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Potential of 11 Pesticides to Initiate Downstream Drift of Stream Macroinvertebrates

Mikhail A. Beketov · Matthias Liess

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Abstract Downstream drift of lotic macroinvertebrates induced by toxicants is a well-known ecologically relevant phenomenon. However, little is known about which toxicants can initiate drift, and potential drift-initiating effects of contaminants are not taken into account in ecotoxicological risk assessment. The aim of the present study was to evaluate potential drift-initiating action of 11 pesticides having different target groups and modes of action. Sublethal concentrations of the pesticides were tested in stream microcosms with amphipods (*Gammarus pulex*), blackfly larvae (*Simulium latigonium*), and mayfly larvae (*Baetis rhodani*). The results show that 6 out of 11 pesticides tested can initiate drift of macroinvertebrates at sublethal concentrations 7–22 times lower than acute LC_{50}s (thiacloprid, imidacloprid, acetamiprid, iprodione, fenvalerate, and indoxacarb). All the toxicants that exhibited drift-initiating action are neurotoxic insecticides belonging to the groups of pyrethroids and neonicotinoids except the fungicide iprodione. The pesticides that did not initiate drift are fungicides (cyprodinil, prochloraz, and azoxystrobin), a juvenile-hormone mimic (fenoxycarb), and a pyrazole insecticide (tebufenpyrad) affecting cell energy production. Remarkably, for all the drift-initiating toxicants, drift of the tested animals was detected within 2 h after contamination. This shows that macroinvertebrate drift can be induced even by short-term pulse exposures to neurotoxic insecticides, at field-relevant concentrations. The present results imply that the possibility of drift-initiating effects of pesticides should be considered within the risk assessment framework for pesticides, as all neurotoxic substances that were investigated did initiate drift at sublethal concentrations.

M. A. Beketov (✉) · M. Liess
Dept. System Ecotoxicology, UFZ – Helmholtz Centre for Environmental Research, Permoserstrasse 15, 04318 Leipzig, Germany
e-mail: mikhail.beketov@ufz.de

At present the ecotoxicological risk assessment of contaminants is mainly based on lethal endpoints derived in standard toxicity tests (e.g., USEPA 1994; OECD 1997). However, many sublethal effects of toxic contaminants are known to be important within the ecological context. For example, downstream drift of stream macroinvertebrates triggered by sublethal toxicant concentrations may result in significant changes of structure of lotic communities, as many of the resident animals may be dislodged and transported to downstream locations (Brittain and Eikeland 1988; Kreutzweiser and Sibley 1991; Schulz and Liess 1999a).

Downstream drift is a common reaction of lotic macroinvertebrates to various types of disturbance, including chemical contamination (Brittain and Eikeland 1988), and has been reported by many authors for stream macroinvertebrates exposed to pesticides. In particular, drift-initiating activity was shown for pyrethroid (Kreutzweiser and Sibley 1991; Davies and Cook 1993; Liess 1993; 1994; Breneman and Pontasch 1994; Schulz and Liess 1999b; Lauridsen and Friberg, 2005), organochlorine (Cuffney et al. 1984; Wallace et al. 1989; Hose et al. 2002), and organophosphate insecticides (Muirhead-Thomson 1978; Liess 1993), lampricides (Dermott and Spence 1984), and copper (Taylor et al. 1994).

Although downstream drift is regarded as a common response to various stress factors, it is known that drift reactions of stream invertebrates may vary significantly among different taxonomic groups (Muirhead-Thomson

1978). Furthermore, the drift-initiating activities of different toxicants are known to be different (Liess 1993; Schulz and Dabrowski 2001). However, knowledge about which toxicants can initiate drift, and at what concentrations, is limited, and the ecotoxicological consequences of drift are only known to a minor extent. As a result, potential drift-initiating effects of contaminants are not taken into account in ecotoxicological environmental risk assessment and registration processes.

The aim of the present study was to evaluate potential drift-initiating activity of 11 pesticides with different modes of action and target groups. The study was designed to reveal which of the 11 considered toxicants have a drift-initiating potential at sublethal concentrations.

Materials and Methods

Experimental Synopsis

Two sets of laboratory experiments were conducted: acute toxicity tests and stream microcosm experiments. Acute toxicity tests were designed to assess toxicity of the pesticides to the test species and to derive median lethal concentrations (LC_{50}). This information was necessary for selecting the sublethal concentrations for the microcosm experiments. Stream microcosm tests were performed in order to assess drift of macroinvertebrates in response to sublethal contamination in a small model stream that mimics natural lotic habitats. In these tests the proportions of drifted (displaced downstream) and not drifted (located upstream) animals were recorded and analysed.

