	0
227	3766812	2-(1-methylpropyl)phenol, methylcarbamate	-6.32	2.86	2.58	1	6.1	6.1	n.a.	6	0
228	4044659	1,4-diisothiocyanatobenzene	-6.40	4.67	1.11	1	5.1	5.1	n.a.	5	0
229	4104750	N-methyl-N-phenylthiourea	-3.36	1.50	0.79	1	0	0	n.a.	1	0
230	6317186	thiocyanic acid, methylene ester	-6.25	0.62	4.44	1	0	5.2	3	5	0
231	6972050	N,N-dimethylthiourea	-3.39	-0.63	2.65	1	7.1	7.1	n.a.	1	0
232	7012375	2,4,4'-PCB	-6.21	5.69	0.05	0	0	0	n.a.	1	0
233	8018017	mancozeb	-5.21	0.62	3.40	1	0	0	n.a.	1	0
234	10605217	carbendazim	-5.54	1.55	2.93	1	6.1	6.1	n.a.	1	0
235	12002481	trichlorobenzene	-4.40	3.93	-0.25	0	0	0	1	1	0
236	15245440	2,4,6-trinitro-1,3-benzenediol	-2.19	1.06	0.00	1	0	0	3	4	0
237	15263533	dithiocarbamate	-7.38	-0.95	6.91	1	0	6.2	n.a.	1	0
238	18259057	2,3,4,5,6-PCB	-7.61	6.98	0.35	0	0	0	n.a.	1	0
239	23564058	thiophanate-methyl	-4.33	1.10	2.11	1	6.1	6.1	n.a.	1	0
240	25154523	nonylphenol	-6.41	5.99	0.00	0	0	0	2	2	0
241	25167833	2,3,4,5-tetrachlorophenol	-5.76	4.09	0.97	0	0	0	n.a.	2/4	0
242	25875518	robenidine	-6.65	3.75	2.15	1	9.1	9.1	3	1	0
243	28249776	thiobencarb	-4.67	3.90	0.05	1	0	0	n.a.	1	0

Table A5. *(continued)*

No.	CAS	Name	log LC_{50} [mol/L]	log K_{ow}	log T_e	CM1	CM2	CM3	4-MOA CM	7-MOA CM	2-MOA CM
244	29232937	pirimiphos-methyl	-9.14	3.44	4.91	1	3.1	3.1	4	6	0
245	32598133	3,3',4,4'-tetrachloro-1,1'-biphenyl	-8.16	6.34	1.45	0	0	0	n.a.	1	0
246	33813206	5,6-dihydro-3H-imidazo[2,1-c] -1,2,4-dithiazole-3-thione	-5.92	1.60	3.27	1	0	6.4	3	5	0
247	33820530	isopropalin	-7.01	5.80	0.76	1	0	0	3	5	0
248	35065271	2,2',4,4',5,5'-hexachloro -1,1'-biphenyl	-8.44	7.62	0.63	0	0	0	n.a.	1	0
249	35367385	diflubenzuron	-7.77	3.59	3.41	1	9.1	9.1	3	1	0
250	35693993	2,2',5,5'-tetrachloro-1,1'-biphenyl	-6.99	6.34	0.27	0	0	0	n.a.	1	0
251	37680652	2,2',5-trichloro-1,1'-biphenyl	-6.67	5.69	0.51	0	0	0	n.a.	1	0
252	37680732	2,4,5,2',5'-PCB	-7.51	6.98	0.25	0	0	0	n.a.	1	0
253	38380073	2,2',3,3',4,4'-PCB	-8.78	7.62	0.97	0	0	0	n.a.	1	0
254	51630581	fenvalerate	-8.13	6.76	1.05	1	0	0	n.a.	3	0
255	52315078	cypermethrin	-9.06	6.38	2.31	1	2.1	2.1	3	3	0
256	52645531	permethrin	-8.23	7.43	0.58	1	2.1	2.1	4	7	0
257	52918635	deltamethrin	-10.09	6.18	3.52	1	2.1	2.1	3	3	0
258	55406536	iodopropynyl butylcarbamate	-6.85	2.45	3.47	1	6.1	6.1	n.a.	1	0
259	57057837	3,4,5-trichloroguaiacol	-5.56	3.27	1.48	0	0	0	n.a.	2/5	0
260	59756604	fluridone	-4.86	4.48	-0.26	1	0	0	n.a.	1/5	0
261	66230044	esfenvalerate	-9.19	6.76	2.12	1	0	0	n.a.	3	0
262	68359375	cyfluthrin	-9.42	5.74	3.22	1	2.1	2.1	3	3	0
263	76738620	paclobutrazol	-4.01	3.36	-0.15	1	0	0	3	1	0
264	91465086	cyhalothrin	-8.72	6.85	1.57	1	0	0	n.a.	3	0
Narcotics											
265	56235	tetrachloromethane	-3.64	2.44	-0.27	0	0	0	1	1	0
266	64175	ethanol	-0.59	-0.14	0.57	0	0	0	1	1	0
267	67641	acetone	-0.62	-0.24	0.45	0	0	0	1	1	0
268	67663	trichlormethane	-2.72	1.52	-0.13	0	0	0	1	1	0
269	67721	hexachloroethane	-4.83	4.03	-0.10	0	0	0	1	1	0
270	71238	1-propanol	-0.93	0.35	0.65	0	0	0	1	1	0
271	71432	benzene	-2.48	1.99	0.51	0	0	0	1	1	0
272	75092	dichlormethane	-2.59	1.34	-0.16	0	0	0	1	1	0
273	76017	pentachloroethane	-3.97	3.11	-0.03	0	0	0	1	1	0
274	78831	2-methyl-1-propanol	-1.82	0.77	0.12	0	0	0	1	1	0
275	78875	1,2-dichloropropane	-3.34	2.25	-0.13	0	0	0	1	1	0

