Ökologische Risikoabschätzung von Pestiziden in kleinen Fließgewässern anhand von Felduntersuchungen in Mittel- und Nordeuropa

Kumulative Dissertationsschrift zur Erlangung des akademischen Grades
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Das Problem der Verunreinigung des Wassers durch Schädlingsbekämpfungsmittel kann nur in einem größeren Zusammenhang verstanden werden, als Teil des Ganzen, zu dem es gehört – der Verunreinigung der gesamten Umwelt des Menschen.

Rachel Carson, Silent Spring, 1962

Die vorliegende kumulative Dissertationsschrift basiert auf den nachfolgend aufgeführten wissenschaftlichen Publikationen:

I. Schäfer, R. B., R. Mueller, W. Brack, K.-D. Wenzel, G. Streck, W. Ruck, and M. Liess. 2007. Determination of 10 particle-associated multiclass polar and semi-polar pesticides from small streams using accelerated solvent extraction. Chemosphere 70: 1952-1960.

II. Gunold, R., R. B. Schäfer, A. Paschke, G. Schüürmann, and M. Liess. 2007. Calibration of the Chemcatcher® passive sampler for monitoring selected polar and semi-polar pesticides in surface water. Environmental Pollution, in press. doi:10.1016/j.envpol.2007.1010.1037.

III. Schäfer, R. B., A. Paschke, B. Vrana, R. Mueller, and M. Liess. 2008. Performance of the Chemcatcher® passive sampler when used to monitor 10 polar and semi-polar pesticides in 16 Central European streams, and comparison with two other sampling methods. Water research, in press. doi:10.1016/j.watres. 2008.01.023.

IV. Schäfer, R. B., T. Caquet, K. Siimes, R. Mueller, L. Lagadic, and M. Liess. 2007. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. Science of the Total Environment 382:272-285.

Die zitierten Beiträge sind in internationalen, englischsprachigen „peer-reviewed“ Fachzeitschriften (sämtliche Impact Factors >2) bereits erschienen oder in Druck und sind in der vorliegenden Arbeit mit Genehmigung der jeweiligen Herausgeber wiedergegeben.

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Inhaltsverzeichnis

Abkürzungsverzeichnis

ACN	acetonitrile
ASE	accelerated solvent extraction
CV	coefficient of variation
DCM	dichloromethane
DIS	deuterated internal standard
EA	ethyl acetate
EDS	event-driven water sampler
GC	gas chromatography
HPLC	high-performance liquid chromatograph
IS	internal standard
K_{OC}	soil organic carbon partition coefficient
K_{ow}	octanol water partition coefficient
LC50	median lethal concentration
LOQ	limit of quantification
MDS	method development samples
MeOH	methanol
MS	mass Spectrometry
PRC	performance reference compound
PS-DVB	polystyrene-divinylbenzene
PTFE	polytetraflouroethylene
RSD	relative standard deviation
R_S	sampling rate
RT	retention time
SEC	size exclusion chromatography
STU	sediment toxic unit
SPE	solid phase extraction
SPS	suspended particles sampler
SPEAR	species at risk
TOC/TIC	total organic/inorganic carbon content
TU	toxic unit
TWA	time-weighted-average
PRC	performance reference compound
WRRL	europäische Wasserrahmenrichtlinie

Summary

Freshwater ecosystems deliver various goods and services for human societies such as provisioning of water and fish resources. Modern agriculture is associated with the release of a large amount of agrochemicals that may enter into streams and result in deterioration of freshwater ecosystems. The present thesis contributes to the assessment of exposure and effects resulting from pesticide input into stream ecosystems and presents one of the largest field studies on the impacts of pesticide contamination in agricultural streams. The field study was conducted in 29 streams of two regions in France and Finland and comprised monitoring of invertebrate community composition and of an important ecosystem process, the leaf-litter breakdown. Furthermore, pesticide monitoring was performed for selected compounds that were previously found at ecotoxicologically relevant concentrations in streams of the study area according to information from local authorities. After the general introduction (Chapter 1), Chapters 2-4 introduce and evaluate sampling and determination methods for pesticide exposure. The results concerning the effects of pesticides in the field studies are presented in Chapter 5. In Chapter 2, a new method for the determination of 10 particle-bound pesticides using accelerated solvent extraction (ASE) is presented. The method developed here showed good results in terms of accuracy and precision in the extraction of polar and semi-polar pesticides belonging to different chemical classes. When applied to the extraction of field samples from streams, the detected concentrations reached levels that may have toxic effects on invertebrate species. In addition, the use of 6 deuterated analogues of the target compounds as internal standards during extraction, cleanup and analysis was examined. The study demonstrates that dissimilarities may occur in the behaviour of target compounds and deuterated analogues for analogues with less than 10 deuterium atoms. The following chapter describes a calibration approach to derive sampling rates for the Chemcatcher® passive sampler equipped with a receiving phase for polar and semi-polar pesticides. Sampling rates are needed to estimate time-weighted average water concentrations from field deployments of non-equilibrium passive samplers. The sampling rates obtained in the experiment were in the range of 0.1 to 0.5 L/day for the study compounds. In general, the experiment demonstrated that the Chemcatcher® passive sampler is suitable for field deployments up to 14 days in the configuration without a diffusion-limiting membrane. In Chapter 4 the provided sampling rates are used to

compute time-weighted average water concentrations for the field deployment of the Chemcatcher® in the French streams. Furthermore, this chapter compares the performance of the Chemcatcher® to the performance of two other sampling systems when used to detect pesticide exposure in streams. The study suggests that sampling waterborne exposure with the Chemcatcher® or an event-driven water sampler gives a more appropriate picture of the contamination with polar and semi-polar pesticides than sampling suspended particles. Chapter 5 is devoted to the effect side, where the risk assessment of the pesticide input in the French and Finnish streams for invertebrate communities is presented. In addition, the impact of pesticides on leaf-litter breakdown was investigated and the applicability of the trait-based Species At Risk (SPEAR) index to detect pesticide stress in large-scale biomonitoring was evaluated. The results of this chapter show that pesticides may alter invertebrate community composition and lead to an impairment of the leaf-litter breakdown at levels that were considered to be protective previously. Moreover, the study demonstrates the suitability of the SPEAR index to detect effects of pesticides when used on biomonitoring data including different biogeographical regions. In conclusion, the thesis adds to the growing evidence that pesticides present an important stressor for aquatic ecosystems and provides tools that can be applied in future studies on the exposure and the effects of pesticides in aquatic systems.

Zusammenfassung

Süßwasserökosysteme stellen für menschliche Gesellschaften verschiedene Ökosystemdienstleistungen wie zum Beispiel Trinkwasser oder Fischressourcen bereit. In der modernen Landwirtschaft kommen große Mengen von Agrochemikalien zum Einsatz, die in die Fließgewässer gelangen und dort zur Schädigung des Ökosystems führen können. Die vorliegende Arbeit trägt wichtige Erkenntnisse zur Abschätzung von Exposition und Effekten bei, die aus dem Eintrag von Pestiziden in Fließgewässerökosysteme resultieren können. Dabei wird eine der umfangreichsten Feldstudien zu den Auswirkungen der Pestizidbelastung in kleinen landwirtschaftlichen Fließgewässern vorgestellt. Die Feldstudie wurde an 29 Bächen in zwei Gebieten Finnlands und Frankreichs durchgeführt und umfaßte die Aufnahme der Zusammensetzung der Invertebratengemeinschaften und eines wichtigen Ökosystemprozesses, dem Blattabbau. Des Weiteren wurde ein Pestizidmonitoring für Verbindungen durchgeführt, die nach den Ergebnissen behördlicher Untersuchungen in ökotoxikologisch relevanten Konzentrationen in den Gewässern vorkommen. Nach der Einleitung (Kapitel 1), werden in den Kapiteln 2-4 neue Methoden zur Erfassung der Pestizidbelastung von Fließgewässern vorgestellt und bewertet. Die Ergebnisse aus der Feldstudie zu den Auswirkungen von Pestiziden werden im Kapitel 5 präsentiert. Im 2. Kapitel wird eine neue Methode zur Erfassung von schwebstoffadsorbierten Pestiziden vorgestellt, bei der die Extraktion mit der beschleunigten Lösemittelextraktion (ASE) durchgeführt wurde. Die Methode zeigte gute Ergebnisse bezüglich Exaktheit und Genauigkeit der Wiederfindung bei der Extraktion 10 polarer und semi-polarer Pestizide verschiedener chemischer Klassen. Bei der Anwendung der Methode auf Schwebstoffproben aus den französischen Bächen wurden Konzentrationen gefunden, die auf Invertebraten toxisch wirken könnten. Zusätzlich wurde in dieser Studie die Geeignetheit von deuterierten Standards der Analyten zur Verwendung als interne Standards während der Extraktion, Aufreinigung und Analytik untersucht. Dabei zeigte sich, daß bei deuterierten Standards mit weniger als 10 Deuteriumatomen Unterschiede im Verhalten zur nicht-deuterierten Verbindung auftreten können. Das folgende Kapitel stellt ein Kalibrationsexperiment zur Ermittlung von Sammelraten für den Chemcatcher® Passivsammler mit polarer Empfängerphase vor. Sammelraten für Passivsammler werden benötigt, um nach der Ausbringung in Gewässern zeitlich-gewichtete

Durchschnittskonzentrationen (engl. TWA) berechnen zu können. Für den Chemcatcher® bewegten sich die Sammelraten im Bereich von 0.1 bis 0.5 L pro Tag. Das Kalibrationsexperiment ergab zudem, daß der Chemcatcher® ohne diffusionslimitierende Membran für Felduntersuchungen mit bis zu 14 Tagen Expositionszeit geeignet ist. Die ermittelten Sammelraten werden im 4. Kapitel verwendet, um TWAs für die Freilandexposition des Chemcatchers® in den französischen Bächen zu berechnen. Ferner wird die Leistungsfähigkeit des Chemcatchers® mit zwei weiteren Verfahren zur Erfassung von gewässerbezogener Pestizidbelastung verglichen. Dabei zeigte sich, daß die Probenahme der Wasserphase mit dem Chemcatcher® oder einem ereignisbezogenen Wasserprobenehmer bei polaren und semipolaren Pestiziden ein angemesseneres Bild der Belastung liefert als die Probenahme der Schwebstoffphase. Das 5. Kapitel ist den Auswirkungen von Pestiziden gewidmet und präsentiert eine Risikoabschätzung für die Auswirkungen der Pestizideinträge in die französischen und finnischen Gewässer. Zusätzlich werden potentielle Effekte auf den Blattabbau untersucht und die Anwendbarkeit des „Gefährdete Arten“ (SPEcies At Risk - SPEAR) Index für das länderübergreifende Biomonitoring beurteilt. Das Hauptergebnis der Studie ist, daß Pestizide selbst in Konzentrationen, die bisher als unbedeutend angesehen wurden, sowohl die Struktur der Invertebratengemeinschaften verändern als auch den Blattabbau hemmen können. Des Weiteren war der SPEAR Indikator geeignet, Effekte von Pestiziden über verschiedene Regionen hinweg nachzuweisen. Zusammenfassend weist diese Arbeit nach, daß Pestizide einen wichtigen Störfaktor für Fließgewässerökosysteme darstellen können und liefert Methoden zur Expositions- und Effektabschätzung die in zukünftigen Studien Anwendung finden könnten.

Kapitel 1: Allgemeiner Teil

Überblick

Die vorliegende kumulative Dissertation hat zum Ziel, einen wichtigen Beitrag zur ökologischen Risikoabschätzung von Pestiziden in kleinen Fließgewässern zu leisten. Dafür werden sowohl in methodischer Hinsicht neue Verfahren für die Expositionsabschätzung und die Effektabschätzung eingeführt bzw. überprüft und bewertet als auch eine Risikoabschätzung auf Basis von eigenen Felduntersuchungen zu den Auswirkungen von Pestiziden präsentiert.

Die Arbeit gliedert sich in 6 Kapitel. Im 1. Kapitel wird ein Überblick über den Stand der Wissenschaft in den verschiedenen Bereichen gegeben, die in dieser Arbeit berührt werden. Zunächst wird in das Biomonitoring in Fließgewässern eingeführt und die Gründe für die Verwendung von Makroinvertebraten als Indikatororganismen werden dargestellt. Danach folgt ein kurzer Überblick über Methoden zur Auswertung von aquatischen Biomonitoringdaten mit Fokus auf der aktuellen Entwicklung bezüglich ökologischer Indizes. Im Anschluss daran findet eine Charakterisierung der Einträge von Pestiziden in Fließgewässer statt, mit den daraus folgenden Anforderungen an Probenahmemethoden zur realistischen Abschätzung der Exposition. In den folgenden Abschnitten wird auf den aktuellen Stand der Entwicklung von Passivsammlern zur kontinuierlichen Erfassung der Exposition eingegangen sowie auf die Extraktion von partikelgebundenen Pestiziden, die ebenfalls zur realistischen Expositionsabschätzung dienen könnten. Schließlich wird der aktuelle Wissensstand bezüglich der Effekte von Pestiziden in Fließgewässerökosystemen skizziert, zum einen bezüglich der Strukturveränderung der Makroinvertebratengemeinschaft und zum anderen bezüglich der funktionalen Änderung von Ökosystemprozessen. Das 1. Kapitel endet mit der Darstellung des Konzeptes und der Ziele dieser Arbeit. An dieser Stelle werden auch die Problemstellungen und Ziele der einzelnen Publikationen dieser kumulativen Dissertation vorgestellt. Die Publikationen folgen in den Kapiteln 2 bis 5. Im Kapitel 6 werden die wichtigsten Ergebnisse kurz zusammengefasst und Perspektiven für weitere Arbeiten gegeben.

Einführung

Grundlagen des Biomonitoring

Fließgewässer stellen für menschliche Gesellschaften eine Vielfalt von Ökosystemdienstleistungen bereit. Sie werden unter anderem zur Trinkwassergewinnung, zum Abwassertransport, zum Fischen und zum Warentransport genutzt (Klee 1991). Aus diesen unterschiedlichen Nutzungsinteressen resultierten in den letzten Jahrhunderten Konflikte (Radkau 2002), denn für einige Nutzungsformen spielt die Reinheit des Wassers eine entscheidende Rolle, während andere menschliche Aktivitäten wiederum zur Verschmutzung der Fließgewässer führen. Dabei verfügen Fließgewässerökosysteme in begrenztem Maße über die Fähigkeit zur Selbstreinigung, das heißt, dass ein gewisse Menge von eingetragener allochthoner organischer Substanz abgebaut werden kann (Lampert und Sommer 1999). Anthropogene Stressoren können aquatische Lebensgemeinschaften verändern, wodurch auch das Funktionieren von Ökosystemprozessen wie z.B. die Selbstreinigungsfähigkeit beeinträchtigt werden kann. Die europäische Wasserrahmenrichtlinie (WRRL) strebt deshalb einen sehr guten oder guten ökologischen Zustand für die europäischen Gewässer an, was sich als keine oder nur geringe Veränderung der Ökosysteme durch anthropogene Stressoren übersetzen lässt (EU 2000). Um den ökologischen Zustand eines Fließgewässers zu beurteilen, gibt es chemische (z.B. Bestimmung der Ammoniumkonzentration) und biologische Monitoringmethoden. Das Biomonitoring beruht konzeptionell auf der Reaktion von Lebewesen auf ihre Umweltbedingungen. Die Wirkung eines bestimmten Stressors kann auf verschiedenen Ebenen der biologischen Organisation beobachtet werden:

1. Suborganismische Ebene (z.B. Veränderungen bei der Enzymaktivität (Sturm et al. 2007) oder der chemischen Signalübertragung).

2. Ebene des einzelnen Organismus (Mortalität (Schulz und Liess 1999b) sowie Veränderungen der Morphologie (Vuori und Kukkonen 2002), des Wachstums, der Reproduktion oder des Verhaltens (Sih et al. 2004))

3. Populationsebene (Veränderungen der Populationsstruktur (Liess et al. 2006) oder der Mortalitätsrate (Schulz und Liess 1999b, Arndt et al. 1987))

4. Ebene der Lebensgemeinschaft (Abweichung der Gemeinschaftszusammensetzung gegenüber einem unbeeinträchtigten Referenzzustand oder die Abnahme des Anteils von empfindlichen Arten (Wright et al. 1998, Stoddard et al. 2006))

5. Ökosystemebene (Änderungen bei Ökosystemprozessen (Gessner und Chauvet 2002, Lecerf et al. 2006))

Bei den Schutzgütern im Natur- und Umweltschutz handelt es sich meist um Populationen, Gemeinschaften, Biodiversität oder Ökosystemfunktionen (MEA 2005). Die Relevanz von suborganismischen oder organismischen Beobachtungen ist für diese aggregierten Ebenen aufgrund der schlechten Übertragbarkeit meist gering (Forbes et al. 2006). Allerdings ist der Nachweis der Kausalität zwischen einem Stressor und Effekten auf den unteren Organisationsebenen leichter (Hyne und Maher 2003). Umgekehrt erlaubt die Beobachtung von Lebensgemeinschaften oder Ökosystemprozessen im Freiland eine realistische Beurteilung der Umweltqualität, während die spezifischen Ursachen für Abweichungen vom Referenzzustand aufgrund von natürlicher Variation und möglichen Kombinationswirkungen von Stressoren nicht einfach zu identifizieren sind (Heugens et al. 2001, Liess et al. 2005). Beim Biomonitoring von Fließgewässern dominiert das Erfassen der Makroinvertebratengemeinschaften, vor allem bei den regelmäßigen behördlichen Monitoringprogrammen (von der Ohe et al. 2007).

Makroinvertebraten als Zeigergruppe beim Biomonitoring

Zur Gruppe der Makroinvertebraten werden alle größeren aquatischen Invertebraten gezählt. Dazu gehören die aquatischen Insekten, Würmer, Mollusken und Krebse. Sie werden zum Beispiel mit einem Surber-Sampler mit einer Maschenweite von 500 µm erfasst (Schwoerbel 1994). Im Fließgewässerökosystem nehmen Makroinvertebraten als Konsumenten eine mittlere Stellung bezüglich der Trophieebene ein und ernähren sich von Bakterien, Detritus, Phytoplankton, Pflanzen, allochthonem organischen Material wie Blättern oder räuberisch von anderen Invertebraten (Cummins 1973). Sie beeinflussen verschiedene Ökosystemprozesse wie den Nährstoffzyklus, die Primärproduktion oder Abbauprozesse (Wallace und Webster 1996). Neben der Relevanz für das Funktionieren des Fließgewässerökosystems sprechen folgende Gründe für die Verwendung von Makroinvertebraten beim Biomonitoring (Bonada et al. 2006):

- Makroinvertebraten kommen weltweit in allen Fließgewässern vor
- sie besitzen eine hohe Artendiversität, wodurch sich ein großes Spektrum von Reaktionen auf Umweltveränderungen ergibt
- sie sind relativ ortsgebunden, so dass der Einfluss von Stressoren noch nach Wochen bis Jahren in der Gemeinschaft zu detektieren ist (Niemi et al. 1990)
- einige Arten zeigen durch Drift eine direkte Reaktion auf den Eintrag eines Schadstoffes (Schulz und Liess 1999a)

- der Aufwand für Felduntersuchungen ist relativ gering und es existiert eine umfangreiche taxonomische Literatur zur Bestimmung der Arten
- bei einigen Arten handelt es sich um die empfindlichsten Glieder der aquatischen Fauna gegenüber bestimmten Schadstoffen

Im Rahmen des Biomonitorings der Makroinvertebratenfauna werden entweder die Abundanzen der unterschiedlichen Arten aufgenommen (quantitatives Monitoring) oder die Gemeinschaft wird lediglich qualitativ charakterisiert, d.h. nur die Anwesenheit eines Taxons wird protokolliert. Damit die Umweltqualität auf Grundlage dieser Beobachtungen beurteilt werden kann, gibt es verschiedene Ansätze um die Informationen zu aggregieren und zu visualisieren. Am bedeutsamsten ist hier die Verwendung von multivariaten statistischen Methoden oder von Indizes. Für das vergleichende Biomonitoring in größeren geografischen Einheiten wie Flusseinzugsgebieten, das im Rahmen der Umsetzung der WRRL stark an Bedeutung gewonnen hat, ist der Einsatz von Indizes, die auf biologischen Merkmalen beruhen, am erfolgversprechendsten (Statzner et al. 2001, Bonada et al. 2006).

Biologische Indexsysteme für aquatische Monitoringdaten

Eine der ersten Methoden zur Beurteilung der biologischen Gewässergüte war der Saprobienindex (DIN 1990). Er wurde zu Beginn des 20. Jahrhunderts entwickelt, um die Belastung der Gewässer mit organischen Verunreinigungen, welche aus der Einleitung von Abwässern resultierte, zu erfassen (Bonada et al. 2006). Das Saprobiensystem weist Organismen anhand ihrer Toleranz gegenüber biologisch abbaubare organische Substanzen einen Saprobienwert zu, der empirisch aus der Beobachtung belasteter und unbelasteter Gewässer abgeleitet wurde. Die Saprobienwerte der Organismen werden dann gewichtet (z.B. nach der Abundanz), in einem Indexwert für die jeweilige Probestelle zusammengefasst (Klee 1991). Die Höhe dieses Indexwertes bestimmt die Zuordnung zu einer Gewässergüteklasse. Der Saprobienindex hat sich als recht erfolgreich für das Monitoring der Belastung mit organischen Verbindungen erwiesen. Durch den flächendeckenden Ausbau von Kläranlagen hat die Bedeutung dieser Belastung für die ökologische Qualität der Fließgewässer in den meisten europäischen Ländern abgenommen (Allan 1995, BMU 2005). Das grundlegende Konzept des Saprobiensystems, nämlich die Klassifizierung der beobachteten Taxa anhand ihrer Empfindlichkeit gegenüber einem Stressor, findet sich jedoch bei vielen anderen Indexsystemen wieder (Wildhaber und Schmitt 1998). Generell wird bei diesen Systemen eine Beeinträchtigung der ökologischen Qualität durch eine signifikante Abweichung des

Indexwertes einer Probestelle vom Indexwert eines Referenzzustandes bzw. durch Unter- oder Überschreitung von definierten Klassengrenzwerten angezeigt. Ein großes Problem ist hierbei die beschränkte geografische Anwendbarkeit, da der Indexwert des Referenzzustandes sowohl in Abhängigkeit vom Fließgewässertyp als auch von der jeweiligen geografischen Region variieren kann (Sandin und Verdonschot 2006, von der Ohe et al. 2007). Diese Variation resultiert zum einen daraus, dass viele Taxa der Makroinvertebratenfauna in ihrer Ausbreitung geografisch limitiert sind, was sich auch in der Einteilung Europas in 25 biogeografische Regionen äußert (Illies 1978). Zum anderen bewirken klimatische Faktoren grundlegende Unterschiede in den Gemeinschaftszusammensetzungen. So ist die Anzahl von unterschiedlichen Gattungen im Mittelmeerraum höher als in Zentraleuropa (Bonada et al. 2007).

In der letzten Dekade haben verschiedene Autoren aufgezeigt, dass Indizes, die auf biologischen Merkmalen beruhen, viel versprechend für das Biomonitoring in größeren Regionen sind (Doledec et al. 1999, Usseglio-Polatera et al. 2000, Haybach et al. 2004, Liess und von der Ohe 2005, Statzner et al. 2005). Die theoretische Grundidee dieser Ansätze ist, dass Taxa aufgrund bestimmter Ausprägungen von physiologischen oder ökologischen Merkmalen empfindlich bzw. tolerant gegenüber einem Stressor sind. Beispielsweise sind Makroinvertebraten mit einem stromlinienförmigen Körperbau und geringer Körpergröße widerstandsfähiger gegenüber erhöhten Fließgeschwindigkeiten z.B. bei Sturmfluten (Statzner et al. 2004). Ein weiteres Beispiel ist, dass sich Arten mit einer hohen Reproduktionsrate besser von den Auswirkungen von toxischem Stress erholen können als Arten mit niedrigeren Reproduktionsraten (Stark et al. 2004, Liess und von der Ohe 2005). Die Auswirkungen eines bestimmten Stressors sollten sich also dadurch nachweisen lassen, dass der Anteil von Arten mit dem unempfindlichen Modus des jeweiligen Merkmals in der Gemeinschaft zunimmt (Naeem und Wright 2003). Dieses Konzept wird in Abbildung 1 veranschaulicht. Hier weist jede Art in der Gemeinschaft (Symbol) drei biologische Merkmale auf (Form, Farbe und Größe). Der hypothetische Stressor wirkt nun analog zu einem Filter auf ein Merkmal (Größe), wodurch der Anteil von Arten mit einer geringen Größe zunimmt (Abbildung 1). Der konzeptionelle Unterschied zu taxonomiebasierten Indizes besteht also darin, dass nicht mehr konkrete Arten im Mittelpunkt der Analyse stehen, sondern die Ausprägung der ökologischen oder physiologischen Merkmale einer Gemeinschaft.

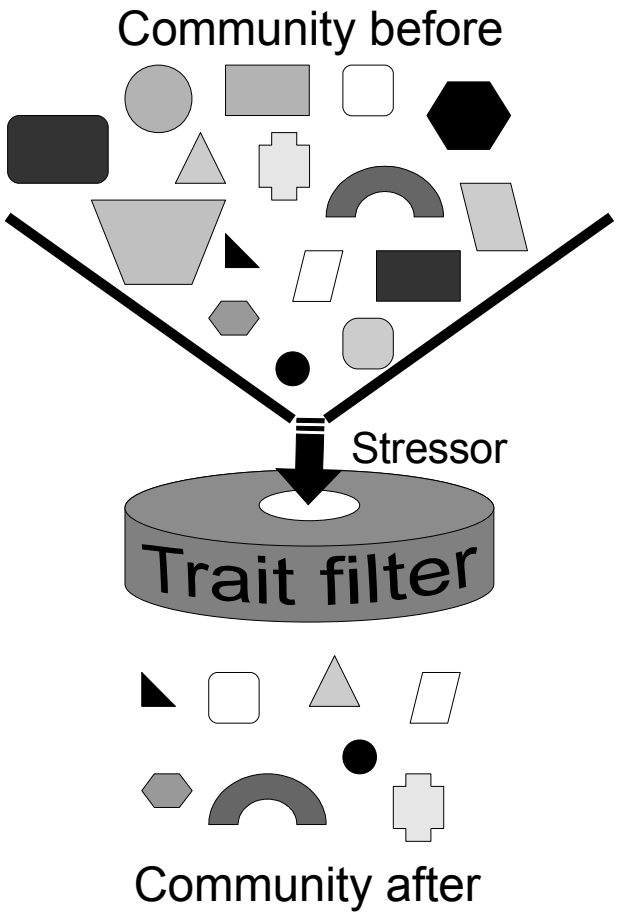

Abbildung 1: Auswirkungen eines Stressors auf die Merkmalsausprägungen in einer Gemeinschaft. Durch den Stressor werden die Arten (Symbole) anhand ihrer Größe selektiert, Farbe und Form werden nicht beeinflusst. Der Stressor wirkt somit analog eines Filters, der den Anteil kleiner Arten in der Gemeinschaft erhöht.

Die bisherigen Arbeiten auf dem Gebiet merkmalsbasierter Indizes haben hauptsächlich gezeigt, dass die Invertebratengemeinschaften an europäischen Referenzstellen eine ähnliche Zusammensetzung bei der Ausprägung ihrer Merkmale aufweisen. Das bedeutet, dass im Gegensatz zu taxonomischen Ansätzen die Referenzstellen über größere Regionen hinweg ähnliche Anteile von Merkmalsausprägungen besitzen (Gayraud et al. 2003, von der Ohe et al. 2007). Weitere Ergebnisse zeigen zudem, dass unterschiedliche Belastungssituationen mit signifikanten Unterschieden bei ausgewählten biologischen Merkmalen korrespondieren. So wurde für den Stressor Salinität nachgewiesen, dass sich der Anteil einiger Merkmalsausprägungen (z.B. lange Generationszeit, Filtrieren als Ernährungstyp) der Invertebratengemeinschaft bei steigender Belastung signifikant verringerte (Piscart et al. 2006). Auch für unterschiedliche Landnutzungsformen (Doledec et al. 2006) konnten signifikante Unterschiede zwischen bestimmten Merkmals-

ausprägungen (z.B. zwischen Reproduktionstypen oder Köperformen) gezeigt werden. Die entsprechenden biologischen Merkmale könnten somit als Index für die Umweltqualität des betrachteten Stressors verwendet werden. Diesbezüglich haben Statzner et al. (2005) einen konzeptionellen Rahmen für die Entwicklung von merkmalsbasierten Indizes für verschiedene Stressoren aufgespannt, indem die Autoren Hypothesen aufgestellt haben, welche biologischen Merkmale bei den verschiedenen Stressoren eine Reaktion zeigen könnten. Bisher existiert nur ein konsistentes Indexsystem: der „Gefährdete Arten“ (SPEecies At Risk -SPEAR) Index (Liess und von der Ohe 2005), der entwickelt wurde um aquatische Biomonitoringdaten hinsichtlich potentiellem Stress durch Pestizide auszuwerten. Der Index beruht auf physiologischen (relative Empfindlichkeit gegenüber toxischen organischen Substanzen wie Pestiziden (von der Ohe und Liess 2004)) und ökologischen Merkmalen (Emergenzzeitpunkt, Wanderungsfähigkeit und Generationszeit) und teilt die beobachtete Invertebratengemeinschaft in empfindliche und tolerante Arten gegenüber dem Stressor „Pestizide“ ein. Der SPEAR Index wurde bei der Auswertung einer Feldstudie an 20 deutschen Fließgewässern angewendet und reagierte im Vergleich zu unbelasteten Referenzstellen bei Probestellen mit Pestizidbelastung mit einer signifikant Abnahme des Anteils an empfindlichen Arten (Liess und von der Ohe 2005).

Charakterisierung von Pestizideinträgen in Bäche in Regionen mit landwirtschaftlicher Landnutzung

In der konventionellen und integrierten Landwirtschaft wird zur Sicherung und Steigerung der Ernteerträge auf organische Insektizide, Herbizide und Fungizide (im Folgenden unter dem Sammelbegriff „Pestizide“ zusammengefasst) zurückgegriffen (Oerke und Dehne 2004). Beispielsweise wurden für Deutschland in den Jahren 1993 bis 2006 für die Anwendung im Inland zwischen 33.660 und 38.880 Tonnen abgegebener Wirkstoffmenge bei der Biologischen Bundesanstalt für Land- und Forstwirtschaft (BBA) registriert (BBA 2002, BVL 2007). Die ausgebrachten Pestizide können über verschiedene Pfade in die Oberflächengewässer gelangen (Bach et al. 2001). Studien zur Relevanz der Eintragspfade haben gezeigt, dass Runoff (engl. Fachbegriff für niederschlagsbedingten Oberflächenabfluss vom Feld) hinsichtlich der Eintragsmenge aber auch der Höhe der Konzentrationen im Gewässer eine der wichtigsten Quellen des Pestizideintrags ist (Wauchope 1978, Liess et al. 1999, Raupach et al. 2001, Neumann et al. 2002). Runoff wird durch starke Regenereignisse ausgelöst und tritt somit nur episodisch auf (Guo et al. 2004). In Abbildung 2 sind der Abfluss (A) und der Verlauf der

Konzentrationen dreier Pestizide in einem Fließgewässer (B) während des ersten Starkregenereignisses (46 mm) 23 Tage nach der Pestizidausbringung dargestellt. Im Fließgewässer wurden nach Einsetzen des Niederschlags Spitzenkonzentrationen von bis zu 8 µg/L gefunden, die nach rund 48 Stunden auf unter 10% der Spitzenkonzentration gefallen waren. Eine monatliche Punktwasserprobe, wie sie bei behördlichen Monitoringprogrammen üblich ist, hat eine hohe Wahrscheinlichkeit, solche Eintragsereignisse nicht zu erfassen (Richards und Baker 1993, Schäfer et al. 2004). Für eine realistische Abschätzung des Expositionsniveaus muss die Probenahmestrategie deswegen an den Charakter dieses ereignisbezogenen Eintragspfades angepasst werden.

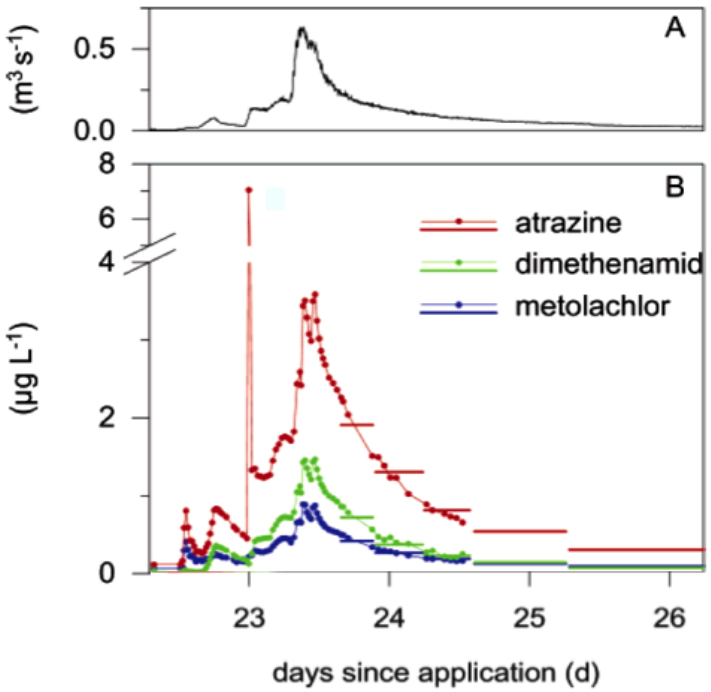

Abbildung 2: Abfluss (A) und Konzentrationen von 3 Pestiziden in einem angrenzenden kleinen Fließgewässer (B) beim ersten Starkregenereignis nach der Pestizidausbringung. Entommen aus Leu et al. (2004) und modifiziert.

Verwendung von Passivsammlern zur Expositionsabschätzung

Eine neuere Entwicklung der letzten zwei Dekaden beim Gewässermonitoring organischer Substanzen ist die Verwendung von Passivsammlern zur kontinuierlichen Erfassung der Gewässerbelastung (Stuer-Lauridsen 2005). Passivsammeln kann als Probenahmemethode definiert werden, bei der die Analyten von dem Probenahmemedium durch freie Diffusion in die Empfängerphase gelangen und dort akkumulieren (Gorecki und Namiesnik 2002). Als Empfängerphase kann z.B. ein Lösungsmittel, ein poröses Sorptionsmittel oder eine Festphasenextraktionsmembran verwendet werden. Passivsammler, die im Freiland ausgebracht werden, bestehen aus

einem Gehäuse, in dem die Empfängerphase fixiert ist und ggf. einer diffusionslimitierenden Membran. Wenn die Sammler für eine lange Zeit im Probenahmemedium belassen werden, nähert sich die Verteilung des Analyten zwischen dem Medium und der Empfängerphase einem thermodynamischen Gleichgewicht (Abbildung 3), welches abhängig von der jeweiligen Substanz und der Empfängerphase ist (Vrana et al. 2005). Es wird davon ausgegangen, dass bis zur Halbwertszeit des Gleichgewichtszustandes eine quasi-lineare Aufnahme in die Empfängerphase des Passivsammlers stattfindet (Abbildung 3). Das heißt, dass sich der Passivsammler bis zu diesem Zeitpunkt in einer zeitlich-integrierenden Aufnahmephase befindet. In diesem Fall kann aus der aufgenommen Masse des Analyten die zeitlich-gewichtete Wasserdurchschnittskonzentration (time-weighted average - TWA) berechnet werden. Allerdings wird für die Berechnung eine substanz- und sammlerspezifische Sammelrate benötigt, die in Kalibrationsexperimenten im Labor bestimmt werden kann. Dabei wird die Aufnahmerate des Sammlers bei konstanter Exposition ermittelt (Booij et al. 2007). Aus den Ergebnissen des Kalibrationsexperiments kann zusätzlich die Dauer abgeleitet werden, die der jeweilige Passivsammler bei Feldversuchen in der zeitlich-integrierenden Aufnahmephase verbleibt. Insbesondere für polare organische Substanzen sind bisher kaum Sammelraten ermittelt worden, so dass der Einsatz von Passivsammlern für diese Substanzen die Durchführung von Kalibrationsexperimenten erfordert (Mills et al. 2007). Im aquatischen Milieu können Passivsammler meist 1 bis 4 Wochen als zeitlich-integrierende Sammler eingesetzt werden, um die Belastung des Gewässers in Form der TWA-Konzentrationen zu bestimmen (Vrana et al. 2005, Tran et al. 2007). Da es eine gewisse Verzögerung gibt bis die Moleküle in die Empfängerphase diffundiert sind, ist ungeklärt, inwiefern auch kurzfristige Eintragsereignisse zum Beispiel durch Runoff erfasst werden (Greenwood et al. 2007).

Wie jede Probenahmetechnik hat die Verwendung von Passivsammlern Vor- und Nachteile. Der große Vorteil gegenüber anderen Probenahmemethoden liegt sicherlich in der Langzeiterfassung der Gewässerbelastung, die zudem mit weniger Arbeits- und Kostenaufwand verbunden ist (Kot et al. 2000). Des Weiteren findet in den Sammlern auch eine direkte Anreicherung im Gewässer statt, was die Detektion von Konzentrationen im Bereich von bis zu wenigen Picogramm pro Liter erlaubt (Vrana et al. 2005). Ein großer Nachteil ist jedoch, dass von der TWA-Konzentration eines Analyten nicht auf die Dauer und Höhe der Spitzenkonzentration geschlossen werden kann, diese erscheint jedoch ökotoxikologisch höchst relevant (Van Straalen 1997).

Allerdings fehlen bisher vergleichende Studien zur Eignung von Passivsammlern und anderen Probenahmemethoden für die Erklärung von ökologischen Effekten. Ein weiterer Nachteil ist, dass die Aufnahmerate von Substanzen von Umweltbedingungen wie Temperatur und Fließgeschwindigkeit beeinflusst wird (Vrana et al. 2007). Dies kann bei Freilandausbringung die Vergleichbarkeit zwischen Gewässern verzerren. Sind die Aufnahme- und Abgabekinetik von Substanzen bei einem Sammler gleich (= isotrop), kann jedoch vor der Ausbringung eine so genannte Performance Reference Compound (PRC) hinzugegeben und aus der Freisetzung dieser Substanz auf die Umweltbedingungen geschlossen werden (Huckins et al. 2002). Ferner erfassen Passivsammler nur den gelösten Anteil der Schadstoffe in der Wasserphase, während die Exposition gegenüber partikelgebundenen Schadstoffen ebenfalls ökotoxikologisch relevant sein kann (Schulz und Liess 2001, Sturm et al. 2007).

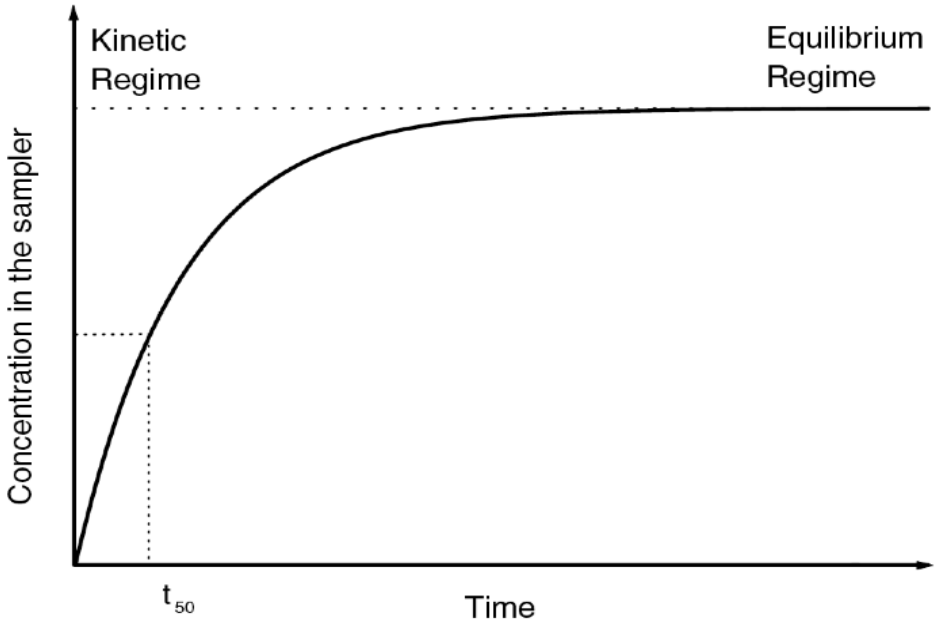

Abbildung 3: Aufnahmekinetik eines Analyten in einen Passivsammler. Bis zur Halbwertszeit (t_{50}) befindet sich der Sammler in der quasi-linearen Aufnahmephase. Entnommen aus Vrana et al. (2005) und modifiziert.

Bestimmung von Schwebstoffkonzentrationen mit der beschleunigten Lösemittelextraktion für die Expositionsabschätzung von Pestiziden

Nach der Ausbringung befinden sich die Pestizide meist auf der Pflanzenoberfläche oder sind an Bodenpartikel adsorbiert. Bei Eintritt eines Starkregenereignisses können sie von den Pflanzen gespült oder partikeladsorbiert in die aquatischen Ökosysteme eingetragen werden. Wegen der langsamen Desorptionskinetik stellt sich bei einigen Substanzen nicht

sofort das auf Grundlage des K_{oc} (Verteilungskoeffizient zwischen dem organischen Kohlenstoff im Boden und Wasser) erwartete Gleichgewicht zwischen der Wasserphase und der Partikelphase ein, so dass die Konzentrationen an den Schwebstoffen höher sind als im Gleichgewichtszustand (Pereira und Rostad 1990, Inoue et al. 2002). Bei bisherigen Untersuchungen lagen Substanzen mit einem K_{oc} von 100 oder größer zumindest partiell partikeladsorbiert vor (Long et al. 1998, Inoue et al. 2002). Im Gewässer können partikeladsorbierte Substanzen dann schrittweise desorbieren und zur Belastung der aquatischen Biozönose beitragen (Mayer und Reichenberg 2006).

Zur Bestimmung der partikeladsorbierten Exposition ist die Extraktion der Analyten von den Schwebstoffen notwendig. Wichtige Indikatoren für die Güte eines Extraktions–verfahrens sind die Exaktheit und Genauigkeit der Wiederfindung. Diese werden dadurch ermittelt, dass mehrere unbelastete Proben mit dem Analyten angereichert und extrahiert werden. Der mittlere Anteil der ursprünglichen Konzentration (in %), der bei der Analyse gefunden wird, entspricht der Exaktheit und die Variation drückt die Genauigkeit des Extraktionsverfahrens aus.

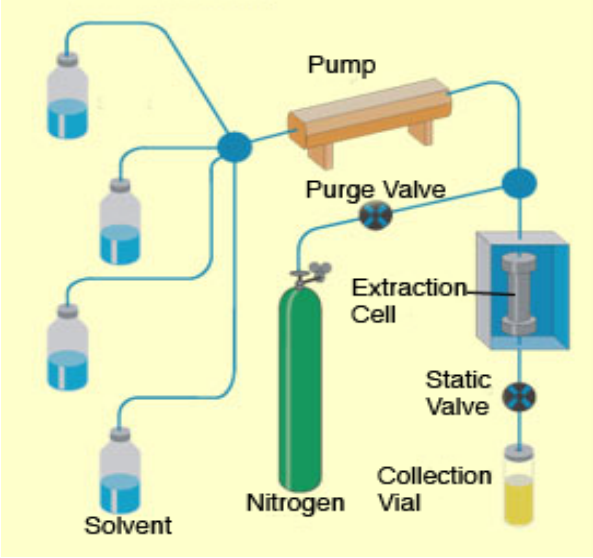

Abbildung 4: Schematische Darstellung der beschleunigten Lösemittelextraktion (ASE). Quelle: Dionex Corporation, http://www1.dionex.com/en-us/instruments/ins7387.html, modifiziert

Eine Extraktionsmethode, die hoch automatisiert und schnell ist, und dabei eine hohe Extraktionseffizienz aufweist, ist die beschleunigte Lösemittelextraktion (accelerated solvent extraction – ASE). Im Vergleich mit anderen Extraktionsmethoden wie z.B. der Soxhlet-Extraktion lieferte die ASE bei der Extraktion von polyzyklischen aromatischen Kohlenwasserstoffen und verschiedenen Pestiziden gleiche oder bessere Ergebnisse in Bezug auf die Extraktionseffizienz (Conte et al. 1997, Fisher et al. 1997, Frost et al. 1997,

Hubert et al. 2001). Hinsichtlich der Umweltfreundlichkeit und Ressourcenschonung des Verfahrens ist hervorzuheben, dass der Lösungsmittelverbrauch mit 20-100 mL pro Probe in Abhängigkeit von der Extraktionsmenge, sehr gering ist.

Der schematische Aufbau der ASE ist in Abbildung 4 dargestellt. Zunächst wird die Probe in die Extraktionszelle gegeben und diese im Gerät platziert. Die Extraktionszelle wird nun automatisch mit dem Lösungsmittel befüllt und der Druck und Temperatur werden entsprechend den gewählten Extraktionsbedingungen erhöht. Der Temperaturbereich geht von Zimmertemperatur bis zu 200°C, der Druckbereich von 3.5 bis 20 MPa. Während die hohe Temperatur dazu dient, die Extraktionsstärke zu verbessern, hindert der hohe Druck das Lösungsmittel am Übergang in die gasförmige Phase (Giergielewicz-Mozajska et al. 2001). Nach der gewählten Extraktionsdauer (üblicherweise im Minutenbereich) wird der Extrakt in ein Auffangfläschchen befördert (Abbildung 4). Wenn die Extraktion nicht vollständig war, kann der Extraktionsprozess mit frischem Lösungsmittel beliebig oft wiederholt werden, häufig werden zwei Extraktionsschritte durchgeführt (Giergielewicz-Mozajska et al. 2001).