The following macroinvertebrates were used in the experimental work: amphipod *Gammarus pulex* (Linnaeus 1758) (Amphipoda, Gammaridae), blackfly larvae *Simulium latigonium* (Rubtsov, 1956) (Diptera, Simuliidae) and mayfly larvae *Baetis rhodani* (Pictet, 1843) (Ephemeroptera, Baetidae). The amphipod *G. pulex* and mayfly larvae *B. rhodani* were collected in a small stream near Pulsnitz city (Saxony, Germany). The stream was considered to be uncontaminated as no arable land is present for several kilometres upstream from the collecting site. Larvae of blackfly *S. latigonium* were obtained from uncontaminated stream mesocosms of the UFZ. After collection, the animals were transferred to the laboratory and kept there for acclimation in a 1:1 mixture of M7 medium (OECD 1997) and water from the stream or mesocosms from which the animals were collected. The animals were maintained at 15 ± 2°C with a photoperiod of 10:14 h (light:dark) during both the acclimation period and the experiments.

Toxicants and Analytical Measurements

The following 11 pesticides were investigated: the pyrethroid insecticide fenvalerate; the neonicotinoid insecticides thiacloprid, imidacloprid, and acetamiprid; the oxadiazine insecticide indoxacarb; the juvenile hormone mimic fenoxycarb; the pyrazole insecticide tebufenpyrad; and the fungicides iprodione, cyprodinil, prochloraz, and azoxystrobin. All toxicants used in this study were analytical-grade powders (Sigma-Aldrich Laborchemikalien, Seelze, Germany). Stock solutions of the toxicant were made in dimethyl sulfoxide (DMSO) with maximum concentration <1% of DMSO in the exposure solutions, as this solvent is known to be nontoxic at this concentration for aquatic invertebrates (Bowman et al. 1981).

Most of the solutions tested in the microcosm experiments were analytically measured. For measurements, 200–300 mL of water was collected 1 h after contamination. Measurements were performed with liquid chromatography by the EN ISO 11369 method (ISO 1997) (liquid chromatograph HPLC system with Diodenarray Detektor II Series 2000, binary pump, autosampler, 30°C column oven, PerkinElmer, Wellesley, MA, USA). The high-performance liquid chromatography (HPLC) columns were LiChrospher 60, RP-select B, 5 µm, (Merck, Darmstadt, Germany). The measurements were performed by Kommunale Wasserwerke Leipzig GmbH (Leipzig, Germany).

Nominal and measured concentrations of the pesticides tested in the microcosm experiments are given in Table 1. The following pesticides were not measured analytically due to technical reasons: acetamiprid, fenvalerate, indoxacarb, cyprodinil, and prochloraz. Toxicant concentrations tested in the acute toxicity tests were not measured.

Acute Toxicity Tests

Active and externally undamaged individuals were selected for these experiments. Ten animals per exposure treatment (including control) were put individually into 100-mL glass beakers each containing 60 mL of a test solution. The duration of the tests was 96 h. Test solutions were not renewed. The animals were not fed during the tests. All the exposure solutions were made using M7 medium (OECD 1997). The main physicochemical parameters of the M7 medium were: pH 7.4, conductivity 600 µS/cm, carbonate hardness approximately 180 mg $CaCO_3$/L. The stock solutions of the toxicants were prepared using DMSO as a carrier. Mortality was monitored daily. The death of tested organisms was defined as absence of any movement (including movement of pleopods of the gammarids).

Table 1 Nominal and measured (in parentheses) concentrations of the pesticides tested in the microcosm experiments (μg/L)

Toxicant	Nominal and measured (in parentheses) pesticide concentration, μg/L		
	Baetis rhodani	*Simulium latigonium*	*Gammarus pulex*
Thiacloprid	0.3 (0.31)	0.3 NM	50 (30.3)
Imidacloprid	1 (0.97)	NA	30 NM
Acetamiprid	0.5 NM	0.5 NM	3 NM
Iprodione	NA	NA	500 (366)
Fenvalerate	0.01 NM	0.01 NM	0.01 NM
Indoxacarb	3 NM	20 NM	300 NM
Azoxystrobin	NA	NA	20 (16.50)
Tebufenpyrad	0.2 NM	NA	3 (2.5)
Fenoxycarb	NA	50 (32.6)	100 NM
Cyprodinil	NA	50 NM	70 NM
Prochloraz	NA	100	100 NM