Table A5. *(continued)*

No.	CAS	Name	log LC_{50} [mol/L]	log K_{ow}	log T_e	CM1	CM2	CM3	4-MOA CM	7-MOA CM	2-MOA CM
276	79005	1,1,2-trichloroethane	-3.09	2.01	-0.09	0	0	0	1	1	0
277	79016	trichloroethene	-3.35	2.47	0.05	0	0	0	1	1	0
278	79345	1,1,2,2-tetrachloroethane	-3.45	2.19	-0.29	0	0	0	1	1	0
279	91203	naphthalene	-4.12	3.17	-0.12	0	0	0	1	1	0
280	95476	o-xylene	-3.78	3.09	0.15	0	0	0	1	1	0
281	95501	1,2-dichlorobenzene	-4.81	3.28	-0.71	0	0	0	1	1	0
282	96184	1,2,3-trichloropropane	-3.72	2.50	-0.29	0	0	0	1	1	0
283	101553	4-bromophenyl-phenylether	-5.84	4.94	-0.33	0	0	0	n.a.	1	0
284	101848	diphenyl ether	-5.46	4.05	-0.71	0	0	0	n.a.	1	0
285	106423	p-xylene	-3.52	3.09	0.41	0	0	0	1	1	0
286	106467	1,4-dichlorobenzene	-4.17	3.28	-0.08	0	0	0	1	1	0
287	107062	1,2-dichloroethane	-2.29	1.83	0.56	0	0	0	1	1	0
288	107211	1,2-ethanediol	-0.48	-1.20	-0.23	0	0	0	n.a.	1	0
289	107415	2-methyl-2,4-pentanediol	-1.22	0.58	0.55	0	0	0	1	1	0
290	108383	m-xylene	-3.43	3.09	0.50	0	0	0	1	1	0
291	108883	toluene	-2.80	2.54	0.66	0	0	0	1	1	0
292	108907	monochlorobenzene	-3.77	2.64	-0.22	0	0	0	1	1	0
293	111706	1-heptanol	-3.22	2.31	0.04	0	0	0	1	1	0
294	111900	2-(2-ethoxyethoxy)ethanol	-1.53	-0.69	-0.84	0	0	0	1	1	0
295	112276	triethylene glycol	-0.46	-1.75	-0.68	0	0	0	n.a.	1	0
296	115208	2,2,2-trichloroethanol	-3.00	1.21	-0.69	0	0	0	n.a.	5	0
297	120821	1,2,4-trichlorobenzene	-4.16	3.93	0.49	0	0	0	1	1	0
298	127184	tetrachloroethene	-4.04	2.97	-0.22	0	0	0	1	1	0
299	142289	1,3-dichloropropane	-2.61	2.32	0.66	0	0	0	1	1	0
300	541731	1,3-dichlorobenzene	-4.18	3.28	-0.09	0	0	0	1	1	0