Ein großes Problem bei der Extraktion von Sedimenten und Schwebstoffen ist die Koextraktion von Substanzen wie z.B. Huminstoffen oder Fulvinsäuren (Concha-Grana et al. 2004). Diese koextrahierten Stoffe können während der Analytik stören, weshalb in den meisten Fällen eine Aufreinigung der Extrakte notwendig ist. Für die Extraktion von polaren und semipolaren Pestiziden von Schwebstoffen wurde bisher noch keine ASE-Methode entwickelt. Auch bezüglich des Aufreinigungsschrittes bei der Bestimmung polarer Substanzen besteht Entwicklungsbedarf (Dabrowska et al. 2003). Schließlich steht ein Vergleich der ökotoxikologischen Relevanz von partikeladsorbierten Pestizideinträgen mit dem Eintrag über die Wasserphase aus.

Effekte von Pestizideinträgen auf das Fließgewässerökosystem

Pestizide können einen wichtigen Stressor für Organismen in Fließgewässern darstellen (Liess et al. 2005). Das betrifft im Besonderen kleinere Fließgewässer (1.-3. Ordnung nach Strahler (1957)), da hier Verdünnungseffekte weniger zum Tragen kommen und die Konzentrationen somit höher sind. Kleinere Fließgewässer spielen eine wichtige Rolle im Wasserhaushalt, zum einen hinsichtlich der Länge des Gewässernetzes (Liess et al. 2001) und zum anderen, weil sie im Austausch mit dem Grundwasser stehen und es dadurch zur Weiterleitung von Verunreinigungen kommen kann (Pereira und Hostettler 1993). Ferner

haben sie eine große ökologische Bedeutung, da sie als Rückzugs- und Laichraum für Fische dienen können und einen wichtigen Energielieferanten für stromabwärts-befindliche Fischgemeinschaften darstellen (Wipfli 2005).

In einer Vielzahl von Studien wurde gezeigt, dass Pestizide die Zusammensetzung der aquatischen Invertebratenfauna verändern können (Heckman 1981, Wallace et al. 1982, Hatakeyama und Yokoyama 1997, Liess und Schulz 1999, Schulz und Liess 1999a, Leonard et al. 2000, Hanazato 2001, Friberg et al. 2003, Jergentz et al. 2004, Anderson et al. 2006). Beispielsweise untersuchten Schulz und Liess (1999) die Auswirkungen von Runoff nach Insektizid-Applikation auf die Makroinvertebratengemeinschaft kleiner Fließgewässer. Insektizidbelasteter Runoff führte zum Verschwinden von 8 der 11 vorher gefundenen Arten und die verbleibenden Arten waren signifikant in ihrer Abundanz reduziert. Runoff außerhalb der Zeit der Insektizid-Anwendung rief diese Effekte nicht hervor (Liess und Schulz 1999, Schulz und Liess 1999a). Ähnliche Ergebnisse lieferte eine Untersuchung der Makroinvertebratenfauna in Flussabschnitten mit und ohne landwirtschaftliche Nutzung von Insektiziden, Herbiziden und Fungiziden (Hatakeyama und Yokoyama 1997). Nach der Pestizidausbringung wurde eine starke Abnahme von Diversität und Populationsdichte in Gewässerabschnitten mit angrenzender landwirtschaftlicher Nutzung beobachtet, während in Abschnitten oberhalb der Ausbringungsflächen diese Effekte nicht auftraten. Eine weitere Studie an drei Fließgewässern in Argentinien zeigte, dass die Makroinvertebratenfauna nur in den beiden Gewässern verändert wurde, in denen Endosulfan in Runoffproben nachgewiesen wurde (Jergentz et al. 2004).

Die bisherigen Untersuchungen zu den Effekten von Pestiziden auf die Invertebratenfauna wurden meist an einer geringen Zahl von Fließgewässern durchgeführt ($n \leq 5$), so dass ungeklärt ist, ob es sich bei den Beobachtungen nur um lokale Phänomene handelt. Ausnahmen bilden eine Studie an 29 dänischen Fließgewässern, die einen Zusammenhang zwischen der Summe der Insektizid-, Herbizid- und Fungizid–konzentrationen im Sediment und der Zusammensetzung der Makroinvertebratenfauna nahe legte (Friberg et al. 2003), und eine Studie an 20 deutschen Fließgewässern, die eine Abnahme des Anteils der empfindlichen Arten (% SPEAR) an der Gemeinschaft mit zunehmenden Pestizidstress aufzeigte (Liess und von der Ohe 2005). Inwiefern durch die Beeinträchtigung der Gemeinschaftsstruktur die Funktionen des Ökosystems verändert werden, wurde jedoch nicht untersucht. Eine wichtige Funktion im aquatischen Ökosystem kleiner Fließgewässer stellt der Abbau von allochthonem organischen

Material dar (Wallace et al. 1997). Dieser Prozess ist auch deshalb relevant, weil flussabwärts befindliche Abschnitte energetisch auf den Eintrag des zerkleinerten organischen Materials angewiesen sind (Vannote et al. 1980, Wipfli 2005). Die Geschwindigkeit des Abbauprozesses von Blättern wird deshalb als funktionsbezogener Index für die Güte der Wasserqualität verwendet (Pascoal et al. 2001, Gessner und Chauvet 2002). Dafür werden Blatttaschen in den Fließgewässern ausgebracht, deren verbleibende Blattmasse in Intervallen oder nach einer bestimmten Zeit bestimmt wird (Benfield 1996). Für verschiedene Stressoren wie Schwermetalle, Eutrophierung (Lecerf et al. 2006) oder Versauerung wurde gezeigt, dass sich die Abbaurate von Blättern in den betroffenen Fließgewässern änderte (Gessner und Chauvet 2002). Zu den Auswirkungen von Pestiziden auf die Blattabbaurate liegt bisher erst eine Studie vor, die bei einem insektizidbehandelten Bach eine Reduktion der Abbaurate im Vergleich zu einem Kontrollbach fand (Chung et al. 1993). Da in dieser Studie das Insektizid direkt in das Gewässer eingebracht wurde, um die Effekte von Moskitokontrollmaßnahmen zu simulieren, ist ungeklärt, ob auch der landwirtschaftlich bedingte Eintrag von Pestiziden diesen Ökosystemprozess beeinflusst.

Konzept und Ziele

Das übergeordnete Ziel der vorliegenden Arbeit ist, einen wichtigen Beitrag zur ökologischen Risikoabschätzung von Pestiziden in Fließgewässerökosystemen zu leisten. Dabei sah die Konzeption des Promotionsprojektes vor, dass die Effekte von Pestizid–einträgen anhand von Felduntersuchungen in zwei unterschiedlichen biogeografischen Regionen mit kontrastierendem Pestizideinsatz untersucht werden sollten (Abbildung 5). Hierfür wurden zwei Regionen in Frankreich und Finnland ausgewählt, da für diese Gebiete Ergebnisse behördlicher Monitoringprogramme und Anwendungsmengendaten vorlagen, die benötigt wurden, um aus den Hunderten von zugelassenen Wirkstoffen potentiell ökotoxikologisch relevante Substanzen zu identifizieren. Für jedes Gebiet wurden die 10 Pestizide mit der höchsten Ökotoxizität gegenüber dem Standardtestorganismus *Daphnia magna* ausgewählt. Die Untersuchungen umfassten zum einen die Aufnahme des ökologischen Gewässerzustandes und zum anderen die Bestimmung der Konzentrationen der ausgewählten Pestizide (Abbildung 5). Dieser zweite Aspekt bildete einen Schwerpunkt der vorliegenden Arbeit, da eine präzise Charakterisierung der Exposition entscheidend ist, um eine kausale Verbindung zu

Effekten herzustellen (Schulz 2004). Wie in Abbildung 5 ersichtlich, wurden drei verschiedene Probenahmemethoden eingesetzt, um die Pestizidbelastung zu bestimmen. Die Auswahl der Methoden orientierte sich an dem Ziel, zum einen unterschiedliche Eintragspfade und zum anderen Belastungen sowohl der Wasser- als auch der Schwebstoffphase zu erfassen. Dabei machte die Methodenauswahl die Entwicklung einer Bestimmungsmethode für partikelgebundene Pestizide (Kapitel 2) und die Kalibration und Anpassung eines Passivsammlers notwendig (Kapitel 3-4).

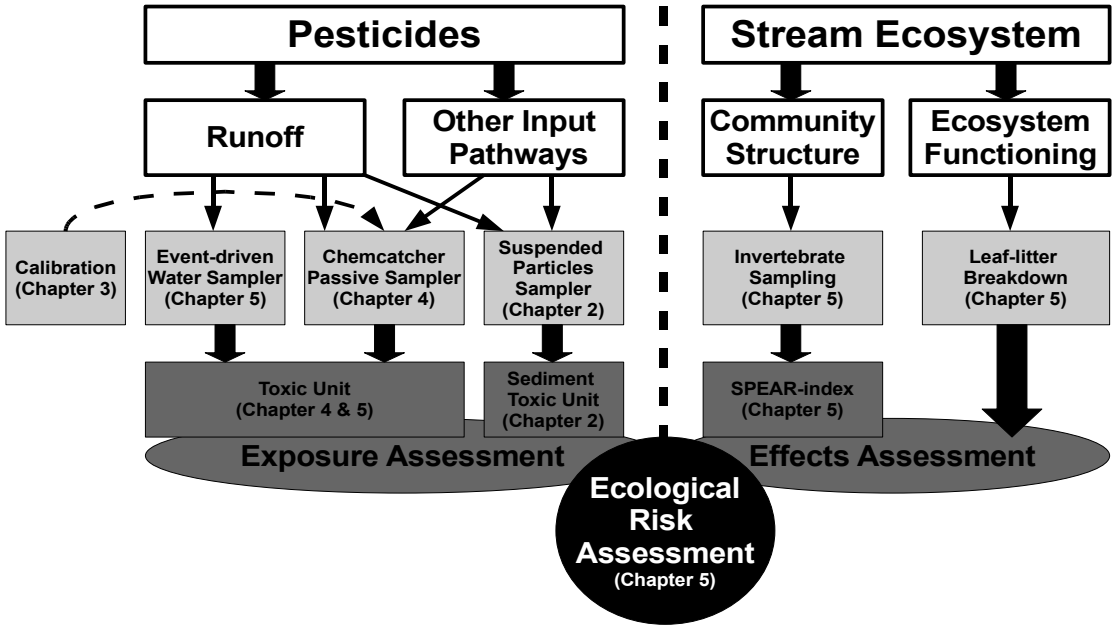

Abbildung 5: Konzeptioneller Ansatz der vorliegenden Dissertation. Weiße Kästen = Prozesse und Entitäten in der Umwelt. Hellgraue Kästen = Monitoringmethoden für den jeweiligen Prozess/Entität. Dunkelgraue Kästen = Datenauswertungsmethoden.

Um die Ergebnisse des chemischen und biologischen Monitorings miteinander in Beziehung setzen zu können, mussten sowohl die Informationen auf der Expositions- wie der Effektseite aggregiert werden. Auf der Effektseite wurde dafür das SPEAR-Konzept verwendet, auf das schon oben eingegangen wurde und im Detail in Kapitel 5 erklärt wird. Zum Vergleich der Pestizidbelastung an den verschiedenen Messstellen wurde das Konzept der Toxischen Einheiten (toxic unit - TU) verwendet, dass die beobachteten Pestizidkonzentrationen anhand der Toxizität der jeweiligen Substanz gegenüber dem Standardtestorganismus *Daphnia magna* standardisiert (Sprague 1970, Liess und von der Ohe 2005). Anhand dieser normierten Werte konnte dann der Zusammenhang zwischen Pestizidtoxizität und Gemeinschaftsstruktur bzw. Blattabbaurate untersucht und die

Abschätzung eines ökologischen Risikos durch Pestizide durchgeführt werden (Abbildung 5). Nähere Informationen und die genaue Analyse sind in Kapitel 5 beschrieben. Abstrakter betrachtet, beschreibt diese Arbeit also auch ein methodisches Konzept, um die Ergebnisse von abiotischen und biotischen Monitoringdaten zusammenzubringen. Im Folgenden werden die Problemstellungen und Ziele der einzelnen Publikationen vorgestellt aus denen sich die kumulative Dissertation zusammensetzt, die Ergebnisse werden in Kapitel 6 zusammengefasst:

Publikation 1 (Kapitel 2): Bestimmung von 10 polaren und semi-polaren Pestiziden verschiedener chemischer Klassen an Schwebstoffen aus Bächen unter Verwendung der beschleunigten Lösemittelextraktion

Originaltitel

„Determination of 10 particle-associated multiclass polar and semi-polar pesticides from small streams using accelerated solvent extraction“ (Schäfer et al. 2007a)

Problemstellung

Die ASE stellt ein effizientes Extraktionsverfahren dar, um matrixgebundene Pestizide zu extrahieren (Hubert et al. 2001). Allerdings existiert bisher noch keine ASE-Methode zur Extraktion und Bestimmung von schwebstoffgebundenen Pestiziden, die verschiedenen chemischen Stoffklassen angehören. Ferner stellt sich das Problem, dass die Höhe der Wiederfindung von Stoffen in Proben mit unterschiedlichen Eigenschaften (z.B. TOC-Gehalt) variieren kann (Steinheimer et al. 1994). Für Freilandproben von verschiedenen Standorten wäre deswegen ein interner Standard wünschenswert, der vor der Extraktion der Probe hinzugegeben wird und die Güte der Extraktion anzeigt.

Ziele

- Entwicklung und Anwendung einer Extraktionsmethode für partikelgebundene polare und semi-polare Pestizide aus Fließgewässern mit der ASE
- Untersuchung der Eignung von deuterierten Standards der Analyten als interne Standards

Publikation 2 (Kapitel 3): Kalibrierung des Chemcatcher® Passivsammlers für das Monitoring von ausgewählten polaren und semipolaren Pestiziden in Oberflächengewässern

Originaltitel

„Calibration of the Chemcatcher® passive sampler for monitoring selected polar and semi-polar pesticides in surface water“ (Gunold et al. 2007)

Problemstellung

Damit nach der Freilandexposition von Passivsammlern die TWA-Konzentration bestimmt werden kann, muss die substanz- und empfängerphasen-spezifische Sammelrate bekannt sein (Booij et al. 2007). Diese kann im Rahmen von Labor-Kalibrationsexperimenten ermittelt werden (Vrana et al. 2006). Für polare und semi-polare Pestizide sind bisher jedoch nur wenige Sammelraten verfügbar. Ferner stellt sich die Frage, ob die Sammelraten aufgrund von physikochemischen Substanzeigenschaften vorhergesagt werden können und somit auf aufwändige Laborexperimente verzichtet werden könnte. Schließlich wurde bisher noch nicht untersucht, ob die Austauschkinetik von Substanzen bei einer Empfängerphase für polare Substanzen isotrop ist. Nur in diesem Fall könnte das Performance Reference Compound (PRC)-Konzept angewendet werden, um Variation in den Umweltbedingungen zwischen verschiedenen Probestellen bei der Freilandausbringung berücksichtigen zu können.

Ziele

- Bestimmung von Sammelraten für den Chemcatcher® Passivsammler
- Beurteilung der Vorhersagbarkeit von Sammelraten auf Basis von physikochemischen Eigenschaften der Analyten
- Beurteilung der Anwendbarkeit des PRC-Konzeptes für polare Substanzen

Publikation 3 (Kapitel 4): Leistungsfähigkeit des Chemcatcher® Passivsammlers zur Bestimmung von 10 polaren und semi-polaren Pestiziden in 16 mitteleuropäischen Fließgewässern und Vergleich mit zwei anderen Probenahmemethoden

Originaltitel

„Performance of the Chemcatcher® passive sampler when used to monitor 10 polar and semi-polar pesticides in 16 Central European streams, and comparison with two other sampling methods“ (Schäfer et al. 2007b)

Problemstellung

Bisher wurden Passivsammler im aquatischen Bereich hauptsächlich eingesetzt, um unpolare Substanzen zu erfassen. Der Chemcatcher® Passivsammler sollte mit entsprechender Empfängerphase für das kontinuierliche Monitoring der Belastung mit polaren Substanzen geeignet sein. Bisher wurden jedoch keine umfangreicheren Feldstudien durchgeführt (Kingston et al. 2000). Speziell die Erfassung von kurzzeitigen Expositionen wurde noch nicht untersucht (Greenwood et al. 2007). Darüber hinaus wurde der Chemcatcher® bisher nicht mit anderen ereignisbezogenen Probenahmemethoden für die Erfassung der Pestizidbelastung verglichen. Ein solcher Vergleich wäre wichtig, um die Geeignetheit verschiedener Probenahmemethoden für die Charakterisierung des Expositionsniveaus beurteilen zu können.

Ziele

- Untersuchung der Eignung des Chemcatcher® Passivsammlers für die Erfassung von runoff-bedingten Pestizideinträgen
- Beurteilung verschiedener Probenahmemethoden zur realistischen Charakterisierung des Expositionsniveaus gegenüber Pestiziden

Publikation 4 (Kapitel 5): Auswirkungen von Pestiziden auf die Gemeinschaftsstruktur und Ökosystemfunktionen in Bächen in landwirtschaftlich genutzten Gebieten in drei biogeografischen Regionen Europas

Originaltitel

„Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe“ (Schäfer et al. 2007c)

Problemstellung

Obwohl verschiedene Untersuchungen gezeigt haben, dass Pestizide Effekte auf die Makroinvertebratengemeinschaften haben können, gibt es bisher wenige Untersuchungen, die über das lokale Niveau hinausgehen. Ferner ist nicht bekannt, ob die Strukturveränderungen in der Invertebratengemeinschaft Auswirkungen auf wichtige Ökosystemprozesse haben. Kürzlich wurde der SPEAR-Index entwickelt, der sich auf ökologische und biologische Merkmale einer Gemeinschaft bezieht, um Pestizidstress nachzuweisen (Liess und von der Ohe 2005). Theoretisch sollte dieser merkmalsbasierte Index zur vergleichenden Beurteilung des Einflusses von Pestiziden auf den Gewässerzustand in verschiedenen biogeografische Regionen geeignet sein, doch das

wurde bisher nicht untersucht.

Ziele

- Abschätzung, ob und ab welchem Belastungsgrad Pestizide die Struktur der Invertebratenfauna und den Abbau von allochthonem organischen Material beeinträchtigen
- Beurteilung der Eignung des SPEAR-Indexes zur Detektion von Pestizidstress über biogeografische Regionen

Literaturverzeichnis Kapitel 1

Allan, J. D. 1995. Stream Ecology. Kluwer Academic Publishers, Dordrecht.

Anderson, B. S., B. M. Phillips, J. W. Hunt, V. Connor, N. Richard, und R. S. Tjeerdema. 2006. Identifying primary stressors impacting macroinvertebrates in the Salinas River (California, USA): Relative effects of pesticides and suspended particles. Environmental Pollution 141:402.

Arndt, U., W. Nobel, und B. Schweizer. 1987. Bioindikatoren: Möglichkeiten, Grenzen und neue Erkenntnisse Ulmer, Stuttgart.

Bach, M., A. Huber, und H. G. Frede. 2001. Modeling pesticide losses from diffuse sources in Germany. Water Science and Technology 44:189-196.

BBA. 2002. Jahresbericht 2002, Berlin; Braunschweig.

Benfield, E. F. 1996. Leaf Breakdown in Stream Ecosystems. Pages 579-589 in F. R. Hauer und G. A. Lamberti, editors. Methods in Stream Ecology. Academic Press, San Diego.

BMU. 2005. Die Wasserrahmenrichtlinie - Ergebnisse der Bestandsaufnahme 2004 in Deutschland. Berlin.

Bonada, N., S. Doledec, und B. Statzner. 2007. Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: implications for future climatic scenarios. Global Change Biology 13:1658-1671.

Bonada, N., N. Prat, V. H. Resh, und B. Statzner. 2006. Developments in aquatic insect biomonitoring: A comparative analysis of recent approaches. Annual Review of Entomology 51:495-523.

Booij, K., B. Vrana, und J. N. Huckins. 2007. Theory, modelling and calibration of passive samplers used in water monitoring. Pages 141-169 in R. Greenwood, G. A. Mills, und B. Vrana, editors. Comprehensive Analytical Chemistry 48: Passive sampling techniques in environmental monitoring. Elsevier, Amsterdam.

BVL. 2007. Absatz an Pflanzenschutzmitteln in der Bundesrepublik Deutschland: Ergebnisse der Meldungen gemäß §19 Pflanzenschutzgesetz für das Jahr 2006. BVL, Braunschweig.

Chung, K., J. B. Wallace, und J. W. Grubaugh. 1993. The Impact of Insecticide Treatment on Abundance, Biomass and Production of Litterbag Fauna in a Headwater Stream: A Study of Pretreatment, Treatment and Recovery. Limnologica 23:93-106.

Concha-Grana, E., M. I. Turnes-Carou, S. Muniategui-Lorenzo, P. Lupez-Mahia, E. Fernandez-Fernandez, und D. Prada-Rodriguez. 2004. Development of pressurized liquid extraction and cleanup procedures for determination of organochlorine pesticides in soils. Journal of Chromatography A 1047:147-155.

Conte, E., R. Milani, G. Morali, und F. Abballe. 1997. Comparison between accelerated solvent extraction and traditional extraction methods for the analysis of the herbicide diflufenican in soil. Journal of Chromatography A 765:121-125.

Cummins, K. W. 1973. Trophic Relations of Aquatic Insects. Annual Review of Entomology 18:183-206.

Dabrowska, H., L. Dabrowski, M. Biziuk, J. Gaca, und J. Namiesnik. 2003. Solid-phase extraction clean-up of soil and sediment extracts for the determination of various types of pollutants in a single run. Journal Of Chromatography A 1003:29-42.

DIN. 1990. Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung; Biologisch-ökologische Gewässeruntersuchung (Gruppe M); Bestimmung des Saprobienindex (M2). Pages 18 in. Deutsches Institut für Normung e.V., Beuth Verlag, Berlin, Germany.

Doledec, S., N. Phillips, M. Scarsbrook, R. H. Riley, und C. R. Townsend. 2006. Comparison of structural and functional approaches to determining landuse effects on grassland stream invertebrate communities. Journal of the North American Benthological Society 25:44-60.

Doledec, S., B. Statzner, und M. Bournard. 1999. Species traits for future biomonitoring across ecoregions: patterns along a human-impacted river. Freshwater Biology 42.

EU. 2000. Richtlinie 2000/60/EG des europäischen Parlaments und des Rates vom 23. Oktober 2000 zur Schaffung eines Ordnungsrahmens für Maßnahmen der Gemeinschaft im Bereich der Wasserpolitik. Amtsblatt der Europäischen Gemeinschaften L327:1-72.

Fisher, J. A., M. J. Scarlett, und A. D. Stott. 1997. Accelerated solvent extraction: An evaluation for screening of soils for selected U.S. EPA semivolatile organic priority pollutants. Environmental Science and Technology 31:1120-1127.

Forbes, V. E., A. Palmqvist, und L. Bach. 2006. The use and misuse of biomarkers in ecotoxicology. Environmental Toxicology and Chemistry 25:272-280.

Friberg, N., M. Lindstrom, B. Kronvang, und S. E. Larsen. 2003. Macroinvertebrate/sediment relationships along a pesticide gradient in Danish streams. Hydrobiologia 494:103-110.

Frost, S. P., J. R. Dean, K. P. Evans, K. Harradine, C. Cary, und M. H. I. Comber. 1997. Extraction of hexaconazole from weathered soils: A comparison between Soxhlet extraction, microwave-assisted extraction, supercritical fluid extraction and accelerated solvent extraction. Analyst 122:895-898.

Gayraud, S., B. Statzner, P. Bady, A. Haybach, F. Scholl, P. Usseglio-Polatera, und M. Bacchi. 2003. Invertebrate traits for the biomonitoring of large European rivers: an initial assessment of alternative metrics. Freshwater Biology 48:2045-2064.

Gessner, M. O., und E. Chauvet. 2002. A case for using litter breakdown to assess functional stream integrity. Ecological Applications 12:498-510.

Giergielewicz-Mozajska, H., L. Dabrowski, und J. Namiesnik. 2001. Accelerated Solvent Extraction (ASE) in the analysis of environmental solid samples - Some aspects of theory and practice. Critical Reviews in Analytical Chemistry 31:149-165.

Gorecki, T., und J. Namiesnik. 2002. Passive sampling. Trac-Trends in Analytical Chemistry 21:276-291.

Greenwood, R., G. A. Mills, B. Vrana, I. J. Allan, R. Aguilar-Martinez, und G. Morrison. 2007. Monitoring of priority pollutants in water using Chemcatcher passive sampling devices. Pages 199-229 in R. Greenwood, G. A. Mills, und B. Vrana, editors. Comprehensive Analytical Chemistry 48: Passive sampling techniques in environmental monitoring. Elsevier, Amsterdam.

Gunold, R., R. B. Schäfer, A. Paschke, G. Schüürmann, and M. Liess. 2007. Calibration of the Chemcatcher® passive sampler for monitoring selected polar and semi-polar pesticides in surface water. Environmental Pollution, in press. doi:10.1016/j.envpol.2007.1010.1037.

Guo, L., C. E. Nordmark, F. C. Spurlock, B. R. Johnson, L. Y. Li, J. M. Lee, und K. S. Goh. 2004. Characterizing dependence of pesticide load in surface water on precipitation and pesticide use for the Sacramento River watershed. Environmental Science and Technology 38:3842-3852.

Hanazato, T. 2001. Pesticide effects on freshwater zooplankton: an ecological perspective. Environmental Pollution 112:1-10.

Hatakeyama, S., und 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.

Haybach, A., F. Schöll, B. König, und F. Kohmann. 2004. Use of biological traits for interpreting functional relationships in large rivers. Limnologica 34:451-459.

Heckman, C. W. 1981. Long-term effects of intensive pesticide applications on the aquatic community in orchard ditches near Hamburg, Germany. Archives of Environmental Contamination and Toxicology 10:393-426.

Heugens, E., A. Hendriks, T. Dekker., N. M. Van Straalen, und W. Amiraal. 2001. A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. Critical Reviews in Toxicology 31:247-284.

Hubert, A., K.-D. Wenzel, W. Engelwald, und G. Schüürmann. 2001. Accelerated solvent extraction - More efficient extraction of POPs and PAHs from real contaminated plant and soil samples. Reviews in Analytical Chemistry 20:101-144.

Huckins, J. N., J. D. Petty, J. A. Lebo, F. V. Almeida, K. Booij, D. A. Alvarez, R. C. Clark, und B. B. Mogensen. 2002. Development of the permeability/performance reference compound approach for in situ calibration of semipermeable membrane devices. Environmental Science and Technology 36:85-91.

Hyne, R. V., und W. A. Maher. 2003. Invertebrate biomarkers: links to toxicosis that predict population decline. Ecotoxicology and Environmental Safety 54:366-374.

Illies, J. 1978. Limnofauna Europaea, A Compilation of the European Freshwater Species with Emphasis on their Distribution and Ecology, 2nd edition. G. Fischer, Jena.

Inoue, T., S. Ebise, A. Numabe, O. Nagafuchi, und Y. Matsui. 2002. Runoff characteristics of particulate pesticides in a river from paddy fields. Water Science and Technology 45:121-126.

Jergentz, S., H. Mugni, C. Bonetto, und R. Schulz. 2004. Runoff-related endosulfan contamination and aquatic macroinvertebrate response in rural basins near Buenos Aires, Argentina. Archives of Environmental Contamination and Toxicology 46:345-352.

Kingston, J. K., R. Greenwood, G. A. Mills, G. M. Morrison, und L. B. Persson. 2000. Development of a novel passive sampling system for the time-averaged measurement of a range of organic pollutants in aquatic environments. Journal of Environmental Monitoring 2:487-495.

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

Kot, A., B. Zabiegala, and J. Namiesnik. 2000. Passive sampling for long-term monitoring of organic pollutants in water. Trac-Trends in Analytical Chemistry 19:446-459.

Lampert, W., und U. Sommer. 1999. Limnoökologie. Thieme, Stuttgart; New York.

Lecerf, A., P. Usseglio-Polatera, J. Y. Charcosset, D. Lambrigot, B. Bracht, und E. Chauvet. 2006. Assessment of functional integrity of eutrophic streams using litter breakdown and benthic macroinvertebrates. Archiv fur Hydrobiologie 165:105-126.

Leonard, A. W., R. V. Hyne, R. P. Lim, F. Pablo, und P. J. Van den Brink. 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.

Leu, C., H. Singer, C. Stamm, S. R. Muller, und R. P. Schwarzenbach. 2004. Simultaneous assessment of sources, processes, and factors influencing herbicide losses to surface waters in a small agricultural catchment. Environmental Science and Technology 38:3827-3834.

Liess, M., C. Brown, P. Dohmen, S. Duquesne, F. Heimbach, J. Kreuger, L. Lagadic, W. Reinert, S. Maund, M. Streloke, und J. Tarazona. 2005. Effects of Pesticides in the Field – EPIF. SETAC Press, Brussels, Belgium

Liess, M., B. J. Pieters, und S. Duquesne. 2006. Long-term signal of population disturbance after pulse exposure to an insecticide: Rapid recovery of abundance, persistent alteration of structure. Environmental Toxicology and Chemistry 25:1326-1331.

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

Liess, M., R. Schulz, N. Berenzen, J. Nanko-Drees, und J. Wogram. 2001. Pesticide contamination and macroinvertebrate communities in running waters in agricultural areas. Umweltbundesamt, Berlin.

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

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

Long, J. L. A., W. A. House, A. Parker, und J. E. Rae. 1998. Micro-organic compounds associated with sediments in the Humber rivers. Science of the Total Environment 210:229-253.

Maltby, L., S. A. Clayton, R. M. Wood, und N. McLoughlin. 2002. Evaluation of the Gammarus pulex in situ feeding assay as a biomonitor of water quality: Robustness, responsiveness, and relevance. Environmental Toxicology and Chemistry 21:361-368.

Mayer, P., und F. Reichenberg. 2006. Can highly hydrophobic organic substances cause aquatic baseline toxicity and can they contribute to mixture toxicity? Environmental Toxicology and Chemistry 25:2639-2644.

MEA. 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC.

Mills, G. A., B. Vrana, I. J. Allan, D. A. Alvarez, J. N. Huckins, und R. Greenwood. 2007. Trends in monitoring pharmaceuticals and personal-care products in the aquatic environment by use of passive sampling devices. Analytical and Bioanalytical Chemistry 387:1153-1157.

Naeem, S., und J. P. Wright. 2003. Disentangling biodiversity effects on ecosystem functioning: Deriving solutions to a seemingly insurmountable problem. Ecology Letters 6:567-579.

Neumann, M., R. Schulz, K. Schäfer, W. Müller, W. Mannheller, und M. Liess. 2002. The significance of entry routes as point and non-point sources of pesticides in small streams. Water Research 36:835-842.

Niemi, G. J., P. DeVore, D. Taylor, A. Lima, und J. Pastor. 1990. Overview of case studies on recovery of aquatic systems from disturbance. Environ. Management 14:571-587.

Oerke, E. C., und H. W. Dehne. 2004. Safeguarding production - losses in major crops and the role of crop protection. Crop Protection 23:275-285.

Pascoal, C., F. Cassio, und P. Gomes. 2001. Leaf breakdown rates: A measure of water quality? International Review of Hydrobiology 86:407-416.

Pereira, W. E., und F. D. Hostettler. 1993. Nonpoint-Source Contamination of the Mississippi River and Its Tributaries by Herbicides. Environmental Science & Technology 27:1542-1552.

Pereira, W. E., und C. E. Rostad. 1990. Occurrence, distributions, and transport of herbicides and their degradation products in the lower Mississippi river and its tributaries. Environmental Science and Technology 24:1400-1406.

Piscart, C., P. Usseglio-Polatera, J. C. Moreteau, und J. N. Beisel. 2006. The role of salinity in the selection of biological traits of freshwater invertebrates. Archiv Fur Hydrobiologie 166:185-198.

Radkau, J. 2002. Natur und Macht. Eine Weltgeschichte der Umwelt. C.H. Beck, München.

Raupach, M. R., P. R. Briggs, P. W. Ford, J. F. Leys, M. Muschal, B. Cooper, und V. E. Edge. 2001. Endosulfan transport: I. Integrative assessment of airborne and waterborne pathways. Journal of Environmental Quality 30:714-728.

Richards, R. P., und D. B. Baker. 1993. Pesticide concentration patterns in agricultural drainage networks in the Lake Erie basin. Environmental Toxicology and Chemistry 12:13-26.

Sandin, L., und P. F. M. Verdonschot. 2006. Stream and river typologies - Major results and conclusions from the STAR project. Hydrobiologia 566:33-37.

Schäfer, R. B., W.-U. Palm, D. Steffen, und W. Ruck. 2004. Pflanzenbehandlungs- und Schädlingsbekämpfungsmittel in niedersächsischen Fließgewässern von 1994 bis 2001. Hydrologie und Wasserbewirtschaftung 48:117-125.

Schäfer, R. B., R. Mueller, W. Brack, K.-D. Wenzel, G. Streck, W. Ruck, and M. Liess. 2007a. Determination of 10 particle-associated multiclass polar and semi-polar pesticides from small streams using accelerated solvent extraction. Chemosphere 70: 1952-1960.

Schäfer, R. B., A. Paschke, B. Vrana, R. Mueller, and M. Liess. 2007b. Performance of the Chemcatcher® passive sampler when used to monitor 10 polar and semi-polar pesticides in 16 Central European streams, and comparison with two other sampling methods Water research, in press. doi:10.1016/j.watres.2008.01.023.

Schäfer, R. B., T. Caquet, K. Siimes, R. Mueller, L. Lagadic, and M. Liess. 2007c. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. Science of the Total Environment 382:272-285.

Schulz, R. 2004. Field Studies on Exposure, Effects, and Risk Mitigation of Aquatic Nonpoint-Source Insecticide Pollution: A Review. Journal of Environmental Quality 33:419-448.

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

Schulz, R., und M. Liess. 1999b. Validity and ecological relevance of an active in situ bioassay using *Gammarus pulex* and *Limnephilus lunatus*. Environmental Toxicology and Chemistry 18:2243-2250.

Schulz, R., und M. Liess. 2001. Acute and chronic effects of particle-associated fenvalerate on stream macroinvertebrates: a runoff simulation study using outdoor microcosms. Archives of Environmental Contamination and Toxicology 40:481-488.

Schwoerbel, J. 1994. Methoden der Hydrobiologie. Fischer, Stuttgart; Jena; New York.

Sih, A., A. M. Bell, und J. L. Kerby. 2004. Two stressors are far deadlier than one. Trends in Ecology & Evolution 19:274-276.

Sprague, J. B. 1970. Measurement of pollutant toxicity to fish, II-Utilizing and applying bioassay results. Water Research 4:3-32.

Stark, J. D., J. E. Banks, und R. Vargas. 2004. How risky is risk assessment: The role that life history strategies play in susceptibility of species to stress. Proceedings of the National Academy of Sciences of the United States of America 101:732-736.

Statzner, B., P. Bady, S. Doledec, und F. Scholl. 2005. Invertebrate traits for the biomonitoring of large European rivers: an initial assessment of trait patterns in least impacted river reaches. Freshwater Biology 50:2136-2161.

Statzner, B., B. Bis, S. Dolédec, und P. Usseglio-Polatera. 2001. Perspectives for biomonitoring at large spatial scales: A unified measure for the functional composition of invertebrate communities in European running waters. Basic and Applied Ecology 2:73-85.

Statzner, B., S. Doledec, und B. Hugueny. 2004. Biological trait composition of European stream invertebrate communities: assessing the effects of various trait filter types. Ecography 27:470-488.

Steinheimer, T. R., R. L. Pfeiffer, und K. D. Scoggin. 1994. Extraction of Atrazine, Cyanazine, Desethylatrazine, Desisopropylatrazine, and Metolachlor from Fortified Western Corn-Belt Soils by Sfe with Co2. Analytical Chemistry 66:645-650.

Stoddard, J. L., D. P. Larsen, C. P. Hawkins, R. K. Johnson, und R. H. Norris. 2006. Setting expectations for the ecological condition of streams: The concept of reference condition. Ecological Applications 16:1267-1276.

Strahler, A. N. 1957. Quatitative analysis of watershed geomorphology. Transactions, American Geophysical Union 38:913-920.

Stuer-Lauridsen, F. 2005. Review of passive accumulation devices for monitoring organic micropollutants in the aquatic environment. Environmental Pollution 136:503-524.

Sturm, A., T. S. Radau, T. Hahn, und R. Schulz. 2007. Inhibition of rainbow trout acetylcholinesterase by aqueous and suspended particle-associated organophosphorous insecticides. Chemosphere 68:605-612.

Tran, A. T. K., R. V. Hyne, und P. Doble. 2007. Calibration of a passive sampling device for time-integrated sampling of hydrophilic herbicides in aquatic environments. Environmental Toxicology and Chemistry 26:435-443.

Usseglio-Polatera, P., M. Bournaud, P. Richoux, und H. Tachet. 2000. Biological and ecological traits of benthic freshwater macroinvertebrates: relationships and definition of groups with similar traits. Freshwater Biology 43:175-205.

Van Straalen, N. M. 1997. How to measure no effect. Part II: Threshold effects in ecotoxicology. Environmetrics 8:249-253.

Vannote, R. L., W. G. Minnshall, K. W. Cummins, J. R. Sedell, und C. E. Cushing. 1980. The river continuum concept. Canadian Journal of and Aquatic Sciences 37:130-137.

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

von der Ohe, P. C., A. Prüß, R. B. Schäfer, M. Liess, E. d. Deckere, und W. Brack. 2007. Water quality indices across Europe - a comparison of the good ecological status of five river basins. Journal of Environmental Monitoring 9:970-978.

Vrana, B., I. J. Allan, R. Greenwood, G. A. Mills, E. Dominiak, K. Svensson, J. Knutsson, und G. Morrison. 2005. Passive sampling techniques for monitoring pollutants in water. TrAC - Trends in Analytical Chemistry 24:845-868.

Vrana, B., G. A. Mills, E. Dominiak, und R. Greenwood. 2006. Calibration of the Chemcatcher passive sampler for the monitoring of priority organic pollutants in water. Environmental Pollution 142:333-343.

Vrana, B., G. A. Mills, M. Kotterman, P. Leonards, K. Booij, und R. Greenwood. 2007. Modelling and field application of the Chemcatcher passive sampler calibration data for the monitoring of hydrophobic organic pollutants in water. Environmental Pollution 145:895-904.

Vuori, K. M., und J. V. K. Kukkonen. 2002. Hydropsychid (Trichoptera, Hydropsychidae) gill abnormalities as morphological biomarkers of stream pollution. Freshwater Biology 47:1297-1306.

Wallace, J. B., S. L. Eggert, J. L. Meyer, und J. R. Webster. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277:102-104.

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

Wallace, J. B., J. R. Webster, und T. F. Cuffney. 1982. Stream Detritus Dynamics: Regulation by Invertebrate Consumers. Oecologia 53:197-200.

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

Wildhaber, M. L., und C. J. Schmitt. 1998. Indices of benthic community tolerance in contaminated Great Lakes sediments: Relations with sediment contaminant concentrations, sediment toxicity, and the sediment quality triad. Environmental Monitoring and Assessment 49:23-49.

Wipfli, M. S. 2005. Trophic linkages between headwater forests and downstream fish habitats: implications for forest and fish management. Landscape and Urban Planning 72:205.

Wright, J. F., M. T. Furse, und D. Moss. 1998. River classification using invertebrates: RIVPACS applications. Aquatic Conservation Marine and Freshwater 8:617-631.

Kapitel 2: Determination of 10 particle-associated multiclass polar and semi-polar pesticides from small streams using accelerated solvent extraction

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Abstract

A new analytical method using accelerated solvent extraction was developed for the determination of 10 particle-associated polar and semipolar pesticides. In addition, 6 deuterated analogues of the target compounds were evaluated as internal standards. The method yielded acceptable accuracy (73 – 103% recovery) and precision (< 25% relative standard deviation) for 8 compounds. Using size exclusion chromatography (SEC) as cleanup step resulted in higher recoveries compared to solid phase extraction (SPE) cleanup. Deuterated standards with 10 or more deuterium atoms performed well as internal standards concerning similar recovery and correlation with the target analytes. The method was employed to extract particle-associated pesticides from 16 streams located in an area with intense agriculture in France. Acetochlor, pirimicarb, tebuconazole, fenpropidin, α-endosulfan and chlorfenvinphos were detected at concentrations up to 1 mg kg^{-1} dry weight. A comparison with aquatic toxicity data indicated potential risk to the benthic fauna exposed to these concentrations of pirimicarb, α-endosulfan and chlorfenvinphos. We suggest that the method presented here be used for the extraction and quantitation of particle-associated polar pesticides.

Introduction

Modern agriculture uses large amounts of pesticides, which may be subject to transport processes that convey them into ground and surface waters (Carabias-Martinez et al., 2000). Field runoff represents one of the most important entry routes for small streams in agricultural areas (Liess et al., 1999; Lopes et al., 2007; Schriever et al., 2007). When runoff occurs, the pesticides are transported on eroded field particles or freely dissolved in the water phase. The distribution between the particle and water phases depends on the soil characteristics and the physico-chemical properties of the compound, such as the soil organic carbon partition coefficient (K_{OC}). Several field studies have shown that pesticides with log K_{OC}-values > 2 are partly or (log K_{OC} > 5) primarily transported on suspended particles (Long et al., 1998; Inoue et al., 2002; Liu et al., 2004). Since pesticides bound to suspended particles may adversely affect the benthic fauna, their determination is relevant for environmental risk assessment (Schulz and Liess, 2001; Jergentz et al., 2004; Sturm et al., 2007).

Accelerated solvent extraction (ASE; also named PLE for pressurized solvent extraction) is a relatively new extraction technique that has been applied successfully in the extraction of pesticide residues from various matrices (Hubert et al., 2001). Compared to traditional methods like ultrasonic or Soxhlet extraction, it has similar or sometimes even higher extraction efficiencies but consumes less solvent and labour time (Hubert et al., 2000). To our knowledge, no study exists on the extraction of pesticides from suspended particles with ASE, although has been shown to give good results in the extraction of herbicides (Kremer et al., 2004), fungicides (Frost et al., 1997) and insecticides (Dabrowska et al., 2003) from soils and sediments. However, these studies focused on few classes of pesticides with a narrow range of polarity, and investigations on the extraction of multiclass pesticides from suspended particles, soils or sediments with ASE are scarce. The major difficulty concerning the extraction from suspended particles is the large amount of organic coextractants, such as humic substances that may interfere with the target analytes and make a cleanup step mandatory (Bergamaschi et al., 1999). However, efficient cleanup methods to separate polar organic contaminants from coextractants are scarce (Dabrowska et al., 2003).

In the present study, we aimed at (1) developing an ASE-based extraction method with subsequent cleanup for the determination of polar and semi-polar pesticides on suspended particles and (2) applying this method to assess the exposure and associated risk of 16

small streams in a region with intense agriculture in France. In addition, we (3) evaluated the use of deuterated analogues of the target compounds as deuterated internal standard (DIS). The analytes were chosen on the basis of ecotoxicological relevance (Schäfer et al., 2007) and comprised 10 mainly polar and semi-polar pesticides belonging to different chemical classes (Table 1).

Methods and materials

Reagents

Acetonitrile (ACN), ethyl acetate (EA), methanol (MeOH), ethanol, dichloro methane (DCM), acetone and anhydrous sodium sulfate were obtained from Merck (Darmstadt, Germany) and were of GC-grade, except ethanol and anhydrous sodium sulfate which were of analytical grade. All compounds, deuterated analogues and the internal standard (IS) triphenyl phosphate (Table 1) were purchased from Dr. Ehrenstorf (Augsburg, Germany) and had a purity of at least 96.5% (except acetochlor 92%). Chromabond HR-P 6-mL SPE cartridges containing 500 mg of adsorbent polystyrene-divinylbenzene (PS-DVB) and Chromabond Easy 6 mL solid phase extraction (SPE) cartridges containing 500 mg bifunctional modified PS-DVB were purchased from Macherey-Nagel (Düren, Germany).

Study region, monitoring and sample treatment

Britanny, located in northwestern France, was chosen as sampling region as agriculture is the main land use type, accounting for 18437 (65.4%) of the 27510 km^2. Overall, 16 sampling sites were selected in small streams (max. width: 5 m, max. depth: 0.8 m) with adjacent agricultural production, and were monitored for suspended particles from April 19th to May 25th in 2005 (Schäfer et al., 2007). For this purpose, a suspended particle sampler was deployed in the streambed, similar to the one described previously (Liess et al., 1996). Briefly, the sampler (Supplementary material, Figure S1) consisted of a 3-L glass bottle (diameter: 12 cm, height: 30 cm) as sedimentation vessel, which was buried in the streambed and was covered by a stainless steel plate (25 × 25 cm). Stream water and the particles, suspended therein could enter the sedimentation vessel through an upstream-directed inlet tube (diameter: 4 cm) attached to the steel plate, with an opening

Table 1: Physicochemical and analytical data for 10 measured compounds, 8 deuterated analogues and the internal standard (IS)

Compound	Type[a]	Class[a]	log K_{ow}[b]	log K_{oc}[b]	RT (min)[c]	Quantifying ion (m/z)	Qualifying ions (m/z)	LOQ (pg µL^{-1})[d]
Carbofuran	I	carbamate	2.32	1.75	6.363	164	164, 149, 131, 122	125
Carbofuran D3[e]	-	-	-	-	6.363	164	164, 149, 131, 123	125
Linuron[f]	H	urea	3.20	2.7	7.722	161	161, 163, 99, 90	125
Linuron D6[e,f]	-	-	-	-	7.738	161	161, 163, 99, 90	125
Pirimicarb D6	-	-	-	-	15.682	244	244, 166, 78	125
Pirimicarb	I	carbamate	1.70	1.9	15.768	238	238, 166, 72	125
Acetochlor D11	-	-	-	-	16.416	173	173, 157, 233	125
Acetochlor	H	chloroacetamide	2.39	2.32	16.615	146	146, 162, 59	125
Alachlor D13	-	-	-	-	16.854	200	200, 173, 172	125
Alachlor	H	chloroacetamide	3.52	2.28	17.101	160	160, 188, 146	125
Fenpropidin	F	piperidine	2.90[a]	3.2[g]	17.696	273	273, 274, 272, 98	125
Chlorfenvinphos D10	-	-	-	-	21.459	271	271, 269, 333	125
Chlorfenvinphos	I	organic phosphorous acid	3.10	2.47	21.668	267	267, 323, 269	125
α-Endosulfan D4	-	-	-	-	22.590	172	172, 237, 235	125
α-Endosulfan	I	organochlorine	3.83	4.13	22.691	241	241, 195, 237	125
Oxadiazon	H	oxadiazole	4.80	3.51	24.492	175	175, 177, 258, 260	125
Tebuconazole D6	-	-	-	-	27.477	256	256, 125, 87	125[h]
Tebuconazole	F	triazole	3.7[a]	3.5[g]	27.537	125	250, 125, 70	125[h]
Triphenyl phosphate[i]	-	-	-	-	27.797	326	326, 325	125

[a]taken from Tomlin (2003), I = insecticide, H = herbicide, F = fungicide [b]taken from Sabljic et al. (1995) [c]Retention time
[d]Limit of quantification [e]excluded from study since not distinguishable from target analytes in mass spectrometry [f]as 3,4-dichloroaniline
[g]estimated with Chemprop 4.1 (http://www.ufz.de/index.php?en=6738) [h]250 and 1000 for some samples with high matrix interference

slit (height: 0.2 cm) located 5 cm above the cover. In contrast, the outlet tube (diameter: 4 cm, upper end cut at a 45° angle) was directed downstream. The efficacy of the sampling system to retain suspended particles decreased with increasing flow rate and with decreasing grain size (Liess et al., 1996).