NA – not assessed because the number of animals was limited

NM – not measured due to technical reasons

Stream Microcosm Experiments: Drift Assessment of Macroinvertebrates

The system of stream microcosms consisted of four glass channels as shown in Fig. 1. Each stream was 1.2 m in length, 10.5 cm in height, and 4.5 cm in width. Mean (± standard error, SE) current velocity was 0.06 m/s (± 0.001) and discharge was 0.07 L/s (± 0.001). The glass channels were installed on a wooden stand such that there was a 2-cm difference in height between the right-hand (upstream) and left-hand (downstream) margins (Fig. 1). The bottom of each channel was covered with white gravel (the white colour was chosen to make detection of the animals easier). Each stream was designed as a closed circulation system. In this system the water flows as follows: from the upstream to the downstream sections of the channel it is propelled by gravity, then it falls down into the 2-L reservoir installed below the downstream margin of the channel, and then it is pumped back to the upstream section through a plastic tube (located above the stream, 5 mm in diameter) by an electric pump. The total amount of water in each microcosm system was 5 L.

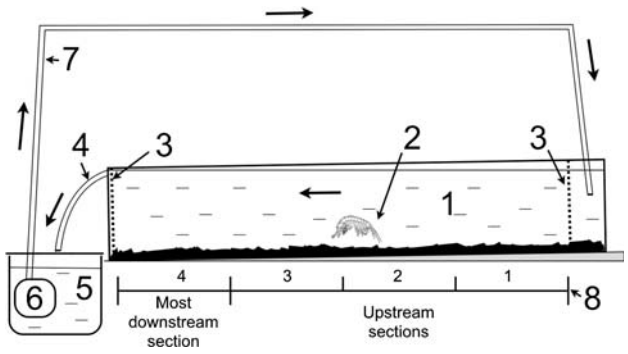

Fig. 1 Scheme of the stream microcosm. Numbers indicate: 1 – stream in glass channel, 2 – tested animal (size is not proportional to the system), 3 – metal nets, 4 – outflow pipe, 5 – water reservoir, 6 – pump, 7 – plastic pipe, 8 – scheme of stream lengthways division (explanations in the text, the thick arrows show the water flow direction)

At both the downstream (near the stream margin, before the out-flow hole) and upstream (10 cm from the stream margin, after the in-flow plastic pipe) ends of each channel metal nets (mesh size 0.5 mm) were installed (Fig. 1). Their purpose was to prevent animals from drifting into the 2-L reservoir or becoming located close to the inflow pipe, as the current in this area differed considerably from that in all other parts of the stream.

To avoid cross-contamination of the test systems with different toxicants, separate sets of all plastic equipment (tubes, pumps) were prepared for each of the tested pesticides. All glassware and metal meshes were washed with acetone and water after each experiment.

Each stream microcosm was divided lengthways into four observation sections of identical size by attaching strips of transparent tape to the outer glass surface (Fig. 1). For each experiment ten animals were located in the most upstream sections of the streams 1 h before contamination. The animals were released into the water column, after which the blackfly larvae attached themselves to the gravel and glass walls of the streams; the mayflies settled on the gravel; and the gammarids were moving near the gravel.

All the toxicants were tested on *G. pulex* and at least one of the two insect species (*B. rhodani* or *S. latigonium*), except iprodione and azoxystrobin that was tested on *G. pulex* only because of the limited number of insect larvae available.

Stock solutions of tested toxicants were pipetted into the upstream section of the streams near the in-flow plastic pipe. After contamination the distribution of animals among the four lengthways observation sections of the streams was recorded with the following time schedule: 0.5, 1, 2, 4, 22, 24, 26, 28, and 48 h after contamination. Locations of the animals were not assessed prior to contamination, since animals were observed to remain in the

upstream section where they were initially located during 1 h before contamination. Observation was performed visually; the number of animals in each section of the stream was recorded. Each experiment was started in the morning (09:00 to 10:00). Velocity and discharge were measured three times in each stream before contamination.

The concentrations used in the drift experiments were approximately ten times lower than the LC_{50} values determined in the acute tests. The results of an experiment were not accepted if there was significant mortality. In such cases the tests were repeated. Similarly, the tests were repeated if a significant difference in stream discharge between the treatments was detected. To assess possible drift-initiating effect of the carrier DMSO, separate experiments were performed with 3 and 0.3 mL/L of this solvent and a control series. This test showed no significant effect of the DMSO on the drift of the tested species.