[a] LC_{50} denotes the experimental lethal concentration 50% towards *Daphnia magna* within 48 h (*23*), K_{ow} the calculated octanol/water partition coefficient (*28*), and T_e the excess toxicity (Eq. 1). CM1, CM2 and CM3 are the presently developed classification models, and 4-MOA CM, 7-MOA CM and 2-MOA-CM denote three MOA-based classification models taken from literature (*11, 12, 22*).

n.a. = not assigned

Table A6. Compounds with 48-h Toxicity to 6 Daphnidae Species in Terms of Log LC_{50}, Log K_{ow}, Log T_e and the Prediction Results of Three Classification Models [a].

No.	CAS	Name	Species	log LC_{50} [mol/L]	log K_{ow}	log T_e	CM1	CM2	CM3
Validation Set1									
1	50293	DDT	*Daphnia pulex*	-8.58	6.79	1.2	0	0	0
4	55389	fenthion	*Daphnia pulex*	-8.35	4.08	3.5	1	3.1	3.1
6	56382	parathion	*Daphnia pulex*	-8.58	3.73	4.1	1	3.1	3.1
8	58899	lindane	*Daphnia pulex*	-5.14	4.26	0.1	0	0	0
14	62533	aniline	*Daphnia pulex*	-5.97	1.08	4.0	1	8.1	8.1
17	62737	dichlorvos	*Ceriodaphnia dubia*	-9.20	0.60	7.7	1	2.1	2.1
18	63252	carbaryl	*Daphnia pulex*	-7.53	2.35	4.3	1	6.1	6.1
20	72208	endrin	*Daphnia pulex*	-7.28	5.45	1.2	0	0	0
23	75070	acetaldehyde	*Ceriodaphnia dubia*	-0.88	-0.17	0.1	0	0	0
38	85018	phenanthrene	*Daphnia pulex*	-5.20	4.34	0.1	0	0	0
42	87865	pentachlorophenol	*Daphnia pulex*	-5.37	4.74	-0.1	0	0	0
49	90028	salicylaldehyde	*Daphnia pulex*	-4.36	2.01	1.5	0	0	0
59	94757	2,4-D	*Daphnia pulex*	-4.84	2.62	1.4	0	0	0
61	95487	o-cresol	*Daphnia pulex*	-4.05	2.06	1.1	0	0	0
90	105373	ethyl propionate	*Daphnia pulex*	-3.17	1.36	0.9	0	0	0
92	105679	2,4-dimethylphenol	*Ceriodaphnia dubia*	-4.43	2.61	1.0	0	0	0
94	106445	p-cresol	*Daphnia pulicaria*	-3.68	2.06	0.8	0	0	0
96	106489	4-chlorophenol	*Ceriodaphnia dubia*	-4.15	2.16	1.2	0	0	0
100	107073	2-chloroethanol	*Daphnia pulex*	-2.15	0.11	1.1	0	0	0
101	107119	allylamine	*Daphnia pulex*	-3.23	0.21	2.0	1	0	0
106	108394	m-cresol	*Daphnia pulicaria*	-3.04	2.06	0.1	0	0	0
110	108952	phenol	*Daphnia pulex*	-3.13	1.51	0.7	0	0	0
116	110861	pyridine	*Daphnia pulex*	-2.14	0.80	0.4	1	0	0
117	111422	2,2'-iminobisethanol	*Daphnia pulex*	-4.64	-1.71	5.3	1	0	0
121	115297	endosulfan	*Daphnia carinata*	-5.93	3.50	1.7	1	0	0
124	116063	aldicarb	*Daphnia laevis*	-6.13	1.36	3.9	1	6.1	6.1
132	121755	malathion	*Daphnia pulex*	-8.22	2.29	5.1	1	3.1	3.1
134	122145	fenitrothion	*Moina macrocopa*	-6.85	3.30	2.8	1	3.1	3.1
135	122349	simazine	*Daphnia pulex*	-3.00	2.40	-0.2	1	0	0
137	123546	2,4-pentanedione	*Daphnia pulex*	-3.30	0.05	2.3	0	0	0
145	141786	ethyl acetate	*Daphnia pulex*	-2.53	0.86	0.8	0	0	0
152	206440	fluoranthene	*Ceriodaphnia dubia*	-6.65	4.93	1.0	0	0	0
154	298000	methyl parathion	*Ceriodaphnia dubia*	-7.94	2.75	4.4	1	3.1	3.1