The suspended particles in the samplers were collected twice at a two-weeks interval, after discarding the water phase in the samplers by gentle decantation. During the second sampling, a sample of the stream-bed sediment (0 – 2 cm depth) was taken with a metal spoon at 3 sites because of insufficient sample volume of suspended particles. In the laboratory, the samples were freeze-dried (temperature: -35°C; pressure: 22 Pa) using a beta2-16 system (Christ, Osterode im Harz, Germany), sieved by 2 mm and stored at -30 °C. Total organic and inorganic carbon content (TOC/TIC) of the samples was determined using a Leco RC-412 instrument (Mönchengladbach, Germany).

Spiking and sample extraction using accelerated solvent extraction

We evaluated the influence of some ASE parameters and solvent type on analytical recoveries. Samples of 8-10 g of suspended particles from an uncontaminated site were spiked (100 µg kg^{-1}) with all target compounds (hereinafter referred to as method development samples (MDS)) and their deuterated analogues (Table 1) and extracted using 22-mL extraction cells on an ASE 200-system (Dionex, Idstein, Germany). No deuterated analogue was used when (1) it was not commercially available (fenpropidin and oxadiazon) or (2) it interfered in mass-spectrometric detection with the target analyte due to similar retention time and qualifying ions (carbofuran and linuron; Table 1). Initially, we tested different solvent mixtures (acetone-DCM (7:3), ACN-MeOH (8:2), EA-ACN (2:1), EA-acetone (2:1)) at 80 °C and 120 °C. EA-ACN (2:1) and EA-acetone (2:1) had highest recoveries (except for fenpropidin where ACN-MeOH (8:2) had highest recoveries), but EA-acetone (2:1) was selected as solvent because of lower matrix coextraction (extracts were less coloured). Subsequently, temperature (80 °C – 140 °C), pressure (7.58 MPa – 12.41 MPa) and static cycle duration (3 – 7 min) were systematically varied to find the optimum for analytical recoveries (not shown). According to the results of these experiments, the extraction of the field samples spiked with deuterated standards (100 µg kg^{-1}) was carried out with two extraction cycles (static time: 6 min.) using EA-acetone (2:1) at 110 °C and 11 MPa.

Post-extraction procedures and sample cleanup

After extraction, the solvent volume (40 – 50 mL) was gently reduced to 1 mL or dryness (for SPE) under nitrogen at 30 °C using a TurboVap II (Caliper Life Sciences, Russelsheim, Germany). SPE and size exclusion chromatography (SEC) were evaluated as cleanup step using MDS extracts. SPE was carried out on a Baker spe-12G glass vacuum manifold (Mallinckrodt Baker, Griesheim, Germany), connected to a Laboport N820 AT.18 vacuum pump (KNF Neuberger, Freiburg, Germany). Chromabond HR-P columns were used for SPE cleanup because of better extraction performance (fewer compounds with recovery < 60%) compared to the Chromabond Easy columns (Supplementary material, Table S1). The column was preconditioned (6 mL MeOH, 6 mL EA), then the water-dissolved (300 mL) MDS extract was taken through (5 mL min^{-1}) and the column was dried for 30 min under vacuum. Subsequently, the column was eluted with 12 mL EA-ACN (1:1) under gravity flow and traces of water were removed with anhydrous sodium sulfate. The eluate was evaporated to 300 µL as described above and 10 µL IS were added prior to analysis.

SEC was performed with a system consisting of a high-performance liquid chromatograph (HPLC) equipped with two Kontron 422 pumps (Kontron Instruments, Neufahrn, Germany), an 535 UV detector (Bio-tek, Neufahrn, Germany), an SF-2120 fraction collector (Advantec MFS, Pleasanton, United States) and a Biobeads S-X3 cleanup column (a chromatographic glass column 70 × 2.7 cm i.d. packed with 55 g of the dry material; Antec GmbH, Sindelsdorf, Germany). DCM was used as eluting solvent (4 mL min^{-1}) and the fraction was collected between 27.5 and 43 min. This collection time window was selected to maximise cleanup efficiency while minimizing losses of the target compounds. Fenpropidin and tebuconazole D6 partly eluted before 27.5 min and were therefore not fully collected (56% and 26.9% reduction in analytical recovery (n = 3), respectively). The collected fraction was evaporated as described above to 1 mL and 50 µL of IS added. Aliquots of this extract were injected for GC/MS. SEC was selected for cleanup of the field samples because of higher analytical recoveries for most compounds and because of higher automation (see *Results and Discussion*).

Pesticide determination and quantification

The compounds were quantified by gas chromatography/mass spectrometry (GC/MS). This was done on an Agilent 6890N gas chromatograph (Agilent Technologies Germany, Boeblingen, Germany) equipped with a MPS2 autosampler, a KAS4 injector, both from

Gerstel (Muehlheim a.d. Ruhr, Germany) and an Agilent 5973 mass selective detector. A HP-5MS capillary column, 30m × 0.25mm × 0.25 µm, from Agilent was used for separation. The column was held at a temperature of 70°C for 2 minutes, heated by 25°C per minute up to 150°C, then by 3°C per minute up to 200°C. The end value of 280°C was achieved by increasing the temperature 8°C per minute and was held constant for 10 minutes. The MS operated in single-ion monitoring (SIM) with one quantifying and three or four qualifying ions (except triphenylphosphate with only two qualifiers) (Table 1). The limit of quantification (LOQ) was defined as the lowest concentration with a signal-to-noise ratio of at least 9. LOQs and retention times (RT) are given in Table 1. External calibration was used for quantitation of MDS. The IS was employed to detect deteriorations in analytical performance. The analytes extracted from field samples were quantified using the deuterated analogue as DIS that exhibited most similar behavior (see below). Due to the absence of a suitable DIS, fenpropidin was quantified in the field samples with external calibration and corrected for the highest observed recovery on MDS (0.3) as a conservative estimate of the real concentration.

Risk assessment of particle-associated pesticide concentrations

Except for α-endosulfan, insufficient sediment toxicity data were available to assess the risk of the measured pesticide concentrations to benthic organisms. Therefore a sediment toxic unit (STU) was computed for each pesticide, site and date (DiToro et al., 1991):

$$\mathrm{STU} = \frac{C_s}{\mathrm{TOC} \times K_{\mathrm{OC}} \times \mathrm{LC50}}$$

where C_s is the particle-associated pesticide concentration (µg kg^{-1}) (Table 3) and LC50 is the water-based 48-h acute median lethal concentration for *Daphnia magna* (µg L^{-1}) (Table 4). The values for K_{OC} were derived from the log K_{OC} as given in Table 1 and the values for the TOC are given in Table 3. However, the computed STUs should be regarded as a worst-case estimate since sediment concentrations are usually lower than suspended particle concentrations (Long et al., 1998).

Data analysis

Pearson's correlation coefficient *r* was computed to indicate the strength of a linear relationship between two compounds followed by a *t*-test to detect significant correlations. Similarity of the relationship among the deuterated standards between two

groups of samples was checked using the Mantel-test on Euclidean distance matrices (Legendre and Legendre, 1998). Simultaneous testing for a significant difference of several means between two groups of samples was done with Hotelling's T^2-test (Johnson and Wichern, 2003). All statistical computations and graphics were created with the open source software package R (www.r-project.org) using version 2.5.1 (for Mac OS X, 10.4.10).

Results and discussion

Recovery study with two different cleanup methods

For extraction with subsequent SEC cleanup, analytical recoveries ranged from 80 to 103% for most compounds (Figure 1). When SPE was used for cleanup, the analytical recoveries were in general lower (37 – 66%). Six compounds with SPE cleanup and two compounds with SEC cleanup exhibited high variation (> 25% relative standard deviation (R.S.D.), see Figure 1). The lower recoveries associated with SPE cleanup presumably resulted from the coextraction of natural organic matter, indicated by deeply coloured extracts, which can bind to the sorbents or analytes (Ridal et al., 1997; Kremer et al., 2004). Irrespective of the cleanup method employed after extraction, fenpropidin and linuron were the analytes with the lowest recoveries. We suggest that fenpropidin either degraded during extraction or that the extraction was not exhaustive because analytical recoveries were higher when the fenpropidin standard was spiked to the sample after extraction (28.9% for SEC cleanup and 60.3% for SPE cleanup). Moreover, losses of fenpropidin occurred during SEC since the analyte was partly eluted prior the collection time window. To our knowledge, there are no other studies on the extraction of fenpropidin. Better recoveries for fenpropidin should be obtained by selecting a different extraction solvent because higher recoveries (76.2%, n = 1) were observed during method development for ACN-MeOH (8:2).

The low recoveries of linuron may originate from both the extraction and the cleanup step. Concerning extraction, Crescenzi et al. (2000) observed up to 100% decomposition of linuron at temperatures higher than 90°C with phosphate-buffered water. During method development when most extraction temperatures were > 100°C, we did not find linuron recoveries > 50% for any of the various solvents and ASE parameters. Nevertheless, other authors reported linuron recoveries with ASE in different grains

from 43.5 to 101.3% at 80°C (Pang et al., 2006) and of 75% in a cucumber matrix at 100°C (Frenich et al., 2005). Concerning cleanup, a reduction in recoveries of linuron up to 53% have been observed in SPE in the presence of coextracted humic acids, possibly by binding of the analyte to the humic acids (Boti et al., 2007). Binding to organic macromolecules could also explain a decrease in linuron concentrations associated with SEC cleanup as this would lead to earlier elution and therefore discarding of the linuron fraction. However, further research would be needed to identify the mechanism responsible for the losses of linuron.

Overall, SEC represented an appropriate cleanup method after ASE. Nevertheless, SPE had higher cleanup power, and therefore analytical sensitivity in terms of LOQ was a factor of 3 to 4 higher. The cleanup strength of SEC could be increased by delaying the collection time window, but depending on the selected analytes, this could compromise the recovery.

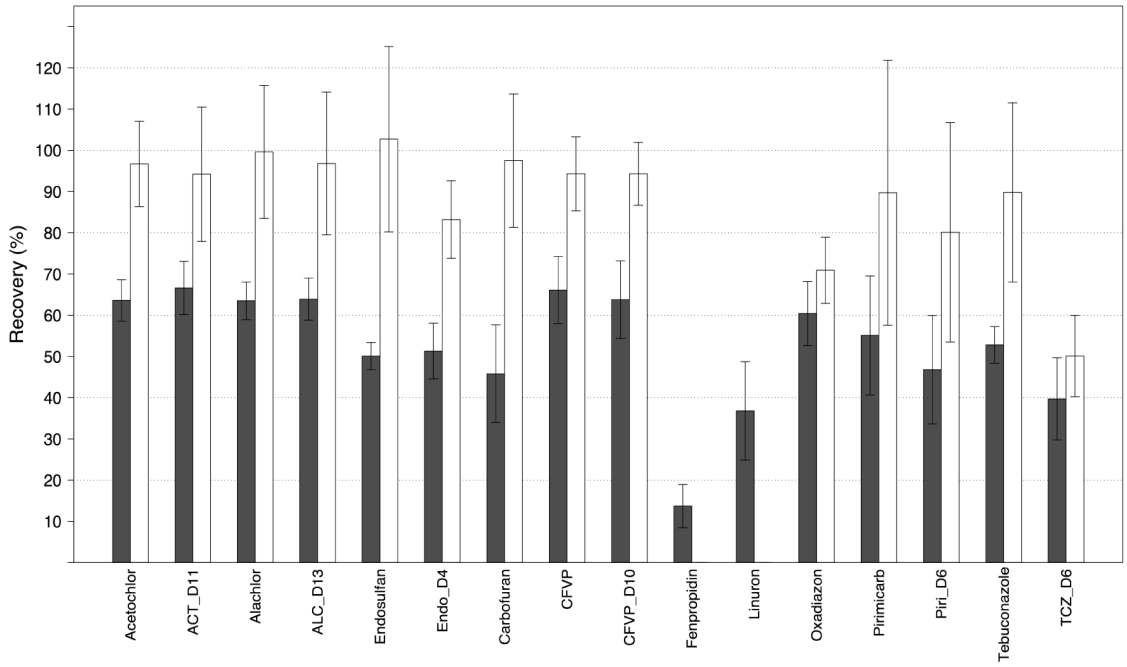

Figure 1: Analytical recoveries ± % of relative standard deviations for SPE (black bars) and SEC (white bars) cleanup after accelerated solvent extraction (n= 6 for both) of MDS. See Table 3 for full names of abbreviated compounds

Table 2: Correlations between the analytical recoveries of the target analytes and the deuterated analogues for SEC as cleanup step (n= 15)[a]

	Chlorfenvinphos D10	**α-Endosulfan D4[d]**	**Tebuconazole D6[b]**	**Alachlor D13**	**Pirimicarb D6**	**Acetochlor D11**
Chlorfenvinphos	**0.93****	0.88**	0.68*	0.59*	0.49	0.53*
α-Endosulfan	0.54*	**0.89****	0.1	0.69**	0.45	0.78**
Tebuconazole[b]	0.69**	0.29	**0.75****	-0.02	0.51	0.01
Alachlor	0.75**	0.76*	0.55	**0.97****	0.64*	0.93**
Pirimicarb	0.72**	0.51	0.58*	0.78**	0.76**	**0.79****
Acetochlor	0.76**	0.81**	0.43	**0.89****	0.55*	0.88**
Carbofuran[c]	**0.76***	0.34	0.4	0.65	0.69	0.74
Oxadiazon	0.69**	**0.93****	0.36	0.84**	0.48	0.87**

[a] except linuron and fenpropidin due to low recoveries in SEC. Results of the extractions from MDS were used as input data. Bold values indicate the deuterated compound that constituted the DIS for the respective analyte. * $p < 0.05$; ** $p < 0.01$. For further details refer to the Methods section.
[b] n= 13 [c] n= 7 [d] n= 10

Performance of deuterated analogues as deuterated internal standards

DIS are used to increase accuracy and precision in the determination of analytes, assuming similar behavior during sample treatment, chromatography and mass spectrometry (Grasso et al., 1998). Perfect similarity between a compound and the deuterated analogue would comprise (1) the same recovery in the analytical method and (2) strong correlation of the measurements (*r* close to 1). In our study, differences in recovery > 10% compared to the target analyte were observed for the deuterated analogues α-endosulfan D4 with SEC cleanup as well as for tebuconazole D6 and pirimicarb D6 for both cleanup steps for the MDS samples (Figure 1). Surprisingly, there is a paucity of studies on the relationship of analytes and their deuterated analogues during extraction (Stokvis et al., 2005). However, a study on the extraction of the drug haloperidol also reported a 28% lower recovery for the deuterated analogue (Hempenius et al., 1999). Two mechanisms have been proposed to explain these differences in behavior: (1) stronger binding of deuterium atoms with the carbon atoms compared to hydrogen atoms may alter the physicochemical properties of the deuterated analogue and (2) an exchange of deuterium atoms for hydrogen atoms may cause losses in the recovery of the deuterated analogue (Stokvis et al., 2005). The latter mechanism would lead to reduced recoveries of the deuterated analogue compared to the associated analyte. Indeed, for all pairs with differences lower recoveries of the deuterated analogue were observed (Table 2). However, for tebuconazole D6 with SEC cleanup the losses can presumably be attributed to elution before collection of the fraction. More studies would be needed to clarify the responsible mechanisms.

Concerning the correlation of the measurements, again tebuconazole D6 and pirimicarb D6 were the only compounds that correlated with $r < 0.85$ to the respective analyte in MDS for SEC cleanup (Table 2). Acetochlor and pirimicarb showed slightly higher correlation with alachlor D13 and acetochlor D11, respectively, compared to their deuterated analogues (Table 2). A high intercorrelation between acetochlor, alachlor and the deuterated analogues was expected due to the similarity in molecule structure and physicochemical properties (Table 1) and was also observed for alachlor with acetochlor D11 ($r = 0.93$). Strong correlations ($r > 0.8$) between compounds and deuterated standards were also reflected in close retention times in chromatography (except oxadiazon with both alachlor D13 and acetochlor D11, see Table 1).

For quantitation of the analytes in field samples we selected the deuterated standard with the highest correlation in MDS as DIS (Table 2). Hence, we assumed the correlations also to be valid for the field samples. This hypothesis was not directly testable; therefore, it was tested if the relationship among deuterated standards was similar for both groups of samples (MDS and field samples with SEC cleanup). Although the mean recoveries of the deuterated compounds were significantly lower for the field samples ($p < 0.01$, $n = 38$, Hotelling's T^2-test; compare Table 3 and Figure 1), the relationship among the deuterated compounds was similar (Mantel statistic $r = 0.89$, $p < 0.01$, Mantel-test). Thus, we suggest that the relationship between the target analytes and the deuterated compounds also remained stable.

The recoveries for the deuterated compounds as DIS in field samples varied widely (R.S.D.s of 20 to 53%) and ranged from values below LOQ to 107% (Table 3). Similar behavior was expected for the target analytes, and this highlights the benefit of using deuterated standards to indicate variation in extraction efficiency or matrix interference when applying an extraction method to samples with different physicochemical properties.

Overall, our results show that deuterated standards, with more than 10 hydrogen atoms replaced by deuterium, performed well in terms of similarity in accuracy, precision (variation) and correlation with the respective analyte. However, inaccuracies may arise when assuming similarity between analytes and the deuterated analogue without testing (for example 44% unexplained variance and about 10% difference in recoveries for pirimicarb-pirimicarb D6).

Pesticide concentrations in the field samples and associated risk for aquatic communities

The field samples were characterized by a high variation in organic carbon content ranging from 0.94 to 16.28%, while the inorganic carbon content was of minor importance with values < 0.32% (Table 3). A previous study showed that increasing TOC resulted in lower recoveries, presumably through higher extraction of interfering matrix compounds (Hrdlicka and Dolinova, 2001). However, we did not find a significant correlation between the recovery of deuterated compounds and the TOC or TIC ($p > 0.05$, $n = 24$ ($n = 15$ for α-endosulfan D4 and $n = 14$ for tebuconazole D6)). Presumably, the reduction of recoveries through matrix interference is more dependent on the particular kind of organic carbon than on the TOC (Zhou et al., 1995).

Table 3: Carbon content, analytical recovery of deuterated equivalents and pesticide concentrations of suspended particles collected in 16 small streams

Sample information			Carbon content		Recovery of deuterated analogues (%)[b]						Concentration (µg kg^{-1} dry weight)[c,d]					
Stream	Date[a]	Sample type	TOC	TIC	CFVP D10	Endo D4	TCZ D6	ALC D13	Piri D6	ACT D11	CFVP	Endo	FPP	TCZ	Piri	ACT
1	6.5.	Particles	4.78	0.02	86.5	30.38	bq	74.1	61.2	87.4	<17.2	<17.2	<17.2	<137.4[e]	<17.2	53.1
1	25.5.	Particles	4.96	0.05	73.4	73.41	17.4	73.8	75.8	76.7	24.2	137.5	<13.2	<13.2	<13.2	66.9
2	6.5.	Particles	5.11	0.02	81.6	bq	bq	67.3	39.9	80.7	<27.1	<27.1	<27.1	<217[e]	<27.1	<27.1
3	6.5.	Particles	8.31	0.01	70.4	22.57	42.9	73.2	97.5	75.7	<21.9	<21.9	195.3	61.1	<21.9	<21.9
3	25.5.	Particles	3.21	0.27	82.5	79.30	27.6	87.7	95.9	89.4	911.9	<22	<22	110.5	<22	<22
4	6.5.	Particles	3.32	0.27	33.6	bq	19.8	30.9	45.0	47.9	<19.8	<19.8	<19.8	<19.8	<19.8	<19.8
4	26.5.	Particles	5.03	0.04	73.8	21.80	16.1	61.8	64.6	68.9	<15.7	<15.7	241.6	<15.7	<15.7	<15.7
5	26.5.	Sediment	1.39	0.00	48.4	bq	26.5	55.3	22.1	58.9	15.8	<3.7	15.2	<29.4[e]	<3.7	21.0
6	6.5.	Particles	6.26	0.07	64.7	bq	bq	51.5	45.5	51.8	<19.7	<19.7	<19.7	<19.7	<19.7	<19.7
6	25.5.	Sediment	0.94	0.00	75.7	bq	bq	66.2	21.4	73.8	<9.7	<9.7	<9.7	<77.7[e]	<9.7	<9.7
7	6.5.	Particles	4.79	0.02	77.7	bq	bq	64.5	40.0	63.8	<28.6	<28.6	<28.6	<28.6	<28.6	<28.6
7	25.5.	Particles	6.71	0.04	99.2	30.00	bq	85.5	71.9	103.4	<276.4[f]	<276.4[f]	<276.4[f]	<2211.2[e,f]	<276.4[f]	<276.4[f]
8	6.5.	Particles	3.17	0.06	68.1	22.31	26.7	59.4	49.1	57.9	<23.6	<23.6	<23.6	<23.6	<23.6	<23.6
8	23.5.	Particles	3.52	0.00	62.8	74.97	6.9	66.8	39.4	70.2	22.2	<9	59.1	22.1	<9	<9
9	6.5.	Particles	5.30	0.05	64.9	61.39	17.4	62.5	43.3	63.9	<13.8	<13.8	<13.8	27.5	50.5	<13.8
9	24.5.	Particles	3.89	0.08	76.4	23.36	17.9	63.7	55.5	65.1	<17.2	<17.2	253.4	<17.2	<17.2	789.7
10	6.5.	Particles	4.15	0.07	104.1	90.52	34.5	86.3	106.8	92.5	953.4	<25.2	<25.2	335.2	<25.2	<25.2
10	25.5.	Particles	4.69	0.16	69.1	63.28	30.3	61.1	90.0	67.8	<33.3	<33.3	<33.3	49.1	<33.3	<33.3
11	19.5.	Particles	12.04	0.02	67.1	bq	bq	53.9	32.8	54.2	<29.9	<29.9	<29.9	<29.9	<29.9	<29.9
12	19.5.	Particles	14.51	0.06	85.0	bq	bq	70.8	65.1	78.8	<39.5	<39.5	<39.5	<315.8[e]	<39.5	<39.5
13	19.5.	Particles	8.26	0.32	84.8	91.11	28.1	85.0	79.1	83.4	72.4	<48	<48	65.0	<48	<48
14	19.5.	Particles	16.28	0.17	67.7	bq	bq	54.6	48.5	60.5	<33.8	<33.8	232.4	<270.2[e]	<33.8	<33.8
15	19.5.	Particles	5.95	0.06	60.7	21.49	bq	52.0	28.9	56.3	<19.3	<19.3	<19.3	<154.2[e]	<19.3	<19.3
16	19.5.	Sediment	1.40	0.00	63.6	63.11	23.8	69.3	13.9	73.0	17.9	14.3	27.3	10.2	<3.1	<3.1
Mean					72.6	51.3	24.0	65.7	55.5	70.9						
R.S.D. (%)					20.4	53.3	37.5	20.0	46.1	19.7						

Abbreviations: TOC = Total organic carbon (%), TIC = Total inorganic carbon (%), CFVP = chlorfenvinphos, Endo = α-endosulfan, TCZ = tebuconazole, ALC = alachlor, ACT = acetochlor, Piri = pirimicarb, FPP = fenpropidin [a]in 2005 [b]bq = below limit of quantification [c]only detected analytes shown [d]"<" indicates sample LOQ based upon the amount of extracted material [e]high limit of quantification due to matrix interferences [f] high limit of quantification due to low amount of sample material

Of the 10 pesticides, chlorfenvinphos, fenpropidin and tebuconazole were found in approximately 25% of the field samples, while acetochlor, α-endosulfan and pirimicarb were found occasionally. The concentrations peaked at 1 mg kg^{-1}, which is in accordance with previous measurements of pesticides on suspended particles in the Koise river, Japan (Inoue et al., 2002) or the Lourens river, South Africa (Schulz, 2001). Nevertheless, real concentrations were probably higher for fenpropidin, α-endosulfan and tebuconazole. In the case of fenpropidin, we assigned the maximum recovery (0.3) observed for MDS but real recovery was presumably lower (see above; compare Figure 1). α-Endosulfan and tebuconazole were detected 2 and 8 times in samples with concentrations of the respective DIS above LOQ, but never in samples with DIS concentrations below LOQ, suggesting the existence of false-negatives in the latter samples.

More pesticides were detected for the second sampling (12 compared to 7) at sites where sample material was available for both dates (n = 8). The measured α-endosulfan concentrations of 14.3 and 137.5 µg kg^{-1} were in the range of values that were reported to have toxic effects on benthic organisms. Jergentz et al. (2004) showed that the macroinvertebrate density and abundance were reduced in two streams after receiving particle-associated α-endosulfan input of 43 and 318 µg kg^{-1}, respectively. Furthermore, particle-associated concentrations between 50 and 200 µg kg^{-1} reduced the abundance and reproduction of a benthic polychaete and copepod (Chandler and Scott, 1991).

Table 4: Maximum sediment toxic units (STU) for the particle–associated analytes detected in 16 streams

Compound	**LC50 (µg L^{-1})[a]**	**Highest particle-associated concentration (µg kg^{-1})[b]**	**Maximum STU**
acetochlor	9000	789.7	0.011
chlorfenvinphos	0.3	911.9	320.9
fenpropidin	500	253.4	0.008
pirimicarb	17	50.5	0.706
tebuconazole	4200	335.2	0.001

[a] taken from Tomlin (2003) [b] dry weight

The concentrations of other compounds were evaluated on the base of STUs, as no sediment toxicity data were available. The STUs were in the range of 1/10,000 to 320 times the LC50 for *Daphnia magna* (Table 4). Assuming a dose-response relationship similar to that in the water column (DiToro et al., 1991), pesticide concentrations > 1/100 to 1/1000 the LC50 for *Daphnia magna* may have effects on the benthic community (Liess and von der Ohe, 2005; Schäfer et al., 2007). Consequently, the observed

concentrations of chlorfenvinphos and pirimicarb would have a high potential to have detrimental effects on benthic organisms (Table 4). However, this is a preliminary evaluation since no reliable sediment toxicity data are available, and the current assessment is based on several simplifications. Clearly, there is a paucity of studies on the effects of particle-associated pesticides.

Conclusions

The method of accelerated solvent extraction and size exclusion chromatography developed here is highly automated and is applicable in routine analysis of suspended particles. However, new methods are needed for the cleanup of polar compounds to improve sensitivity.

As internal standard, deuterated analogues with more than 10 deuterium atoms are recommended. Further investigations should address the moderate match of lower deuterated analogues with the target analytes.

Overall, particle-associated contaminants deserve much more attention in the exposure and toxicity assessment of aquatic ecosystems, as observed concentrations may represent an important stressor for aquatic ecosystems.

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References

Bergamaschi, B.A., Baston, D.S., Crepeau, K.L., Kuivila, K.M., 1999. Determination of pesticides associated with suspended sediments in the San Joaquin river, California, USA, using gas chromatography-ion trap mass spectrometry. Toxicol. Environ. Chem. 69, 305-319.

Boti, V.I., Sakkas, V.A., Albanis, T.A., 2007. Measurement uncertainty arising from trueness of the analysis of two endocrine disruptors and their metabolites in environmental samples - Part II. Solid-phase extraction from environmental natural waters. J. Chromatogr. A 1146, 148-156.

Carabias-Martinez, R., Rodriguez-Gonzalo, E., Sanchez San Roman, F.J., Fernandez-Laespada, M.E., 2000. Evaluation of surface- and ground-water pollution due to herbicides in agricultural areas of Zamora and Salamanca (Spain). J. Chromatogr. A 869, 471-480.

Chandler, G.T., Scott, G.I., 1991. Effects of sediment-bound endosulfan on survival, reproduction and larval settlement of meiobenthic polychaetes and copepodes. Environ. Toxicol. Chem. 10, 375-382.

Crescenzi, C., Di Corcia, A., Nazzari, M., Samperi, R., 2000. Hot phosphate-buffered water extraction coupled on-line with liquid chromatography/mass spectrometry for analyzing contaminants in soil. Anal. Chem. 72, 3050-3055.

Dabrowska, H., Dabrowski, L., Biziuk, M., Gaca, J., Namiesnik, J., 2003. Solid-phase extraction clean-up of soil and sediment extracts for the determination of various types of pollutants in a single run. J. Chromatogr. A 1003, 29-42.

DiToro, D.M., Zarba, C.S., Hansen, D.J., Berry, W.J., Swartz, R.C., Cowan, C.E., Pavlou, S.P., Allen, H.E., Thomas, N.E., Paquin, P.R., 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partinioning. Environ. Toxicol. Chem. 10, 1541-1583.

Frenich, A.G., Vidal, J.L.M., Salvador, I.M., Lopez-Lopez, T., 2005. Determination of multiclass pesticides in food commodities by pressurized liquid extraction using GC-MS/MS and LC-MS/MS. Anal. Bioanal. Chem. 383, 1106-1118.

Frost, S.P., Dean, J.R., Evans, K.P., Harradine, K., Cary, C., Comber, M.H.I., 1997. Extraction of hexaconazole from weathered soils: A comparison between Soxhlet extraction, microwave-assisted extraction, supercritical fluid extraction and accelerated solvent extraction. Analyst 122, 895-898.

Grasso, P., Benfenati, E., Terreni, M., Pregnolato, M., Natangelo, M., Pagani, G., 1998. Deuterated internal standards for gas chromatographic mass spectrometric analysis of polar organophosphorus pesticides in water samples. J. Chromatogr. A 822, 91-99.

Hempenius, J., Steenvoorden, R.J.J.M., Lagerwerf, F.M., Wieling, J., Jonkman, J.H.G., 1999. 'High throughput' solid-phase extraction technology and turbo ionspray LC-MS-MS applied to the determination of haloperidol in human plasma. J. Pharm. Biomed. Anal. 20, 889-898.

Hrdlicka, A., Dolinova, J., 2001. Automated hot solvent extraction and HPLC determination of atrazine and its degradation products in soil. J. Liq. Chromatogr. Rel. Technol. 24, 721-734.

Hubert, A., Wenzel, K.-D., Engelwald, W., Schüürmann, G., 2001. Accelerated solvent extraction - More efficient extraction of POPs and PAHs from real contaminated plant and soil samples. Rev. Anal. Chem 20, 101-144.

Hubert, A., Wenzel, K.D., Manz, M., Weissflog, L., Schüürmann, G., Engewald, W., 2000. High extraction efficiency for POPs in real contaminated soil samples using accelerated solvent extraction. Anal. Chem. 72, 1294-1300.

Inoue, T., Ebise, S., Numabe, A., Nagafuchi, O., Matsui, Y., 2002. Runoff characteristics of particulate pesticides in a river from paddy fields. Water Sci. Technol. 45, 121-126.

Jergentz, S., Mugni, H., Bonetto, C., Schulz, R., 2004. Runoff-related endosulfan contamination and aquatic macroinvertebrate response in rural basins near Buenos Aires, Argentina. Arch. Environ. Contam. Toxicol. 46, 345-352.

Johnson, R.A., Wichern, D.W., 2003. Applied Multivariate Statistical Analysis. Prentice Hall.

Kremer, E., Rompa, M., Zygmunt, B., 2004. Extraction of acidic herbicides from soil by means of accelerated solvent extraction. Chromatographia 60, S169-S174.

Legendre, P., Legendre, L., 1998. Numerical Ecology. Elsevier, Amsterdam.

Liess, M., Schulz, R., Liess, M.H.-D., Rother, B., Kreuzig, R., 1999. Determination of insecticide contamination in agricultural headwater streams. Water Res. 33, 239-247.

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

Liess, M., von der Ohe, P.C., 2005. Analyzing effects of pesticides on invertebrate communities in streams. Environ. Toxicol. Chem. 24, 954-965.

Liu, W., Gan, J.J., Lee, S., Kabashima, J.N., 2004. Phase distribution of synthetic pyrethroids in runoff and stream water. Environ. Toxicol. Chem. 23, 7-11.

Long, J.L.A., House, W.A., Parker, A., Rae, J.E., 1998. Micro-organic compounds associated with sediments in the Humber rivers. Sci. Total Environ. 210, 229-253.

Lopes, I., Moreira-Santos, M., da Silva, E.M., Sousa, J.P., Guilhermino, L., Soares, A., Ribeiro, R., 2007. In situ assays with tropical cladocerans to evaluate edge-of-field pesticide runoff toxicity. Chemosphere 67, 2250-2256.

Pang, G.F., Liu, Y.M., Fan, C.L., Zhang, J.J., Cao, Y.Z., Li, X.M., Li, Z.Y., Wu, Y.P., Guo, T.T., 2006. Simultaneous determination of 405 pesticide residues in grain by accelerated solvent extraction then gas chromatography-mass spectrometry or liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 384, 1366-1408.

Ridal, J.J., Fox, M.E., Sullivan, C.A., Maguire, R.J., Mazumder, A., Lean, D.R.S., 1997. Evaluation of automated extraction of organochlorine contaminants from freshwater. Anal. Chem. 69, 711-717.

Sabljic, A., Gusten, H., Verhaar, H., Hermens, J., 1995. Qsar Modeling of Soil Sorption - Improvements and Systematics of Log K-Oc Vs Log K-Ow Correlations. Chemosphere 31, 4489-4514.

Schäfer, R.B., Caquet, T., Siimes, K., Mueller, R., Lagadic, L., Liess, M., 2007. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. Sci. Total Environ. 382, 272-285.

Schriever, C.A., von der Ohe, P.C., Liess, M., 2007. Estimating pesticide runoff in small streams. Chemosphere 68, 2161-2171.

Schulz, R., 2001. Comparison of spraydrift- and runoff-related input of azinphos-methyl and endosulfan from fruit orchards into the Lourens River, South Africa. Chemosphere 45, 543-551.

Schulz, R., Liess, M., 2001. Acute and chronic effects of particle-associated fenvalerate on stream macroinvertebrates: a runoff simulation study using outdoor microcosms. Arch. Environ. Contam. Toxicol. 40, 481-488.

Stokvis, E., Rosing, H., Beijnen, J.H., 2005. Stable isotopically labeled internal standards in quantitative bioanalysis using liquid chromatography/mass spectrometry: necessity or not? Rapid Commun. Mass Spectrom. 19, 401-407.

Sturm, A., Radau, T.S., Hahn, T., Schulz, R., 2007. Inhibition of rainbow trout acetylcholinesterase by aqueous and suspended particle-associated organophosphorous insecticides. Chemosphere 68, 605-612.

Tomlin, C.D.S., 2003. The pesticide manual, a world compendium BCPC Publications, Hampshire, UK.

Zhou, J.L., Rowland, S., Mantoura, R.F.C., 1995. Partition of synthetic pyrethroid insecticides between dissolved and particulate phases. Water Res. 29, 1023-1031.

Supplementary material

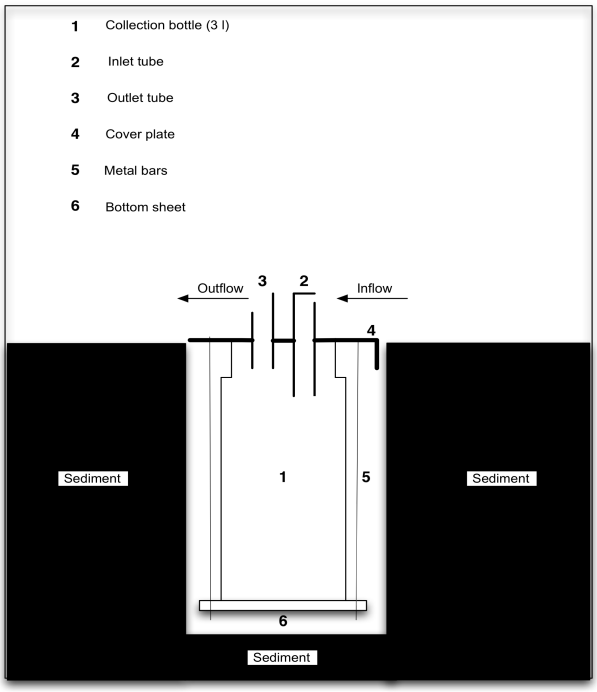

Figure S1: Suspended particles sampler

Table S1: Analytical recovery of compounds in SPE of 300 mL spiked (1 µg L^{-1}) tap water with two different SPE-columns.

	Chromabond Easy (n= 3)		Chromabond HR-P (n=4)	
	Recovery (%)	R.S.D. (%)	Recovery	R.S.D. (%)
Acetochlor	79.9	7.8	92.0	4.2
Acetochlor D11	94.8	4.8	nm	nm
Alachlor	93.9	3.1	92.3	3.9
Alachlor D13	93.7	3.6	90.0	3.8
α-Endosulfan	77.5	6.1	81.5	2.1
α-Endosulfan D4	77.9	3.1	80.5	3.4
Carbofuran	99.1	11.4	47.1	8.2
Chlorfenvinphos	96.0	7.9	93.3	10.5
Chlorfenvinphos D10	96.9	13.3	91.1	9.0
Fenpropidin	bd	bd	108.3	5.1
Linuron[a]	125.5	9.4	125.3	31.9
Oxadiazon	86.1	2.2	85.4	2.2
Pirimicarb	96.8	5.1	90.8	2.0
Pirimicarb D6	99.0	9.8	88.8	3.8
Tebuconazole	37.7	30.0	61.5	4.9
Tebuconazole D6	24.7	27.7	62.9	5.2

nm = not measured; bd = below limit of quantification, [a]as 3,4-dichloroaniline.

Kapitel 3: Calibration of the Chemcatcher® passive sampler for monitoring selected polar and semi-polar pesticides in surface water

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Abstract

Passive sampling is a powerful method for continuous pollution monitoring, but calibration experiments are still needed to generate sampling rates in order to estimate water concentrations for polar compounds. We calibrated the Chemcatcher® device with an uncovered SDB-XC Empore disk as receiving phase for 12 polar and semi-polar pesticides in aquatic environments in flow-through tank experiments at two water flow velocities (0.135 m/s and 0.4 m/s). In the 14-day period of exposure the uptake of test substances in the sampler remained linear, and all derived sampling rates R_s were in the range of 0.1 to 0.5 L/day. By additionally monitoring the release of two preloaded polar pesticides from the SDB-XC disks over time, very high variation in release kinetics was found, which calls into question the applicability of performance reference compounds. Our study expands the applicability of the Chemcatcher® for monitoring trace concentrations of pesticides with frequent occurrence in water.

Introduction

The widespread use of agrochemicals is connected with a frequent input of these substances into surface waters. Continuous monitoring of the water quality is therefore necessary. Conventional methods for the monitoring of surface waters rely on discontinuous grab sampling at fixed time intervals and on expensive analytical efforts. However, these methods may miss spontaneous and periodic fluctuations in the concentration of a pollutant (Guo et al., 2004, Kreuger, 1998, Liess et al., 1999). Furthermore, the concentration of trace contaminants may lie below the detection limit if only small sample volumes are available.

Passive samplers represent a promising alternative as they continuously accumulate pollutants, allowing the assessment of time-weighted-average (TWA) concentrations. The widespread facilities for the application of passive samplers to monitor different pollutants was reviewed previously (Gorecki and Namiesnik, 2002, Leon-Gonzalez and Perez-Arribas, 2000, Vrana et al., 2005). However, most passive sampling devices for organic compounds in water focus on hydrophobic substances (Vrana et al., 2006a). By contrast, the Chemcatcher® passive sampler allows both hydrophobic and hydrophilic compounds to be sampled when taking into account the appropriate receiving phases, in terms of Empore disks for solid phase extraction (SPE) (Kingston et al., 2000). Furthermore, a diffusion-limiting membrane can be used optionally to extend the time until the receiving phase reaches equilibrium with the surrounding medium. To derive time-weighted average (TWA) concentrations from the accumulated contaminant mass during field exposure, compound-specific sampling rates are required (Kingston et al., 2000). For selected polar compounds, calibration experiments have to be conducted to determine sampling rates. Tran et al. (2007) calibrated the Chemcatcher® with Empore SDB-XC and SDB-RPS disks as receiving phases and polyethersulfone membranes for 8 polar herbicides. Stephens et al. (2005) calibrated the C_{18} Empore disk and the SDB-RPS Empore disk without membrane for 4 polar herbicides. Nevertheless, calibration data are scarce for different classes of herbicides and absent for polar insecticides and fungicides.

In the present study, we calibrated the Chemcatcher® with the SDB-XC Empore disk as receiving phase for 10 selected polar and semi-polar pesticides, using a laboratory flow-through system. In addition, atrazine and hexazinone were included in the experiment for the purpose of comparison with previous calibration studies (Stephens et al., 2005, Tran et al., 2007). Furthermore, we evaluated the applicability of polar performance reference

compounds (PRC), usually employed to account for between-site variation of environmental variables (Booij et al., 2002, Vrana et al., 2006a). The samplers were exposed over a period of 14 days to two different current velocities, both typical of small lowland agricultural streams (Schäfer et al., 2007).

Theory

The only driving force for the accumulation of compounds in the receiving phase of passive samplers in aquatic environments is the difference in chemical potential (Vrana et al., 2006a). Various transport steps govern the uptake of an organic compound from water to the receiving phase of a passive sampler. The first step is the diffusion of the analyte from the water phase surrounding the sampler to a stagnant aqueous boundary layer located above the receiving phase (Vrana et al., 2007). Subsequently, there are several transport steps between the different compartments of the sampling system, from the boundary layer through a possible biofilm layer via a diffusion-limiting membrane to the receiving phase. For a given compound, the uptake rate (the increase of accumulated analyte mass per time period) depends on the material of the receiving phase, the material of a possible membrane and the volume of the receiving phase. Furthermore, the uptake rate varies with the physicochemical properties of the target analytes (Stephens et al., 2005), water temperature (Vrana et al., 2006a) and the current velocity of the aquatic environment (Vrana and Schüürmann, 2002), as these factors influence the transport of chemicals to the stagnant aqueous boundary layer. Moreover, biofouling may affect the uptake rate (Booij et al., 2006). PRCs can be used to account for variability in uptake kinetics due to physicochemical parameters and biofouling, if uptake and release kinetics are isotropic (Huckins et al., 2002).

After deployment of the sampling device, a linear uptake of chemicals over time continues approximately until half-saturation of the receiving phase is reached (Vrana et al., 2005). As exposure time increases further, the uptake decreases and approaches equilibrium partitioning with the medium, i.e. the state in which uptake and offload rates are equal. In this situation the sampler only responds to changes of the surrounding analyte concentration with a delay determined by the transfer kinetics between sampler and medium.

Under linear uptake conditions the relationship between the accumulated mass of an analyte on the sampler and its concentration in the surrounding water is given by the following equation (Kingston et al., 2000):

$$m_s = C_W * R_s * t \quad (1)$$

where m_s is the accumulated mass after exposure time t, C_w the time-weighted average (TWA) concentration of the analyte in the water phase in the dimension mass/volume, and R_s the sampling rate. If fabrication blank (and trip blank) investigations yield initial non-zero background concentration of target substance in the sampler, this has to be considered in Eq. (1) and the equations following below. The sampling rate R_s is the product of overall mass transfer coefficient towards the sampler and its surface area and represents the substance-specific volume of water extracted per unit of time by the sampling device.

During calibration experiments the samplers are exposed for a defined time to a constant analyte concentration in the water. Hence, the sampling rate can be derived by re-arranging Equation (1) to:

$$R_s = \frac{m_s}{C_W * t} \quad (2)$$

With the known sampling rate R_s for an analyte, its time-weighted average (TWA) concentration in the water phase during exposure time can be calculated by:

$$C_W = \frac{m_s}{R_s * t} \quad (3)$$

The release of accumulated substances from the receiving phase can be described with the following equation:

$$m_s(t) = m_s(0) * e^{(-k_e * t)} \quad (4)$$

where $m_s(t)$ is the mass of accumulated compounds in the receiving phase after the exposure time t, $m_s(0)$ is the amount of analytes added to the receiving phase before exposure, and k_e is the overall exchange rate constant.

Experimental

Materials and chemicals

Empore SDB-XC disks were purchased from 3M (St. Paul, USA). Chromabond HR-P cartridges were obtained from Macherey-Nagel (Düren, Germany). All pesticide standards were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and had a purity of at least 97 %, except chlorfenvinphos (95 % purity) and acetochlor (92 % purity). Solvents (HPLC-grade – acetone, methanol, ethyl acetate, acetonitrile / analytical grade – ethanol, 2-propanol) and sodium sulfate, sodium chloride (both for analysis) were obtained from Merck (Darmstadt, Germany). Water was pumped with a Sigma/1 diaphragm-metering pump obtained from ProMinent (Heidelberg, Germany). The carousel device was driven by an adjustable electric stirring machine, type RZR 2051 (Heidolph, Kelheim, Germany). Reagents were added using a Wellchrom K-120 HPLC-pump obtained from Knauer (Berlin, Germany). Solvent evaporation was done with a TurboVap 2 (Zymark, Hopkington, USA).