Drift was assessed as the proportion of individuals in the most downstream section (the fourth section of the four sections outlined, Fig. 1) and all other sections of the stream microcosm considered as a single upstream section (first to third sections, Fig. 1).

Data Analyses

The median lethal concentrations (LC_{50}) were calculated by the Trimmed Spearman–Karber method (Hamilton et al. 1977) using the program Spearman (Montana State University, Bozeman, MT, USA). This method is a nonparametric statistical procedure that is effective for different concentration–response curve shapes including non-monotonic results.

In the stream microcosm experiments, drift was assessed as the proportion of individuals in the most downstream section compared with that in all other upstream sections considered as a single section (downstream/upstream proportion). Significance of differences ($p < 0.05$) from the respective controls was assessed as the proportion of drifted/not drifted individuals for each observational time-point by contingency tables with the chi-square test. This analysis was used because an individual was considered as a unit of replication, since the animals were randomly selected for the experiments (Zar 1996). Statistical analyses were performed using Prism 4.0c for Macintosh (GraphPad Software Inc.).

Results

Acute Toxicity Tests

Acute toxicity tests were used to derive the median lethal concentrations (LC_{50}), which are necessary for comparisons with the drift-initiating sublethal concentrations. The LC_{50} values are summarised in Table 2. The insect species *B. rhodani* and *S. latigonium* were more sensitive than the amphipod *G. pulex* to all the tested pesticides except fenvalerate, for which sensitivity of the insects and crustaceans were equal (Table 2).

Stream Microcosm Experiments: Drift Assessment of Macroinvertebrates

The results of the microcosm experiments showed that 6 (thiacloprid, imidacloprid, acetamiprid, iprodione, fenvalerate, and indoxacarb) out of 11 tested pesticides can initiate drift of stream-dwelling macroinvertebrates at concentrations that cause no significant mortality of the tested macroinvertebrates (Fig. 2). Although the concentrations used in the experiments were planned to be approximately ten times lower than the acute LC_{50} values (Table 2), in some cases they were lower or higher than

Table 2 Median lethal concentrations LC_{50} values and respective 95% confidence intervals in parentheses (μg/L)

Toxicant	LC_{50} for 96 h (95% confidence interval), μg/L		
	Baetis rhodani	*Simulium latigonium*	*Gammarus pulex*
Thiacloprid	4.60 (3.74–5.66)	NA	350 (210–570)
Imidacloprid	*8.49 (4.45–16.20)	3.73 (1.54–9.05)	270 (170–450)
Acetamiprid	NA	3.73 (1.54–9.05)	50.0 (30.0–90.0)
Iprodione	NA	480 (360–220)	3460 (2090–5720)
Fenvalerate	NA	0.12 (0.04–0.37)	0.17 (0.09–0.34)
Indoxacarb	*48.5 (NR)	NA	2520 (1330–4770)
Azoxystrobin	NA	NA	270 (170–450)
Tebufenpyrad	*2.69 (1.41–5.12)	NA	24.1 (11.1–52.5)
Fenoxycarb	NA	550 (NR)	1730 (970–3100)
Cyprodinil	NA	NA	690 (460–1040)
Prochloraz	NA	NA	2180 (1140–4180)

* LC_{50} for 48 h ($\geq$ 10% mortality in the control after 48 h)

NA – not assessed because the number of animals was limited

NR – confidence intervals are not reliable

this level, because precise LC_{50} values were not known at the time of the experiment. As a result, the range of the concentrations at which drift was observed in the experiments is 7–22 times lower than the respective acute LC_{50} values. However, the threshold values of the drift-initiating concentrations remained to be evaluated, as the present experiments were not designed to derive such threshold values.

All the toxicants that exhibited a drift-initiating effect are neurotoxic insecticides, except the fungicide iprodione. The pesticides that did not initiate drift were the fungicides cyprodinil, prochloraz, and azoxystrobin, the juvenile hormone mimic fenoxycarb, and the pyrazole insecticide tebufenpyrad, which affects cell energy production.

Notably, for all the toxicants exhibiting drift-initiating activity the drift of the tested animals was already detected within 2 h after contamination. Maximum drift percentages were detected 4 h after contamination (Fig. 2). During subsequent observation periods (22–48 h after contamination) the drift responses became less pronounced.