Table A6. *(continued)*

No.	CAS	Name	Species	log LC_{50} [mol/L]	log K_{ow}	log T_e	CM1	CM2	CM3
157	333415	diazinon	*Daphnia pulex*	-8.59	3.86	4.0	1	3.1	3.1
158	470906	chlorfenvinfos	*Ceriodaphnia dubia*	-8.95	4.15	4.1	1	0	0
175	609198	3,4,5-trichlorophenol	*Ceriodaphnia dubia*	-5.70	3.45	1.5	0	0	0
195	1014706	simetryn	*Moina macrocopa*	-3.82	2.90	0.1	1	0	0
199	1563662	carbofuran	*Daphnia pulex*	-6.74	2.30	3.6	1	6.1	6.1
202	1582098	trifluralin	*Daphnia pulex*	-6.14	5.31	0.2	1	0	0
206	1912249	atrazine	*Daphnia pulex*	-3.72	2.82	0.1	1	0	0
218	2668248	2-methoxy-4,5,6-trichlorophenol	*Ceriodaphnia dubia*	-5.10	3.27	1.0	0	0	0
222	2921882	clorpyrifos	*Daphnia pulex*	-8.48	4.66	3.1	1	3.1	3.1
227	3766812	2-(1-methylpropyl)phenol, methylcarbamate	*Moina macrocopa*	-6.32	2.86	2.7	1	6.1	6.1
241	25167833	tetrachlorophenol	*Daphnia pulex*	-5.36	4.09	0.5	0	0	0
243	28249776	thiobencarb	*Ceriodaphnia dubia*	-5.70	3.90	1.1	1	0	0
256	52645531	permethrin	*Daphnia pulex*	-7.70	7.43	-0.3	1	2.1	2.1
264	91465086	cyhalothrin	*Ceriodaphnia dubia*	-9.18	6.85	1.7	1	0	0
Validation Set 1 continued / Validation Set 2									
301	51036	piperonyl butoxide	*Ceriodaphnia dubia*	-5.71	4.29	0.7	0	0	0
302	57556	propylene glycol	*Ceriodaphnia dubia*	-0.90	-0.78	0.7	0	0	0
303	72435	methoxychlor	*Ceriodaphnia dubia*	-7.39	5.67	1.1	0	0	0
304	76448	heptachlor	*Daphnia pulex*	-6.95	5.86	0.4	0	0	0
305	88062	dowicide 2S	*Ceriodaphnia dubia*	-4.69	3.45	0.5	0	0	0
306	93721	silvex	*Daphnia pulex*	-5.05	3.68	0.6	0	0	0
307	108463	resorcinol	*Daphnia pulicaria*	-3.04	1.03	1.1	0	0	0
308	145733	endothall	*Ceriodaphnia dubia*	-3.59	1.89	0.8	0	0	0
309	330541	diuron	*Daphnia pulex*	-5.22	2.67	1.7	1	0	0
310	709988	propanil	*Ceriodaphnia dubia*	-4.75	2.88	1.1	1	0	0
311	959988	alpha-endosulfan	*Daphnia carinata*	-6.21	3.50	1.9	1	0	0
312	1031078	endosulfan sulfate	*Daphnia carinata*	-5.75	3.64	1.3	1	0	0
313	1194656	dichlobenil	*Daphnia pulex*	-4.67	2.83	1.0	1	0	0
314	1563388	carbofuran phenol	*Ceriodaphnia dubia*	-6.01	2.90	2.3	0	0	0
315	1646873	2-methyl-2-(methylsulfinyl)propionaldehyde, O-(methylcarbamoyl)oxime	*Daphnia laevis*	-6.34	-0.78	6.1	1	6.1	6.1
316	1646884	aldoxycarb	*Daphnia laevis*	-5.34	-0.67	5.0	1	6.1	6.1
317	1836755	nitrofen	*Ceriodaphnia dubia*	-6.12	4.32	1.1	1	0	0