Selection of compounds and physicochemical properties

Compounds selected for the study represented those identified as ecotoxicologically relevant in a previous field study (Schäfer et al., 2007). These comprised 10 polar and semi-polar insecticides, fungicides and herbicides from different chemical groups such as carbamates, organic phosphorous acids, chloroacetamides, ureas, piperidines, triazoles, oxadiazoles and organochlorines. Hexazinone and atrazine were included in the study for the purpose of comparison with previous calibration studies. Pirimicarb-D6 and chlorfenvinphos-D10 were selected as PRCs for investigation of the offload behaviour. Water solubilities (Tomlin, 2003) of the compounds exhibited high variability (Table 1) referring to the different areas of application.

Sampler design and treatment of the receiving phase

The Chemcatcher passive sampler was used as described by Kingston et al. (2000). We employed an SDB-XC Empore disk as receiving phase (47 mm diameter; 15.9 cm² surface area). To allow for the detection of short-term fluctuations in the analyte concentration during potential field deployment, no diffusion-limiting membrane was used. The open side of the Chemcatcher was sealed with a copper mesh (mesh size 5 mm), to prevent mechanical damage and suppress biofouling (Vrana et al., 2005).

Before use the SDB-XC Empore disk was conditioned with 10 mL acetone, 10 mL 2-propanol and 10 mL methanol. After rinsing with 20 mL water (HPLC-grade), 100 mL tap water with a spiking solution (pirimicarb-D6 and chlorfenvinphos-D10 – 1µg/L) was taken through the disk. Finally, the conditioned and spiked disks were placed in the Chemcatcher body, which was subsequently filled with tap water, closed and stored at 4°C until exposure (< 72 hours).

After exposure the SDB-XC Empore disks were taken off the PTFE-body, dried under vacuum for about 15 minutes and subsequently eluted twice with 10 mL acetonitrile/methanol (1:1). The eluate was gently evaporated to dryness under nitrogen in a 200 mL evaporation vial and resolved with 300 µL acetonitrile. Prior to analysis 5 µL triphenyl phosphate were added as internal standard.

Table 1: Physicochemical properties of the selected pesticides

Pesticide	log K_{ow}[a]	Type	Mw[b,c] (g/mol)	Water solubility[b](mg/L)
hexazinone	1.20[b]	herbicide	252.3	33000
pirimicarb	1.70	insecticide	238.3	3000
carbofuran	2.32	insecticide	221.3	320
acetochlor	2.39	herbicide	269.8	223
atrazine	2.61	herbicide	215.7	33
fenpropidin	2.90[b]	fungicide	273.5	530
chlorfenvinphos	3.10	insecticide	359.6	121
linuron	3.20	herbicide	249.1	63.8
alachlor	3.52	herbicide	269.8	170
tebuconazole	3.70[b]	fungicide	307.8	36
α-endosulfan	3.83[b]	insecticide	406.9	0.32
oxadiazon	4.80	herbicide	345.2	1

[a] data from Sabljic et al. (1995) [b] data from Tomlin (2003) [c] molecular weight

Experimental setup

The experiment was conducted in a flow-through system consisting of a 20-L vessel with a carousel where 14 passive samplers were placed on two levels (see Figure 1), as described previously by (Vrana et al., 2006a). Both the samplers and the carousel were made of polytetraflouroethylene (PTFE). The carousel was driven by an electric stirring motor with adjustable rotation speed and simulated water flow velocity by moving the installed passive samplers through the water. Additionally, the rotation of the carousel facilitated the dispersion of the stock solution in the water. Tap water was used due to a conductivity (515 µS/cm) similar to streams (100 – 500 µS/cm) and kept at 14.25°C by

air conditioning (the whole equipment was placed inside a climatic test cabinet). The rotation speed of the system was set to 0.135 m/s and 0.4 m/s; both temperature and flow velocities corresponded to average conditions of streams in a field study (Schäfer et al., 2007). The pesticide concentration in the vessel was kept constant with a continuous in- and outflow of water (5 L/h) and stock solution of methanol-dissolved pesticides (100 µg/L each pesticide) using an HPLC-pump (5 mL/h). The pesticide water concentration was monitored with 500-mL water samples taken every 72 h.

During the calibration experiment, two samplers were removed every two days (one of each storey of the carousel device) and replaced by empty Chemcatcher bodies to keep the hydrodynamic conditions constant. Prior to the initiation of a new experiment, the vessel, the carousel and the samplers were washed with ethanol to remove biofilms.

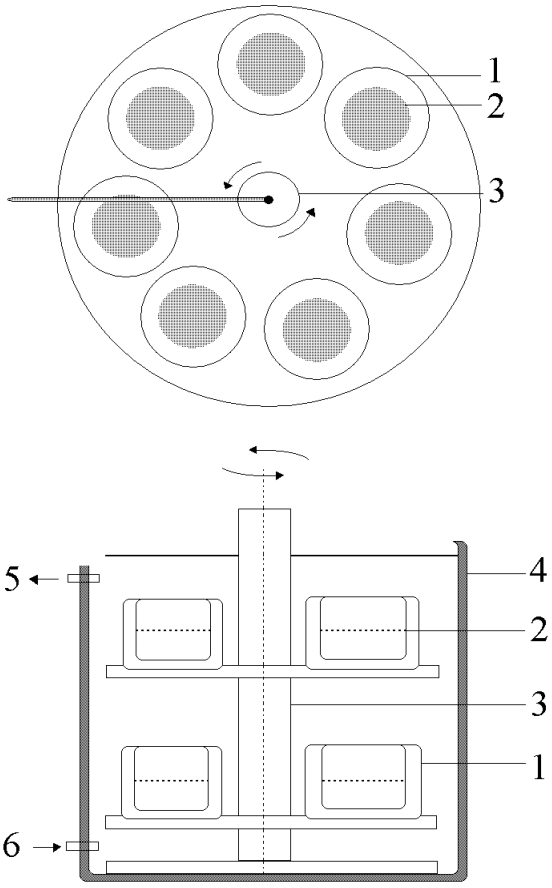

Figure 1: Exposure tank and carousel device of the flow-through system for the calibration of the Chemcatcher. 1 = sampler; 2 = receiving phase; 3 = carousel device; 4 = glass vessel; 5 = out-flow; 6 = in-flow

Treatment of water samples

The conductivity of water samples was increased up to 50 mS/cm with sodium chloride to

prevent dissociation of the compounds. Subsequently, the sample was taken through a preconditioned (10 mL methanol/ethyl acetate 1:1) Chromabond HR-P cartridge (5 mL/min) and dried under vacuum for 30 minutes. The column was eluted with 12 mL acetonitrile/ethyl acetate (1:1) under gravity flow and traces of water were removed with sodium sulfate. The eluate was handled as described above.

Evaluation of analyte recovery and degradation

Analytical recovery of the compounds (Table 1) in solid-phase extraction was investigated using 500 mL HPLC-grade water spiked with 100 ng of each pesticide, which was handled like water samples as described above.

For evaluation of potential degradation of the substances on the receiving phase SDB-XC disks were spiked with a stock solution of 1µg of all pesticides except hexazinone and atrazine as described above and stored at 4°C for 21 days. Analyte degradation was checked after 14 and 21 days and derived from comparison of the respective recoveries with recoveries in direct extraction.

Instrumental analysis

All samples were measured on an Agilent 6890N (Agilent Technologies Germany, Boeblingen, Germany) gas chromatograph with an HP-5MS column (30 m x 0.25 mm x 0.25 mm) linked to a Pegasus III time-of-flight (TOF) mass spectrometer (Leco, Mönchengladbach, Germany). The samples were transferred with an MPS2-ALEX-autosampler obtained from Gerstel (Mühlheim, Germany) and injected with a CIS-4-injection system (Gerstel) at a temperature of 280°C in splitless mode. The column was kept at a temperature of 95°C for 1.5 minutes, heated by 20°C per minute up to 190°C, then 5°C per minute up to 230°C. The end value of 300°C was achieved by increasing the temperature 25°C per minute and was held constant for 20 minutes.

Statistical data evaluation

Linear regression models with zero intercept were employed for all pesticides, with the accumulated mass of the respective analyte as response variable and exposure time as explanatory variable. R_s values were derived from the slope of the regression lines (see Equation 2), where C_w was corrected for the respective analyte recovery (Vrana et al., 2006a). The uncertainties in the sampling rates R_S were expressed in terms of coefficient

of variation (CV) and computed according to the law of error propagation using the CVs of the uptake slope parameters and the analyte concentrations $C_{w.}$ The slope of deuterated PRCs was evaluated with nonlinear regression analysis using Equation 4. The exchange rate constant k_e of the uptake curves was estimated using nonlinear regression fit with:

$$m_s = a(1 - e^{(-k_e * t)}) \quad (5)$$

where a is a constant, and all other variables are defined as described above. A paired sample t-test was performed to check for differences between the complete sets of R_s-values for the two current velocities. All statistical analyses and graphs were created with Origin for Windows (version 7.5, Microcal Software).

Results

Degradation of pesticides on the receiving phase

All pesticides except carbofuran and linuron showed no degradation on the receiving phase during storage. Carbofuran recoveries decreased from 78% (CV = 3.3%, n = 4) to 64% (CV = 9.4%, n = 4) after 14 days and to 47% (CV = 11.6%, n = 4) after 21 days. Recovery of linuron was 81% (CV = 12.4%, n = 4) when eluted directly, decreasing to 49% (CV = 15.7%, n = 4) after 14 days and to 27% (CV = 9.1%, n = 4) after 21 days.

Table 2: Recovery in solid phase extraction (SPE) and mean concentration in water

Pesticide	SPE Recovery (n = 3)		v = 0.135 m/s[b] C_w[c] (n = 9)		v = 0.4 m/s[b] C_w[c] (n = 8)	
	%	CV[a]	ng/L	CV[a]	ng/L	CV[a]
acetochlor	84	15.4	84.6	7.6	91.2	18.7
alachlor	81.2	14.2	55.4	15.8	63.9	17.8
atrazine	84.9	14.6	108.3	8.2	127.6	24
carbofuran	83.8	25.4	147.7	7.1	167.8	22
chlorfenvinphos	116.2	9.5	91.6	12.4	121.2	18.5
α-endosulfan	76.4	8.7	80.9	14.2	101.6	11.6
fenpropidin	79.2	14.3	80.3	16.5	93.5	19.3
hexazinone	120.9	7.6	73.3	20.3	114	24.5
linuron	80.6	10.3	79.3	18.8	135.9	19.3
oxadiazon	93.9	9.1	75.7	20.6	100.4	18.6
pirimicarb	88.7	12.7	88.6	18.7	101.8	21.9
tebuconazole	70	4.2	115.4	23.6	138.6	18.9

[a] coefficient of variation [b] flow velocity [c] mean analyte concentration in water

Recoveries and analyte concentrations

Analyte recoveries from spiked water samples ranged for all compounds from 68 to 121% with CVs between 4 and 15% except carbofuran (CV = 25%) (Table 2).

The aqueous analyte concentrations ranged from 55 to 148 ng/L during the calibration with 0.135 m/s and from 64 to 168 ng/L at 0.4 m/s current velocity. CVs ranged from 7 to 24% and from 12 to 25%, respectively (Table 2). At 0.135 m/s hexazinone, oxadiazon and tebuconazole varied most in analyte concentration with CVs above 20%. At the faster current velocity hexazinone, pirimicarb, carbofuran and atrazine exhibited higher variation than the other compounds (CV > 20%).

Uptake kinetics

The linear regression models were significant ($p < 0.05$) for all pesticides in each experiment. Typical results are shown in Figure 2.

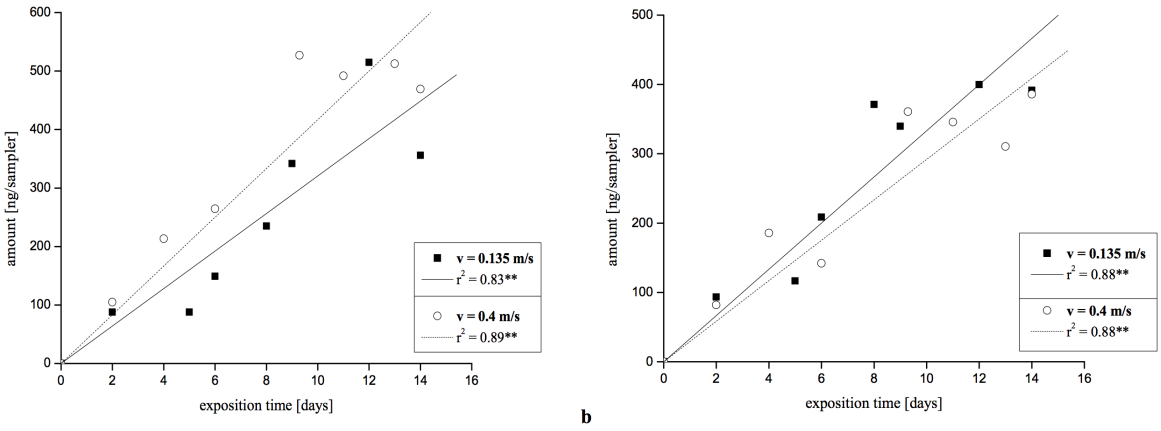

Figure 2: Linear regression lines for the uptake of chlorfenvinphos (2a) and pirimicarb (2b) on the SDB-XC receiving phase. v = flow velocity; ** = $p < 0.01$.

The uptake kinetics of all investigated pesticides remained approximately linear during the exposure time. The sampling rates ranged from 0.1 to 0.5 L/day with slightly higher values under lower flow conditions (Table 3). A paired-sample t-test showed a significant difference between the sampling rates of the two current velocities ($t = -2.2152$, $p = 0.049$; $n = 12$). The CVs at 0.135 m/s were between 10 and 30 % and ranged from 10 to 23% at 0.4 m/s. There was a 3.5-fold increase from the lowest (linuron) to the highest (oxadiazon) sampling rate under slow flow conditions and a 2.5-fold increase at 0.4 m/s for the same compounds. The sampling rate of pirimicarb increased most from fast to slow current velocity, with a factor of 1.3, while chlorfenvinphos changed least with the

factor 1.02 (Table 3). Tebuconazole decreased most in sampling rate with a reduction of current velocity (factor 0.8).

Table 3: Sampling rates in the flow-through experiment

Pesticide	v = 0.135 m/s[b]		v = 0.4 m/s[b]	
	R_s[c] (L/day)	CV[a] (%)	R_s[c] (L/day)	CV[a] (%)
acetochlor	0.35	10.3	0.34	15.5
alachlor	0.32	16.5	0.31	11.7
atrazine	0.28	10	0.22	10
carbofuran	0.13	14.8	0.14	23.3
chlorfenvinphos	0.35	15.7	0.34	14.1
α-endosulfan	0.42	16.6	0.38	10.8
fenpropidin	0.3	19	0.27	17.8
hexazinone	0.26	24.2	0.21	15.9
linuron	0.12	21.5	0.13	17.8
oxadiazon	0.44	22.6	0.34	12.2
pirimicarb	0.38	20	0.29	11.4
tebuconazole	0.19	30.1	0.24	12.4

[a] coefficient of variation [b] flow velocity [c] sampling rate

Dependence of sampling rates on physicochemical properties

Although not significant, the sampling rates increased with the logarithmic octanol/water partition coefficients (log K_{ow}) of the compounds (Figure 3) ($p = 0.39$, $r^2 = 0{,}08$ for 0.135 m/s and $p = 0.2$, $r^2 = 0{,}16$ for 0.4m/s; $n = 12$). Removal of carbofuran and linuron slightly improved the relationship ($p = 0.34$, $r^2 = 0{,}11$ for 0.135 m/s and $p = 0.11$, $r^2 = 0{,}28$ for 0.4 m/s; $n = 10$). Sampling rates decreased with higher solubility (not shown) but the relationship was also not significant ($p = 0.74$ for 0.135 m/s; $p = 0.5$ for 0.4 m/s; $n = 12$).

Offload kinetics

For pirimicarb-D6 a nonlinear regression fit with Equation (4) could be obtained, but certain observations deviated strongly from the regression curve ($r^2 = 0.17$ for 0.135 m/s; $r^2 = 0.53$ for 0.4 m/s; $n = 8$). For chlorfenvinphos-D10 no acceptable regression fit was obtained for the low flow velocity ($r^2 = 0.03$ for 0.135 m/s; $r^2 = 0.43$ for 0.4 m/s; $n = 8$). The exchange rate constants k_e derived from the offload curves of pirimicarb-D6 and chlorfenvinphos-D10 were 0.02 d^{-1} (CV= 88%) and not evaluable for 0.135 m/s and 0.055 d^{-1} (CV= 38%) and 0.02 d^{-1}(CV= 48%) for 0.4 m/s, respectively. The estimation of

k_e from the uptake curves resulted in up to threefold higher values for pirimicarb-D6 (0.065 d^{-1} (CV= 80%) for 0.135 m/s and 0.09 d^{-1} (CV= 66%) for 0.4 m/s) and not reliable values for chlorfenvinphos-D10 (0.03 d^{-1} (CV= 287%) for 0.135 m/s and 0.01 d^{-1} (CV= 48%) for 0.4 m/s.)

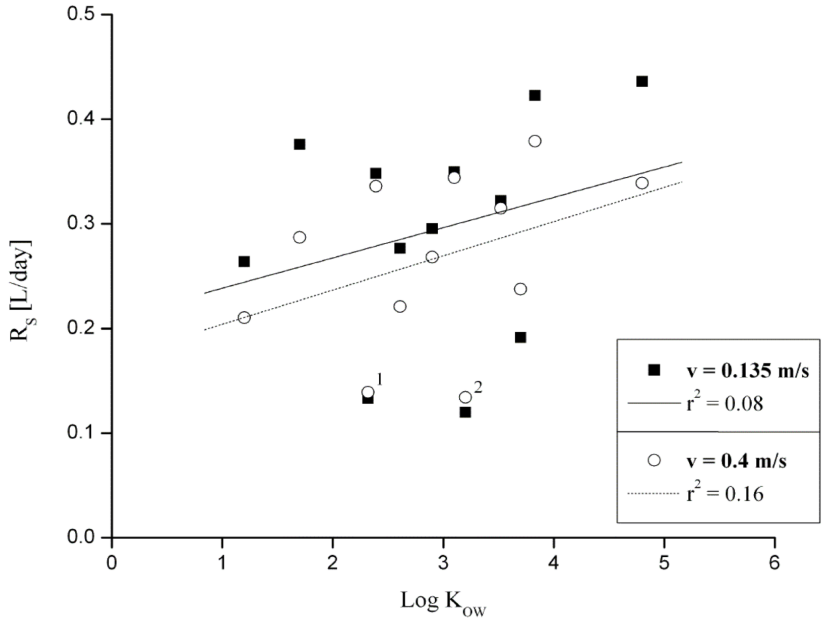

Figure 3: Relationship between sampling rates R_s and log $K_{ow.}$ Data taken from Table 1 and Table 3. 1 = carbofuran; 2 = linuron.

Discussion

Degradation of pesticides on the receiving phase

The decrease of recovery rates of linuron and carbofuran from the receiving phase over storage time indicated degradation on the SDB-XC disk. The main abiotic degradation pathway of carbofuran in water is base-catalysed hydrolysis to carbofuran-phenol, as described by Yu et al. (1974). Further products are 2,3-dihydro-2,2-dimethyl-benzofuran-4,7-diol, 3-ketocarbofuran (Yu et al., 1974) and 3-hydroxy-carbofuran-7-phenol (Chiron et al., 1996). It follows that under acidic and neutral condition, no significant hydrolysis is to be expected. Indeed, it was reported that in the dark at neutral pH, no degradation of carbofuran was observed during exposure times relevant to our experiment (Iesce et al., 2006, Munch and Frebis, 1992). By constrast, Iesce et al. (2006) reported a half-life of 3 hours for hydrolysis in the dark for carbofuran under alkaline

conditions (pH 9). Nevertheless, a study on the stability of carbofuran on a C_{18}-Empore disk reported up to 30% decrease in recoveries after 14 days of storage at temperatures up to 55°C (Cobb et al., 2006). These latter findings suggest that the Empore disk supports microbial activity and associated biodegradation of the compound. In our experiments we observed similar degradation rates, and in fact biofilms had developed on the disk to a visible extent after 14 to 21 days.

Degradation of linuron yielded 3,4-dichloroaniline as the major degradation product but was shown to be relatively persistent with a half-life of 945 days in water at pH values from 5 to 9 (Geißbuhler, 1975). However, Munch and Frebis (1992) reported a slight decrease in average recoveries (5%) when stored for 14 days at 4°C. On the SDB-XC Empore disks we observed a decrease of up to 49% recovery after 14 days. Again we assume that this is due to microbial degradation, as persistence in water was shown to be high.

As the Chemcatcher may be deployed up to 4 weeks, degradation of linuron and carbofuran is likely to occur in the field if the compounds are sampled from the stream. Hence, degradation should be taken into account when monitoring these two compounds, especially at high pH values (Bailey et al., 1996, Seiber et al., 1978).

Average concentrations during calibration experiments

During the calibration experiment several compounds exhibited high variation (Table 2). Average water concentrations in the first experiment (0.4 m/s) ranged from 64 to 168 ng/L, and variation was a little higher, up to 25% (n = 4). This was due to a two-fold increase of the analyte concentrations in the middle of the experiment (day 9) with a slight subsequent decrease. Since analyte concentrations were controlled only at 72-h intervals, we have no information about how long the concentrations were elevated. When presupposing a short time (< 12 h), the resulting average water concentrations would be reduced and therefore the computed sampling rates at 0.4 m/s were higher.

Overall the water concentrations were quite stable at 100 ng/L. The higher average concentrations at 0.4 m/s may have resulted from inaccuracies of the water pump resulting in lower water inflow while analyte input remained constant.

Uptake kinetics at different flow velocities

In our calibration experiment, we mostly observed lower sampling rates of analytes at faster flow velocities, with R_s values being 6.1 % smaller on an average. Under the investigated conditions with high and probably turbulent flow, no influence of the current velocity on the uptake kinetics was expected since the uptake should be governed solely by the sampler's resistance to mass transfer and not primarily by diffusion through the aqueous boundary layer. By contrast, this would be expected for non-polar compounds and was demonstrated by Vrana and Schüürmann (2002) for SPMDs for very slow flow (0.0006 m/s, 0.0028 m/s). However, they found flow-independent sampling rates for a hydrophobic compound (log K_{ow} = 5.5) when current velocities increased to 0.0114 m/s, and slightly decreasing R_s values for lindane (log K_{ow} = 3.8).

The sampling rates in our study exhibited significant differences when compared in pairs for the two current velocities (paired sample t-test, t = -2.2152, p = 0.049; n = 12). However, after removal of the elevated water concentration at 0.4 m/s, the R_s values would not be significantly different (paired-sample t-test, t = 1.2283, p = 0.2450; n = 12). Hence we suggest that the differences between the sampling rates can be attributed to variability in the analyte concentrations in water between the experiments rather than to differences in uptake kinetics. The influence of flow velocity on the sampling rate seems to play a minor role for hydrophilic substances, and other calibrations were run with stirred water only (Alvarez et al., 2007). Nevertheless more studies are needed regarding the influence of current velocity on the sampling rate, as this is a very important environmental variable in field deployments.

Comparison of sampling rates

An overview of our sampling rates and those of previous studies is given in Table 4. The sampling rates of Stephens et al. (2005) for hexazinone and atrazine were two-fold and four-fold higher compared to our values (Table 4). This may be due to a higher temperature in their experiment as pointed out by Vrana et al. (2006a, 2007) for other compounds. Furthermore, they used different receiving phases (SDB-RPS and C_{18} Empore disks). The SDB-RPS phase contains additional sulfonic acid functional groups to improve mass transfer compared to the SCB-XC disk, which can explain the higher sampling rates. However, higher sampling rates resulted in a shorter linear uptake phase of the sampler. Thus the advantage of employing a SDB-XC Empore disk would be a

longer exposure period until equilibrium is reached. When a diffusion-limiting membrane is employed, further prolongation of this period is possible, but the sampling rates of the SDB-XC disks for atrazine decreased by a factor of 10 (Tran et al., 2007). This is in accordance with a study of Kingston et al. (2000) where the use of a membrane resulted in a five-fold decrease of the accumulated mass of atrazine on a C_{18} disk.

The R_S value of endosulfan was 0.379 L/day at a flow velocity of 0.4 m/s in our experiment. Vrana et al. (2006a) found sampling rates between 0.06 to 0.15 L/day for endosulfan with a C_{18} Empore disk as receiving phase, covered with a LDPE membrane and exposed to flow velocities of 0, 0.4 and 0.7 m/s. In recent studies on polar organic chemical integrative sampler disks (POCIS), sampling rates of several pesticides were similar to those of our study and ranged from 0.026 L/day for Carbofuran to 0.3 L/day for Pirimicarb (Alvarez et al., 2007). Our results demonstrate that the Chemcatcher can be adapted according to the desired time of exposure.

Table 4: Comparison of our sampling rates and previous studies

Setup Conditions				**Pesticides calibrated in several studies with R_S values (L/day)**			
Receiving phase	Membrane	T (°C)	v (m/s)	atrazine	hexazinone	endosulfan	Reference
SDB-XC	PES[a]	23	0.004	0.023			Tran et al. (2007)
C18	-	22	0.14	1.4	0.74		Stephens et al. (2005)
SDB-RPS	-	22	0.14	1.2	0.6		Stephens et al. (2005)
SDB-XC	-	14	0.135	0.28	0.26		This study
SDB-XC	-	14	0.4			0.38	This study
C18	LDPE[b]	11	0.4			0.08	Vrana et al. (2005)

[a] PES – polyethersulfone [b] LDPE – low density polyethylene

Relationship between sampling rates and physicochemical properties

Vrana et al. (2006a) demonstrated a strong linear relationship between log K_{ow} and the sampling rate for non-polar substances (log K_{ow} = 3.5 - 7). For more polar pesticides (log K_{ow} = 1.2 – 2.85) Stephens et al. (2005) suggested an increasing sampler-water equilibrium-partitioning coefficient log K_{sw}, which is proportional to the sampling rate, with increasing log K_{ow} (Stephens et al., 2005). In our experiment, no significant relationship was found between log K_{ow} and sampling rate (Figure 3). The low correlation between the sampling rate and log K_{ow} in our study may be explained by the uptake mechanism to the receiving phase, as this is adsorption for the SDB-XC disk in contrast

to partitioning for C_{18} disks (Mills et al., 2007).

Offload kinetics

PRCs present a relevant method of coping with between-site variation of environmental variables and biofouling that may result in differences in the analyte uptake during field deployment (Huckins et al., 2002). Although we did not discover a significant effect of flow velocity on the sampling rate, PRCs for polar pesticides would be useful to account for in situ variation of other environmental factors such as biofouling or water temperature during field deployment. The application of PRCs relies on isotropic behaviour of uptake and release kinetics (Gorecki and Namiesnik, 2002). Several studies demonstrated isotropic kinetics for hydrophobic compounds and different receiving phases (Vrana et al., 2006a, Vrana et al., 2007, Vrana et al., 2006b). In studies on PRCs for polar compounds, isotropic behaviour was found for triclopyr (log K_{ow} = -0.45) for the SDB-XC Empore disk (Tran et al., 2007) and for desisopropylatrazine (log K_{ow} = 1.15) for the POCIS (Mazzella et al., 2007). Both studies employed a polyethersulfone diffusion-limiting membrane. In our study, the nonlinear regression fit of the offload curve was bad compared to these studies. Furthermore, we did not observe a clear elimination of the PRCs from the receiving phase for low-flow conditions (Figure 4a and 4b). Due to the high variation in exchange rate constants, the isotropy of uptake and offload kinetics could not be confirmed reliably for the uncovered receiving phase.

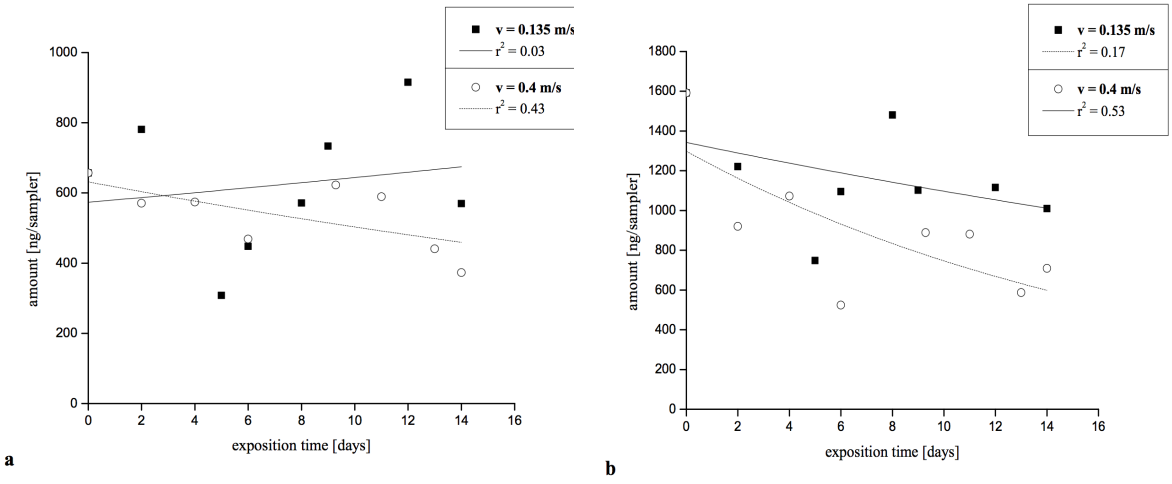

Figure 4: Nonlinear regression fit for offload curves of the performance reference compounds chlorfenvinphos-D10 (4a) and pirimicarb-D6 (4b) from the SDB-XC receiving phase. v = flow velocity.

A possible explanation for the differences to previous studies is that the PRCs are not loaded homogeneously over the thickness of the receiving phase. This may result in high variation when no diffusion-limiting membrane is used. Another explanation was given by Mills et al. (2007), who described the use of PRCs with polar receiving phases as problematic because their accumulation is rather determined by adsorption than by partitioning. Thus, uptake and offload kinetics could not be governed by the same mass transfer law. However, the use of a diffusion-limiting membrane may overcome this problem as demonstrated by Tran et al. (2007) and Mazzella et al. (2007), with being the limiting step of the mass transfer between sampler and surrounding medium. Furthermore, the use of a diffusion-limiting membrane may reduce biofouling due to a lower surface area compared to the Empore disk. Nevertheless, the membrane would limit the sampling rate, and brief fluctuations of analyte concentration during exposure may be missed.

Conclusions

The Chemcatcher passive sampler with SDB-XC Empore disc as receiving phase is suitable for the use in time-integrative water monitoring of polar compounds, and we provide sampling rates for future field studies. Flow velocity is of minor importance in field deployments for rates between 0.15 and 0.4 m/s, as this variable seems to not affect the sampling rates. By contrast, degradation on the receiving phase is relevant and needs to be accounted for, because the derived TWA concentrations would underestimate real exposure otherwise. Furthermore, new techniques are needed to account for between-site differences in environmental conditions, as PRCs may not be applicable for polar receiving phases without membranes.

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References

Alvarez, D.A., Huckins, J.N., Petty, J.D., Jones-Lepp, T., Stuer-Lauridsen, F., Getting, D.T., Goddard, J.P., Gravell, A., 2007. Comprehensive Analytical Chemistry 48: Tool for monitoring hydrophilic contaminants in water: polar organic chemical integrative sampler (POCIS). Greenwood, R., Mills, G.A. and Vrana, B. (eds), pp. 171-197, Elsevier, Amesterdam.

Bailey, H.C., DiGiorgio, C., Kroll, K., Miller, J.L., Hinton, D.E., Starrett, G., 1996. Development of procedures for identifying pesticide toxicity in ambient waters: Carbofuran, diazinon, chlorpyrifos. Environmental Toxicology and Chemistry 15 (6), 837-845.

Booij, K., Smedes, F., van Weerlee, E.M., 2002. Spiking of performance reference compounds in low density polyethylene and silicone passive water samplers. Chemosphere 46 (8), 1157-1161.

Booij, K., van Bommel, R., Mets, A., Dekker, R., 2006. Little effect of excessive biofouling on the uptake of organic contaminants by semipermeable membrane devices. Chemosphere 65 (11), 2485-2492.

Chiron, S., Torres, J.A., FernandezAlba, A., Alpendurada, M.F., Barcelo, D., 1996. Identification of carbofuran and methiocarb and their transformation products in estuarine waters by on-line solid phase extraction liquid chromatography-mass spectrometry. International Journal of Environmental Analytical Chemistry 65 (1-4), 37-52.

Cobb, J.M., Mattice, J.D., Senseman, S.A., Dumas, J.A., Mersie, W., Riley, M.B., Potter, T.L., Mueller, T.C., Watson, E.B., 2006. Stability of pesticides on solid-phase extraction disks after incubation at various temperatures and for various time intervals: interlaboratory study. Journal of AOAC International 89 (4), 903-912.

Geißbuhler, H., 1975. The substituted ureas. In: Kearney, P.C., Kaufman, D.D. (Eds.), Herbicides - Chemistry, degradation and mode of action. Marcel Dekker Inc., New York, NY, USA, pp. 349-376.

Gorecki, T., Namiesnik, J., 2002. Passive sampling. TrAC-Trends in Analytical Chemistry 21 (4), 276-291.

Guo, L., Nordmark, C.E., Spurlock, F.C., Johnson, B.R., Li, L.Y., Lee, J.M., Goh, K.S., 2004. Characterizing dependence of pesticide load in surface water on precipitation and pesticide use for the Sacramento River watershed. Environmental Science and Technology 38 (14), 3842-3852.

Huckins, J.N., Petty, J.D., Lebo, J.A., Almeida, F.V., Booij, K., Alvarez, D.A., Clark, R.C., Mogensen, B.B., 2002. Development of the permeability/performance reference compound approach for in situ calibration of semipermeable membrane devices. Environmental Science and Technology 36 (1), 85-91.

Iesce, M.R., della Greca, M., Cermola, F., Rubino, M., Isidori, M., Pascarella, L., 2006. Transformation and ecotoxicity of carbamic pesticides in water. Environmental Science and Pollution Research 13 (2), 105-109.

Kingston, J.K., Greenwood, R., Mills, G.A., Morrison, G.M., Persson, L.B., 2000. Development of a novel passive sampling system for the time-averaged measurement of a range of organic pollutants in aquatic environments. Journal of Environmental Monitoring 2 (5), 487-495.

Kreuger, J., 1998. Pesticides in stream water within an agricultural catchment in southern Sweden, 1990-1996. The Science of the Total Environment 216, 227-251.

Leon-Gonzalez, M.E., Perez-Arribas, L.V., 2000. Chemically modified polymeric sorbents for sample preconcentration. Journal Of Chromatography A 902 (1), 3-16.

Liess, M., Schulz, R., Liess, M.H.-D., Rother, B., Kreuzig, R., 1999. Determination of insecticide contamination in agricultural headwater streams. Water Research 33 (1), 239-247.

Mazzella, N., Dubernet, J.-F., Delmas, F., 2007. Determination of kinetic and equilibrium regimes in the operation of polar organic chemical integrative samplers. Application to the passive sampling of the polar herbicides in aquatic environments. Journal Of Chromatography A 1154, 42-51.

Mills, G.A., Vrana, B., Allan, I., Alvarez, D.A., Huckins, J.N., Greenwood, R., 2007. Trends in monitoring pharmaceuticals and personal-care products in the aquatic environment by use of passive sampling devices. Analytical And Bioanalytical Chemistry 387 (4), 1153-1157.

Munch, D.J., Frebis, C.P., 1992. Analyte stability studies conducted during the national pesticide survey. Environmental Science and Technology 26 (5), 921-925.

Sabljic, A., Gusten, H., Verhaar, H., Hermens, J., 1995. Qsar modelling of soil sorption. Improvements and systematics of log KOC vs. log KOW correlations. Chemosphere 31 (11), 4489-4514.

Schäfer, R.B., Caquet, T., Siimes, K., Mueller, R., Lagadic, L., Liess, M., 2007. Effects of pesticides on community structure and ecosystem functions in agricultural headwater streams of France and Finland. Science of the Total Environment 382 (2-3), 272-285.

Seiber, J.N., Catahan, M.P., Barril, C.R., 1978. Loss of carbofuran from rice paddy water - Chemical and physical factors. Journal of Environmental Science and Health Part B 13 (2), 131-148.

Stephens, B.S., Kapernick, A., Mueller, J., Eaglesham, G., 2005. Aquatic passive sampling of herbicides on naked particle loaded membranes: Accelerated measurement and empirical estimation of kinetic parameters. Environmental Science and Technology 39 (22), 8891-8897.

Tomlin, C.D.S., 2003. The Pesticide Manual, A World Compendium, Incorporating the agrochemicals handbook. Crop Protection Publications, Alton, Hampshire, UK, pp. 1344 p.

Tran, A.T.K., Hyne, R.V., Doble, P., 2007. Calibration of a passive sampling device for time-integrated sampling of hydrophilic herbicides in aquatic environments. Environmental Toxicology and Chemistry 26 (3), 435-443.

Vrana, B., Allan, I.J., Greenwood, R., Mills, G.A., Dominiak, E., Svensson, K., Knutsson, J., Morrison, G., 2005. Passive sampling techniques for monitoring pollutants in water. TrAC - Trends in Analytical Chemistry 24 (10), 845-868.

Vrana, B., Mills, G.A., Dominiak, E., Greenwood, R., 2006a. Calibration of the Chemcatcher passive sampler for the monitoring of priority organic pollutants in water. Environmental Pollution 142 (2), 333-343.

Vrana, B., Mills, G.A., Kotterman, M., Leonards, P., Booij, K., Greenwood, R., 2007. Modelling and field application of the Chemcatcher passive sampler calibration data for the monitoring of hydrophobic organic pollutants in water. Environmental Pollution 145 (3), 895-904.

Vrana, B., Paschke, A., Popp, P., 2006b. Calibration and field performance of membrane-enclosed sorptive coating for integrative passive sampling of persistent organic pollutants in water. Environmental Pollution 144 (1), 296-307.

Vrana, B., Schüürmann, G., 2002. Calibrating the uptake kinetics of semipermeable membrane devices in water: Impact of hydrodynamics. Environmental Science and Technology 36 (2), 290-296.

Yu, C.C., Booth, G.M., Hansen, D.J., Larsen, J.R., 1974. Fate of carbofuran in a model ecosystem. Journal of Agricultural and Food Chemistry 22 (3), 431-434.

Kapitel 4: Performance of the Chemcatcher® passive sampler when used to monitor 10 polar and semi-polar pesticides in 16 Central European streams, and comparison with two other sampling methods

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Abstract

We investigated the performance of the Chemcatcher®, an aquatic passive sampling device consisting of a sampler body and an Empore® disk as receiving phase, when used to monitor acetochlor, alachlor, carbofuran, chlorfenvinphos, α-endosulfan, fenpropidin linuron, oxadiazon, pirimicarb and tebuconazole in 16 Central European streams. The Chemcatcher®, equipped with an SDB-XC Empore® disk, detected seven of the aforementioned pesticides with a total of 54 detections. The time-weighted average (TWA) concentrations reached up to 1 µg/L for acetochlor and alachlor. Toxic units derived from these concentrations explained reasonably well the observed ecological effects of pesticide stress, measured with the SPEAR index. In a follow-up analysis, we compared the Chemcatcher® performance with two other sampling systems. The results obtained with the Chemcatcher® closely matched those of the event-driven water sampler. By contrast, the TWA concentrations were not significantly correlated with concentrations on suspended particles. We conclude that the Chemcatcher® is suitable for the monitoring of polar organic toxicants and presents an alternative to conventional spot

sampling in the monitoring of episodically occurring pollutants.

Introduction

The monitoring of pesticide concentrations in surface waters is an inevitable step for the environmental risk assessment of pesticides. For these compounds, field runoff represents a relevant input path into streams in agricultural areas (Liess et al., 1999; Neumann et al., 2002). Runoff events occur discontinuously in association with heavy precipitation, and runoff-related pesticide exposure may have adverse effects on invertebrate communities (Leonard et al., 2000; Liess and von der Ohe, 2005). Since most pesticide concentrations during runoff events decrease to background levels within hours to a few days, routine water monitoring which mainly relies on spot (bottle) sampling at fixed intervals is likely to miss a great proportion of relevant events (Richards and Baker, 1993; Leu et al., 2004). Hence environmental monitoring techniques are needed that allow for detection of runoff-related peak exposure and that are labour- and cost-efficient at the same time.

Continuous water sampling represents an alternative to spot sampling. Throughout the last decade, passive sampling devices using various receiving phases have been employed successfully for continuous monitoring of various pollutants in surface waters (Stuer-Lauridsen, 2005; Vrana et al., 2005). The Chemcatcher® passive sampler with polar receiving phase and the polar organic chemical integrative sampler (POCIS) performed well in the monitoring of polar organic contaminants (Escher et al., 2006; Alvarez et al., 2007). Nevertheless, there is a paucity of studies addressing the monitoring of short-term pollution events with passive samplers (Greenwood et al., 2007). Furthermore, to our knowledge only one study demonstrated a relationship between pesticide concentrations determined by passive samplers and effects on aquatic communities (Leonard et al., 2000). The establishment of such a relationship is hampered by the fact that time-weighted average (TWA) concentrations are obtained from passive sampling devices, whereas peak concentrations are required to assess potential acute ecotoxicological effects. In this study we present results of a field study at 16 sampling sites using the Chemcatcher® passive sampler to detect the polar and semi-polar pesticides acetochlor, alachlor, carbofuran, chlorfenvinphos, α-endosulfan, fenpropidin linuron, oxadiazon, pirimicarb and tebuconazole. The compounds were chosen on the basis of their ecotoxicological relevance in the sampling region (Schäfer et al., 2007a). In addition, we examine the extent to which the TWA concentrations can be related to a community-

based biotic index – the Species At Risk (SPEAR)-index – designed to detect effects of pesticides on benthic invertebrates (Liess and von der Ohe, 2005).

Since several sampling systems have been proposed to assess runoff-related pesticide exposure, there is also a need to compare the performance of different sampling systems. Therefore, another objective of this study was to compare the performance of the Chemcatcher® to the performances of two other sampling systems: an event-driven water sampler (EDS) and a suspended-particle sampler (SPS) (Technical drawings of all sampling methods can be found in the supplementary data). Both methods have been proposed and used to catch runoff events in previous studies (Liess et al., 1996; Liess et al., 1999; Schulz et al., 2001; Liess and von der Ohe, 2005) and were deployed at the same sampling sites as the passive samplers in this study (Schäfer et al., 2007a; Schäfer et al., 2007b). The comparison of the Chemcatcher® to these sampling methods comprised the following criteria: (1) number of pesticides detected and (2) the total number of detections above the limit of quantitation. Since sampling methods should deliver results that are relevant to assess effects on biota, we included as criteria also (3) the ability to explain variation in the SPEAR index.

Materials and methods

Study area

Brittany, located in northwestern France, was chosen as the sampling region since (1) agriculture is the predominant land-use type there with 23.5% of the area (27510 km2) being used for corn (19.2%), vegetable (2.6%), oil-seed (1.2%) and potato (0.5%) production and (2) in Western Europe pesticide usage is the highest globally in terms of expenditures per area (Oerke and Dehne, 2004). A total of 16 sampling sites in small agricultural streams (max. width: 5 m, max. depth: 0.8 m) were selected on the basis that they were expected to exhibit a gradient in pesticide contamination (Schäfer et al., 2007a).

Preparation, deployment and extraction of the passive sampler

The Chemcatcher® passive sampling device (University Portsmouth, UK; commercially available at Alcontrol AB, Linkoping, Sweden) was employed for continuous water monitoring as described by Kingston et al. (2000). The Chemcatcher® consists of a

polytetrafluorethylene (PTFE) sampler body and for the purpose of this study was equipped with SDB-XC Empore® disks (3M, Neuss, Germany) as receiving phase (47 mm diameter; 15.9 cm² surface area) containing polystyrene-divinylbenzene (PS-DVB) as sorbent.

Before use the SDB-XC Empore® disk was conditioned with 10 mL acetone (HPLC-grade), 10 mL 2-propanol (analytical-grade) and 10 mL methanol (HPLC-grade) obtained from Merck (Darmstadt, Germany). The conditioned disks were placed in the Chemcatcher® body, which was subsequently filled with purified water, closed and stored in zip-lock bags at 4°C until exposure (< 48 hours). To obtain a rapid response to concentration changes no diffusion-limiting membrane was used. Procedural blanks were stored non-exposed throughout the whole study period.

The Chemcatcher® devices were deployed at the 16 sampling sites on 9-11th of May for 10 to 13 days (Figure 1), prior to a period with expected heavy precipitation according to the local weather forecast (www.meteofrance.com). The samplers were fixed to steel bars approximately 15 cm below the water surface. The open side of the Chemcatcher® was sealed with a copper mesh (mesh size 5 mm) to prevent mechanical damage and suppress biofouling (Vrana et al., 2005). It was directed towards the stream bottom. Four sites were equipped in duplicate and one in triplicate to assess variability of the pesticide uptake. A field blank was exposed to the air during deployment and retrieval of samplers to account for potential airborne pollution.

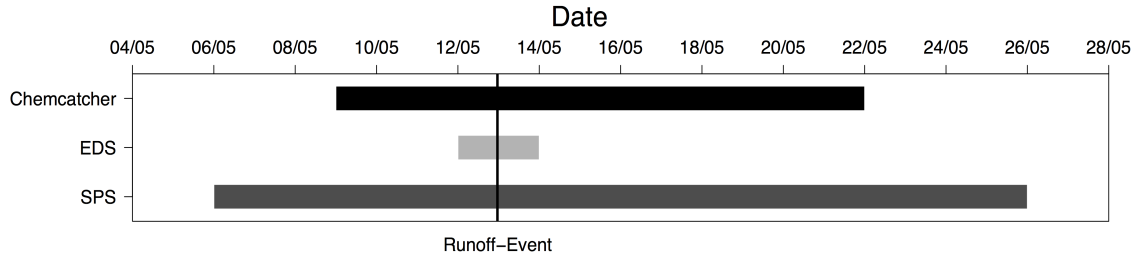

Figure 1: Sampling scheme for the three monitoring methods in 16 French streams. "Runoff-Event" indicates a heavy precipitation event (> 10 mm/day).