Discussion

Although a number of studies have shown that various toxicants can initiate drift of stream macroinvertebrates, none of them was intended to reveal which toxicants do initiate drift and which do not. Nevertheless, the studies by Liess (1993) and Schulz and Dabrowski (2001) have shown that the potential to trigger downstream drift is unequal for different substances.

The present results suggest that neurotoxic insecticides have pronounced drift-initiating potential, as all of the tested neurotoxicants initiated drift in stream microcosms (five substances, Fig. 2). This is in agreement with previously published investigations, most of which have demonstrated a drift-initiating action of neurotoxic insecticides such as pyrethroids, organochlorines, and organophosphates (Muirhead-Thomson 1978; Cuffney et al. 1984; Wallace et al. 1989; Kreutzweiser and Sibley 1991; Davies and Cook 1993; Liess 1993; 1994; Breneman and Pontasch 1994; Schulz and Liess 1999b; Hose et al. 2002; Lauridsen and Friberg, 2005).

The neurotoxic insecticides that initiated drift in the present study were the pyrethroid fenvalerate and the neonicotinoids thiacloprid, imidacloprid, and acetamiprid. As mentioned above,the drift-initiating action of pyrethroids has been shown previously by a number of studies. In contrast, the drift-initiating potential of neonicotinoids had not previously been investigated. Little is known about the potential environmental effects of this new class of insecticides (Beketov and Liess 2008). However, previously the authors observed pronounced drift of *Simulium latigonium* in large outdoor stream mesocosms contaminated with thiacloprid at a concentration below the respective acute LC_{50} value (unpublished data, experiment description in Beketov et al. submitted).

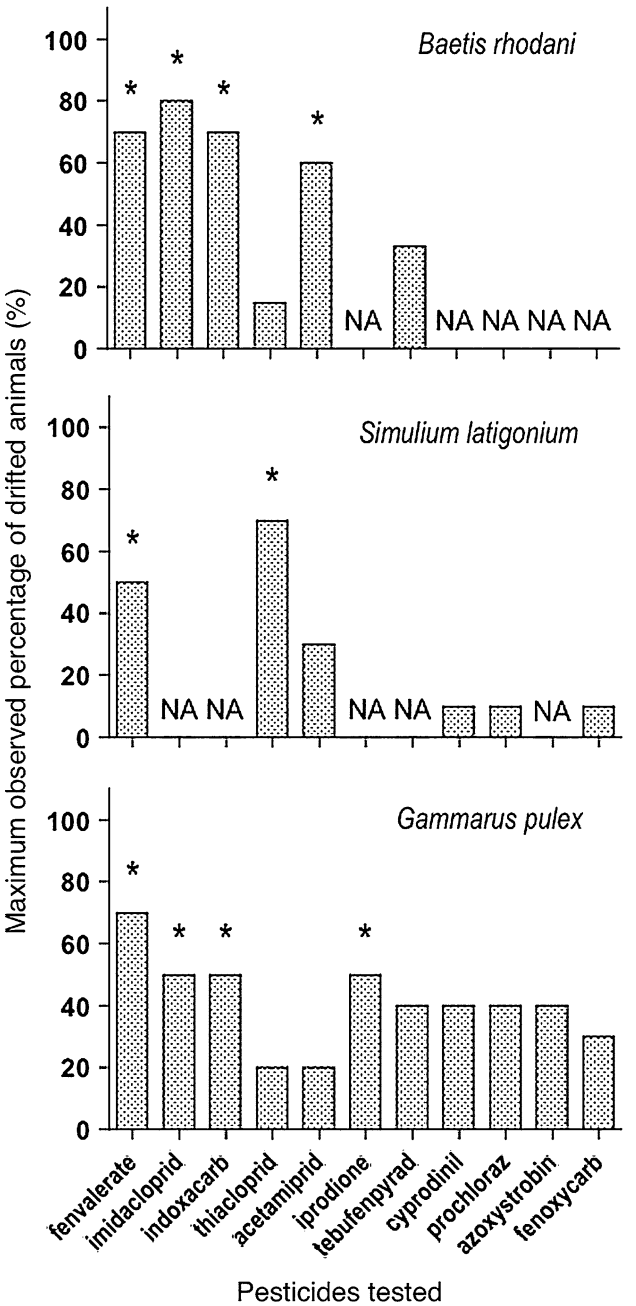

Fig. 2 Maximum observed drift responses of *Baetis rhodani*, *Simulium latigonium*, and *Gammarus pulex* to the sublethal concentrations (approximately 10 times below the acute LC_{50} values) of 11 investigated pesticides observed during 4 h after contamination (percentage of drifted animals as a difference from control). Asterisks indicate significant ($p < 0.05$) differences from the respective controls assessed as proportion of drifted individuals by contingency tables, chi-square test. The drift-initiating toxicants are grouped towards the left side of the graph