Table A6. *(continued)*

No.	**CAS**	**Name**	**Species**	**log LC_{50} [mol/L]**	**log K_{ow}**	**log T_e**	**CM1**	**CM2**	**CM3**
318	2212671	molinate	*Ceriodaphnia dubia*	-4.83	2.91	1.1	1	0	0
319	7786347	mevinphos	*Ceriodaphnia dubia*	-8.37	-0.24	7.6	1	1.1	1.1
320	8001352	toxaphene	*Daphnia pulex*	-7.44	6.79	0.1	0	0	0
321	8003347	pyrethrum	*Daphnia pulex*	-7.12	6.15	0.3	0	0	0
322	15972608	lasso	*Daphnia pulex*	-4.44	3.37	0.3	1	0	0
323	19666309	oxadiazon	*Moina macrocopa*	-5.80	4.81	0.3	1	0	0
324	21087649	metribuzin	*Ceriodaphnia dubia*	-3.78	1.49	1.4	1	0	0
325	33213659	beta-endosulfan	*Daphnia carinata*	-6.30	3.50	1.9	1	0	0
326	51218452	metolachlor	*Ceriodaphnia dubia*	-4.25	3.24	0.2	1	0	0
327	95737681	pyriproxyfen	*Daphnia carinata*	-6.60	5.55	0.4	1	0	0

[a] LC50 denotes the experimental lethal concentration 50% towards 6 daphniidae species within 48 h (*23*), K_{ow} the calculated octanol/water partition coefficient (*28*), and T_e the excess toxicity (Eq. 1). CM1, CM2 and CM3 are the presently developed classification models.
n.a. = not assigned

Lebenslauf

Name: Peter Carsten von der Ohe
Geburtsdatum und Ort: 20.04.1973 Bremen

Promotion

06/01 – 01/05 Doktorand am Umweltforschungszentrum Leipzig–Halle GmbH, im Department Chemische Ökotoxikologie unter Betreuung von PD Matthias Liess und unter Betreuung von Prof. Dr. Georg Rüppell, Zoologisches Institut, Technische Universität Braunschweig.

Thema der Doktorarbeit: „Ecotoxicological Assessment of Organic Pollutants with Regard to their Direct and Indirect Effects on Freshwater Macroinvertebrates of small Headwater Streams“

Studium

05/01	Diplom Biologe (univ.)
05/00 – 05/01	Diplomand am Zoologischen Institut in Braunschweig, *Arbeitsgruppe Limnologie,* Thema: „Ökologische von Makroinvertebraten als Indikator für die Pestizidbelastung kleiner Fließgewässer“,:
10/95 – 04/00	Student der TU Braunschweig im Fachbereich Biowissenschaften und Psychologie, Prüfungen in Ökologie, Zoologie und Botanik.

Zivildienst

01/94 – 03/95	Fahrer für „Essen auf Rädern“ bei der Arbeiterwohlfahrt in Achim

---

Schule

05/93	Allgemeine Hochschulreife
08/90 – 05/93	Gymnasium in Achim,
07/85 – 07/90	Realschule in Achim
08/83 – 06/85	Orientierungsstufe in Baden
08/79 – 07/83	Grundschule in Uesen

Veröffentlichungen Allgemeiner Art

Totschek, K., Steiner, S., Von der Ohe, P.C. 1999. Ganztagsbeobachtungen des Basstölpel Sula bassana: Helgoland am 17. Juni 1998, von 3.30-0.00. *Seevögel* **20**: 123-127.