After exposure, the passive samplers were filled with stream water from the respective site, closed and stored in zip-lock bags at 4°C in the dark. In the laboratory, the SDB-XC Empore® disks were carefully taken off the PTFE-body, dried under vacuum using a vacuum manifold for about 15 minutes and subsequently eluted twice with 10 mL acetonitrile/methanol. The eluate was gently evaporated to dryness under nitrogen at 30°C in a 200 mL evaporation vial using a TurboVap 2 concentration workstation (Zymark,

Hopkington, USA) and redissolved with 200 µL acetonitrile. Prior to analysis 5 µL triphenyl phosphate (TPP) were added as internal standard (IS).

Chemical analysis

The selected compounds (Table 1) were quantified using an Agilent 6890N (Agilent Technologies Germany, Boeblingen, Germany) gas chromatograph (GC) equipped with a MPS2 autosampler, a CAS4 inlet (both from Gerstel, Mühlheim a.d. Ruhr, Germany) and an Agilent 5973 mass selective detector (MSD). The limit of quantification (LOQ) of the GC-MSD was 125 pg/µL for all compounds. The sample LOQs differed between the sampling methods and between compounds for the Chemcatcher (Table 1). Typical total ion chromatograms are given in Figure 2.

Table 1: Physicochemical and analytical data for 10 measured pesticides

Compound	Type[a]	Class[a]	log K_{ow}[b]	log K_{oc}[b]	LOQ CC (ng/L)[c,d]	LOQ EDS (ng/L)[c]	LOQ SPS (µg/kg)[c,e]	LOQ calc. (µg/kg)[c,f]	LC50 (µg/L)[a,g]
Acetochlor	H	chloro-acetamide	2.39	2.32	5.1	25	12.5	0.26	9000
Alachlor	H	chloro-acetamide	3.52	2.28	5.4	25	12.5	0.24	10000
α-Endosulfan	I	organo-chlorine	3.83	4.13	3.6	25	12.5	16.86	75
Carbofuran	I	carbamate	2.32	1.75	10.4	25	12.5	0.07	38.6
Chlorfenvinphos	I	organic phosphorous acid	3.10	2.47	5.2	25	12.5	0.37	0.3
Fenpropidin	F	piperidine	2.90[a]	3.20[i]	4.1	25	12.5	1.98	500
Linuron[h]	H	urea derivative	3.20	2.70	4.3	25	12.5	0.63	120
Oxadiazon	H	oxadiazole	4.80	3.51	3.5	25	12.5	4.04	2400
Pirimicarb	I	carbamate	1.70	1.90	4.5	25	12.5	0.10	17
Tebuconazole	F	triazole	3.70[a]	3.50[i]	6.1	25	12.5[j]	3.95	4200

[a] taken from Tomlin (2003), I = Insecticide, H = Herbicide, F = Fungicide [b] taken from Sabljic et al. (1995) [c] LOQ = limit of quantification for a sample obtained with the respective method [d] CC = Chemcatcher®; computed for 14-day exposure
[e] for extraction of 10g of suspended particles [f] Sample LOQ for suspended particles that would correspond to the level of the EDS LOQ assuming equilibrium partitioning, computed according to: $\text{LOQ calc.} = \text{LOQ EDS} * K_{OC} * f_{OC}$ where f_{OC} is the mass fraction of organic carbon (assuming f_{OC} = 5%) [g] LC50 for *Daphnia magna*
[h] quantificated as 3,4-dichloroaniline [i] estimated with Chemprop 4.1 (http://www.ufz.de/index.php?en=6738) [j] 25 and 100 for some samples with high matrix interference

Calculation of passive sampler TWA concentrations

From the field-exposed passive samplers, the accumulated mass of each compound per sampler is obtained. To calculate TWA concentrations, a substance-specific sampling rate R_s, expressed in equivalent volume of sampled water per day, is required. For the compounds of this study, the sampling rates were previously determined in a laboratory flow-through experiment and found to range from 0.1 to 0.5 L/d (Gunold et al., 2007). In addition, this calibration study showed that the Chemcatcher® remained in the linear integrative uptake regime for up to 14 days. Using the sampling rates of this study, the TWA concentrations for the sites in our study were calculated according to:

$$C_W = \frac{m_s}{R_s * t} \quad (1)$$

where C_w is the TWA concentration of the respective analyte in the water phase in the dimension mass/volume and m_s is the accumulated mass after exposure time t. The procedural blank and the field blank yielded zero background contamination and had therefore not to be considered in Equation (1).

The calculated TWA concentrations should be regarded as approximation only, because between-site variation in water temperature and biofouling were not taken into account, as the performance reference compound (PRC) concept (Huckins et al., 2002) was not applicable (Gunold et al., 2007).

Linking exposure to the SPEAR index

We examined the extent to which the TWA concentrations determined with the Chemcatcher® can explain variation in the SPEAR index. Briefly, the SPEAR index predicts the effects of organic toxicants on the invertebrate community of a site, based upon traits of benthic invertebrates such as voltinism, migration potential, emergence time and physiological sensitivity (Liess and von der Ohe, 2005). Practically, these traits are used to classify the observed macroinvertebrate community of each sampling site into taxa potentially sensitive or tolerant towards organic toxicants. Subsequently, the SPEAR index value for a respective site is derived by computing the relative abundance of sensitive species in a community. Details on the sampling of the benthic invertebrates and on the computation of the SPEAR index are given in Schäfer et al. (2007a). To assess and standardize the toxicity of the measured TWA concentrations, a log-transformed maximum toxic unit (TU) was computed using the 48-h acute median lethal concentration

(LC50) for Daphnia magna (Table 1) as described by Schäfer et al. (2007a). A TU value of -5 was assigned to a site if no pesticide was found, corresponding to unpolluted sites in a previous study (Liess and von der Ohe, 2005).

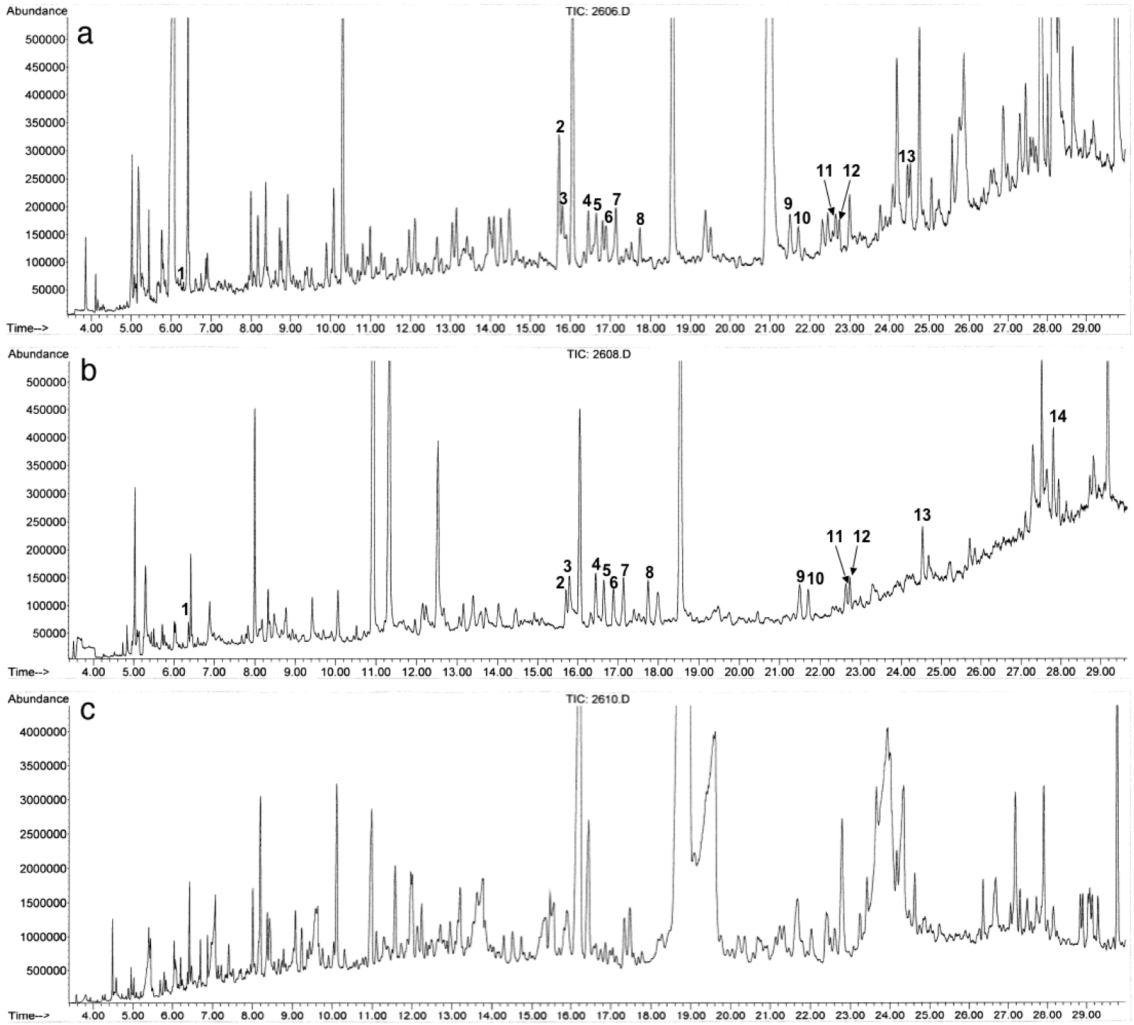

Figure 2: Typical total-ion chromatograms for (a) the event-driven water sampler (EDS), (b) the Chemcatcher®, and (c) the suspended-particle sampler (SPS). The samples were spiked with 1 µg/L (SPS 100 µg/kg) of pesticide standards. Deuterated internal standards were only used for comparison of the EDS and Chemcatcher®. Please note the different scaling of the y-axis for the SPS chromatogram. Analytes: 1: carbofuran, 2: pirimicarb D6, 3: pirimicarb, 4: acetochlor D11, 5: acetochlor, 6: alachlor D13, 7: alachlor, 8: fenpropidin, 9: chlorfenvinphos D10, 10: chlorfenvinphos, 11: α-endosulfan D4, 12: α-endosulfan, 13: oxadiazon, 14: tebuconazol

Description of the event-driven water sampler (EDS)

The EDS was designed to catch peak concentrations during pesticide runoff. The sampling system set into the streams consisted of a 1-L glass bottle fixed to a steel bar and was mounted approximately 5 cm above normal water level (Liess et al., 2001; Schulz et al., 2001). After a heavy rain event (> 10 mm precipitation/ 24 h) the filled sample bottles were retrieved and water samples were solid-phase-extracted using

6 ml Chromabond HR-P columns containing 500 mg of polystyrene-divinylbenzene (PS-DVB), purchased from Macherey-Nagel (Düren, Germany), according to the method described in Schäfer *et al.* (2007a). The eluates were treated as described for the Chemcatcher®. The EDS monitoring results reported here refer to a single heavy-rain event (>10 mm/day) during the study period that occurred between 12th and 13th of May (Figure 1). The TUs of this method were taken from Schäfer *et al.* (2007a).

Description of the suspended-particle sampler (SPS)

The SPS was designed to sample suspended particles and consisted of a 3-L sedimentation vessel that was buried in the streambed. Suspended particles that entered therein could settle down (Liess et al., 1996). The sampled suspended material was collected at two-week intervals, freeze-dried and passed through a 2-mm sieve to remove needles, sticks and leaf parts. Approximately 10 g (dry weight) of the sample were extracted using accelerated solvent extraction (ASE 200–system from Dionex, Idstein, Germany; extraction parameters: two 6-min-cycles with ethyl acetate-acetone (2:1) at 110°C and 11 MPa) with subsequent size exclusion chromatography (SEC) cleanup (Biobeads S-X3 cleanup column from Antec GmbH, Sindelsdorf, Germany) as described by Schäfer *et al.* (2007b). Due to matrix interferences the collected fraction in SEC was not evaporated further than to 1000 µl and subsequently, 50 µl TPP were added as IS. To obtain comparable data sets, we used the results of the sampling period between 6th to 23-26th of May for this method (Figure 1). A maximum sediment toxic unit was computed from the suspended particle concentrations as described in Schäfer *et al.* (2007b). Log-transformed sediment toxic units are referred to as STU.

Data analysis

Pearson's correlation coefficient *r* was calculated to indicate the similarity of two sampling methods followed by a *t*-test to detect significant correlations. Observations that were below LOQ for a compound at a certain site and for all sampling methods were excluded from analysis. In case an observation below LOQ corresponded to a measurement above LOQ in another sampling method, the observation below LOQ was replaced by the half the LOQ. This substitution by a constant proved to be most reliable for small data sets in a comparative study (Clarke, 1998). Linear models were constituted (1) to analyse if the linear regression for two sampling methods differed significantly between sites or compounds which were included as covariate factors, and (2) to examine the explanatory power of TU (STU for SPS) for variation in the SPEAR index.

Due to the low number of replicates (2 and 3) we calculated the relative range (RR) as dispersion measure for the TWA concentrations:

$$\text{RR (\%)} = \frac{(\max(X) - \min(X))}{\bar{X}} \quad (2)$$

where X are the observations for the respective compound at a certain site and $\bar{X}$ is the mean of X. The RR is a more conservative estimate of the sample dispersion compared to the relative standard deviation. All statistical computations and graphics were created with the open-source software package R (www.r-project.org) using version 2.6 (for Mac OS X, 10.4.10).

Results

Pesticide monitoring with the Chemcatcher® passive sampler

At the 16 sites, seven of the 10 target pesticides were found with the Chemcatcher® passive sampler (Table 2); those not detected were chlorfenvinphos, α-endosulfan and fenpropidine. Both chloroacetamide herbicides - acetochlor and alachlor - were detected most frequently above the LOQ and had the highest TWA concentrations, reaching up to 1 µg/L. Tebuconazole and pirimicarb were found only occasionally and had the lowest TWA concentrations. The TWA concentrations exhibited high variation at three of the five sampling sites with up to 150% in terms of relative range (RR) (Table 2). The other sites showed medium (< 50% RR) and low (< 30% RR) variation for the majority of the compounds.

The TUs for the sites ranged from -2.4, corresponding to 1/250 the LC50 of *Daphnia magna*, to -5 (Table 2). The TU-values explained reasonably well variation in the SPEAR index (r^2 = 0.5, $p < 0.01$, n = 16) (Table 3), indicating effects of pesticides on the abundance of sensitive invertebrate taxa.

Comparison of the three sampling methods concerning pesticide monitoring

All pesticides of the monitoring program were found in the water samples of the EDS and this sampling method yielded also a slightly higher number of total detections compared to the Chemcatcher® (Table 3). Nevertheless, the pesticide concentrations found by the two water sampling methods were significantly correlated (r = 0.79, $p < 0.01$, n = 75).

Table 2: Time-weighted average concentrations in ng/L (± relative range[a] where replicates available) of pesticides determined with the Chemcatcher® passive sampler as well as TUs and STUs for the three sampling methods.[b]

Site	Acetochlor	Alachlor	Carbofuran	Linuron	Oxadiazon	Pirimicarb	Tebuconazole	TU CC[c]	TU EDS[c,d]	STU SPS[c,e]
1	1158	184	124	54	10	bq	bq	-2.5	-0.4	0.7
2	14	7	21	bq	bq	bq	bq	-3.3	-2.2	-5.0
3	18	198	bq	37	bq	bq	bq	-3.5	-2.7	2.5
4	196	40	36	9	7	bq	bq	-3.0	-2.5	-2.2
5	219	96	127	48	8	bq	6	-2.5	-2.0	1.1
6	60 (± 148%)	12 (± 99%)	bq	16 (± 94%)	4 (±72%)	5 (± 86%)	bq	-2.6	-2.5	-5.0
7	37	132	92	57	bq	8	bq	-3.5	-2.1	-5.0
8	454 (± 102%)	681 (± 99%)	159 (± 27%)	41 (± 116%)	9 (± 103%)	bq	bq	-2.4	-0.8	0.9
9	486 (± 29%)	1233 (± 14%)	52 (± 22%)	22 (± 25%)	bq	bq	15 (± 10%)	-2.9	-2.6	-2.0
10	388 (± 55%)	182 (± 44%)	20 (± 13%)	66 (± 48%)	26 (± 95%)	6 (± 26%)	11 (± 33%)	-3.3	-2.8	-4.1
11	20	14	bq	bq	bq	12	bq	-3.2	-2.6	-5.0
12	bq	bq	bq	bq	bq	bq	bq	-5.0	-5.0	-5.0
13	16 (± 120%)	24 (± 139%)	bq	bq	bq	bq	bq	-5.0	-4.7	1.0
14	bq	bq	bq	bq	bq	bq	bq	-5.0	-5.0	-2.7
15	bq	bq	bq	bq	bq	bq	bq	-5.0	-5.0	-5.0
16	bq	bq	bq	bq	bq	bq	bq	-5.0	-5.0	1.2

[a] n = 2, except site 8 (n = 3). Calculated using Equation 2. [b] bq = below limit of quantification; chlorfenvinphos, α-endosulfan and fenpropidine are not displayed because all observations were below limit of quantification. [c] calculated with LC50 values taken from Tomlin (2003), see Table 1. [d] calculated from data given in Schäfer *et al.* (2007a). [e] calculated from data given in Schäfer *et al.* (2007b).

The concentrations determined with the EDS were in general a factor of 4 to 5 higher than the Chemcatchers' TWA concentrations (Figure 3). The linear regression model, encompassing EDS' concentrations as explanatory variable and the Chemcatchers' concentrations as response variable, was not significantly different between sites or compounds (analysis of variance of the models with and without the covariate factors, *F*-test, $p > 0.05$). For the log-transformed pesticide concentrations inclusion of the covariate compounds in the linear model did increase the amount of explained variance significantly (analysis of variance, *F*-test, $p < 0.01$). However, separate linear regression models for each compound yielded only two significant relationships (*t*-test, $p < 0.05$) (Figure 4).

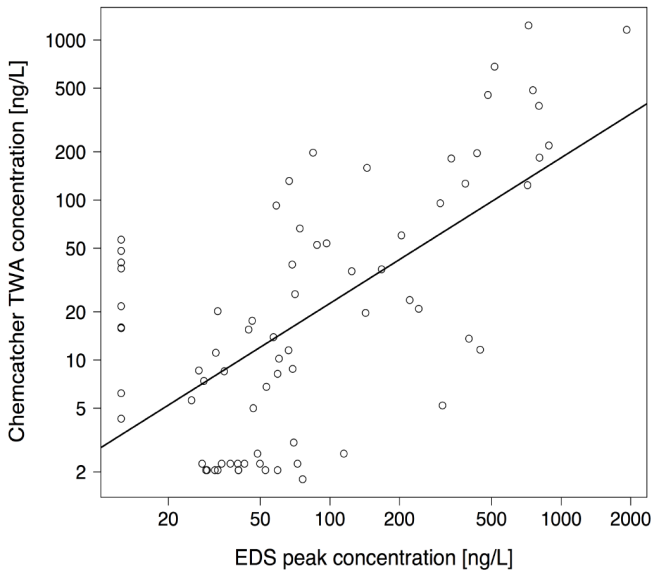

Figure 3: Relationship between the Chemcatcher® TWA concentrations and the EDS peak concentrations in 16 agricultural streams, on a double logarithmic scale. Observations that were below LOQ for both sampling methods were excluded from analysis. Model parameters: $r^2 = 0.4$, $p < 0.01$, $n = 75$. Model parameters for non log-transformed concentration: $r^2 = 0.62$, $p < 0.01$, $n = 75$.

In the suspended particles sampled with the SPS, only 5 of the 10 pesticides were observed; any of the compounds alachlor, carbofuran, linuron, oxadiazon and pirimicarb was found. The total number of pesticide detections (22) in the particulate phase was significantly reduced (χ^2-test with Bonferroni correction, $p < 0.05$) compared to both water phase methods (Table 3). No significant correlations were observed between water concentrations derived from the EDS and the Chemcatcher® on the one hand and the suspended particle concentrations monitored with the SPS on the other hand ($r = 0.05$ and 0.08, $p > 0.05$, $n = 76$ and 72, respectively).

Comparison of the three sampling methods concerning effects assessment

The STUs calculated on the basis of suspended particle concentrations were higher than the TUs based on water concentrations, with a maximum STU value of 2.5 corresponding to 321 times the LC50 for *Daphnia magna*. For water concentrations, the TUs peaked at -0.42, equivalent to 1/2.5 the LC50 value for *Daphnia magna* (Table 2). The TUs of the two water sampling methods were very similar, indicated by a *r* of 0.94 ($p < 0.01$, $n = 16$). The SPEAR index was reasonably well explained by the toxic units of the EDS and the Chemcatcher® whereas no significant linear relationship was observed between STUs and SPEAR (Table 3).

Discussion

Using the Chemcatcher® for the monitoring of polar and semi-polar pesticides

The Chemcatcher® passive sampler equipped with a SDB-XC Empore® disk detected all compounds included in the monitoring program except fenpropidine, chlorfenvinphos and α-endosulfan, although these compounds were found in samples obtained by the other sampling methods. In general, the Chemcatcher® should be suitable for detecting these substances, as they showed above average uptake-rates in the samplers' receiving phase in a calibration study (Gunold et al., 2007). The non-detections with the Chemcatcher® are not likely to result from too low concentrations because in the EDS samples, the concentrations of fenpropidine, chlorfenvinphos and α-endosulfan were not lower than those of the other monitored compounds. An explanation for the non-detection with the Chemcatcher® is that the period of exposure to these pesticides was shorter than in the case of the other compounds detected, resulting in a TWA concentration below LOQ. Since we have no temporal resolution of the water concentrations over the course of the runoff event, this issue remains unresolved. The levels of the TWA concentrations observed with the Chemcatcher® are in good agreement with another field study on 7 sites in southern England using the POCIS passive sampler, where concentrations up to 1 µg/L were reported for Diuron (Alvarez et al., 2004). Concerning variation in TWA concentrations for replicate deployments of passive samplers, some studies reported similar findings (Stuer-Lauridsen, 2005; Alvarez et al., 2007), while another study with the Chemcatcher® found lower variability (relative standard deviation (RSD) < 20%, n =2), though the exposure time was 3-fold reduced compared to our study (Escher et al.,

2006). Variation in the rate of uptake into the receiving phase may result from differences in biofouling and environmental conditions such as temperature or current velocity. Since environmental conditions are nearly identical within a single sampling point, we suggest that the variation in our study resulted from the high biofouling that was observed on the samplers after deployment (Greenwood et al., 2007). Therefore, new techniques are needed for polar passive samplers that help to reduce variability during field exposure, such as the PRC approach for non-polar compounds (Alvarez et al., 2007).

Table 3: Comparison of the three sampling systems in 16 French sites.

Sampling method	Number of different pesticides detected	Total detections above the LOQ	Explanatory power for the SPEAR index[b]
Chemcatcher®	7	54	$r^2 = 0.50$ ($p < 0.01$)
EDS	10	66	$r^2 = 0.38$ ($p = 0.01$)
SPS	5	22[a]	$r^2 = 0.01$ ($p > 0.05$)

[a] Significantly lower than the total detections by the other methods in multiple comparison tests (χ^2-test with Bonferroni correction, $p < 0.05$). [b] Linear regression with the respective TUs/STUs as explanatory variable and SPEAR as response variable.

The derived TUs could reasonably well explain variation in the SPEAR index (Table 3). This suggests that variation in the composition of the invertebrate community could partly be attributed to pesticide stress.and hence that the relative abundance of taxa classified as sensitive according to the SPEAR approach is reduced due to pesticides. A link between TWA concentrations and ecological effects was also found in two other studies (Leonard et al., 2000; Escher et al., 2006). Firstly, runoff-related endosulfan concentrations in passive samplers deployed in the Namoi river in Australia could be linked to the decline in invertebrate population densities (Leonard et al., 2000). Moreover, the Chemcatcher® was successfully employed to monitor herbicides and assess phytotoxicity in a small-scale field study in Australia (Escher et al., 2006). However, caution should be taken when relating TWA concentrations to effects on biota because no distinction can be made between a low-level chronic contamination and a short-term peak contamination on the basis of TWA concentrations. In a situation in which both chronic contamination and peak contamination are present, no link may be found between TWA concentrations and ecological effects. Furthermore, the relationship between TWA concentrations and biotic metrics will most likely not hold in situations in which more than one peak event occurs during the exposure time. Nevertheless, passive samplers with a polar receiving phase may constitute a labour- and cost-efficient tool for field monitoring of polar organic toxicants when the exposure characteristics are known and episodic events are rare.

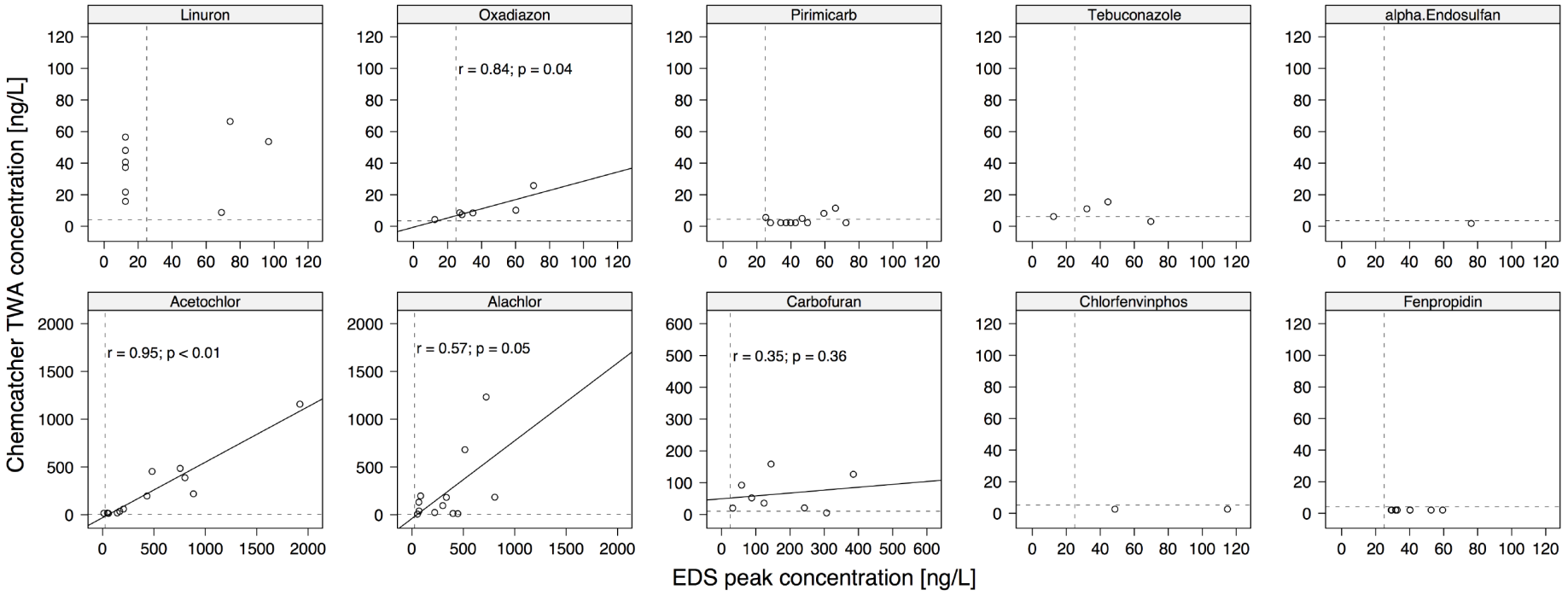

Figure 4: Relationship between the Chemcatcher® TWA concentrations and the EDS peak concentrations in 16 agricultural streams, for single compounds. Observations that were below LOQ for both sampling methods were excluded from analysis. Dashed lines indicate LOQ. *r* = Pearson's correlation coefficent. Regression lines are shown for > 3 observations above LOQ for both methods.

Comparison of the Chemcatcher® with the event-driven water sampler

The Chemcatcher® passive sampler had a slightly lower number of total detections than the EDS (Table 3) but the concentrations were closely related ($r = 0.79$, $p < 0.01$, $n = 75$). Since the EDS sampled only one precipitation-driven runoff event (Figure 1) the similarity of the TWA and EDS concentrations suggests that this event was the most relevant source of the pesticides sampled with the Chemcatcher®. Thus our findings emphasize the relevance of field runoff as input path for pesticides in aquatic ecosystems and hence are in accordance with the results of previous studies in streams of Germany (Liess et al., 1999; Neumann et al., 2002). On average, the TWA concentrations were 4- to 5-fold lower than the EDS concentrations (Figure 3). The concentrations determined with the EDS were assumed to represent peak concentrations during runoff (Liess et al., 2001; Schulz et al., 2001). Assuming that concentrations following runoff events drop to below 10% of the peak water concentration within 1 to 4 days (Richards and Baker, 1993; Leu et al., 2004), one would expect the TWA water concentrations to be in the range of 1/12 to 4/12 of the EDS concentrations, based on an average exposure time of 12 days (Equation 1). Furthermore, this should be dependent on physicochemical properties of investigated pesticides and thus lead to significant differences between compounds. Indeed, we observed a significant difference in the relationship between TWA and peak concentrations for different compounds, though only for log-transformed concentrations. Furthermore, the slopes of the regression lines were different in separate linear regressions for the various compounds (Figure 4). Nevertheless, we are aware that more extensive data are needed to prove these differences between compounds.

Comparison of the Chemcatcher® with the suspended-particles sampler

Only five pesticides were detected on the suspended particles sampled with the SPS, and the total number of detections was significantly lower compared to the Chemcatcher® (Table 3). This may be explained by the polarity of the study compounds in view of the fact that the pesticides not detected had a log $K_{ow} < 3.1$ except for oxadiazon (Table 1). Moreover, the smaller number of observations related to the SPS samples may be partly due to the LOQ, because it was a factor of 3 to 180 higher than the corresponding LOQs of the water samplers except for α-endosulfan, when assuming equilibrium partitioning between water and particulate phase (see LOQ calc., Table 1). The LOQ for the SPS could only be improved by stronger preconcentration of the eluate or extracting an increased mass of suspended particles. Besides the fact that the amount of sample

material from SPS was rather limited, both possibilities were hampered by the high magnitude of matrix coextraction masking the analyte peaks (Figure 2). Thus a more efficient size exclusion chromatography or solid phase extraction cleanup method for polar pesticides would be needed to achieve a lower LOQ (Dabrowska et al., 2003; Schäfer et al., 2007b).

Consequently, the particle-associated pesticide concentrations exhibited no significant correlation with the TWA concentrations or the EDS peak concentrations which refer to the dissolved water phase. This low similarity was also expressed by the proportion of cases (18/22) in which pesticides were found on suspended particles but not in samples collected by either the Chemcatcher® or the EDS. Similarly, no clear relationship between particle-associated contaminants and water concentrations was found in a 1-year monitoring study of 30 organic pesticides in six rivers in the UK (Long et al., 1998). Furthermore, high variability of the pesticide distribution between particulate and water phase was observed in tributaries of the Mississippi river (Pereira and Rostad, 1990) and in a field experiment on the release of six organic pesticides from a heavy clay soil during precipitation events (Brown et al., 1995). The contaminant distribution between particulate and water phase is influenced by environmental conditions, physicochemical properties and site-specific conditions that may explain the observed variation: (1) size of suspended particles, (2) composition and structure of organic matter in the particles (Zhou et al., 1995), (3) runoff-water flow rate (Gouy et al., 1999) and (4) lag time between pesticide application and runoff event. This variation in the pesticide partitioning between particulate and dissolved phase (Brown et al., 1995; Long et al., 1998) along with the high LOQ can explain why the results of the sampling with the SPS and the Chemcatcher® were very different.

Although the SPS samples indicated much higher pesticide stress in terms of STU compared to the TUs derived from the TWA and peak concentrations, no significant relationship could be established to the SPEAR index. By contrast, other studies demonstrated significant linear relationships between STUs derived from bed sediments and the benthic community tolerance metrics (Wildhaber and Schmitt, 1998) or macroinvertebrate community composition (Friberg et al., 2003). The differing results of our study most likely result from monitoring suspended particle concentrations instead of bed-sediment concentrations. Suspended particles in field runoff usually have much higher contaminant concentrations than bed sediments and are rarely in equilibrium with the water phase, rendering questionable the application of the STU approach (Liess et al.,

1996; Long et al., 1998). In the present study, results from passive sampling and event-driven water sampling were more informative when used to explain variation in the invertebrate community. We propose that water concentrations are more likely to explain effects of episodic events with polar toxicants, whereas the effects of chronic exposure to hydrophobic compounds may be predicted from analysis of the sediment phase. However, this should be tested in future studies, and passive samplers in different configurations can be useful tools for such studies.

Conclusions

- The Chemcatcher® can be employed for continuous water sampling of polar organic toxicants for up to 14 days.
- The Chemcatcher® configured with a SDB-XC Empore® and without diffusion-limiting membrane represents a promising method for the monitoring of short-term exposure that conventional spot water sampling is likely to miss.
- Given the increasing attention that is paid to polar substances, a method similar to the performance reference compound concept is needed to account for variation in the passive sampling of polar compounds.
- Exposure assessment with the Chemcatcher® passive sampler yields results similar to water sampling but differs from suspended-particles sampling.
- In large-scale studies with frequently recurring pollution events, the Chemcatcher® is more labour- and cost-efficient than event-driven water sampling.

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References

Alvarez, D.A., Huckins, J.N., Petty, J.D., Jones-Lepp, T., Stuer-Lauridsen, F., Getting, D.T., Goddard, J.P. and Gravell, A., 2007. Tool for monitoring hydrophilic contaminants in water: polar organic chemical integrative sampler (POCIS). Greenwood, R., Mills, G.A. and Vrana, B. (eds): Comprehensive Analytical Chemistry 48: Passive sampling techniques in environmental monitoring, pp. 171-197, Elsevier, Amsterdam.

Alvarez, D.A., Petty, J.D., Huckins, J.N., Jones-Lepp, T.L., Getting, D.T., Goddard, J.P. and Manahan, S.E., 2004. Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. Environmental Toxicology and Chemistry 23 (7), 1640-1648.

Brown, C.D., Hodgkinson, R.A., Rose, D.A., Syers, J.K. and Wilcockson, S.J., 1995. Movement of pesticides to surface waters from a heavy clay soil. Pesticide Science 43 (2), 131-140.

Clarke, J.U., 1998. Evaluation of censored data methods to allow statistical comparisons among very small samples with below detection limit observations. Environmental Science & Technology 32 (1), 177-183.

Dabrowska, H., Dabrowski, L., Biziuk, M., Gaca, J. and Namiesnik, J., 2003. Solid-phase extraction clean-up of soil and sediment extracts for the determination of various types of pollutants in a single run. Journal Of Chromatography A 1003 (1-2), 29-42.

Escher, B.I., Quayle, P., Muller, R., Schreiber, U. and Mueller, J.F., 2006. Passive sampling of herbicides combined with effect analysis in algae using a novel high-throughput phytotoxicity assay (Maxi-Imaging-PAM). Journal of Environmental Monitoring 8 (4), 456-464.

Friberg, N., Lindstrom, M., Kronvang, B. and Larsen, S.E., 2003. Macroinvertebrate/sediment relationships along a pesticide gradient in Danish streams. Hydrobiologia 494 (1-3), 103-110.

Gouy, V., Dur, J.C., Calvet, R., Belamie, R. and Chaplain, V., 1999. Influence of adsorption-desorption phenomena on pesticide run-off from soil using simulated rainfall. Pesticide Science 55 (2), 175-182.

Greenwood, R., Mills, G.A., Vrana, B., Allan, I.J., Aguilar-Martinez, R. and Morrison, G., 2007. Monitoring of priority pollutants in water using Chemcatcher passive sampling devices. Greenwood, R., Mills, G.A. and Vrana, B. (eds): Comprehensive Analytical Chemistry 48: Passive sampling techniques in environmental monitoring, pp. 199-229, Elsevier, Amsterdam.

Gunold, R., Schäfer, R.B., Paschke, A., Schüürmann, G. and Liess, M., 2007. Calibration of the Chemcatcher passive sampler for monitoring selected polar and semi-polar pesticides in surface water. Environmental Pollution, in press, doi: 10.1016/j.envpol.2007.10.037.

Huckins, J.N., Petty, J.D., Lebo, J.A., Almeida, F.V., Booij, K., Alvarez, D.A., Clark, R.C. and Mogensen, B.B., 2002. Development of the permeability/performance reference compound approach for in situ calibration of semipermeable membrane devices. Environmental Science and Technology 36 (1), 85-91.

Kingston, J.K., Greenwood, R., Mills, G.A., Morrison, G.M. and Persson, L.B., 2000. Development of a novel passive sampling system for the time-averaged measurement of a range of organic pollutants in aquatic environments. Journal of Environmental Monitoring 2 (5), 487-495.

Leonard, A.W., Hyne, R.V., Lim, R.P., Pablo, F. and Van den Brink, P.J., 2000. Riverine endosulfan concentrations in the Namoi River, Australia: Link to cotton field runoff and macroinvertebrate population densities. Environmental Toxicology and Chemistry 19 (6), 1540-1551.

Leu, C., Singer, H., Stamm, C., Muller, S.R. and Schwarzenbach, R.P., 2004. Simultaneous assessment of sources, processes, and factors influencing herbicide losses to surface waters in a small agricultural catchment. Environmental Science and Technology 38 (14), 3827-3834.

Liess, M., Schulz, R., Berenzen, N., Nanko-Drees, J. and Wogram, J., 2001. Pesticide contamination and macroinvertebrate communities in running waters in agricultural areas. UBA Texte 65, Umweltbundesamt, Berlin, pp. 227.

Liess, M., Schulz, R., Liess, M.H.-D., Rother, B. and Kreuzig, R., 1999. Determination of insecticide contamination in agricultural headwater streams. Water Research 33 (1), 239-247.

Liess, M., Schulz, R. and Neumann, M., 1996. A method for monitoring pesticides bound to suspended particles in small streams. Chemosphere 32 (10), 1963-1969.

Liess, M. and von der Ohe, P.C., 2005. Analyzing effects of pesticides on invertebrate communities in streams. Environmental Toxicology and Chemistry 24 (4), 954-965.

Long, J.L.A., House, W.A., Parker, A. and Rae, J.E., 1998. Micro-organic compounds associated with sediments in the Humber rivers. Science of the Total Environment 210 (1-6), 229-253.

Neumann, M., Schulz, R., Schäfer, K., Müller, W., Mannheller, W. and Liess, M., 2002. The significance of entry routes as point and non-point sources of pesticides in small streams. Water Research 36 (4), 835-842.

Oerke, E.C. and Dehne, H.W., 2004. Safeguarding production - losses in major crops and the role of crop protection. Crop Protection 23 (4), 275-285.

Pereira, W.E. and Rostad, C.E., 1990. Occurrence, distributions, and transport of herbicides and their degradation products in the lower Mississippi river and its tributaries. Environmental Science and Technology 24 (9), 1400-1406.

Richards, R.P. and Baker, D.B., 1993. Pesticide concentration patterns in agricultural drainage networks in the Lake Erie basin. Environmental Toxicology and Chemistry 12 (1), 13-26.

Sabljic, A., Gusten, H., Verhaar, H. and Hermens, J., 1995. QSAR Modeling of Soil Sorption - Improvements and Systematics of Log Koc vs. Log Kow Correlations. Chemosphere 31 (11-12), 4489-4514.

Schäfer, R.B., Caquet, T., Siimes, K., Mueller, R., Lagadic, L. and Liess, M., 2007a. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. Science of the Total Environment 382 (2-3), 272-285.

Schäfer, R.B., Mueller, R., Brack, W., Wenzel, K.-D., Streck, G. and Liess, M., 2007b. Determination of 10 particle-associated multiclass polar and semi-polar pesticides from small streams using accelerated solvent extraction. Chemosphere 70: 1952-1960.

Schulz, R., Peall, S.K.C., Dabrowski, J.M. and Reinecke, A.J., 2001. Current-use insecticides, phosphates and suspended solids in the Lourens River, Western Cape, during the first rainfall event of the wet season. Water SA 27 (1), 65-70.

Stuer-Lauridsen, F., 2005. Review of passive accumulation devices for monitoring organic micropollutants in the aquatic environment. Environmental Pollution 136 (3), 503-524.

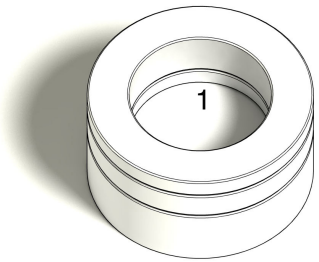
Tomlin, C.D.S., 2003. The pesticide manual, a world compendium BCPC Publications, Hampshire, UK.

Vrana, B., Allan, I.J., Greenwood, R., Mills, G.A., Dominiak, E., Svensson, K., Knutsson, J. and Morrison, G., 2005. Passive sampling techniques for monitoring pollutants in water. TrAC - Trends in Analytical Chemistry 24 (10), 845-868.

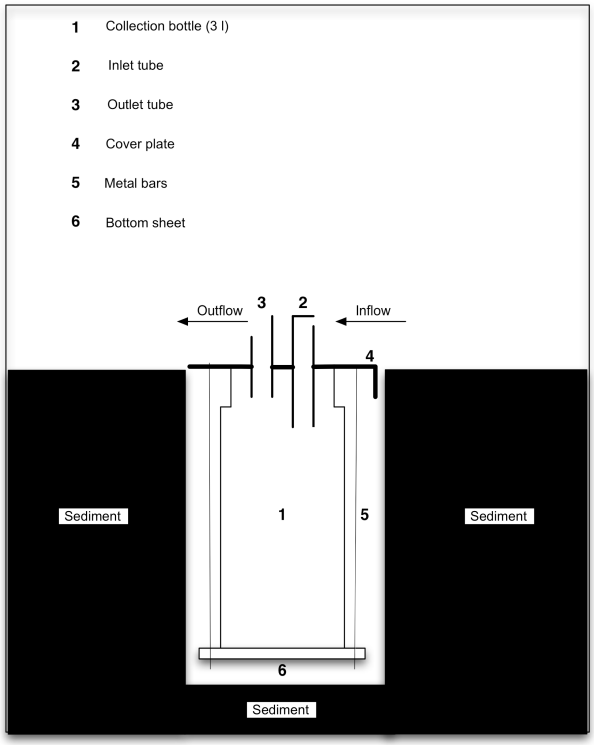
Wildhaber, M.L. and Schmitt, C.J., 1998. Indices of benthic community tolerance in contaminated Great Lakes sediments: Relations with sediment contaminant concentrations, sediment toxicity, and the sediment quality triad. Environmental Monitoring and Assessment 49 (1), 23-49.

Zhou, J.L., Rowland, S. and Mantoura, R.F.C., 1995. Partition of synthetic pyrethroid insecticides between dissolved and particulate phases. Water Research 29 (4), 1023-1031.

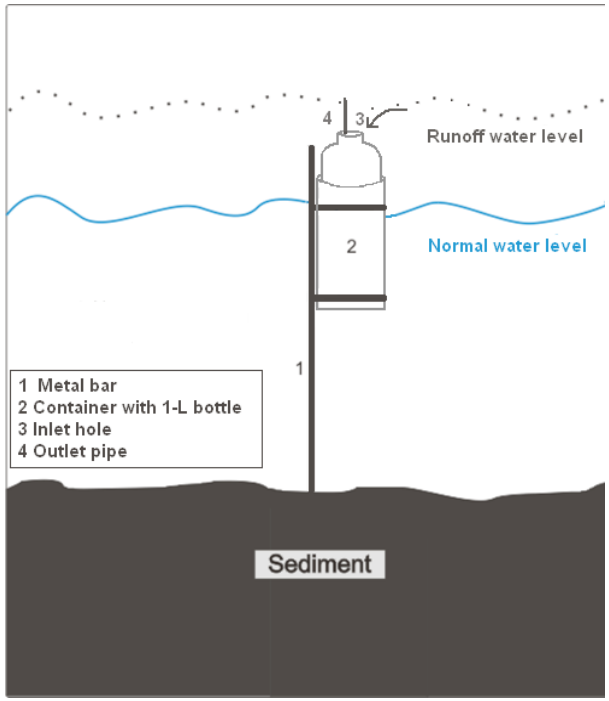
Supplementary material



Chemcatcher® passive sampler with SDB-XC receiving phase (1). The receiving phase was directed towards the stream bottom during deployment.



Suspended-particle sampler (SPS)



Event-driven water sampler (EDS)

Kapitel 5: Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe

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Abstract

There is a paucity of large-scale field investigations on the effects of organic toxicants on stream macroinvertebrate community structure and ecosystem functions. We investigated a total of 29 streams in two study areas of France and Finland for pesticide exposure, invertebrates and leaf litter breakdown. To link pesticide exposure and community composition we applied the trait-based Species At Risk (SPEAR) indicator system.

In the French region, pesticide stress was associated with a decrease in the relative abundance and number of sensitive species in the communities. The presence of undisturbed upstream reaches partly compensated the effects of pesticide contamination. Functional effects of pesticides were identified by a 2.5-fold reduction of the leaf litter breakdown rate that was closely correlated with the structural changes in the contaminated streams. No effects of pesticides were observed in Finnish streams since contamination with pesticides was very low.

In a follow-up analysis, the SPEAR approach successfully discriminated between reference and contaminated sites across different biogeographical regions, also including

results of a previous field study in North Germany. Furthermore, change of the community structure was detectable at a concentration range as low as 1/100 to 1/1000 the acute 48h-LC50 of *Daphnia magna*. Our findings demonstrate that pesticides may influence the structure and function of lotic ecosystems and that the SPEAR approach can be used as a powerful tool in biomonitoring over large spatial scales.

Introduction

Pesticides represent a relevant stressor for many aquatic and terrestrial species (Liess et al., 2005b). They have been shown to potentially affect all groups of organisms in aquatic ecosystems: e.g. microorganisms (DeLorenzo et al., 2001), invertebrates (Castillo et al., 2006), plants (Frankart et al., 2003) and fish (Grande et al., 1994).