The rare examples of drift-initiating action of lampricides (Dermott and Spence 1984) and copper (Taylor et al. 1994) support the observation of this study that not only neurotoxic insecticides can trigger drift of stream macroinvertebrates. However, to our knowledge the physiological action of the fungicide iprodione, for which the drift response was found in the present study, has not been investigated for invertebrates.

The interpretation of the results for the six pesticides for which drift-initiating action was not detected in the present study should take into account that a single concentration was tested per each pesticide. Hence, the possibility of finding drift-initiating action of these toxicants at concentrations higher than those tested in this study should not be excluded. However, at such higher concentrations mortality and immobilisation may mask initiation of drift.

The tested concentrations of the pesticides thiacloprid, imidacloprid, acetamiprid, fenvalerate, azoxystrobin, fenoxycarb, and cyprodinil can be considered as field relevant (Table 1). These concentrations are within the concentration ranges of these chemicals found in surface waters (Schaefer et al 1987; Liess et al. 1999; Schmuck 2001; Liess and Ohe 2005; Vega et al 2005; Schäfer et al 2007). Although no information about the water concentrations of indoxacarb and tebufenpyrad was found in literature, the tested concentrations of these two pesticides are most likely field relevant except the high indoxacarb concentration tested with *G. pulex* (300 μg/L, Table 1), because these concentrations are within the usual ranges of insecticide concentrations in surface waters (e.g., Liess and Ohe 2005; Schäfer et al. 2007) and close to the predicted environmental concentrations (PEC) derived in FOCUS exposure models for surface waters. Thus, the PEC values used in an environmental risk assessment in the context of the European Union (EU) Active Substances Program are 0.34 μg/L for tebufenpyrad applied for apples and pears and 4.7 μg/L for indoxacarb applied for pome fruit [Umweltbundesamt (German Federal Environmental Agency), personal communication]. Only the fungicides iprodione and prochloraz were tested at concentrations, which are notably higher than the field water concentrations reported previously, which were 0.1 μg/L (Urbatzka et al. 2007) and 2 μg/L (Kreuger 1998), respectively.

Concerning the dynamic of the drift, all the toxicants that exhibited drift-initiating activity triggered the drift of the tested animals within 2 h after contamination. This implies that macroinvertebrate drift can be initiated by short-term, pulse exposures to neurotoxic insecticides, which are typical of flowing waters (e.g., Liess et al. 1999; Liess and Ohe 2005).

It is currently unclear how to value organismal drift in the field situation. On one hand, drift can strongly reduce population density at concentrations far below the LC_{50} values, as animals may become dislodged and carried downstream in considerable amounts (Brittain and Eikeland 1988; Kreutzweiser and Sibley 1991; Schulz and Liess 1999a). On the other hand, individuals may protect themselves by avoiding exposure through drift. For instance, the presence of sensitive gammarids in periodically contaminated streams may only be explained by their high drifting mobility in conjunction with their ability to move rapidly upstream (Liess and Ohe 2005). This suggests that at the organism level drift may be an avoidance behaviour (Liess 1994) resulting in an effective protection of individuals (Schulz and Liess 1999b). However, at the community level this may contribute to an alteration of the taxonomic structure (Liess and Ohe 2005) and also affect functional properties of exposed communities, e.g., by reducing leaf decomposition (Schäfer et al. 2007).

In conclusion, the present results imply that the possibility of drift-initiating effects of pesticides should be considered in the ecotoxicological risk assessment of pesticides, since many of these toxicants can initiate drift of stream macroinvertebrates already at sublethal concentrations (e.g., 7–22 times lower than the respective acute LC_{50} values, as shown in the present study). Particularly the drift-initiating potential of neurotoxic insecticides should be taken into account, because this group of pesticides exhibited strong drift-initiating effects on stream-dwelling insects and crustaceans. In environmental risk assessment, the detecting of the drift-initiation action of pesticides can be performed using a standardised laboratory test system such as the microcosms applied in the present study.

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