Although some field studies demonstrated effects of heavy metals on the aquatic community structure at the regional scale, there is a paucity of such investigations for organic toxicants, encompassing more than one stream or river (Clements et al., 2000; Beasley et al., 2003; Maret et al., 2003). Furthermore, the effects of current-use pesticides on important stream ecosystem functions such as leaf litter breakdown (Wallace et al., 1997) are still largely unknown.

The establishment of a causal relationship between a stressor and effects can be hampered by natural variability, as every sampling site exhibits a unique combination of environmental variables and species (Liess et al., 2005b). In addition, confounding factors like the occurrence of other anthropogenic or natural stressors can mask the effects of a particular stressor. The use of species traits, such as generation time or dispersal capacity, represents an interesting approach towards encompassing both natural variability and confounding factors (Statzner et al., 2005). As most stressors or environmental gradients affect only certain trait modalities, called response traits, trait-based approaches may be used to identify the effects of a specific stressor e.g. pesticides. At the community level, the relative abundance or number of species with certain trait modalities would probably decrease thus making it possible to interpret and/or predict community change (Statzner et al., 2005). Recently, Liess and von der Ohe (2005) developed a trait-based concept with which to distinguish pesticide effects on freshwater macroinvertebrates from the influence of other environmental variables. This concept, called Species At Risk (SPEAR), classifies macroinvertebrates according to their vulnerability towards pesticides into

sensitive species (SPEAR) and tolerant species (SPEnotAR), as evaluated by selected ecological and physiological traits. The authors successfully employed this approach in a field study on 20 streams in North Germany, where the relative abundance of SPEAR in a community declined with increasing pesticide stress. Furthermore, pesticide stress was the most important explanatory variable for different community-based SPEAR-endpoints (Liess et al., 2005a). In another study, Schriever et al. (2007) demonstrated that the highest correlation between the fraction of sensitive species and various environmental parameters was obtained for a modelled indicator of pesticide exposure, called runoff potential.

In the present study we aimed at investigating if (1) the use of the SPEAR concept in biomonitoring may be extended beyond North Germany to different biogeographical regions (Illies, 1978) and (2) pesticides have effects not only on the structure but also on the functioning of aquatic ecosystems. Therefore, we conducted field investigations in two regions of France and Finland in which the macroinvertebrate communities, leaf litter breakdown, pesticides and physico-chemical characteristics of 29 streams were monitored during the period of pesticide application. Considering the differences in agricultural practices and especially pesticide use between these countries, we were also able to examine whether the effects of pesticides on non-target organisms are dependent on usage patterns or if the invertebrate communities adapt accordingly. To our knowledge, this is the first study that comparatively investigates pesticide effects in different biogeographical regions.

To further evaluate the performance of the SPEAR approach in large-scale biomonitoring we analyzed its ability to discriminate reference and contaminated sites across different biogeographical regions, also including the sites of the previous field study in North Germany (Liess et al., 2005a).

Methods

Study area and sampling schedule

France and Finland were selected as study countries because they belong to different biogeographical regions (Illies, 1978) and exhibit contrasting pesticide use with an average of approximately 6 and 0.8 kg annually applied active ingredient per hectare,

respectively (EUROSTAT, 2002). This difference partly stems from the lower prevalence of pests in Finland since the northward dispersal of many pests is averted by the colder climate. In France, Brittany in the northwest was chosen as study area because the local authorities reported frequent regional and temporal contamination of streams with pesticides from 2002 to 2004 (DIREN, 2005). A total of 16 sampling sites in first- to third-order lowland streams (Strahler, 1957) were selected which were expected to exhibit a gradient in pesticide contamination based upon the analysis of local authorities' monitoring data (Regional Agency for Agriculture and Forestry (DRAF) Bretagne, personal communication). Since Finnish agriculture is mainly localized in the southern part of the country, this region was chosen for the specification of sampling sites. 13 sites were selected in first- to third-order lowland streams covering different areas of South Finland.

All streams in the two regions of France and Finland were selected to match the physical properties of those sampled during a previous field study in North Germany (Liess et al., 2005a): no drying up in summer; no dredging in the present or past year; presence of adjacent fields with vegetable, corn or oil-seed crops; average stream current velocity ranging between 0.1 and 0.5 m/s; maximum stream depth of 0.8 m. Furthermore, the sites were checked in field survey and with maps (France: IGN 1:25,000 maps, Finland: Maanmittauslaitos 1:50,000 maps) to have no waste-water treatment plants, industrial facilities or mining drainage upstream. Thus, pollution other than from agricultural sources was unlikely. The location of all sampling sites is displayed in Figure 1.

The sites were sampled before (14-19 April 2005 in France, 3-9 July 2005 in Finland) and during (19-26 May 2005 in France, 1-6 August 2005 in Finland) the estimated period of maximum pesticide contamination, according to the monitoring data from local authorities (France: DRAF Bretagne, Finland: Finnish Environment Institute (SYKE), personal communication). However, the timing of pesticide application varies and the sites may therefore have received pesticide input before the initiation of sampling. This holds especially for France, where strong rain was recorded three days before the first sampling date, possibly leading to pesticide runoff.If stated below, we also included in the analysis the results of the study in the German region for April and May, averaged for the 3 years of study (Liess et al., 2005a). We are aware that the results of non-randomly chosen, single regions cannot be extrapolated to the country level. However, for the ease of reading we refer to the respective region with the countries' name throughout this paper.

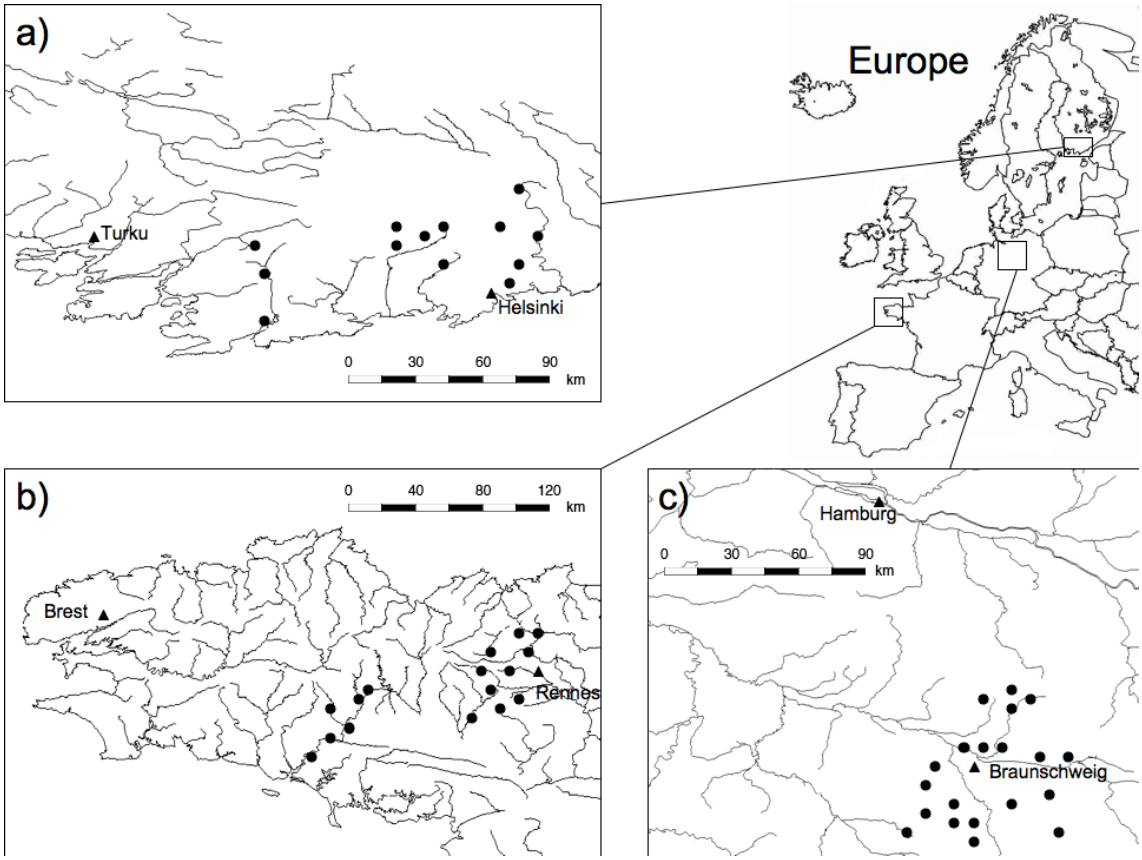

Figure 1: Map of sampling sites and large rivers in Finland (a), France (b) and Germany (c). Sampling streams are not displayed due to scale. Regional maps were created using ESRI World Basemap Data and the European map was created with R (packages: maps and mapdata).

Physico-chemical and geographical parameters

Concentrations of oxygen, ammonium, nitrite, nitrate and orthophosphate in the stream water as well as temperature, pH and stream current velocity were measured as described in Liess and von der Ohe (2005). Water conductivity was determined on site with a device Multi 340i device of WTW (Weilheim, Germany). Total water hardness was measured in the field with an Aquamerck test (precision: 1°dH; Merck, Darmstadt, Germany). Suspended matter was collected in suspended-matter samplers (Liess et al., 1996), measured biweekly and converted into a volume (ml) per week. In-stream structure, depth, width, tailing and buffer strip width were investigated in a 50-m reach upstream and downstream from the sampling site.

Previous studies demonstrated that the presence of forested upstream stretches that are undisturbed in terms of agricultural activities positively influenced downstream habitat quality and partly compensated for the effects of pesticides (Liess et al., 2005a; Schriever et al., 2007). Therefore we inspected the French and Finnish streams upstream of each

sampling site in field survey or with maps (France: IGN 1:25,000 maps, Finland: Maanmittauslaitos 1:50,000 maps) for the presence of riparian forests. If double-sided riparian forests at least 100 m in length were present in the 3-km reach upstream of a sampling site, we categorized it as having an undisturbed upstream reach. Modification of these criteria such as different upstream distances (2 or 4 km) or the presence of single-sided instead of double-sided forest stretches had no appreciable effects on the results of the present study. An overview of the stream characteristics, including the sites previously investigated in Germany (Liess et al., 2005a) is given in Table 1.

Pesticide monitoring and chemical analysis

The substances for the screening programmes in France and Finland were selected by (1) identifying potential compounds based upon the analysis of previous regulatory monitoring programmes (France: DRAF Bretagne, Finland: Finnish Environment Institute (SYKE), personal communication) and (2) ranking them according to their toxicity, indicated by the 48-h acute median lethal concentration (LC50) for *Daphnia magna* as given in Tomlin (2001). The 10 most toxic pesticides included in the respective screening program were mainly non-polar (log K_{ow} > 4) for Finland and polar to semi-polar (log K_{ow} < 4) for France (Table 2). The sampling methods were arranged to catch runoff-induced exposure because this is a major input path for pesticides in small streams (Neumann et al., 2002). They were locally adapted due to differences in polarity and expected concentration levels of the compounds.

In France, runoff-triggered water samplers (Liess et al., 1999b) were deployed and retrieved after heavy rain events (> 10 mm precipitation per day). The water samples were subsequently solid-phase-extracted using 6 ml Chromabond HR-P columns (Macherey-Nagel, Düren, Germany). Analytes trapped on the columns were extracted with 10 ml of 1:1 acetonitrile-ethylacetate and the extract gently evaporated under nitrogen to 0.3 ml. Residue analysis was conducted on an Agilent 6890N (Agilent Technologies Germany, Boeblingen, Germany) gas chromatograph (GC) linked to an Agilent 5973 mass selective detector (MSD).

In Finland, continuous water passive sampling was performed with low-density polyethylene (LDPE) strips (Booij et al., 2003), which were deployed in each stream at the beginning of the study and exposed for 28 days. LDPE strips were extracted by soaking in 500 ml n-hexane for 48 h.

Table 1: Descripitive statistics of environmental parameters at the study sites in France, Finland and Germany.

		France[5]					Finland[5]					Germany[6]				
Parameter	unit	Mean	SD	% SD	Min.	Max.	Mean	SD	% SD	Min.	Max.	Mean	SD	% SD	Min.	Max.
Water temperature[1]	°C	12.58	1.21	9.65	10.55	15.40	17.74	1.89	10.64	13.30	20.10	13.30	3.00	22.56	3.50	19.50
pH[1]		6.94	0.30	4.34	6.59	7.60	6.97	0.34	4.93	6.45	7.65	7.90	0.34	4.30	6.80	8.60
Ammonium	mg/L	0.07	0.10	143.85	0.00	0.38	0.11	0.28	261.77	0.00	1.00	0.07	0.21	300.00	0	1.75
Nitrite	mg/L	0.06	0.05	78.77	0.00	0.13	0.05	0.14	273.62	0.00	0.50	0.15	0.13	86.67	0.01	0.80
Nitrate	mg/L	15.63	7.83	50.09	0.00	27.50	0.00	0.00	0.00	0.00	0.00	3.40	9.20	270.59	0.50	47.5
Orthophosphate	mg/L	1.01	0.91	90.75	0.13	3.50	0.20	0.23	114.53	0.00	0.88	0.19	0.13	68.42	0.00	0.60
Hardness[1]	°dH	6.28	2.13	33.89	3.00	9.00	4.54	0.97	21.32	3.00	6.50	not measured				
Conductivity	µS/cm	183.23	87.72	47.87	86.00	387.00	160.54	58.96	36.72	76.00	277.00	not measured				
Oxygen	mg/L	10.63	0.98	9.21	8.80	12.10	10.21	2.55	24.99	5.86	15.80	10.20	2.20	21.57	3.40	13.80
Current velocity	m/s	0.31	0.14	46.32	0.10	0.73	0.27	0.10	37.00	0.15	0.50	0.17	0.09	52.94	0.02	0.50
Depth	m	0.27	0.11	40.27	0.15	0.60	0.27	0.11	43.09	0.10	0.40	0.16	0.10	62.50	0.04	0.60
Width	m	2.61	1.04	39.73	1.00	4.50	2.12	0.77	36.30	1.00	3.00	1.30	0.44	33.85	0.50	2.50
Tailing	%	48.28	18.11	37.52	17.50	80.00	63.08	22.78	36.11	30.00	90.00	not measured				
Twigs	%	6.63	2.63	39.70	3.50	12.50	8.46	4.27	50.51	0.00	15.00	not measured				
Free substrate[2]	%	64.09	15.84	24.71	30.00	78.00	72.31	14.52	20.08	45.00	85.00	not measured				
Allochthonous leafs	%	8.28	3.38	40.83	5.00	15.00	2.69	6.96	258.40	0.00	25.00	20.00	28.0	140.00	0.00	100.00
Submersed plants[2]	%	14.75	16.36	110.88	2.50	57.50	5.00	8.16	163.30	0.00	30.00	8.00	11.0	137.50	0.00	50.00
Emersed plants	%	4.13	4.15	100.69	0.00	15.00	9.23	9.97	107.99	0.00	30.00	5.00	9.00	180.00	0.00	65.00
Filamentous algea	%	2.13	2.96	139.20	0.00	10.00	2.31	2.59	112.42	0.00	5.00	1.00	4.00	400.00	0.00	25.00
Boulder (> 20 cm)	%	6.25	8.06	129.00	0.00	35.00	3.08	3.25	105.70	0.00	10.00	0.00	0.00	0.00	0.00	0.00
Cobble (5-20 cm)	%	7.50	6.83	91.08	0.00	20.00	13.46	12.81	95.16	5.00	50.00	2.00	7.00	350.00	0.00	30.00
Gravel (1-5 cm)[4]	%	15.31	10.24	66.89	5.00	30.00	13.46	8.75	65.02	0.00	30.00	5.00	10.0	200.00	0.00	40.00
Grit (0,1-1 cm)	%	21.25	9.40	44.23	10.00	50.00	16.92	10.52	62.14	5.00	30.00	other classification				
Sand (0,01-0,1 cm)	%	27.19	13.54	49.79	10.00	55.00	28.46	13.75	48.32	10.00	60.00	24.00	37.0	154.17	0.00	100.00
Clay and silt (< 0,01)[3,4]	%	22.81	15.70	68.83	0.00	50.00	24.62	18.54	75.30	5.00	65.00	55.00	46.0	83.64	0.00	100.00
Suspended matter	ml/wk	180.39	150.45	83.40	28.27	565.49	221.48	198.0	89.42	0.00	565.49	161.0	69.0	42.86	77.0	294.00
Buffer strip width[3]	m	11.56	7.85	67.88	0.00	20.00	2.60	0.97	37.31	2.00	5.00	not measured				
Altitude	m	67.53	41.00	60.71	22.00	143.00	32.69	19.80	60.57	-5.00	68.00	not measured				

[1] intercorrelation in France (Spearman's rho > 0.8) [2] intercorrelation in France (Spearman's rho = -0.836) [3] intercorrelation in France (Spearman's rho = -0.817)
[4]intercorrelation in Finland (Spearman's rho = -0.802) [5] measured twice in 2005 [6] measured between 1998 and 2000; taken from the field study of Liess & von der Ohe (2005)

Table 2: Characteristics and measurement results of pesticides in French+Finnish streams.

Compound	Monit. program	Type [1,2]	Class[2]	LC50 (µg/L)[2]	Log K_{ow}[2]	Max. conc. (µg/L)[3]	Max. TU
acetochlor	France	H	chloroacetamide	9000	4.14	1.920	-3.67
alachlore	France	H	chloroacetamide	10000	3.09	0.806	-4.09
α-endosulfan	France	I	organochlorine	75	4.74	0.076	-2.99
carbofuran	France	I	carbamate	38.6	1.52	0.715	-1.73
chlorfenvinphos	France	I	organic phosphorous acid	0.3	3.85	0.115	-0.42
fenpropidine	France	F	piperidine	500	2.59	0.059	-3.93
linuron	France	H	urea	120	3.00	0.097	-3.09
oxadiazon	France	H	oxadiazole	2400	4.91	0.071	-4.53
pirimicarb	France	I	carbamate	17	1.70	0.072	-2.37
tebuconazole	France	F	triazole	4200	3.70	0.070	-4.78
α-cypermethrin	Finland	I	pyrethroid	0.15	6.94	n.d.	n.d.
α-endosulfan	Finland	I	organochlorine	75	4.70	n.d.	n.d.
azoxystrobin	Finland	F	strobilurine	259	2.50	n.d.	n.d.
cyprodinil	Finland	F	pyrimidine	10	3.90	n.d.	n.d.
deltamethrin	Finland	I	pyrethroid	3.5	6.20	n.d.	n.d.
λ-cyhalothrin	Finland	I	pyrethroid	0.38	7.00	n.d.	n.d.
malathion	Finland	I	organic thio–phosphorous acid	1	2.75	n.d.	n.d.
sulfotep	Finland	I	organic thio–phosphorous acid	2	3.99	n.d.	n.d.
τ-fluvalinate	Finland	I	pyrethroid	1	6.70	n.d.	n.d.
trifluralin	Finland	F	dinitroaniline	245	4.80	0.001[4]	-4.34

[1] H = Herbicide, F = Fungicide and I = Insecticide [2] taken from Tomlin (2001)
[3] n.d. = not detected [4] Time-weighted average concentration

The extract was gently evaporated to 0.3 ml under nitrogen. Residue analysis was performed on an Agilent 6890N GC linked to a Pegasus III time-of-flight (TOF) mass spectrometer (Leco, Mönchengladbach, Germany). Time-weighted average (TWA) water concentrations for the LDPE samplers were calculated according to distribution coefficients from Booij et al. (2003). TWA water concentrations were converted to peak water concentrations by multiplying the TWA concentrations by a factor of 10 (Schäfer, R.B., Paschke, A. and Liess, M., *unpublished data*). Although the sampling methods differed, we think that the outcomes are comparable as the results of passive sampling and runoff-triggered water sampling correlated very high (Pearson's r=0.995) in another study on the French streams (Schäfer et al., in preparation).

Calculation of toxicity levels

To compare the toxicity associated with the pesticide concentrations measured in the different sites, toxic units (TU) were computed from the peak water concentrations determined for each site (Liess et al., 2005a):

$$TU_{(D.magna)} = \max_{i=1}^{n}(\log(C_i/LC50_i)) \quad (1)$$

where $TU_{(D.\ magna)}$ is the maximum toxic unit of the *n* pesticides detected at the considered site, C_i is the concentration (µg/L) of pesticide *i* and $LC50_i$ is the 48h-LC50 of pesticide *i* for *Daphnia magna* (µg/L) as given in Tomlin (2001). Although peak water concentrations may have been underrated due to a delayed response of the sampling system, we assume the computed $TU_{(D.\ magna)}$ to be a conservative measure of pesticide toxicity since (1) pesticide concentrations usually decrease strongly within 24h during runoff but the 48h-LC50 of *Daphnia magna* was used for toxicity assessment (Richards et al., 1993) and (2) only the maximum toxic unit was considered instead of the sum toxicity of the pesticides detected at the respective site. If no pesticide was found a TU-value of -5 was assigned to that site, corresponding to the value found for unpolluted streams in a previous study (Liess et al., 2005a).

Macroinvertebrate sampling

Four replicate samples (surface ca. 0.12 m^2 per sample) of different substrates were taken on each sampling date with a 500-µm mesh-size Surber Sampler (Hydro-Bios, Kiel, Germany) and preserved with formalin (ca. 4% vol.). The invertebrates were sorted out, counted and identified to the lowest possible taxonomic level, which was genus for most taxa. A list of the taxa found in France and Finland along with their frequency in the samples is given in the supplementary material.

SPEAR-index calculation and endpoints

The identified taxa were classified into SPEAR and SPEnotAR according to ecological and physiological traits as described in Liess and von der Ohe (2005). Since life-cycle traits such as emergence time and voltinism are dependent on the biogeographical region (e.g. Central and Northern Europe), the classification for a particular taxon may differ between regions. The available data and region-dependent classification information are compiled in a database which is publicly available and comprises about 1000 macroinvertebrate taxa (Liess et al., 2006). After classification into SPEAR and

SPEnotAR the relative abundance of taxa which are potentially sensitive towards pesticides in a community, was computed for each site and date:

$$\%\mathrm{SPEAR}_{(\mathrm{abundance})} = \frac{\sum_{i=1}^{n} \log(x_i + 1) * y}{\sum_{i=1}^{n} \log(x_i + 1)} * 100 \quad (2)$$

where n is the number of taxa, x_i is the abundance of taxon *i* and y is: 1 if taxon *i* is classified as SPEAR, otherwise 0. Similarly, we calculated the values for another community endpoint $\%\mathrm{SPEAR}_{(\mathrm{PM\ abundance})}$, where classification of taxa relies only on physiological sensitivity (P) and migration ability (M) to exclude biogeographical bias of this index. $\%\mathrm{SPEAR}_{(\mathrm{PM\ abundance})}$ was used to examine the applicability of the SPEAR concept across different regions. The endpoint $\%\mathrm{SPEAR}_{(\mathrm{number})}$, which indicates the relative number of sensitive taxa, was computed for each site and date by:

$$\%\mathrm{SPEAR}_{(\mathrm{number})} = \frac{\sum_{i=1}^{n} y}{n} * 100 \quad (3)$$

Finally, the endpoint $\mathrm{SPEAR}_{(\mathrm{abundance\ during/before})}$ was computed by dividing the $\%\mathrm{SPEAR}_{(\mathrm{abundance})}$ during the period of maximum pesticide input in streams (France: May, Finland: August) by $\%\mathrm{SPEAR}_{(\mathrm{abundance})}$ before this period (France: April, Finland: July). Time periods were estimated on the basis of information from local authorities and as reported elsewhere (Liess et al., 1999a; Liess et al., 2005a).

Leaf litter breakdown

Three gram of air-dried *Alnus glutinosa* leaves at abscission were anchored to the streams in coarse (mesh size: app. 6 mm; polyethylene bag size: 20 x 20 cm) and fine (mesh size: 50 µm; nylon cylinder size: 15 cm length, 7.5 cm diameter) enclosures. Leaves in the coarse bags were accessible to invertebrates whereas leaves in the fine bags were not and served as control for microbial degradation and leaching (Gessner et al., 2002). Triplicate coarse and fine bags were deployed after the first sampling for approximately 21 days in 12 randomly selected sites in France and in 8 sites in Finland. To correct for handling losses three coarse and fine bags were treated the same way as the others but returned immediately to the laboratory after a brief deployment in the stream. After retrieval of the bags, the remaining litter was washed, oven-dried to a constant mass at 60° C (24 to

48 h), reweighed and averaged for each type of bags for every station. The remaining leaf mass $W_t(s)$ in grams for station s after time t was obtained by summing up the handling-loss corrected weight of the remaining litter at site s from the coarse enclosures and the loss due to microbial degradation and leaching at site s derived from the fine enclosures (for details see Benfield (1996)). The exponential leaf breakdown rate k_s was computed by:

$$k_s = \frac{-\ln\left(\frac{W_t(s)}{W_0(s)}\right)}{t_s} \quad (4)$$

where $W_0(s)$ is the initial leaf mass in grams at site s and t_s is the deployment time for the considered site (other variables as defined above) (Benfield, 1996).

Data analysis

The data analysis was performed separately for the Finnish and French sites if not otherwise indicated. Prior to analysis, the average values for the two sampling dates were calculated for all variables that were measured twice at each site, in order to avoid temporal pseudoreplication. However, we also conducted the analyses for each single measurement date. The results broadly confirmed the findings of the analyses performed for the combined data set and they are therefore not shown. Hierarchical cluster analysis of environmental variables using Spearman's rho as similarity measure was performed to check for collinearity and redundancy among environmental variables (McGarigal et al., 2000). Environmental variables that exhibited strong correlation with other variables (Spearman's rho > 0.8; see Table 1) but were implausible an explanation of differences in macroinvertebrate assemblages, on the basis of common ecological knowledge (Allan, 1995), were removed from the data set to avoid misspecification of the linear model (Flack et al., 1987; McGarigal et al., 2000).

Multiple linear regression was applied to identify the environmental variables that were best suited to explain the different SPEAR metrics and the leaf decomposition rate *k*. We weighted the sites in the regression according to the total log (x+1) abundance of species (only for SPEAR metrics). We employed manual model building, defining models on the basis of expert judgement and automatic model building starting with the null model (no explanatory variable included) or the full model (all explanatory variables included). The statistical procedure was backward and forward entering of variables with Akaike's

Information Criterion as stepwise model selection criterion (Akaike, 1974). Model simplification was performed using t-test for significance of single variables and analysis of variance (ANOVA) with F-test for significance of the complete model. Models with different numbers of parameters were compared with the F-test. Goodness of fit was assessed with the adjusted r^2 (r^2 for models with only one explanatory variable). Analysis of covariance (ANCOVA) with t-test was applied to check for significant differences in slope or intercept for factors in regression. Model checking included: heteroscedasticity, normal distribution of residuals and influence of single observations using residual-leverage plots and Cook's distance. We applied hierarchical-partitioning to determine the relative importance of independent explanatory variables in the linear models (Chevan et al., 1991).

To detect effects of pesticide input on SPEAR metrics or *k* in a single country, the respective values were split according to the $TU_{(D.\ magna)}$ into sites potentially receiving ($TU_{(D.\ magna)} > -3.5$) and potentially not-receiving ($TU_{(D.\ magna)} < -3.5$) pesticide input. Welch's t-test for unequal variances was used to compare the means of the two groups.

For the comparison of SPEAR metrics across countries, the respective observations of France, Finland and Germany were grouped according to their $TU_{(D.\ magna)}$ as:

- reference ($TU_{(D.\ magna)} < -3.5$)
- lightly contaminated ($-3.5 < TU_{(D.\ magna)} < -2.25$)
- and heavily contaminated sites ($TU_{(D.\ magna)} > -2.25$)

The class boundaries (-3.5 and -2.25) were chosen to make the sample sizes as even as possible for all countries. However, the use of different class boundaries (-4 and -2) yielded the same results. To detect significant differences between means of groups, non-parametric ANOVA with Kruskal-Wallis test was conducted, followed by a non-parametric multiple-comparisons-test of the Behrens-Fischer type (Munzel et al., 2001).

All statistical computations and graphics were created with the open source software package R (R Development Core Team, 2006) using version 2.4.0 (for Mac OS X, 10.4.8) with appropriate additional packages (hier.part, Hmisc, npmc, maps and mapdata).

Results

Characterization of investigated streams and communities

Water temperature, pH, hardness and oxygen exhibited only slight variation (up to 34% standard deviation) among the sampling sites in each study area, while streambed substrate composition showed the largest variability (up to 400% standard deviation) (Table 1). The French and German streams were very similar concerning most stream characteristics but differed in the clay and silt content of the substrate. In contrast, the French and Finnish sites were very similar regarding substrate composition but mainly differed in water chemistry characteristics. A total of 94 different taxa with an average of 27 taxa per stream were identified in the 13 Finnish streams; the values for the 16 French streams were 110 and 33, respectively (see supplementary material). In the study of Liess and von der Ohe (2005) 129 different taxa and an average of 24 taxa per sampling site were found in the German streams, applying the same level of taxonomic identification as in the present study.

In Finland, *Asellus aquaticus*, Chironomidae spp., *Dryopoidea* spp., *Leuctra fusca*, Limoniidae spp., Oligochaeta spp. and Simuliidae spp. were found in more than 85% of the samples. The most common taxa (> 85% of samples) in France were *Baetis rhodani*, Elmidae spp., *Ephemerella ignita*, *Erpobdella* spp., *Gammarus pulex*, *Hydropsyche* spp. and Oligochaeta spp.. In the German study, Chironomidae spp., *Erpobdella octoculata*, *Glossiphonia complanata*, Tubificidae spp., *Gammarus pulex* and *Limnephilus lunatus* were present in more than 85% of the samples.

Pesticide monitoring

In Finland, only the fungicide trifluraline was detected; it had a maximum TWA water concentration of 1.11 ng/L (Table 2), but resulted in a neglectable $TU_{(D.\ magna)}$ for the Finnish streams (Figure 2). In contrast, all pesticides of the monitoring program were detected in samples from French streams (Table 2). They were identified and quantified after the only strong rain event (20 to 30 mm rainfall between May 12 and 13, 2005 (Meteo France, 2006)) that occurred in the study area during the sampling period. For the French sampling sites pesticide concentrations with $TU_{(D.\ magna)}$ values up to -0.42 were observed (Figure 2). For the German streams $TU_{(D.\ magna)}$ values up to -0.71 were reported (Liess et al., 2005a).

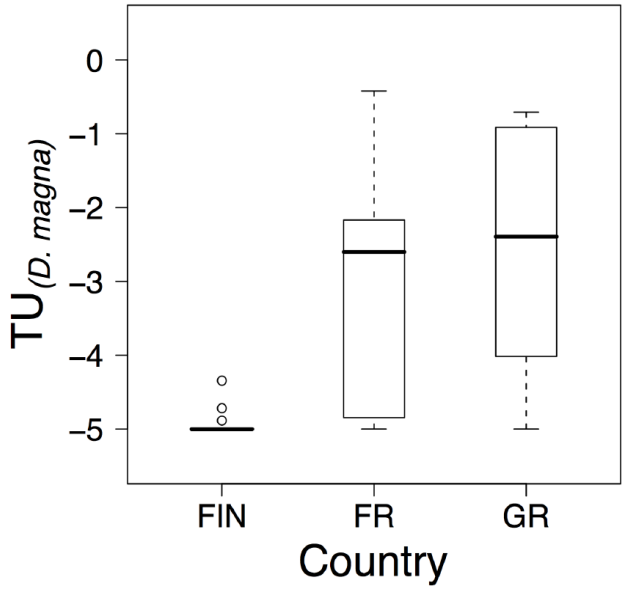

Figure 2: Box-Whisker plot of Toxic Unit$_{(Daphnia\ magna)}$ for the sites in the study areas of France (FR), Germany (GR) and Finland (FI).

Relationship between environmental variables and SPEAR metrics

For the Finnish streams, no significant linear model could be established for %SPEAR$_{(abundance)}$, and stream depth was the only variable to explain %SPEAR$_{(number)}$ (Table 3). Neither %SPEAR$_{(abundance)}$ nor %SPEAR$_{(number)}$ was significantly different for streams with and without undisturbed upstream reaches (Welch's t-test, P = 0.439 and P = 0.696). For the French streams, variability in %SPEAR$_{(abundance)}$ was best explained by TU$_{(D.\ magna)}$ and the factor undisturbed upstream reach (Table 3). The negative relationship between %SPEAR$_{(abundance)}$ and TU$_{(D.\ magna)}$ is illustrated in Figure 3.

Values for %SPEAR$_{(abundance)}$ and %SPEAR$_{(number)}$ were significantly reduced for streams which received pesticide input (Welch's t-test, both $P < 0.001$). Pesticide-impacted streams with undisturbed upstream reaches had significantly higher %SPEAR$_{(abundance)}$ and %SPEAR$_{(number)}$ compared to impacted streams which lacked these reaches (Welch's t-test, P = 0.017 and $P < 0.001$).

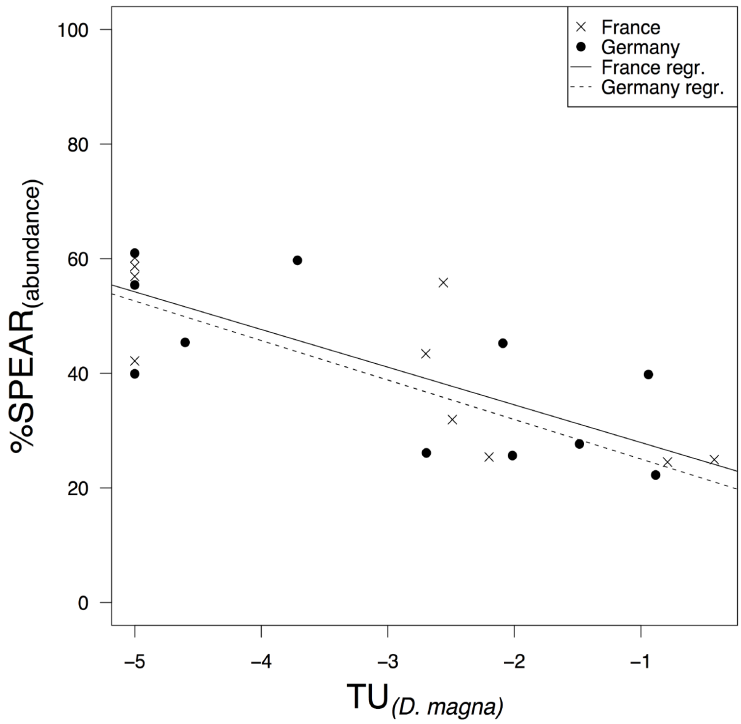

Figure 3: Relation between the benthic invertebrate community structure expressed as $\%SPEAR_{(abundance)}$ and the Toxic $Unit_{(Daphnia\ magna)}$ of the sites with undisturbed upstream reaches in France and Germany. Linear regression lines are significant with $P < 0.001$, $r^2 = 0.61$ and 0.64 for French (n=10) and German streams (n=11), respectively. The slopes and intercept are not significantly different (analysis of covariance, $P = 0.581$).

Relationship between leaf-litter decomposition and environmental variables

The leaf-litter breakdown coefficient *k* for the Finnish streams ranged from 0.001 to 0.046 and *k* was highly correlated with water temperature (Table 3). For the French streams, *k* values between 0.008 and 0.067 were measured. The breakdown rate was significantly different between streams potentially receiving (0.0252±0.0049 s.e.) and not receiving pesticide input (0.0588±0.0008 s.e.) (Welch's t-test, $P < 0.001$). $TU_{(D.\ magna)}$ and the factor undisturbed upstream reach could explain a significant part of the variation in *k* (Table 3), where *k* responded positively to the presence of undisturbed upstream reaches and negatively to an increase of pesticide stress. However, the best-fit model for *k* only comprised the variables $\%SPEAR_{(abundance)}$ and % of sand on the stream bottom (Table 3). This indicated that pesticide stress had no direct effect on the leaf litter breakdown but mediated through its negative effect on sensitive species. The relationship between $\%SPEAR_{(abundance)}$ and *k* is displayed in Figure 4.

Table 3: Summary statistics of linear models to explain SPEAR-endpoints and leaf litter breakdown rate *k* in French and Finnish streams.

		Model statistics			Relative importance of explanatory variable in best-fit linear model (%)[a]							
Response variable	**Country**	**adj. r^2**	**n**	**P**	**Toxic unit**	**Undisturbed upstream reach**	**Stream depth**	**% of filamentous algae**	**Water temperature**	**Current velocity**	**%SPEAR$_{(abundance)}$**	**% of sand**
%SPEAR$_{(abundance)}$	Finland	—	13	> 0.05	—	—	—	—	—	—	not explanatory variable	—
%SPEAR$_{(number)}$	Finland	0.56[b]	13	0.003	—	—	100	—	—	—		—
%SPEAR$_{(abundance)}$	France	0.64	16	< 0.001	61.80	31.20	—	—	—	—		—
%SPEAR$_{(number)}$	France	0.98	16	< 0.001	14.30	29.90	—	19.70	21.40	14.70		—
k	Finland	0.88[b]	8	< 0.001	—	—	—	—	100	—	—	—
k	France	0.44[c]	12	0.041	51.75	48.25	—	—	—	—	—	—
k	France	0.94	12	< 0.001	—	—	—	—	—	—	79.8	20.2

[a] determined in hierarchical partitioning (Chevan and Sutherland, 1991) [b] r^2 not adjusted for one explanatory variable [c] not best-fit model

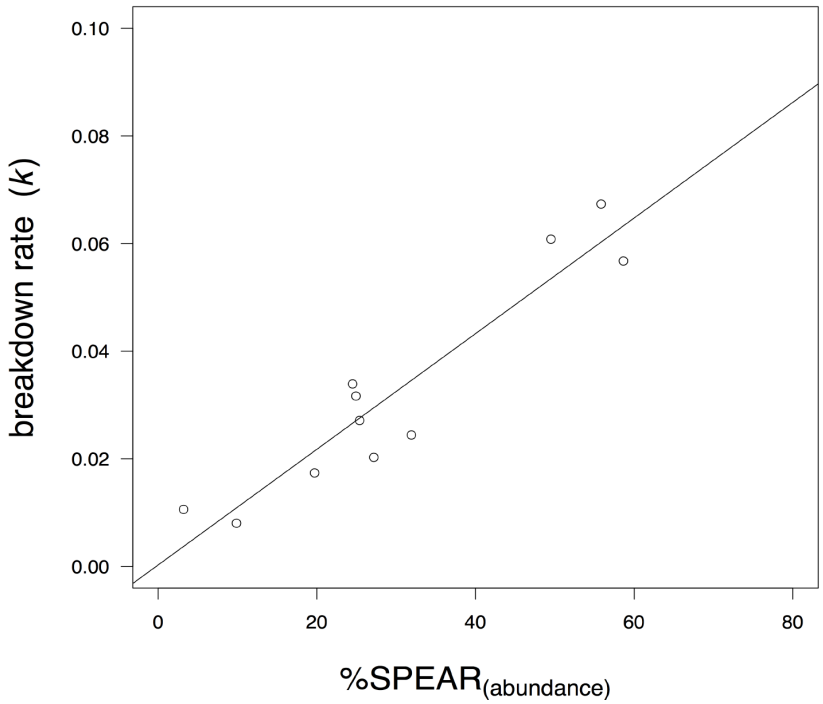

Figure 4: Relation between leaf litter decomposition and $\%SPEAR_{(abundance)}$ for 11 streams in Brittany, France. Linear regression was significant with $P < 0.001$ and $r^2 = 0.89$.

Comparison of $\%SPEAR_{(PM\ abundance)}$ across geographical areas

For Germany, France and Finland, the means of $\%SPEAR_{(PM\ abundance)}$, a metric excluding traits with biogeographical variability, were significantly different when grouped by $TU_{(D.\ magna)}$ (Kruskal-Wallis-test, $\chi^2_{6,49} = 26.32$, $P < 0.001$). All the reference sites in the three countries ($TU < -3.5$) exhibited about the same mean level of $\%SPEAR_{(PM\ abundance)}$ with 46, 52 and 54% for France, Finland and Germany, respectively. Pairwise comparisons showed that values from reference sites were significantly different ($P < 0.05$) from the values recorded in the highly contaminated sites in France and Germany ($TU > -2.25$) (Figure 5). For all countries, neither the reference sites nor the highly contaminated sites were significantly different ($P < 0.05$) from the mean $\%SPEAR_{(PM\ abundance)}$ of lightly polluted streams ($-3.5 < TU < -2.25$). Nevertheless, a clear decline of the $\%SPEAR_{(PM\ abundance)}$ was visible for the group of lightly polluted sites compared to reference sites. Furthermore, when the data from all countries were pooled, the difference between lightly polluted and reference sites showed to be significant in multiple comparisons ($P = 0.003$).

%SPEAR$_{(PM\ abundance)}$ was lower for France than for Germany concerning the groups of lightly and highly contaminated streams (Figure 5), because of the higher TU$_{(D.\ magna)}$ values of the French sites in each group. No difference was observed in regression analysis (not shown, but compare Figure 3).

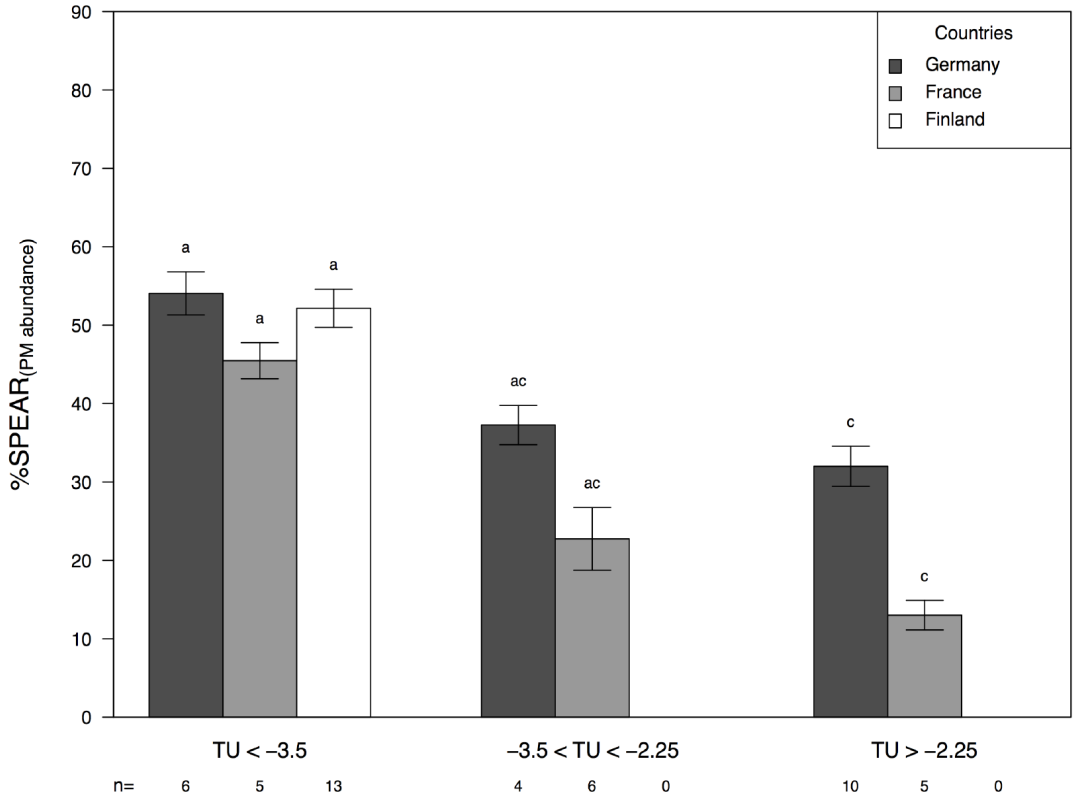

Figure 5: Relation between %SPEAR$_{(PM\ abundance)}$ and toxic unit$_{(Daphnia\ magna)}$ (TU) for the study sites in France, Finland and Germany with the sample sizes n. Different letters over the bars indicate significant differences in multiple comparison post-hoc test (non-parametric Behrens-Fischer test, $P < 0.05$). Error bars show standard error.

Temporal change of %SPEAR$_{(abundance)}$

For Finland, the average change of the communities from July to August, SPEAR$_{(abundance\ during/before)}$, for all streams was 1±0.06 standard error (s.e.). Hence, no change in the community structure occurred in the period of pesticide application. Similarly, French streams that were not subject to pesticide input (TU$_{(D.\ magna)}$ < -3.5) had an average SPEAR$_{(abundance\ during/before)}$ of 1.01±0.08 s.e., while stations which received pesticide inputs (TU$_{(D.\ magna)}$ > -3.5) had a mean SPEAR$_{(abundance\ during/before)}$ of 0.92± 0.06 s.e.. However,

$SPEAR_{(abundance\ during/before)}$ values for the two groups were not significantly different (Welch's t-test, P = 0.215). The average values of $SPEAR_{(abundance\ during/before)}$ for the German streams were 0.92±0.06 s.e. and 0.74 ±0.08 s.e. for streams potentially not receiving and receiving pesticide input, respectively. These values were significantly different at P = 0.048 (Welch's t-test).

The pooled data for community change in the period of pesticide application ($SPEAR_{(abundance\ during/before)}$) of all regions were significantly different concerning the grouping by $TU_{(D.\ magna)}$ (Kruskal-Wallis-test, $\chi^2_{2,49}$ = 6.94, P < 0.031). In multiple comparison tests, the medium (P = 0.032) and highly contaminated sites (P = 0.025) differed significantly from the reference sites, indicating an acute response of $\%SPEAR_{(abundance)}$ from pre- to pesticide-application period.

Discussion

Linking pesticide input to community composition

In the present study we conducted field investigations in a region of each France and Finland to examine whether current-use pesticides would affect freshwater macroinvertebrate communities. Pesticide stress in terms of $TU_{(D.\ magna)}$ was almost absent in the Finnish streams, and the variation in the lotic macroinvertebrate community could not be attributed to the presence of these contaminants. The results of our pesticide measurements are in agreement with those of a Finnish governmental screening programme from 2004 to 2005 (Finnish Environment Institute (SYKE), personal communication), and we do not know of any field study on Finnish streams reporting pesticide concentrations at a level potentially toxic to invertebrates. The low pesticide input in Finnish streams compared to the French may be attributed to the reduced pesticide application rate, different compound classes and geological factors, e.g. higher organic carbon content of the Finnish soils (European Commission Joint Research Centre, Institute for Environment and Sustainability, personal communication). We suppose that the results presented here are representative for the general situation in the agricultural region of Finland, since we sampled streams in different areas of this region. Nevertheless, a more thorough investigation including more compounds, sampling sites and a longer sampling period could alter this appraisal.

For the French streams, we found a clear relationship between pesticide stress and community composition as indicated by $\%SPEAR_{(abundance)}$. Among the environmental variables, pesticide stress, indicated by $TU_{(D.\ magna)}$, best explained the results for $\%SPEAR_{(abundance)}$. Furthermore, we observed a decline in the relative abundance of sensitive taxa after pesticide runoff, although it was not significant. This may be due to effects on the communities before the beginning of our study.

The concentrations presumed to cause an effect in the French streams, resulted in $TU_{(D.\ magna)}$-values up to -0.42 (Table 2) and are in accordance with those reported elsewhere. Liess and Schulz (1999) showed that pesticide-contaminated runoff water with $TU_{(D.\ magna)}$ up to 0.38 was the main cause for the decline in abundances of several macroinvertebrate species in a small headwater stream. The study of Liess and von der Ohe (2005) demonstrated strong causality between a $TU_{(D.\ magna)}$ larger than -3 and a decline of SPEAR. Castillo et al. (2006) stated change in the invertebrate community structure associated with an exposure to pesticide concentrations in the surface water up to a $TU_{(D.\ magna)}$ of -0.73. Finally, a review of mesocosm studies reported effects on the macroinvertebrate community above a $TU_{(D.\ magna)}$ of -2 (Van Wijngaarden et al., 2005).

The relationship between the relative abundance of sensitive taxa and pesticide stress was similar for the French and German streams (Figure 3). Concurrently, geographical and physico-chemical variables varied and approximately 35% of the taxa found in France were not recorded in the German streams. Hence, the response of traits to pesticide stress was not affected by geographical and taxonomical differences. The insensitivity of traits to a range of environmental gradients was also reported by Charvet et al. (2000). This issue warrants further investigation, especially in South and non-European regions, because if the dose-response relationship between traits and pesticide stress could be extrapolated to a wider geographical scale, it would constitute a powerful tool for an effect assessment on the continental scale.

Effects of pesticides on leaf litter decomposition

In our study on the French streams, we found a significant decrease in leaf litter decomposition rates for leaves of *A. glutinosa* due to pesticide stress. The decomposition rates for streams potentially not receiving pesticide input (0.0588±0.0008 s.e.) are in the range of values reported for relatively pristine streams in Portugal during summer (0.051

to 0.064) (Graca et al., 2001). Comparison of the ratio of breakdown coefficients at sites potentially receiving and sites potentially not receiving pesticide input (0.42) with the threshold values proposed by Gessner and Chauvet (2002) confirms the functional impairment caused by pesticides (ratio < 0.5 indicates severe impairment). Chung et al. (1993) also reported a ratio of 0.35 of breakdown rates of rhododendron leaves for a pesticide-treated stream and a reference stream.

A reduction in $\%SPEAR_{(abundance)}$ and $\%SPEAR_{(number)}$ (not shown) was closely related to a decrease in leaf litter processing rates (Figure 4). This may be explained by the fact that 2/3 of the shredder taxa classified according to (Merritt et al., 1996) belong to SPEAR. Maltby et al. (2002) also reported high positive correlation (Pearson's r = 0.83) between physiological effects of pesticides on a shredder species and impairment of leaf litter processing. To sum up, pesticide input in the streams of Brittany, France also affected an important ecosystem process, leaf breakdown, probably mediated through the adverse effects on number and abundance of SPEAR taxa.

Undisturbed upstream reaches enhance quality of impacted streams

The presence or absence of riparian forest parts in the 3-km upstream reach explained a significant part of the variation in the SPEAR endpoints for the French sampling sites. The presence of undisturbed upstream reaches lead to significantly higher values of $\%SPEAR_{(abundance)}$ and $\%SPEAR_{(number)}$ at contaminated sites. Other authors also reported the relevance of undisturbed upstream reaches for recovery from disturbance. A study on the Suna river (Japan) attributed the recovery of several invertebrate species after pesticide spraying to recolonisation from unsprayed upstream reaches within a 5-km distance (Hatakeyama et al., 1997). Liess and von der Ohe (2005) demonstrated the recovery of pesticide-impacted communities when riparian forest reaches was available in the 4 km upstream reach. A recent study, linking exposure modelling with macroinvertebrate composition of 360 streams investigated over a 17-year period in North Germany, showed that the presence of forest parts in the 1.5 km upstream reach facilitated recovery of the relative abundance of SPEAR taxa after modelled pesticide contamination (Schriever et al., 2007).

Two mechanisms could explain this positive impact of undisturbed upstream reaches. First, undisturbed upstream reaches may provide recolonization pools from which species could drift downstream to the impacted reach (Waters, 1972). Second, input of woody

debris and leaf litter from the riparian forest might supply more energy for the downstream reaches and thus increase number and abundance of taxa (Wallace et al., 1995; Bond et al., 2006). The latter mechanism should also increase the number and abundance of sensitive species at slightly or non-contaminated sites. However, Schriever et al. (2007) reported no significant difference in $\%SPEAR_{(abundance)}$ for low-contamination streams with and without undisturbed upstream reaches in the period before pesticide application. In the present study also, we did not find significant differences in abundance or number of SPEAR taxa for uncontaminated sites having and not having undisturbed upstream reaches in Finland. Therefore, current evidence indicates that recovery should mainly be attributed to instream recolonization by macroinvertebrates from undisturbed upstream reaches, although a more thorough study is still necessary to clarify this issue. However, regardless the underlying mechanism, undisturbed upstream reaches clearly enhance recovery of impacted reaches and this poses a valuable management tool for freshwater conservation in agricultural areas.

Derivation of an effect threshold for pesticides

For the pooled data of the German, French and Finnish streams, a significant reduction of sensitive taxa was observed for $TU_{(D.\ magna)}$ values higher than -3.5. However, this value is only a rough estimate for a threshold value because there were hardly any observations for $TU_{(D.\ magna)}$ in the interval [-3,-5). Furthermore, regarding the dose-response relationship between $TU_{(D.\ magna)}$ and $\%SPEAR_{(abundance)}$ in France and Germany (Figure 3) it remains open, if an effect threshold exists or if the relationship is continuously linear up to a $TU_{(D.\ magna)}$ of -5. Thus, more field data are needed especially for low values of $TU_{(D.\ magna)}$ to clarify this issue. Nevertheless, our data indicate effects of pesticides in the $TU_{(D.\ magna)}$ range of -2 and -3. Two other field studies also reported shifts in the invertebrate assemblages for $TU_{(D.\ magna)}$ between -2 and -3 (Berenzen et al., 2005; Liess et al., 2005a). In contrast, we do not know of any mesocosm study where effects below a $TU_{(D.\ magna)}$ of -2 were found (Van Wijngaarden et al., 2005). However, most studies on stream mesocosms just deal with a single pulse exposure while in the field repeated input of many different pesticides frequently occurs (Van Wijngaarden et al., 2005). Recently, a laboratory study with *D. magna* showed that a repeated exposure to dimethoate and pirimicarb significantly increased mortality (Andersen et al., 2006). In addition, multiple stressors may occur in areas with intense agriculture (e.g. pesticides with a different mode

of action, chronic ammonium exposure, eutrophication) and act additively or synergistically, which is commonly not incorporated in mesocosm studies (Heugens et al., 2001). For example, organophosphorous pesticides that were also detected in the French streams (Table 2) have been demonstrated to elicit greater-than-additive responses in combination with various herbicides (Lydy et al., 2005).

We suggest that the community change at $TU_{(D.\ magna)}$ values < -2 may have resulted from the long-term propagation of sublethal effects. This hypothesis is supported by the fact that the relative abundance of sensitive taxa exhibited only a small acute response to pesticide stress in lightly contaminated streams (17±10.1 % reduction in $\%SPEAR_{(abundance)}$). Sublethal effects like reduced fecundity or delayed emergence are known to appear up to a $TU_{(D.\ magna)}$ of -4 (Liess, 2002) and if they cause a competitive disadvantage for sensitive species this may result in community change (Fleeger et al., 2003). However, this remains open to discussion, as another explanation would be that the communities of the lightly contaminated streams may have not recovered from past impacts (Harding et al., 1998).

Overall, our investigation gives rise to the concern that the effect threshold for pesticides in the field is below a $TU_{(D.\ magna)}$ of -2, which is currently regarded as protective, for example in the legislation on pesticides in the European Union (EEC, 1991).

Conclusions

This study showed that the structure and function of aquatic ecosystems may be impaired by pesticides. We suggest that effects may also occur below levels that are commonly thought to be protective. This highlights the importance of field studies since effects at these levels have not been observed in artificial systems. It is noteworthy that no effects were detectable in the Finnish study area under low pesticide usage.

A very important result for risk managers is that undisturbed upstream reaches improve the quality of impaired downstream reaches. This could constitute a valuable measure for future risk mitigation in addition to other innovations in agricultural practice.

Furthermore, current risk assessment would take a great step forward when implementing ecological knowledge. The use of biological traits in biomonitoring could be a starting

point and may prove superior to taxonomically based approaches. The trait-based SPEAR concept was capable of discriminating between the effects of pesticides and those of confounding factors and natural variation over large spatial scales. Thus, the results from the regional investigations may be extrapolated to other biogeographical regions in Central and North Europe. However, more studies are needed in non-European regions to assess the potential for extrapolation beyond Europe. For example, the concept could easily be applied to field observations from North America, as a database on invertebrate traits is available (Vieira et al., 2006).

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References

Akaike H. A new look at the statistical model identification. IEEE Transactions on Automatic Control 1974; 19: 716-723.

Allan JD. Stream Ecology. Kluwer Academic Publishers, Dordrecht, 1995, 400 p.

Andersen TH, Tjornhoj R, Wollenberger L, Slothuus T, Baun A. Acute and chronic effects of pulse exposure of Daphnia magna to dimethoate and pirimicarb. Environmental toxicology and chemistry 2006; 25: 1187-1195.

Beasley G, Kneale PE. Investigating the influence of heavy metals on macroinvertebrate assemblages using Partial Canonical Correspondence Analysis (pCCA). Hydrology and Earth System Sciences 2003; 7: 221-233.

Benfield EF. Leaf Breakdown in Stream Ecosystems. In: Hauer FR, Lamberti GA, editors. Methods in Stream Ecology. Academic Press, San Diego, 1996, pp. 579-589.

Berenzen N, Kumke T, Schulz HK, Schulz R. Macroinvertebrate community structure in agricultural streams: impact of runoff-related pesticide contamination. Ecotoxicology and Environmental Safety 2005; 60: 37-46.

Bond NR, Sabater S, Glaister A, Roberts S, Vanderkruk K. Colonisation of introduced timber by algae and invertebrates, and its potential role in aquatic ecosystem restoration. Hydrobiologia 2006; 556: 303-316.

Booij K, Hofmans HE, Fischer CV, Van Weerlee EM. Temperature-dependent uptake rates of nonpolar organic compounds by semipermeable membrane devices and low-density polyethylene membranes. Environmental Science and Technology 2003; 37: 361-366.

Castillo LE, Martinez E, Ruepert C, Savage C, Gilek M, Pinnock M, Solis E. Water quality and macroinvertebrate community response following pesticide applications in a banana plantation, Limon, Costa Rica. Science of the Total Environment 2006; 367: 418-432.

Charvet S, Statzner B, Usseglio-Polatera P, Dumont B. Traits of benthic macroinvertebrates in semi-natural french streams: An initial application to biomonitoring in Europe. Freshwater Biology 2000; 43: 277-296.

Chevan A, Sutherland M. Hierarchical Partitioning. American Statistician 1991; 45: 90-96.

Clements WH, Carlisle DM, Lazorchak JM, Johnson PC. Heavy metals structure benthic communities in Colorado mountain streams. Ecological Applications 2000; 10: 626-638.

DeLorenzo ME, Scott GI, Ross PE. Toxicity of pesticides to aquatic microorganisms: A review. Environmental Toxicology and Chemistry 2001; 20: 84-98.

DIREN REA. Les produits phytosanitaires dans les eaux superficielles http://www.bretagne.ecologie.gouv.fr/Eau/Tableaux_Bord/Tab-Bord_2003/Eaux_douces/phyto.htm, 2005.

EEC. Council Directive of 15 July 1991 concerning the placing of plant protection products on the market. . In: Office for Official Publications of the European Communities, 1991, 137.

EUROSTAT SOotEC. Use of plant protection products in the European Union Office for Official Publication of the European Union, Luxembourg. 2002, 122.

Flack VF, Chang PC. Frequency of Selecting Noise Variables in Subset Regression-Analysis - a Simulation Study. American Statistician 1987; 41: 84-86.

Fleeger JW, Carman KR, Nisbet RM. Indirect effects of contaminants in aquatic ecosystems. Science of the Total Environment 2003; 317: 207-233.

Frankart C, Eullaffroy P, Vernet G. Comparative effects of four herbicides on non-photochemical fluorescence quenching in Lemna minor. Environmental and Experimental Botany 2003; 49: 159-168.

Gessner MO, Chauvet E. A case for using litter breakdown to assess functional stream integrity. Ecological Applications 2002; 12: 498-510.

Graca MAS, Ferreira RCF, Coimbra CN. Litter processing along a stream gradient: The role of invertebrates and decomposers. Journal of the North American Benthological Society 2001; 20: 408-420.

Grande M, Andersen S, Berge D. Effects of pesticides on fish. Norvegian Journal of Agricultural Sciences 1994; 195-209.

Harding JS, Benfield EF, Bolstad PV, Helfman GS, Jones III EBD. Stream biodiversity: The ghost of land use past. Proceedings of the National Academy of Sciences of the United States of America 1998; 95 14843-14847.

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

Heugens E, Hendriks A, Dekker. T, Van Straalen NM, Amiraal W. A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. Critical Reviews in Toxicology 2001; 31: 247-284.

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

Liess M. Population response to toxicants is altered by intraspecific interaction. Environmental Toxicology and Chemistry 2002; 21: 138-142.

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

Liess M, von der Ohe PC. Analyzing effects of pesticides on invertebrate communities in streams. Environmental Toxicology and Chemistry 2005a; 24: 954-965.

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

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

Liess M, von der Ohe PC, Schriever CA, Schäfer RB, Beketov MA. Online database of species at risk (SPEAR database). In: 2006.

Liess M, Brown C, Dohmen P, Duquesne S, Heimbach F, Kreuger J, Lagadic L, Reinert W, Maund S, Streloke M, Tarazona J. Effects of Pesticides in the Field – EPIF. SETAC Press, Brussels, Belgium 2005b, 136 p.

Lydy MJ, Austin KR. Toxicity assessment of pesticide mixtures typical of the Sacramento-San Joaquin Delta using Chironomus tentans. Archives of Environmental Contamination and Toxicology 2005; 48: 49-55.

Maltby L, Clayton SA, Wood RM, McLoughlin N. Evaluation of the Gammarus pulex in situ feeding assay as a biomonitor of water quality: Robustness, responsiveness, and relevance. Environmental Toxicology and Chemistry 2002; 21: 361-368.

Maret TR, Cain DJ, MacCoy DE, Short TM. Response of benthic invertebrate assemblages to metal exposure and bioaccumulation associated with hard-rock mining in northwestern streams, USA. Journal of the North American Benthological Society 2003; 22: 598-620.

McGarigal K, Cushman S, Stafford S. Multivariate Statistics for Wildlife and Ecology Research. Springer, New York, 2000, 284 p.

Merritt RW, Cummins KW. Trophic Relations of Macroinvertebrates. In: Hauer FR, Lamberti GA, editors. Methods in Stream Ecology. Academic Press, San Diego, 1996, pp. 453-474.

Meteo France. Temps du mois http://www.meteofrance.com/FR/climat/dpt_tempsdumois.jsp?LIEUID=DEPT35, 2006.

Munzel U, Hothorn LA. A unified approach to simultaneous rank test procedures in the unbalanced one-way layout. Biometrical Journal 2001; 43: 553-569.

Neumann M, Schulz R, Schäfer K, Müller W, Mannheller W, Liess M. The significance of entry routes as point and non-point sources of pesticides in small streams. Water Research 2002; 36: 835-842.

R Development Core Team. R: A language and environment for statistical computing, reference index version 2.4.1 www.r-project.org, 2006.

Richards RP, Baker DB. Pesticide concentration patterns in agricultural drainage networks in the Lake Erie basin. Environmental Toxicology and Chemistry 1993; 12: 13-26.

Schriever CA, Hansler-Ball M, Holmes C, Maund S, Liess M. Agricultural intensity and landscape structure: influences on the macroinvertebrate assemblages of small streams in northern Germany. Environmental Toxicology and Chemistry 2007; 26: 346-357.

Statzner B, Bady P, Doledec S, Scholl F. Invertebrate traits for the biomonitoring of large European rivers: an initial assessment of trait patterns in least impacted river reaches. Freshwater Biology 2005; 50: 2136-2161.

Strahler AN. Quatitative analysis of watershed geomorphology. Transactions, American Geophysical Union 1957; 38: 913-920.

Tomlin CDS. The e-Pesticide Manual (Twelfth Edition) on CD - Version 2.1. In: The British Crop Protection Council, 2001.

Van Wijngaarden RPA, Brock TCM, Van Den Brink PJ. Threshold levels for effects of insecticides in freshwater ecosystems: A review. Ecotoxicology 2005; 14: 355-380.

Vieira NKM, Poff NL, Carlisle DM, Moulton SR, Koski ML, Kondratieff BC. A database of lotic invertebrate traits for North America: U.S. Geological Survey Data Series 187. In: 2006.

Wallace JB, Eggert SL, Meyer JL, Webster JR. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 1997; 277: 102-104.

Wallace JB, Whiles MR, Eggert S, Cuffney TF, Lugthart GJ, Chung K. Long term dynamics of coarse particulate organic matter in three Appalachian Mountain streams. Journal of the North American Benthological Society 1995; 14: 217-232.

Waters TF. The drift of stream insects. Annual Review of Entomology 1972; 17: 253-272.

Supplementary data

Taxa list for France (a) and Finland (b) with SPEAR classification and abundance metrics.

a) French taxa

Order	Taxon	SPEAR[1]	$SPEAR_{(PM)}$[1]	Mean abundance (per 0.5 m^2)	Standard deviation	Minimum abundance (per 0.5 m^2)	Maximum abundance (per 0.5 m^2)	Presence in number of sampling sites
Anisoptera	Aeshnidae sp.	0	0	1.00	0.00	1	1	2
Trichoptera	Agapetus sp.	1	1	13.50	15.16	1	42.5	8
Trichoptera	Allogamus sp.	1	1	10.40	3.60	4.5	13.5	5
Ephemeroptera	Ameletus inopinatus	1	1	4.00	0.00	4	4	1
Plecoptera	Amphinemura sp.	1	1	2.00	0.00	2	2	1
Trichoptera	Anabolia nervosa	0	0	2.17	1.61	1	4	3
Basommatophora	Ancylus fluviatilis	0	0	12.19	10.44	4	34	8
Isopoda	Asellus aquaticus	0	1	19.50	30.60	1	65	4
Diptera	Athericidae spp.	1	1	3.81	1.77	1	6.5	8
Trichoptera	Athripsodes spp.	1	1	13.67	13.57	1	42	12
Ephemeroptera	Baetis rhodani	0	1	29.97	18.96	5.5	77.5	15
Basommatophora	Bathyomphalus contortus	0	0	1.00	0.00	1	1	1
Hirudinae	Batracobdella paludosa	0	0	1.00	0.00	1	1	1
Mesogastropoda	Bythinella spp.	0	0	8.67	7.37	2	20	6
Mesogastropoda	Bythinia sp.	0	0	6.33	6.66	2	14	3
Ephemeroptera	Caenis sp.	1	1	12.25	10.92	4	31	6

Order	Taxon	SPEAR[1]	$SPEAR_{(PM)}$[1]	Mean abundance (per 0.5 m^2)	Standard deviation	Minimum abundance (per 0.5 m^2)	Maximum abundance (per 0.5 m^2)	Presence in number of sampling sites
Zygoptera	Calopteryx spp.	1	1	6.44	6.62	1	22.5	9
Plecoptera	Capnia sp.	1	1	1.00	0.00	1	1	1
Ephemeroptera	Centroptilum luteolum	1	1	1.00	0.00	1	1	1
Diptera	Ceratopogonidae sp.	1	1	6.75	6.55	1	15	4
Trichoptera	Chaetopteryx spp.	1	1	16.39	18.07	1	52	9
Diptera	Chironomidae spp.	0	0	476.47	1240.27	13.5	5087.5	16
Plecoptera	Chloroperla sp.	1	1	26.00	0.00	26	26	1
Coleoptera	Chrysomelidae sp.	0	0	1.00	0.00	1	1	1
Zygoptera	Coenagrionidae sp.	1	1	7.00	0.00	7	7	1
Anisoptera	Cordulegaster spp.	0	0	5.80	6.03	2	16.5	5
Coleoptera	Curculionidae spp.	0	0	2.40	2.61	1	7	5
Coleoptera	Dryopidae spp.	0	0	27.07	19.89	3	56	7
Turbellaria	Dugesia sp.	0	0	1.00	0.00	1	1	1
Coleoptera	Dytiscidae spp.	0	0	12.28	14.20	2	49	9
Ephemeroptera	Ecdyonurus sp.	1	1	3.50	2.12	2	5	2
Amphipoda	Echinogammarus berilloni	0	0	136.67	208.91	8	527.5	6
Amphipoda	Echinogammarus sp.	0	0	257.50	208.60	110	405	2
Coleoptera	Elmidae spp.	0	0	48.47	44.36	4	139.5	15
Diptera	Empididae sp.	1	1	4.00	3.61	1	8	3
Ephemeroptera	Ephemera danica	1	1	8.17	6.21	1	17	6
Ephemeroptera	Ephemera lineata	1	1	12.00	0.00	12	12	1
Ephemeroptera	Ephemerella ignita	1	1	88.37	68.63	2	232.5	15

Order	Taxon	SPEAR[1]	$SPEAR_{(PM)}$[1]	Mean abundance (per 0.5 m^2)	Standard deviation	Minimum abundance (per 0.5 m^2)	Maximum abundance (per 0.5 m^2)	Presence in number of sampling sites
Hirudinae	Erpobdella spp.	0	0	13.36	10.88	2	43.5	14
Amphipoda	Gammarus pulex	0	0	116.86	111.03	3.5	375	14
Amphipoda	Gammarus sp.	0	0	28.00	0.00	28	28	1
Heteroptera	Gerridae sp.	0	0	2.71	2.36	1	7	7
Hirudinae	Glossiphoniidae spp.	0	0	4.20	2.35	1	8	10
Trichoptera	Goera pilosa	1	1	4.00	4.24	1	7	2
Coleoptera	Gyrinidae spp.	0	0	2.17	1.00	1	3.5	9
Ephemeroptera	Habrophlebia spp.	1	1	17.31	10.84	1	35.5	8
Hirudinae	Haementeria costata	0	0	10.00	0.00	10	10	1
Trichoptera	Halesus spp.	1	1	11.50	9.78	2.5	37.5	10
Coleoptera	Haliplidae sp.	0	0	4.75	3.20	2	8	4
Hirudinae	Helobdella sp.	0	0	4.00	0.00	4	4	1
Hirudinae	Helobdella stagnalis	0	0	7.00	0.00	7	7	1
Trichoptera	Holocentropus sp.	1	1	1.00	0.00	1	1	1
Hydracarina	Hydracarina spp.	1	1	19.67	28.04	2	52	3
Coleoptera	Hydraenidae sp.	0	0	25.00	0.00	25	25	1
Coleoptera	Hydrophilidae sp.	0	0	6.00	7.07	1	11	2
Trichoptera	Hydropsyche spp.	0	0	24.07	21.68	4	74.5	14
Trichoptera	Hyporhyacophila sp.	1	1	1.00	0.00	1	1	2
Plecoptera	Isoperla sp.	1	1	21.60	23.37	1	60	5
Trichoptera	Ithytrichia lamellaris	1	1	18.00	0.00	18	18	1
Trichoptera	Lepidostoma hirtum	1	1	16.40	30.00	1	70	5
Plecoptera	Euleuctra geniculata	1	1	4.00	0.00	4	4	1

Order	Taxon	SPEAR[1]	$SPEAR_{(PM)}$[1]	Mean abundance (per 0.5 m^2)	Standard deviation	Minimum abundance (per 0.5 m^2)	Maximum abundance (per 0.5 m^2)	Presence in number of sampling sites
Plecoptera	Leuctra sp.	1	1	18.43	22.90	5	70	7
Trichoptera	Limnephilus spp.	1	1	3.06	4.69	1	15.5	9
Diptera	Limoniidae spp.	1	1	19.32	38.75	2	135	11
Trichoptera	Lype sp.	1	1	1.33	0.58	1	2	3
Heteroptera	Mesovelia furcata	0	0	1.00	0.00	1	1	1
Trichoptera	Mystacides sp.	1	1	2.33	0.58	2	3	3
Trichoptera	Nemotaulius punctatolineatus	1	1	1.00	0.00	1	1	1
Plecoptera	Nemoura sp.	1	1	1.00	0.00	1	1	1
Trichoptera	Neureclipsis bimaculata	1	1	1.00	0.00	1	1	1
Trichoptera	Notidobia sp.	1	1	4.50	4.95	1	8	2
Heteroptera	Notonectidae sp.	0	0	6.00	4.24	3	9	2
Trichoptera	Odontocerum albicorne	1	1	1.00	0.00	1	1	1
Trichoptera	Oecetis sp.	1	1	1.75	0.96	1	3	4
Oligochaeta	Oligochaeta spp.	0	0	48.63	32.16	16.5	129	16
Trichoptera	Oxyethira sp.	1	1	1.00	0.00	1	1	1
Ephemeroptera	Paraleptophlebia sp.	1	1	3.60	1.95	1	6	5
Trichoptera	Pararhyacophila sp.	1	1	2.00	1.41	1	4	4
Plecoptera	Perlodes sp.	1	1	60.00	0.00	60	60	1
Trichoptera	Philopotamus sp.	1	1	1.50	0.00	1.5	1.5	1
Basommatophora	Physa sp.	0	0	10.50	0.00	10.5	10.5	1
Basommatophora	Physidae sp.	0	0	2.00	0.00	2	2	1
Basommatophora	Planorbidae spp.	0	0	10.71	10.71	1	38.5	12

Order	Taxon	SPEAR[1]	$SPEAR_{(PM)}$[1]	Mean abundance (per 0.5 m^2)	Standard deviation	Minimum abundance (per 0.5 m^2)	Maximum abundance (per 0.5 m^2)	Presence in number of sampling sites
Anisoptera	Platycnemis sp.	1	1	6.00	3.04	2.5	8	3
Trichoptera	Plectrocnemia sp.	1	1	1.50	0.00	1.5	1.5	1
Trichoptera	Polycentropus sp.	1	1	7.00	5.58	1	14	4
Mesogastropoda	Potamopyrgus antipodarum	0	0	7.83	8.37	3	17.5	3
Isopoda	Proasellus meridianus	0	0	16.05	14.65	1	51.5	11
Ephemeroptera	Procloeon bifidum	1	1	10.00	0.00	10	10	1
Plecoptera	Protonemura sp.	1	1	4.43	4.04	1	10	7
Diptera	Psychodidae sp.	1	1	1.00	0.00	1	1	1
Lepidoptera	Pyralidae sp.	1	1	4.25	5.85	1	13	4
Basommatophora	Radix spp.	0	0	6.64	7.56	1	23	7
Diptera	Rhagionidae sp.	1	1	1.00	0.00	1	1	1
Ephemeroptera	Rhithrogena sp.	1	1	14.17	13.51	1	28	3
Trichoptera	Rhyacophila sp.	1	1	5.50	5.26	1	13	4
Coleoptera	Scirtidae sp.	0	0	1.50	0.71	1	2	2
Trichoptera	Sericostoma spp.	1	1	22.78	16.34	4	48.5	9
Megaloptera	Sialis spp.	1	1	11.50	11.60	4.5	32	5
Trichoptera	Silo sp.	1	1	12.63	13.97	4	33.5	4
Diptera	Simuliidae spp.	0	0	26.27	17.49	6	63.5	13
Plecoptera	Siphonoperla sp.	1	1	18.40	10.72	4.5	32	5
Veneroida	Sphaerium spp.	0	0	21.17	33.10	1	120	12
Basommatophora	Stagnicola sp.	0	0	3.00	1.41	2	4	2
Diptera	Tabanidae spp.	1	1	2.06	1.67	1	6	9
Trichoptera	Tinodes sp.	1	1	17.00	0.00	17	17	1

Order	Taxon	SPEAR[1]	$SPEAR_{(PM)}$[1]	Mean abundance (per 0.5 m²)	Standard deviation	Minimum abundance (per 0.5 m²)	Maximum abundance (per 0.5 m²)	Presence in number of sampling sites
Diptera	Tipulidae sp.	1	1	2.67	1.53	1	4	3
Unionoida	Unionidae sp.	0	0	1.00	0.00	1	1	1
Heteroptera	Veliidae sp.	0	0	6.33	4.51	2	11	3
Trichoptera	Wormaldia sp.	1	1	2.00	0.00	2	2	1

[1] 1 = Species at risk, 0 = Species not at risk.

b) Finnish taxa

Order	Species	SPEAR[1]	$SPEAR_{(PM)}$[1]	Mean abundance (per 0.5 m²)	Standard deviation	Minimum abundance (per 0.5 m²)	Maximum abundance (per 0.5 m²)	Presence in number of sampling sites
Lepidoptera	Acentria ephemerella	1	1	1.50	1.00	1	3	4
Anisoptera	Aeshnidae sp.	0	0	1.00	0.00	1	1	1
Trichoptera	Agrypnia sp.	1	1	2.00	1.73	1	4	3
Trichoptera	Anabolia sp.	1	1	2.83	2.88	1	8.5	6
Basommatophora	Ancylus sp.	0	0	4.50	0.71	4	5	2
Heteroptera	Arctocorixa sp.	1	1	15.00	0.00	15	15	1
Isopoda	Asellus aquaticus	0	1	41.15	50.39	1.5	169	13
Trichoptera	Athripsodes sp.	0	1	3.00	1.00	2	4	3
Ephemeroptera	Baetis digitatus	0	1	5.00	0.00	5	5	1
Ephemeroptera	Baetis fuscatus	0	1	41.00	44.58	4	90.5	3
Ephemeroptera	Baetis niger	0	1	3.60	2.97	1	8	5
Ephemeroptera	Baetis rhodani	0	1	39.55	53.95	1.5	178.5	10

Order	Species	SPEAR[1]	$SPEAR_{(PM)}$[1]	Mean abundance (per 0.5 m^2)	Standard deviation	Minimum abundance (per 0.5 m^2)	Maximum abundance (per 0.5 m^2)	Presence in number of sampling sites
Ephemeroptera	Baetis subalpinus	0	1	9.25	7.42	4	14.5	2
Ephemeroptera	Baetis vernus	0	1	20.94	21.82	1	62	9
Trichoptera	Brachycentrus subnubilus	0	1	1.50	0.71	1	2	2
Plecoptera	Capnia atra	0	1	1.00	0.00	1	1	1
Trichoptera	Chaetopteryx sp.	1	1	3.17	0.29	3	3.5	3
Diptera	Chironomidae spp.	0	0	47.81	33.20	6	122	13
Ephemeroptera	Cloeon inscriptum	0	1	13.00	14.14	3	23	2
Anisoptera	Corduliidae sp.	0	0	4.00	0.00	4	4	1
Heteroptera	Corixa sp.	1	1	1.00	0.00	1	1	1
Coleoptera	Curculionidae sp.	0	0	2.00	0.00	2	2	1
Plecoptera	Diura bicaudata	1	1	2.00	0.00	2	2	1
Diptera	Dixa sp.	1	1	1.00	0.00	1	1	1
Diptera	Dixidae sp.	1	1	10.00	0.00	10	10	1
Coleoptera	Donaciinae sp.	0	0	1.00	0.00	1	1	1
Coleoptera	Dryopoidea spp.	0	0	32.13	50.06	1	171.5	12
Coleoptera	Dytiscidae spp.	0	0	6.14	5.50	1	15	11
Diptera	Empididae sp.	1	1	2.00	1.41	1	3	2
Ephemeroptera	Ephemera danica	1	1	1.50	0.50	1	2	3
Ephemeroptera	Ephemera vulgata	1	1	4.75	3.33	2	9.5	4
Ephemeroptera	Ephemerella ignita	1	1	27.07	37.84	1	101.5	7
Hirudinae	Erpobdella spp.	0	0	7.70	11.73	1	39.5	10
Amphipoda	Gammarus pulex	0	0	254.10	462.84	2	1075	5
Heteroptera	Gerridae spp.	0	0	1.75	0.50	1	2	4

Order	Species	SPEAR[1]	$SPEAR_{(PM)}$[1]	Mean abundance (per 0.5 m^2)	Standard deviation	Minimum abundance (per 0.5 m^2)	Maximum abundance (per 0.5 m^2)	Presence in number of sampling sites
Hirudinae	Glossiphoniidae spp.	0	0	1.93	1.40	1	5	7
Trichoptera	Goera pilosa	0	1	1.00	0.00	1	1	1
Anisoptera	Gomphidae sp.	0	0	3.00	2.83	1	5	2
Coleoptera	Gyrinidae sp.	0	0	1.25	0.50	1	2	4
Hirudinae	Haementeria sp.	0	0	1.67	0.58	1	2	3
Trichoptera	Halesus spp.	1	1	4.38	5.60	1	17.5	8
Coleoptera	Haliplidae sp.	0	0	1.00	0.00	1	1	1
Hirudinae	Helobdella sp.	0	0	2.00	1.15	1	3	4
Coleoptera	Helodes sp.	0	0	3.00	0.00	3	3	1
Ephemeroptera	Heptagenia sp.	1	1	4.83	5.80	1	11.5	3
Diptera	Hexatomini sp.	1	1	2.00	0.00	2	2	1
Trichoptera	Hydatophylax infumatus	0	1	1.00	0.00	1	1	2
Hydracarina	Hydracarina spp.	1	1	2.08	2.42	1	7	6
Trichoptera	Hydropsyche spp.	0	0	48.80	51.49	1	149	10
Trichoptera	Ironoquia dubia	0	1	1.00	0.00	1	1	1
Trichoptera	Lepidostoma hirtum	1	1	23.75	25.10	6	41.5	2
Plecoptera	Leuctra digitata	1	1	7.50	3.54	5	10	2
Plecoptera	Leuctra fusca	1	1	22.27	21.52	1	70	11
Plecoptera	Leuctra sp.	1	1	6.50	0.71	6	7	2
Trichoptera	Limnephilus flavicornis	0	1	5.00	0.00	5	5	1
Trichoptera	Limnephilus spp.	0	1	2.60	1.82	1	5	5
Diptera	Limoniidae spp.	1	1	17.23	11.43	2	38	13
Basommatophora	Lymnaea sp.	0	0	1.00	0.00	1	1	1

Order	Species	SPEAR[1]	$SPEAR_{(PM)}$[1]	Mean abundance (per 0.5 m^2)	Standard deviation	Minimum abundance (per 0.5 m^2)	Maximum abundance (per 0.5 m^2)	Presence in number of sampling sites
Trichoptera	Lype sp.	1	1	2.33	1.53	1	4	3
Heteroptera	Mesovelia furcata	0	0	4.00	0.00	4	4	1
Ephemeroptera	Metretopus borealis	0	1	17.00	22.72	1	43	3
Trichoptera	Molannodes tinctus	0	1	2.00	0.00	2	2	1
Trichoptera	Nemotaulius punctatolineatus	1	1	2.00	0.00	2	2	1
Plecoptera	Nemoura cinerea	1	1	8.80	10.08	1	26	5
Plecoptera	Nemoura dubitans	0	1	2.00	0.00	2	2	1
Trichoptera	Notidobia ciliaris	1	1	2.33	1.21	1	4	6
Oligochaeta	Oligochaeta spp.	0	0	31.04	20.92	6.5	84	13
Trichoptera	Oligostomis reticulata	0	1	3.00	2.29	1	5.5	3
Ephemeroptera	Paraleptophlebia sp.	1	1	3.50	0.00	3.5	3.5	1
Lepidoptera	Parapoynx stratiotata	1	1	2.00	1.73	1	4	3
Turbellaria	Phagocata sp.	0	0	180.00	0.00	180	180	1
Trichoptera	Phryganea sp.	1	1	2.00	0.00	2	2	1
Basommatophora	Physa sp.	0	0	2.00	0.00	2	2	1
Eulamellibranchia	Pisidium sp.	0	0	1.00	0.00	1	1	1
Turbellaria	Planariidae sp.	0	0	3.00	0.00	3	3	1
Basommatophora	Planorbidae sp.	0	0	1.00	0.00	1	1	1
Trichoptera	Plectrocnemia sp.	1	1	1.75	0.35	1.5	2	2
Trichoptera	Polycentropus sp.	1	1	3.75	1.06	3	4.5	2
Trichoptera	Potamophylax rotundipennis	0	1	5.94	5.91	1	19.5	8

Order	Species	SPEAR[1]	$SPEAR_{(PM)}$[1]	Mean abundance (per 0.5 m^2)	Standard deviation	Minimum abundance (per 0.5 m^2)	Maximum abundance (per 0.5 m^2)	Presence in number of sampling sites
Ephemeroptera	Procloeon bifidum	1	1	6.75	6.18	1	17	6
Basommatophora	Radix sp.	0	0	2.00	1.00	1	3	3
Trichoptera	Rhyacophila sp.	0	1	14.40	29.96	1	68	5
Trichoptera	Sericostoma personatum	1	1	7.25	3.89	4.5	10	2
Megaloptera	Sialis fuliginosa	0	1	4.00	2.83	2	6	2
Megaloptera	Sialis lutaria	0	1	11.00	0.00	11	11	1
Megaloptera	Sialis morio	0	1	10.75	12.37	2	19.5	2
Heteroptera	Sigara sp.	1	1	2.00	1.73	1	4	3
Diptera	Simuliidae spp.	0	0	134.50	348.83	1	1286	13
Eulamellibranchia	Sphaerium sp.	0	0	3.50	3.39	1	8.5	4
Diptera	Tabanidae spp.	1	1	2.67	2.09	1	6.5	6
Diptera	Tipulidae spp.	1	1	4.00	4.47	1	13	6
Turbellaria	Turbellaria sp.	0	0	4.50	0.71	4	5	2
Eulamellibranchia	Unionidae sp.	0	0	3.00	0.00	3	3	1
Heteroptera	Veliidae sp.	0	0	1.00	0.00	1	1	1

[1] 1 = Species at risk, 0 = Species not at risk.

Kapitel 6: Zusammenfassung der wichtigsten Ergebnisse und Ausblick

In der vorliegenden Arbeit wurden zwei neue Methoden für die Erfassung der Pestizidexposition in kleinen Fließgewässern eingeführt und überprüft sowie eine Abschätzung der Effekte des Pestizideintrags für zwei beispielhafte Regionen in Frankreich und Finnland vorgenommen. Des Weiteren wurde die Eignung des SPEAR Index, der auf ökologischen und physiologischen Merkmalen von Makroinvertebraten beruht, für das vergleichende Biomonitoring größerer geografischer Einheiten untersucht. Im Folgenden werden zunächst die Ergebnisse dargestellt und dann in Hinblick auf Forschungsfragen diskutiert, die sich an diese Arbeit anschließen.

Zusammenfassung der Ergebnisse

Entwicklung und Anwendung einer Extraktionsmethode für 10 schwebstoffadsorbierte polare und semipolare Pestizide

Um die Belastung der Schwebstoffe aus 16 französischen Bächen mit 10 ausgewählten Pestiziden zu bestimmen (Tabelle 1), wurde eine neue analytische Methode zur Extraktion von schwebstoffadsorbierten polaren und semipolaren Pestiziden entwickelt (Kapitel 2). Für die Extraktion wurde die beschleunigte Lösemittelextraktion (ASE) verwendet und in Bezug auf die Wiederfindungsraten optimiert. Als optimale Extraktionsparameter wurden ein Lösungsmittelgemisch aus Ethylacetat-Aceton (2:1) und 2 Extraktionszyklen (jeweils 6 Minuten) bei 110°C und 11 MPa identifiziert. Die Wiederfindungen mit diesen Extraktionsparametern lagen zwischen 71 und 103% mit relativen Standardabweichungen unter 25% für 8 der 10 Analyten, wenn die Größenausschlusschromatographie als Aufreinigungsmethode verwendet wurde (Tabelle 1). Die Aufreinigung der Extrakte mit Festphasenextraktion lieferte im Allgemeinen geringere Wiederfindungen (13 – 67%).

Da die Wiederfindungen bei Proben mit unterschiedlichen physikochemischen Eigenschaften schwanken können, wurde die Eignung von 6 deuterierten Analyten zur Verwendung als interne Standards untersucht. Die Wiederfindungen von 3 deuterierten Standards mit 10 oder mehr Deuteriumatomen (Tabelle 1) unter verschiedenen

Extraktionsbedingungen korrelierten mit einem Pearson's *r* zwischen 0.88 und 0.97 (n = 15) mit den entsprechenden Ausgangsverbindungen. Die Differenzen in den Wiederfindungsraten zwischen diesen deuterierten Verbindungen und den zugehörigen Analyten lagen unter 4%. Dementsprechend eignen sich diese Verbindungen als interne Standards, um mögliche Abweichungen in der Wiederfindung bei der Extraktion und Aufreinigung von Feldproben anzuzeigen. Die deuterierten Verbindungen mit 4 oder 6 Deuteriumatomen wiesen hingegen bis zu 40% Differenz zu den Wiederfindungsraten der zugehörigen Analyten auf (Tabelle 1) und korrelierten in ihren Wiederfindungen nur mit einem Pearson's *r* zwischen 0.76 und 0.89 (n = 15) mit den entsprechenden Analyten.

Tabelle 1: Physikochemische Eigenschaften von 10 Pestiziden sowie 6 deuterierten Standards und Wiederfindungen (WF) mit relativer Standardabweichung (rel. Std.) für die ASE mit Festphasenextraktion (SPE) oder Größenausschluss-chromatographie (SEC) als Aufreinigungsmethode

Verbindung	**Typ**[a]	**Verbindungsklasse**[a]	**log K_{oc}**[b]	**SPE WF (%)**	**SPE rel. Std. (%)**	**SEC WF (%)**	**SEC rel. Std. (%)**
Acetochlor	H	Chloracetamid	2.32	63.6	7.9	96.7	10.7
Acetochlor D11	-	-	-	66.6	9.7	94.2	17.3
Alachlor	H	Chloracetamid	2.28	63.5	7.2	99.6	16.2
Alachlor D13	-	-	-	63.9	8	96.8	17.9
Carbofuran	I	Carbamat	1.75	45.8	25.9	97.5	16.6
Chlorfenvinphos	I	Organophosphorsäure	2.47	66.1	12.3	94.3	9.5
Chlorfenvinphos D10	-	-	-	63.8	14.7	94.3	8.1
α-Endosulfan	I	Organochlor	4.13	50.1	6.6	102.7	21.9
α-Endosulfan D4	-	-	-	51.3	13.2	83.2	11.3
Fenpropidin	F	Piperidin	3.2[d]	13.7	38.4	0	-
Linuron[e]	H	Carbamid	2.7	36.8	32.4	0	-
Oxadiazon	H	Oxadiazol	3.51	60.4	12.9	70.9	11.3
Pirimicarb	I	Carbamat	1.9	55.1	26.2	89.7	35.8
Pirimicarb D6	-	-	-	46.8	28.1	80.1	33.2
Tebuconazol	F	Triazol	3.5[d]	52.8	8.4	89.8	24.2
Tebuconazol D6	-	-	-	39.7	25.1	50.1	19.7

[a] aus Tomlin (2003), I = Insektizid, H = Herbizid, F = Fungizid [b] aus Sabljic et al. (1995)
[c] als 3,4-Dichloranilin bestimmt [d] mit Chemprop 4.1 berechnet (http://www.ufz.de/index.php?en=6738)

Die entwickelte Extraktionsmethode wurde zur Extraktion von Schwebstoffproben aus 16 französischen Bächen angewendet, die in zweiwöchigen Intervallen innerhalb von 4 Wochen gesammelt worden waren. Dabei wurden die Substanzen Acetochlor, Pirimicarb, Tebuconazol, Fenpropidin, α-Endosulfan and Chlorfenvinphos detektiert.

Acetochlor und Chlorfenvinphos wurden in Konzentrationen von bis zu 1 mg/kg, Fenpropidin und Tebuconazol wurden mit bis zu 350 µg/kg und α-Endosulfan und Pirimicarb wurden mit bis zu 140 µg/kg Trockengewicht gefunden. Ein Vergleich mit Toxizitätsdaten für Makroinvertebraten hatte zum Ergebnis, dass die Konzentrationen von Pirimicarb, α-Endosulfan und Chlorfenvinphos ökotoxikologische Effekte haben könnten, wenn ein Verteilungsgleichgewicht zwischen Wasserphase und Schwebstoffphase angenommen wird. **Zusammenfassend konnte eine Extraktionsmethode mit der ASE für die Bestimmung von schwebstoffadsorbierten polaren und semipolaren Pestiziden unterschiedlicher chemischer Stoffklassen entwickelt und erfolgreich auf Freilandproben angewendet werden.**

Kalibrierung des Chemcatcher® Passivsammlers zur Bestimmung von 10 polaren und semipolaren Pestiziden und Einsatz in 16 kleinen französischen Fließgewässern

Damit nach einer Freilandexposition des Chemcatcher® Passivsammlers zeitlich-gewichtete Durchschnittskonzentrationen (TWA-Konzentrationen) für Analyten bestimmt werden können, werden für den Chemcatcher® der jeweiligen Empfängerphase substanzspezifische Sammelraten benötigt. Zur Ermittlung von Sammelraten wurden deshalb zwei Laborexperimente durchgeführt, in denen jeweils 14 Chemcatcher® in einem Durchflusssystem unter zwei unterschiedlichen Fließgeschwindigkeiten (0.135 m/s und 0.4 m/s) gegenüber 12 polaren und semipolaren Pestiziden mit einer konstanten Wasserkonzentration von 100 µg/L exponiert wurden (Kapitel 3). Innerhalb der jeweils 14-tägigen Experimente blieben die Aufnahmeraten in die Empfängerphase der Passivsammler (SDB-XC Empore Disk) für alle Substanzen linear, was bedeutet, dass der Chemcatcher® in der beschriebenen Konfiguration bis zu 14 Tage als integrativer Passivsammler bei Freilanduntersuchungen eingesetzt werden kann. Die Sammelraten der Substanzen reichten von 0.13 bis 0.44 L/Tag und waren leicht höher für die niedrigere Fließgeschwindigkeit (Tabelle 2). Es wurde kein signifikanter Zusammenhang zwischen den Sammelraten und dem log K_{OW} oder der Löslichkeit gefunden.

Ein Problem bei Freilanduntersuchungen mit Passivsammlern stellen mögliche Unterschiede in den Umweltbedingungen zwischen Probestellen dar, weil sie die Aufnahmeraten von Substanzen in den Passivsammler beeinflussen können. Bei isotroper Austauschkinetik kann das Performance Reference Compound (PRC)-Konzept verwendet werden (Huckins et al. 2002), um den Einfluss der Umweltbedingungen bei der Berechnung der TWA-Konzentrationen berücksichtigen zu können. Deswegen wurde im Rahmen des Kalibrationsexperimentes die Abgabekinetik von zwei beispielhaften

deuterierten polaren Insektiziden (Pirimicarb D6 und Chlorfenvinphos D10) von der Empfängerphase untersucht. Die Abgabekinetik beider Substanzen wies eine hohe Variabilität von bis zu 250% Standardabweichung auf, was die Anwendung des PRC-Konzeptes für polare Empfängerphasen ohne diffusionslimitierende Membran in Frage stellt.

Bei der Freilandexposition des Chemcatchers® über einen Zeitraum von 10-13 Tagen in 16 französischen Bächen (Kapitel 4) wurden 7 der 10 ausgewählten Verbindungen detektiert (Tabelle 2). Dabei wurden die beiden Herbizide Acetochlor und Alachlor mit TWA-Konzentrationen von bis zu 1230 ng/L gefunden, während die anderen Substanzen maximale TWA-Konzentrationen von 159 ng/L aufwiesen. Insgesamt zeigte sich beim Kalibrationsexperiment und der Freilandexposition, dass der Chemcatcher® mit einer SDB-XC Empore Disk als Empfängerphase geeignet ist, polare und semipolare Substanzen zu erfassen.

Tabelle 2: Physikochemische Daten, Sammelraten (R_s) mit Variationskoeffizienten (CV) aus zwei Kalibrationsexperimenten mit unterschiedlichen Fließgeschwindigkeiten (v) und maximale TWA-Wasserkonzentration in 16 französischen Bächen für 10 ausgewählte Pestizide

Pestizid	log K_{ow}[a]	Typ[b]	Löslichkeit[b] (mg/L)	v = 0.14 m/s		v = 0.4 m/s		Max. TWA Konz. (ng/L)[c]
				R_s (L/d)	CV (%)	R_s (L/d)	CV (%)	
Pirimicarb	1.70	I	3000	0.32	16.5	0.31	11.7	12
Carbofuran	2.32	I	320	0.28	10	0.22	10	159
Acetochlor	2.39	H	223	0.13	14.8	0.14	23.3	1158
Fenpropidin	2.90[b]	F	530	0.42	16.6	0.38	10.8	nn
Chlorfenvinphos	3.10	I	121	0.3	19	0.27	17.8	nn
Linuron	3.20	H	63.8	0.26	24.2	0.21	15.9	66
Alachlor	3.52	H	170	0.12	21.5	0.13	17.8	1233
Tebuconazol	3.70[b]	F	36	0.44	22.6	0.34	12.2	15
α-Endosulfan	3.83[b]	I	0.32	0.38	20	0.29	11.4	nn
Oxadiazon	4.80	H	1	0.19	30.1	0.24	12.4	26

[a] entnommen aus Sabljic et al. (1995) [b] entnommen aus Tomlin (2003), I = Insektizid, H = Herbizid, F = Fungizid c nn = nicht nachgewiesen

Vergleich von drei Probenahmemethoden für die Erfassung der Pestizidbelastung in kleinen Fließgewässern

Um die Geeignetheit zur Erfassung der Pestizidbelastung von kleinen Fließgewässern zu beurteilen, wurden drei verschiedene Probenahmemethoden für einen Zeitraum von 2 Wochen miteinander verglichen (Kapitel 4). Bei den Probenahmemethoden handelte es

sich um den Chemcatcher® Passivsammler mit einer SDB-XC Empore Disk als Empfängerphase, einen Schwebstoffsammler bei dem die Extraktion wie oben beschrieben (S.141) erfolgte und einen ereignisbezogenen Wasserprobenehmer (Technische Zeichnungen aller Probenahmesysteme auf S.101). Der ereignisbezogene Wasserprobenehmer nahm jeweils bei einer signifikanten Erhöhung des Wasserspiegels des entsprechenden Fließgewässers (> 5-10 cm) eine 1-L Wasserprobe, da vorherige Studien gezeigt hatten, dass die Erhöhung des Wasserspiegels mit dem Eintrag von Pestiziden über Runoff einherging (Schulz et al. 2001, Liess und von der Ohe 2005).

Mit dem ereignisbezogenen Wasserprobenehmer wurden alle 10 ausgewählten Pestizide und mit dem Chemcatcher® 7 der 10 Substanzen gefunden (Tabelle 2). An den Schwebstoffen wurden im entsprechenden Zeitraum nur die 5 Verbindungen Acetochlor, Tebuconazol, Fenpropidin, α-Endosulfan und Chlorfenvinphos nachgewiesen. Die absolute Anzahl an Detektionen über der Bestimmungsgrenze war in der Wasserphase mit 66 für den ereignisbezogenen Wasserprobenehmer und mit 54 für den Chemcatcher® signifikant höher (χ^2-Test mit Bonferroni-Korrektur, $p < 0.05$) als an den Schwebstoffen, wo nur 22 Detektionen oberhalb der Bestimmungsgrenze gefunden wurden. Dabei wurde auch festgestellt, dass die TWA-Konzentrationen des Passivsammlers und die Wasserkonzentrationen des ereignisbezogenen Wasserprobenehmers signifikant korrelierten (Pearson's $r = 0.79$, $p > 0.01$, $n = 75$). Dieser enge Zusammenhang zeigt, dass die hauptsächliche Belastung, die der Passivsammler in den 2 Wochen registrierte, in Verbindung mit der Erhöhung des Wasserspiegels durch ein starkes Regenereignis auftrat, was wiederum auf die Relevanz des Eintragspfades Runoff hinweist.

Die Pestizidkonzentrationen in der Wasserphase waren auch relevanter für die Erklärung der Variation des SPEAR-Index, der biologische Effekte durch Pestizidstress anzeigt. So konnten die TWA-Konzentrationen des Chemcatchers® 50% und die Konzentrationen des ereignisbezogenen Wasserprobenehmers 38% der Varianz des SPEAR-Index erklären, während die Schwebstoffkonzentrationen nur 1% dieser Varianz erklären konnten.

Insgesamt ist also die Beprobung der Wasserphase geeigneter, um die Belastung mit polaren und semipolaren Pestiziden zu bestimmen. Außerdem zeigte die Untersuchung, dass der Chemcatcher® in der Lage ist, episodische Eintragsereignisse wie pestizidbelasteten Runoff zu erfassen.

Effekte von Pestiziden auf das Ökosystem kleiner Fließgewässer in zwei beispielhaften Gebieten mit intensiver Landwirtschaft

Im Rahmen der vorliegenden Arbeit wurden Felduntersuchungen zu den Auswirkungen von Pestiziden auf Fließgewässerökosysteme in zwei unterschiedlichen biogeografischen Regionen mit kontrastierendem Pestizideinsatz durchgeführt (Kapitel 5). Hierfür wurden zwei Regionen in Frankreich und Finnland ausgewählt, da für diese Gebiete Ergebnisse behördlicher Monitoringprogramme und Anwendungsmengendaten vorlagen, die benötigt wurden, um aus den Hunderten von zugelassenen Wirkstoffen potentiell ökotoxikologisch relevante Substanzen zu identifizieren. Für jedes Gebiet wurden die 10 Pestizide mit der höchsten Ökotoxizität gegenüber dem Standardtestorganismus *Daphnia magna* ausgewählt (Tabelle 3). Die Untersuchungen umfassten zum einen die Aufnahme des ökologischen Gewässerzustandes durch Beprobung der Makroinvertebratengemeinschaft sowie Messung der Blattabbaurate und zum anderen die Bestimmung der Konzentrationen der ausgewählten Pestizide.

In den finnischen Fließgewässern wurde nur eine der zehn ausgewählten Substanzen (das Fungizid Trifluralin) detektiert, die aber keine nachweisbaren Effekte auf die Makroinvertebratengemeinschaften oder den Blattabbau hatte. In der französischen Region wurden alle der zehn ausgewählten Pestizide gefunden (Tabelle 3). Mit steigendem Pestizidstress, gemessen in toxischen Einheit (TU) für *Daphnia magna*, nahm die Abundanz und Artenzahl der mit dem SPEAR-Konzept als empfindlich klassifizierten Makroinvertebraten ab (siehe S.111 für Details zur Methode). Die Verfügbarkeit von unbelasteten Gewässerabschnitten flussaufwärts konnte die Auswirkungen von Pestiziden auf die empfindlichen Arten signifikant verringern (Welch's t-Test, $p < 0.01$, $n = 16$), vermutlich dadurch, dass diese Gewässerabschnitte als Rekolonisationsreservoir dienten. Das Ausmaß der strukturellen Änderung in der Invertebratengemeinschaft in den französischen Fließgewässern korrelierte eng mit der Abnahme einer Ökosystemfunktion, dem Blattabbau (Pearson's $r = 0.94$, $p < 0.01$, $n = 11$).

Im Anschluss wurde untersucht, inwieweit das SPEAR-Konzept geeignet ist, um Biomonitoringdaten aus verschiedenen biogeografischen Regionen vergleichend zu analysieren. Dafür wurden auch die Ergebnisse von Felduntersuchungen in Norddeutschland einbezogen (Liess und von der Ohe 2005). Es zeigte sich, dass die SPEAR-Indizes von Referenzgewässern und belasteten Gewässern über die drei Regionen in Finnland, Frankreich und Deutschland hinweg signifikant unterschiedlich waren (Behrens-Fischer post-hoc Test, $p < 0.05$, $n = 49$). Des Weiteren wurden signifikante

Effekte von Pestiziden auf die Invertebratengemeinschaft im Bereich zwischen 1/100 bis 1/1000 des LC50 für *Daphnia magna* festgestellt (Behrens-Fischer post hoc Test, $p < 0.01$, $n = 49$), ein Expositionsniveau das bisher als unproblematisch galt.

Zusammenfassend zeigen die Ergebnisse, dass Pestizide die Struktur der Invertebratenfauna verändern können, woraus eine Beeinträchtigung von wichtigen Ökosystemfunktionen resultieren kann. Außerdem ist der SPEAR-Index geeignet, um ökologische Effekte über große geografische Regionen hinweg zu identifizieren.

Tabelle 2: Physikochemische Eigenschaften, Toxizität und maximal gemessene Konzentrationen (MK) mit der zugehörigen toxischen Einheit für *Daphnia magna* (TU) für die ausgewählten Substanzen im finnischen und französischen Monitoringprogramm (MP)

Pestizid	MP[a]	Typ[b,c]	Verbindungs-klasse[b,d]	log K_{ow}[b]	LC50 (µg/L)[b,e]	MK (µg/L)[f]	TU[f]
Acetochlor	FR	H	Chloracetamid	4.14	9000	1.920	-3.67
Alachlor	FR	H	Chloracetamid	3.09	10000	0.806	-4.09
α-Endosulfan	FR	I	Organochlor	4.74	75	0.076	-2.99
Carbofuran	FR	I	Carbamat	1.52	38.6	0.715	-1.73
Chlorfenvinphos	FR	I	OPS	3.85	0.3	0.115	-0.42
Fenpropidin	FR	F	Piperidin	2.59	500	0.059	-3.93
Linuron	FR	H	Carbamid	3.00	120	0.097	-3.09
Oxadiazon	FR	H	Oxadiazol	4.91	2400	0.071	-4.53
Pirimicarb	FR	I	Carbamat	1.70	17	0.072	-2.37
Tebuconazol	FR	F	Triazol	3.70	4200	0.070	-4.78
α-Cypermethrin	FIN	I	Pyrethroid	6.94	0.15	nn	nn
α-Endosulfan	FIN	I	Organochlor	4.70	75	nn	nn
Azoxystrobin	FIN	F	Strobilurin	2.50	259	nn	nn
Cyprodinil	FIN	F	Pyrimidin	3.90	10	nn	nn
Deltamethrin	FIN	I	Pyrethroid	6.20	3.5	nn	nn
λ-Cyhalothrin	FIN	I	Pyrethroid	7.00	0.38	nn	nn
Malathion	FIN	I	OPS	2.75	1	nn	nn
Sulfotep	FIN	I	OPS	3.99	2	nn	nn
τ-Fluvalinate	FIN	I	Pyrethroid	6.70	1	nn	nn
Trifluralin	FIN	F	Dinitroanilin	4.80	245	0.001[g]	-4.34

[a] FR = Frankreich, FIN = Finnland [b] entnommen aus Tomlin (2001) [c] H = Herbizid, F = Fungizid und I = Insektizid [d] OPS = Organophosphorsäure [e] LC50 für *Daphnia magna* [f] nn = nicht nachgewiesen [g] TWA-Konzentration

Diskussion der wichtigsten Ergebnisse und Ausblick

Neue Methoden für die Expositionsabschätzung von Pestiziden

Im Rahmen der vorliegenden Arbeit wurde eine neue Extraktionsmethode für partikeladsorbierte Pestizide entwickelt und eine Passivsammlermethode mit dem Chemcatcher® für polare Pestizide kalibriert und angewendet. Im Vergleich zu Probenahmemethoden für die Wasserphase konnte die Erfassung der schwebstoffadsorbierten Pestizide keinen Beitrag zur Aufklärung von Effekten liefern (Kapitel 4, S.92). Diesbezüglich gilt es in zukünftigen Studien zu klären, ob die Erklärungskraft der schwebstoff- oder sedimentadsorbierten Pestizidkonzentrationen für unpolare Substanzen mit einem log $K_{OC} > 3$ oder für chronische Effekte höher wäre. Eine große Herausforderung stellt diesbezüglich auch die Entwicklung einer effizienteren Aufreinigungsmethode für polare und semi-polare Substanzen dar, da die Bestimmungsgrenzen aufgrund der starken Matrixbelastung von Schwebstoff- und Sedimentproben vergleichsweise hoch waren. Inwiefern hier die Möglichkeiten der Dialyse mit der beschleunigten Lösemittelextraktion (ASE) genutzt werden könnten, wäre ein Ansatzpunkt für zukünftige Studien (Wenzel et al. 2004).

Ferner stellt sich die grundsätzliche Frage, ob zur Beurteilung der Exposition nicht direkt Biota extrahiert werden sollten (Nödler 2007). Letztendlich ist es die Konzentration im Organismus die ökotoxikologisch relevant ist und bei einer ausreichend großen Menge an Probenmaterial würde die Extraktion der Organismen ein realistischeres Bild der Belastungssituation abgeben (Fent 1998). Somit wäre ein weiterer Ansatz die Eignung der entwickelten Extraktionsmethode für die Extraktion von Biota zu überprüfen.

Ein wichtiges Ergebnis dieser Arbeit ist, dass der Chemcatcher® Passivsammler geeignet ist, die Belastung mit polaren und semipolaren Substanzen im Allgemeinen und speziell bei Runoff-Ereignissen zu erfassen. Allerdings scheint das Performance Reference Compound-Konzept (Huckins et al. 2002), das zur Berücksichtigung von Unterschieden in den Umweltbedingungen bei Feldexpositionen eingesetzt wird, nicht anwendbar zu sein, zumindest nicht ohne die Verwendung einer diffusionslimitierenden Membran (Tran et al. 2007). Die Verwendung einer solchen Membran könnte auch das Problem des Biofouling der Empfängerphase lösen. Inwiefern bei Verwendung einer Membran allerdings noch ein kurzfristiges Eintragsereignis wie Runoff erfasst wird, müsste in zukünftigen Studien geklärt werden.

Im Vergleich mit einem ereignisbezogenen Wasserprobenehmer, der schon in verschiedenen Feldstudien eingesetzt wurde (Schulz et al. 2001, Liess und von der Ohe 2005), detektierte der Chemcatcher® eine etwas geringere Anzahl von Substanzen. Bezüglich der Erklärung der Variabilität des SPEAR-Index ergaben sich daraus aber keine nennenswerten Unterschiede zum ereignisbezogenen Wasserprobenehmer. Die gute Erklärungsleistung der zeitlich-gewichteten Durchschnittskonzentrationen (TWA) des Chemcatcher® für ökologische Effekte ergab sich vermutlich aus dem Umstand, dass Runoff der hauptsächliche Eintragspfad war. In Situationen, wo neben episodischer Exposition auch eine kontinuierliche Belastung mit der gleichen Verbindung vorkommt, wäre die Erklärungsleistung der TWA-Konzentrationen wahrscheinlich gering, da sich keine eindeutige Beziehung zur akuten oder chronischen Konzentration herstellen ließe.

Nichtsdestotrotz stellt das Passivsammeln ein effektives Instrument zur kontinuierlichen Gewässerüberwachung dar. Auch für größere Freilandmonitoringprogramme, bei denen die Proben aus dem ereignisbezogenen Wasserprobennehmer nicht unmittelbar nach einem episodischen Ereignis eingesammelt werden können, stellen Passivsammler eine Alternative dar. Schließlich wird mit dem Einsatz von Passivsammlern häufig auch eine wesentlich geringere Nachweisgrenze als bei Wasserproben erreicht, so dass sie für das Screening von neuen Problemstoffen in der Umwelt eingesetzt werden könnten (Muir und Howard 2006).

Finnland als Leitbild für die Reduktion der Pestizidbelastung in anderen europäischen Länden?

Für das europäische Gewässermanagement legen die Ergebnisse der Feldstudien nahe, dass eine Pestizidanwendung, wie sie in der finnischen Landwirtschaft praktiziert wird, keine nachhaltige Schädigung der Gewässerfauna erwarten lässt. Als Hauptursache für die geringe Belastung der finnischen Gewässer wurde die niedrige Ausbringungsmenge angenommen, die in Finnland im Vergleich zu anderen europäischen Ländern um bis zu einen Faktor 10 kleiner ist (EUROSTAT 2002). Eine Untersuchung in zwei Regionen in Kalifornien identifizierte ebenfalls die Ausbringungsmenge von Pestiziden als primäre Prädiktorvariable der Konzentration und Toxizität von Pestiziden in Gewässern (Hunt et al. 2006). Auch eine Studie zur Fracht und Konzentration von 25 Pestiziden in schwedischen Fließgewässern erklärte den Großteil der Variabilität in diesen Variablen mit Unterschieden in der Ausbringungsmenge (Kreuger und Tornqvist 1998). Die geringe Ausbringungsmenge in Finnland resultiert aus der geografischen Lage, die mit einer

vergleichsweise niedrigen Präsenz von potentiellen Schädlingen für die Landwirtschaft einhergeht (Patterson et al. 1999). Daraus folgt, dass die finnische Ausbringungsmenge von Pestiziden nicht ohne weiteres auf mittel- und südeuropäische Regionen mit stärkerem Vorkommen an Schädlingen übertragbar ist, wenn Einbußen bei den Ernteerträgen vermieden werden sollen. Eine empirische Untersuchung in verschiedenen Entwicklungsländern zeigte jedoch, dass die Ausbringungsmengen um 42% verringert werden konnten, ohne Einbußen bei den Ernteerträgen hervorzurufen wenn geänderte landwirtschaftliche Nutzungspraktiken wie das integrierte Schädlingsmanagement oder das integrierte Nährstoffmanagement angewendet wurden (Pretty et al. 2006). Inwiefern diese Ansätze auch in der europäischen Landwirtschaft Anwendung finden könnten, müsste näher untersucht werden.

Allerdings können die Pestizideinträge in Gewässer nicht nur durch eine Verringerung der Ausbringungsmenge sondern auch durch Maßnahmen zur Reduktion des Austrags der Pestizide vom Feld reduziert werden (Abbildung 1). Im Rahmen der vorliegenden Arbeit wurde bis auf die Aufnahme der Feldrandstreifenbreite nicht untersucht, inwiefern solche Maßnahmen zur geringen Belastung der finnischen Gewässer beitrugen. Allerdings fiel auf, dass bei zwei Feldern mit relativ insektizidintensiven Früchten (Zuckerrüben) ein Streifen mit Getreide den Abstand zum Gewässer vergrößerte. Ansonsten waren die Randstreifen bei den finnischen Gewässern im Durchschnitt nicht breiter als bei den französischen. Zukünftige Studien müssten eine Befragung der Landwirte in das Untersuchungsdesign integrieren, um die Anwendung und Wirkung solcher Maßnahmen zu untersuchen. Die Effektivität von Maßnahmen zur Reduktion der Einträge in die Gewässer wie z.B. das Anlegen von Feldrandstreifen oder die zeitliche Anpassung der Ausbringung wurde kürzlich in einer Metastudie untersucht (Reichenberger et al. 2007). Dabei zeigte sich, dass die Wirksamkeit der Maßnahmen unter anderem vom jeweiligen Substanzspektrum abhängt. So sind z.B. Randstreifen primär zur Verringerung des Austrags von unpolaren Substanzen effektiv. Diesbezüglich schließt sich auf Basis der Ergebnisse dieser Arbeit auch die Frage an, inwieweit das angewendete Substanzspektrum in Finnland (eher unpolar) für geringere Einträge in die finnischen Gewässer verantwortlich ist im Vergleich zu den in Frankreich eingesetzten Wirkstoffen (eher polar). Offen ist vor allem, ob ein geringerer Eintrag von unpolaren Substanzen die Effekte aufgrund der meist höheren Toxizität dieser Verbindungen kompensieren kann (von der Ohe et al. 2005).

Insgesamt kann auf Grundlage der Ergebnissen der vorliegenden Arbeit geschlossen

werden, dass in den finnischen Fließgewässern im Allgemeinen keine negativen Auswirkungen durch Pestizide zu erwarten sind. Allerdings kann die finnische Situation aufgrund der mit der geografischen Situation verbundenen Randbedingungen nur eingeschränkt als Leitbild für andere europäische Regionen fungieren. Ohnehin stellt es eine Wertentscheidung dar, ob ein solcher Umweltzustand erreicht werden soll oder die Kosten für dieses Schutzniveau für andere Regionen zu hoch wären (Boesten et al. 2007).

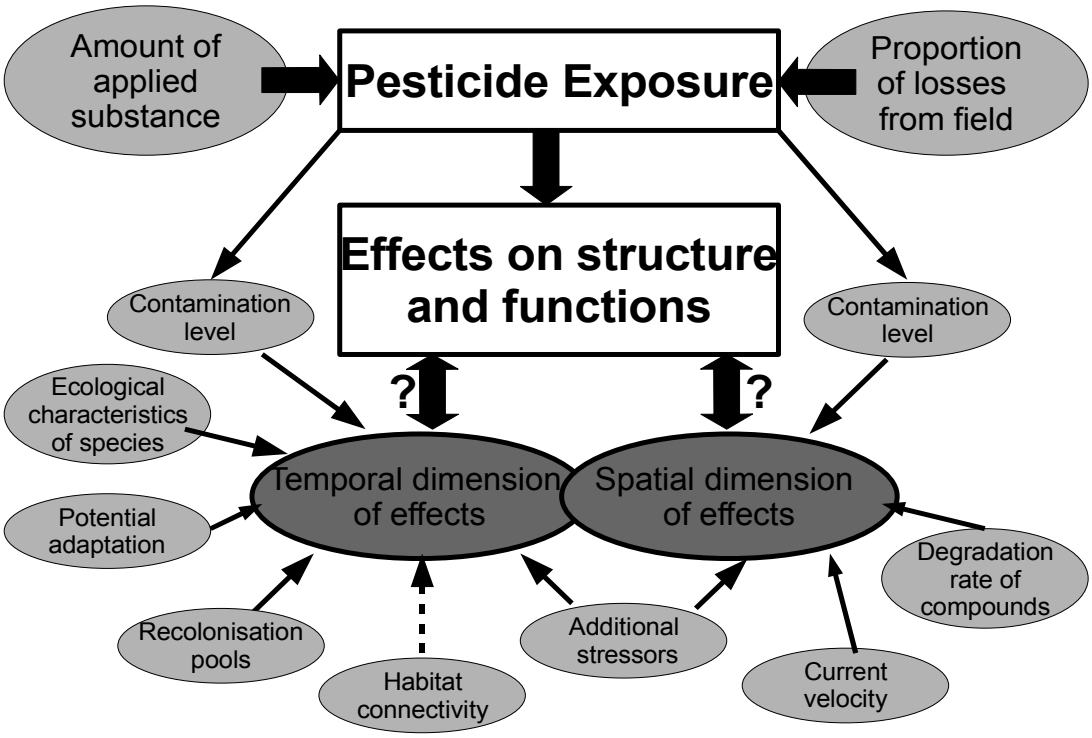

Abbildung 1: Einflussfaktoren für die räumliche und zeitliche Dimension der Effekte von Pestiziden.

Folgerungen und offene Fragen aus der Studie an französischen Fließgewässern über die Gefährdung von Fließgewässerökosystemen durch Pestizide

Die Ergebnisse einiger Feldstudien inklusive dieser Arbeit zeigen, dass die gegenwärtige Praxis der Pestizidanwendung Effekte auf die Invertebratenfauna kleiner Fließgewässer haben kann (vgl. S.25). Die vorliegende Arbeit kommt außerdem zum Ergebnis, dass auch unterhalb einer toxischen Einheit von 1/100 des LC50 für *Daphnia magna* Veränderungen der Struktur der Makroinvertebratengemeinschaft auftreten können, was wiederum in einer Beeinträchtigung von Ökosystemfunktionen resultieren kann. Bisher wurde davon ausgegangen, dass unterhalb der Schwelle von 1/100 der TU für *Daphnia magna* keine nachteiligen Effekte auftreten. Deshalb wurde diese Schwelle als zulässiges Expositionsniveau bei der Zulassung von Pestiziden in der EU festgelegt (EEC 1991).

Auf die Gründe, warum in den meisten Mesokosmenstudien keine Effekte bei einem Expositionsniveau von 1/100 der TU für *Daphnia magna* gefunden wurden, wurde bereits in Kapitel 5 (S.124) ausführlich eingegangen. Ein häufiger Vorwurf gegenüber Felduntersuchungen ist, dass Effekte bei geringen Konzentrationen lediglich das Artefakt einer unterschätzten Höchstkonzentration seien. In Bezug auf diese Arbeit sei dazu angemerkt, dass selbst eine Unterschätzung der Höchstkonzentration im Gewässer um den Faktor 2 nur eine Veränderung der TU um den Faktor 1.3 ($\log_{10}$ 2) zur Folge hätte. Eine solche Unterschätzung der Höchstkonzentrationen würde also das Ergebnis, dass Effekte unterhalb der Schwelle von 1/100 der TU für *Daphnia magna* auftreten, nicht grundsätzlich verändern. Allerdings zeigte das Verhältnis zwischen den Durchschnittskonzentrationen in den Passivsammlern und den Spitzenkonzentration die mit dem ereignisbezogenen Probenehmer ermittelt wurden ohnehin, dass die Spitzenkonzentrationen nicht wesentlich unterschätzt wurden (S.95).

Eine wichtige Fragestellung die sich in Bezug auf die Effekte anschließt, ist die Frage, welcher zeitlichen und räumlichen Dimension die beobachteten Effekte angehören. Zu beiden Aspekten gibt es bisher nur wenige Freilanduntersuchungen. Bezüglich der zeitlichen Dimension zeigte eine Untersuchung, bei der ein Fließgewässer intentionell mit Methoxychlor kontaminiert wurde, dass die Wiedererholung der geschädigten Gemeinschaft bis zu fünf Jahre dauern kann (Wallace et al. 1986). Eine weitere Studie in der Region Braunschweig kam zu dem Ergebnis, dass innerhalb von einem Jahr keine vollständige Wiedererholung von einer Schädigung durch Pestizide erfolgte (Liess und von der Ohe 2005). Generell determinieren verschiedene Faktoren die Dauer der Wiedererholung, wobei die Relevanz der einzelnen Faktoren bisher noch nicht vergleichend untersucht wurde (Schäfer und Liess 2005) (Abbildung 1). Ein wichtiger Faktor für die Wiedererholung ist die Anwesenheit von flussaufwärts gelegenen unbelasteten Gewässerabschnitten, was auch die vorliegende Arbeit zeigte. Diese Abschnitte können die Effekte von Pestiziden auf die Gemeinschaftsstruktur vermutlich durch Wiederbesiedelung puffern (Hatakeyama und Yokoyama 1997, Liess und von der Ohe 2005), die genauen ökologischen Mechanismen sind allerdings ungeklärt. Da viele Invertebraten sich per Flug ausbreiten, könnte auch das Vorhandensein von unbelasteten Fließgewässern in der Nähe belasteter Gewässer zur Wiederbesiedelung und damit zur Abfederung von Pestizideffekten beitragen (Jansson et al. 2007). Weitere Faktoren, welche die Dauer der Wiedererholung beeinflussen, sind die ökologischen Eigenschaften der betroffenen Arten (Calow et al. 1997, Liess und von der Ohe 2005) und eine mögliche

Anpassung an die Störung durch Pestizide (Heckman 1981, Landis et al. 1996). Schließlich müsste eine realistische Beurteilung des Wiedererholungspotentials auch die Wirkung von anderen Stressoren auf die aquatische Biozönose einbeziehen (Heugens et al. 2001).

Bezüglich der räumlichen Dimension der Effekte existieren kaum Untersuchungen. So wurde bisher nicht untersucht, auf welcher Länge des betroffenen Gewässers Effekte durch Pestizide auftreten. Wichtige Faktoren, die die räumliche Dimension der Schädigung beeinflussen, sind die Höhe der Kontamination, die Persistenz der Substanzen und die Fließgeschwindigkeit, da diese Faktoren bestimmen wie weit die Pestizide transportiert werden. Die Relevanz dieser Faktoren für die räumliche Dimension der Effekte zeigte sich beim Sandozunfall im Rhein (Heil 1990).

Des Weiteren ist nicht bekannt, inwiefern die Effekte durch Pestizide auf kleinere Fließgewässer beschränkt sind. Bis auf Unfälle wurden bisher die Effekte von Pestiziden in größeren Gewässern aus mehreren Gründen kaum untersucht:

- Durch die Vielzahl von Stressoren und der hohen Fluktuation der Konzentrationen ist das Herstellen von einer eindeutigen kausalen Verbindung zwischen einem einzelnen Stressor und der Gemeinschaftsstruktur fast unmöglich (Allan 1995)
- Flüsse bestehen im Querschnitt aus einer Vielzahl von Habitaten mit heterogener Verteilung der Arten, so dass auch die Effekte innerhalb des Querschnitts vermutlich unterschiedlich ausfallen würden (Lake 2000)
- Ein repräsentatives Monitoring der Invertebratengemeinschaft ist wesentlich aufwändiger als in kleinen Fließgewässern (Flotemersch et al. 2006)
- Es lassen sich nur schwer unbelastete Referenzgewässer mit ähnlichen Eigenschaften finden, da es nur eine begrenzte Anzahl von größeren Fließgewässern in einer Region gibt. Aufgrund der geringeren Stichprobe wäre die statistische Teststärke, signifikante Unterschiede zu finden, dann niedrig.

Gleichwohl ergab eine Auswertung von Punktwasserproben in norddeutschen Flüssen, dass bis zu 20% der Proben eine TU von größer als 1/10 des LC50 für *Daphnia magna* aufwiesen, was eine Konzentration darstellt bei der sowohl in Mesokosmen- als auch Freilandstudien deutliche Effekte beobachtet wurden (Van Wijngaarden et al. 2005). Da zur Zeit häufig noch großes Unwissen bezüglich der Ursachen des schlechten ökologischen Zustands von Fließgewässern besteht (Posthuma und De Zwart 2006), wären auch Studien an größeren Fließgewässern dringend notwendig, um die Relevanz von Pestiziden auf der Ebene des Einzugsgebietes beurteilen zu können.

Identifikation von Stressoren mit merkmalsbasierten Indizes

Die Invertebratenfauna wird durch verschiedene Stressoren beeinflusst und wie auch die vorliegende Arbeit aufzeigt, können Pestizide ein relevanter Stressor sein. Im Rahmen einer Feldstudie an 20 Fließgewässern im Raum Braunschweig wurde der SPEAR-Index eingeführt, um Effekte von Pestiziden nachzuweisen (Liess und von der Ohe 2005). Die Ergebnisse dieser Arbeit bestätigen, dass dieser Index brauchbar ist, um Effekte von Pestiziden vor dem Hintergrund der natürlichen Variabilität von Lebensgemeinschaften zu identifizieren. Bemerkenswert war dabei insbesondere, dass die Dosis-Wirkungsbeziehung zwischen Pestizidstress und dem SPEAR-Index in verschiedenen biogeografischen Regionen nicht signifikant unterschiedlich war. Auch für das Biomonitoring über größere geografische Regionen hinweg erwies sich der Index als anwendbar, da:

- Referenzstellen in drei Regionen in Mittel- und Nordeuropa sich nicht signifikant unterschieden,
- und die Referenzstellen aller Regionen signifikant unterschiedliche Indexwerte im Vergleich mit belasteten Probestellen zeigten.

Dieses Ergebnis korrespondiert mit den Resultaten anderer Studien, die ein ähnliches Profil der Merkmalausprägungen von Invertebratengemeinschaften an europäischen Referenzstellen aufzeigten (Charvet et al. 2000, Statzner et al. 2005). Daran schließen sich verschiedene Fragestellungen an.

Zunächst stellt sich die Frage, ob die Dosis-Wirkungsbeziehung zwischen Pestizidstress und dem SPEAR-Index auf südeuropäische Regionen übertragen werden kann. Dabei ist insbesondere zu berücksichtigen, dass viele kleine Fließgewässer in Südeuropa zeitweise trocken fallen. Inwiefern dadurch die Auswirkungen von toxischem Stress verstärkt werden oder aber umgekehrt toxischer Stress vergleichsweise unbedeutend ist, sollte in zukünftigen Studien untersucht werden. Die Ergebnisse wären auch für nordeuropäische Regionen relevant, denn für diese wird in einigen Klimaszenarien eine Zunahme von Trockenheit vorhergesagt (Arnell 1999, Milly et al. 2005).

Als weitere Fragestellung schließt sich die Eignung von Indizes, die auf ökologischen und physiologischen Merkmalen beruhen, für andere Stressoren an. Besonders im Kontext der Umsetzung der europäischen Wasserrahmenrichtlinie (WRRL) ist die Identifizierung der Ursachen für die Abweichung vom guten ökologischen Zustand ein dringendes Problem. In zwei Studien zu den Auswirkungen von Landnutzung und Salinität wurde bereits

gezeigt, dass merkmalsbasierte Indizes ein effektives Instrument zur Identifikation dieser Stressoren darstellen könnten (Doledec et al. 2006, Piscart et al. 2006). Dabei wäre für Stressoren wie Salinität oder Schwermetalle die Entwicklung eines Indexsystems analog zum SPEAR-Index möglich, da eine Vielzahl von Daten zur physiologischen Toleranz von Invertebraten (Kefford et al. 2003, von der Ohe und Liess 2004) und zu den ökologischen Merkmalen für europäische und nordamerikanische Arten vorliegen (Vieira et al. 2006, Statzner et al. 2007). Für andere Stressoren wie z.B. Habitatdegradation durch Kanalisierung oder Ausbaggerung dürfte die Entwicklung von merkmalsbasierten Indikatoren schwieriger sein, denn hier wird die Merkmalszusammensetzung von Gemeinschaften sich vermutlich schon zwischen verschiedenen Flusstypen unterscheiden, da hier Veränderungen in der Zielvariable (Geomorphologie) schon konstitutiv für Unterschiede zwischen Fließgewässertypen sind (Lorenz et al. 2004). Für solche Stressoren müssten also Referenzzustände in Abhängigkeit von den jeweiligen Fließgewässertypen definiert werden. Ingesamt sind auf diesem Gebiet also einige Ansatzpunkte zur Entwicklung effektiver Instrumente für die Identifikation von Stressoren vorhanden und der Autor hofft, mit dieser Arbeit einen Anstoß zu weiteren Studien geleistet zu haben.

Literaturverzeichnis Kapitel 6

Allan, J. D. 1995. Stream Ecology. Kluwer Academic Publishers, Dordrecht.

Arnell, N. W. 1999. The effect of climate change on hydrological regimes in Europe: a continental perspective. Global Environmental Change-Human and Policy Dimensions 9:5-23.

Boesten, J. J. T. I., H. Kopp, P. I. Adriaanse, T. C. M. Brock, und V. E. Forbes. 2007. Conceptual model for improving the link between exposure and effects in the aquatic risk assessment of pesticides. Ecotoxicology and Environmental Safety 66:291-308.

Calow, P., R. M. Sibly, und V. Forbes. 1997. Risk assessment on the basis of simplified life-history scenarios. Environmental Toxicology and Chemistry 16:1983-1989.

Charvet, S., B. Statzner, P. Usseglio-Polatera, und B. Dumont. 2000. Traits of benthic macroinvertebrates in semi-natural french streams: An initial application to biomonitoring in Europe. Freshwater Biology 43:277-296.

Doledec, S., N. Phillips, M. Scarsbrook, R. H. Riley, und C. R. Townsend. 2006. Comparison of structural and functional approaches to determining landuse effects on grassland stream invertebrate communities. Journal of the North American Benthological Society 25:44-60.

EEC. 1991. Council Directive of 15 July 1991 concerning the placing of plant protection products on the market. . Pages 137 in. Office for Official Publications of the European Communities.

EUROSTAT, S. O. o. t. E. C. 2002. Use of plant protection products in the European Union Office for Official Publication of the European Union, Luxembourg.

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

Flotemersch, J. E., K. Blocksom, J. J. Hutchens, und B. C. Autrey. 2006. Development of a standardized Large River Bioassessment Protocol (LR-BP) for macroinvertebrate assemblages. River Research and Applications 22:775-790.

Hatakeyama, S., und 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.

Heckman, C. W. 1981. Long-term effects of intensive pesticide applications on the aquatic community in orchard ditches near Hamburg, Germany. Archives of Environmental Contamination and Toxicology 10:393-426.

Heil, K. H. 1990. Die Auswirkungen des Sandoz-Unfalls auf die Biozönose des Rheins. Pages 11-26 in G. Friedrich und R. Kinzelbach, editors. Limnologie aktuell: Biologie des Rheins. Gustav Fischer, Stuttgart, New York.

Heugens, E., A. Hendriks, T. Dekker., N. M. Van Straalen, und W. Amiraal. 2001. A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. Critical Reviews in Toxicology 31:247-284.

Huckins, J. N., J. D. Petty, J. A. Lebo, F. V. Almeida, K. Booij, D. A. Alvarez, R. C. Clark, und B. B. Mogensen. 2002. Development of the permeability/performance reference compound approach for in situ calibration of semipermeable membrane devices. Environmental Science and Technology 36:85-91.

Hunt, J. W., B. S. Anderson, B. M. Phillips, R. S. Tjeerdema, N. Richard, V. Connor, K. Worcester, M. Angelo, A. Bern, B. Fulfrost, und D. Mulvaney. 2006. Spatial relationships between water quality and pesticide application rates in agricultural watersheds. Environmental Monitoring and Assessment 121:245-262.

Jansson, R., C. Nilsson, und B. Malmqvist. 2007. Restoring freshwater ecosystems in riverine landscapes: the roles of connectivity and recovery processes. Freshwater Biology 52:589-596.

Kefford, B. J., P. J. Papas, und D. Nugegoda. 2003. Relative salinity tolerance of macroinvertebrates from the Barwon River, Victoria, Australia. Marine and Freshwater Research 54:755-765.

Kreuger, J., und L. Tornqvist. 1998. Multiple regression analysis of pesticide occurrence in streamflow related to pesticide properties and quantities applied. Chemosphere 37:189-207.

Lake, P. S. 2000. Disturbance, patchiness, and diversity in streams. Journal of the North American Benthological Society 19:573-592.

Landis, W. G., R. A. Matthews, und G. B. Matthews. 1996. The layered and historical nature of ecological systems and the risk assessment of pesticides. Environmental Toxicology and Chemistry 15:432-440.

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

Lorenz, A., C. K. Feld, und D. Hering. 2004. Typology of streams in Germany based on benthic invertebrates: Ecoregions, zonation, geology and substrate. Limnologica 34:379-389.

Milly, P. C. D., K. A. Dunne, und A. V. Vecchia. 2005. Global pattern of trends in streamflow and water availability in a changing climate. Nature 438:347-350.

Muir, D. C. G., und P. H. Howard. 2006. Are there other persistent organic pollutants? A challenge for environmental chemists. Environmental Science & Technology 40:7157-7166.

Nödler, K. 2007. Asellus aquaticus als Passivsammler lipophiler organischer Schadstoffe in belasteten Gewässern. Master thesis. University Duisburg, Duisburg.

Patterson, D. T., J. K. Westbrook, R. J. V. Joyce, P. D. Lingren, und J. Rogasik. 1999. Weeds, insects, and diseases. Climatic Change 43:711-727.

Piscart, C., J. C. Moreteau, und J. N. Beisel. 2006. Monitoring changes in freshwater macroinvertebrate communities along a salinity gradient using artificial substrates. Environmental Monitoring and Assessment 116:529-542.

Posthuma, L., und D. De Zwart. 2006. Predicted effects of toxicant mixtures are confirmed by changes in fish species assemblages in Ohio, USA, Rivers. Environmental Toxicology and Chemistry 25:1094-1105.

Pretty, J. N., A. D. Noble, D. Bossio, J. Dixon, R. E. Hine, F. de Vries, und J. I. L. Morison. 2006. Resource-conserving agriculture increases yields in developing countries. Environmental Science & Technology 40:1114-1119.

Reichenberger, S., M. Bach, A. Skitschak, und H. G. Frede. 2007. Mitigation strategies to reduce pesticide inputs into ground- and surface water and their effectiveness; A review. Science of the Total Environment 384:1-35.

Schäfer, R. B., und M. Liess. 2005. Langzeitwirkungen von Pflanzenschutzmitteln im Freiland. Pages 217-243 in B. L. f. Wasserwirtschaft, editor. Münchner Beiträge zur Abwasser-, Fischerei und Flussbiologie. Oldenbourg, München.

Sabljic, A., Gusten, H., Verhaar, H., Hermens, J., 1995. Qsar Modeling of Soil Sorption - Improvements and Systematics of Log K-Oc Vs Log K-Ow Correlations. Chemosphere 31, 4489-4514.

Schulz, R., S. K. C. Peall, J. M. Dabrowski, und A. J. Reinecke. 2001. Current-use insecticides, phosphates and suspended solids in the Lourens River, Western Cape, during the first rainfall event of the wet season. Water SA 27:65-70.

Statzner, B., P. Bady, S. Doledec, und F. Scholl. 2005. Invertebrate traits for the biomonitoring of large European rivers: an initial assessment of trait patterns in least impacted river reaches. Freshwater Biology 50:2136-2161.

Statzner, B., N. Bonada, und S. Doledec. 2007. Conservation of taxonomic and biological trait diversity of European stream macroinvertebrate communities: a case for a collective public database. Biodiversity and Conservation 16:3609-3632.

Tomlin, C.D.S., 2003. The pesticide manual, a world compendium BCPC Publications, Hampshire, UK.

Tran, A. T. K., R. V. Hyne, und P. Doble. 2007. Calibration of a passive sampling device for time-integrated sampling of hydrophilic herbicides in aquatic environments. Environmental Toxicology and Chemistry 26:435-443.

Van Wijngaarden, R. P. A., T. C. M. Brock, und P. J. Van Den Brink. 2005. Threshold levels for effects of insecticides in freshwater ecosystems: A review. Ecotoxicology 14:355-380.

Vieira, N. K. M., N. L. Poff, D. M. Carlisle, S. R. Moulton, M. L. Koski, und B. C. Kondratieff. 2006. A database of lotic invertebrate traits for North America: U.S. Geological Survey Data Series 187. Retrieved: December 22 2006, from http://pubs.water.usgs.gov/ds187.

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

von der Ohe, P. C., R. Kuhne, R. U. Ebert, R. Altenburger, M. Liess, und G. Schuurmann. 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.

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

Wenzel, K. D., B. Vrana, A. Hubert, und G. Schuurmann. 2004. Dialysis of persistent organic pollutants and polycyclic aromatic hydrocarbons from semipermeable membranes. A procedure using an accelerated solvent extraction device. Analytical Chemistry 76:5503-5509.

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Schäfer, R.: Pestizide in niedersächsischen Fließgewässern – Hildesheim: Niedersächsisches Landesamt für Ökologie, Reihe „Oberirdische Gewässer“ 19/2003, 48 Seiten

Erklärung

Hiermit versichere ich, dass ich die eingereichte Dissertation selbstständig und ohne unerlaubte Hilfsmittel verfasst habe. Anderer als der von mir angegebenen Hilfsmittel und Schriften habe ich mich nicht bedient. Alle wörtlich oder sinngemäß den Schriften anderer Autorinnen oder Autoren entnommenen Stellen habe ich kenntlich gemacht.

Die vorliegende Arbeit wurde weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zwecke der Promotion oder eines Prüfungsverfahrens vorgelegt.

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